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Pharmaceutical Sciences **RESEARCH ARTICLE……!!!**

SIMULTANEOUS DETERMINATION AND VALIDATION OF LEVOFLOXACIN AND PHENYLPROPANOLAMINE BY AREA UNDER CURVE AND Q – ABSORBANCE RATIO METHODS IN BULK AND PHARMACEUTICAL FORMULATIONS

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KEYWORDS:

Levofloxacin, Phenylpropanolamine, Area under Curve Method, Q – Absorbance ratio Method.

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ABSTRACT

In the present work two simple and sensitive UV spectrophotometric methods have been developed for the simultaneous estimation of Levofloxacin and Phenylpropanolamine in bulk drug and Pharmaceutical formulations. Here distilled water is used as solvent throughout the experiments. Beer's law obeyed in the concentration range of 3-15µg/ml and 15-75µg/ml for Levofloxacin and Phenylpropanolamine respectively. Method A: Area under curve method is based on the measurement of area at selected analytical wavelength ranges and performing the analysis using "Cramer's Rule" and "Matrix method". Two analytical wavelength ranges selected were 282 to 292nm and 251 to 261nm for the estimation of Levofloxacin and Phenylpropanolamine with r^2 = 0.9993, % RSD = 0.3845-1.3806 and r^2 = 0.9996, %RSD = 0.2269-1.1236, for Levofloxacin and Phenylpropanolamine respectively. LOD and LOQ was found to be 0.1559µg/mL and 0.4724µg/mL for Levofloxacin and 0.5612µg/ml and 1.7007µg/mL for Phenylpropanolamine respectively. Method B: Q – Absorbance ratio method is based the measurement of absorbances at two selected wavelengths. One being the λ_{max} of one of the two components (λ_2) and other being a wavelength of equal absorptivity of the two components (λ_1), i.e. an Isoabsorptivity point. Here we selected 287nm as one wavelength, which is the λ_{max} of Levofloxacin and another wavelength 267nm, which is the iso absorptive point of both drugs with r^2 = 0.9995, %RSD = 0.2234-1.3771 and r^2 = 0.9995, %RSD = 0.4576-1.3996, for Levofloxacin and Phenylpropanolamine respectively. LOD and LOQ was found to be 0.1505µg/ml and 0.4563µg/ml for Levofloxacin and 0.7249µg/ml and 2.1969µg/ml for Phenylpropanolamine respectively.

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INTRODUCTION:

Levofloxacin[1-10] 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 [1-10] 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 individually 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[11-16].

MATERIALS 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

100mg of Levofloxacin (LEVO) and Phenylpropanolamine (PPA) was weighed and transferred to two different 100 ml volumetric flask. Drug was dissolved in 50 ml distilled water by ultrasonication and volume was made upto the mark with distilled water to obtained final concentration of 1000µg/ml (stock A).

From the above stock A solution, 10ml of aliquot was pipetted out into two different 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 from 3-15µg/ml for LEVO and 15-75µg/ml for PPA.

Preparation of sample solution

20 tablets which contains both LEVO and PPA were weighed and powdered. The tablet powder equivalent to 100 mg of PPA 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 Estimation:

Method A: Area under curve:

The AUC (area under curve) method is applicable when there is no sharp peak or when broad spectra are obtained. It involves calculation of the integrated value of absorbance with respect to the wavelength between the two selected wavelengths 282 and 292nm (LEVO) and 251and 261nm (PPA). The area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. This wavelength range is selected on the basis of repeated observations, so as to get the linearity between the area under curve and concentration.

Method B: Q – Absorbance Ratio:

Absorption ratio method uses the ratio of absorptions of two selected wavelength, one of which is iso-absorptive point and other being the λmax of one of the two components. From the overlain spectra of two drugs (as shown in fig:6), it shows that LEVO and PPA having isoabsorptive point at 267 nm. The second wavelength used is 287 nm, which is the λmax of LEVO. The absorptivity coefficient were calculated using calibrations curve.

A set of two equations were framed using the mean absorptivity.

$$Cx = \frac{Qm - Qy}{Qx - Qy} * \frac{A_1}{ax_1}$$

$$Cy = \frac{Qm - Qx}{Qy - Qx} * \frac{A_1}{ay_1}$$

$$Q_m = \frac{\text{Absorbance of Sample solution at 287nm } (\lambda_2)}{\text{Absorbance of Sample solution at 267nm } (\lambda_1)}$$

$$Q_x = \frac{\text{Absorbance of LEVO at 287nm}}{\text{Absorbance of LEVO at 267nm}}$$

$$Q_y = \frac{\text{Absorbance of PPA at 287nm}}{\text{Absorbance of PPA at 267nm}}$$

Where, Qx and Qy are value of LEVO and PPA respectively, ax_1 and ay_1 are absorptivity value at iso-absorptive point for LEVO and PPA.

VALIDATION PARAMETER:

Linearity

In Method A the linearity of Area under curve method was found to be 3-15µg/ml and 15-75µg/ml with correlation coefficients of 0.9993 at 282-292nm for LEVO and 0.9996 at 251-261nm for PPA respectively. Calibration curves are shown in (fig: 4 and 5)

In Method B the linearity of Q-Absorbance ratio method was found to be 3-15µg/ml and 15-75µg/ml with correlation coefficient of 0.9995 at 267nm for PPA (Isoabsorptivity) and 0.9995 at 287nm for LEVO respectively. Calibration curves are shown in (fig:7 and 8). Summary of validation parameters by developed methods as shown in Table no 1.

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 analytical drug 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.

RESULTS AND DISSCUSION:

The selected drugs LEVO and PPA in Bulk and Formulation were estimated by using both Area under curve and Q-Absorbance ratio of UV spectrophotometric methods as per ICH guidelines. The linearity range in both methods for LEVO and PPA was 3-15µg/ml and 15-75µg/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 were validated by recovery studies and was found to be significant and within specification limits, with % recovery 99-102%. The assay results were found to be within the acceptable limits.

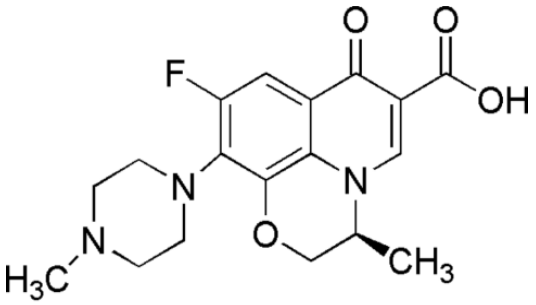

Fig 1 : Chemical structure of Levofloxacin.

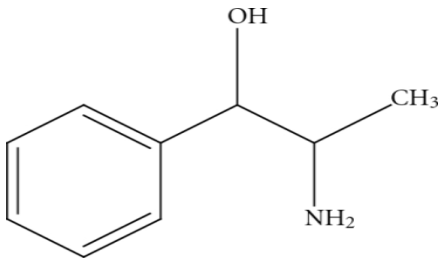

Fig 2: Chemical structure of Phenylpropanolamine.

Fig 3: Spectra of LEVO and PPA for Area under curve method at 282-292nm and 251-261nm

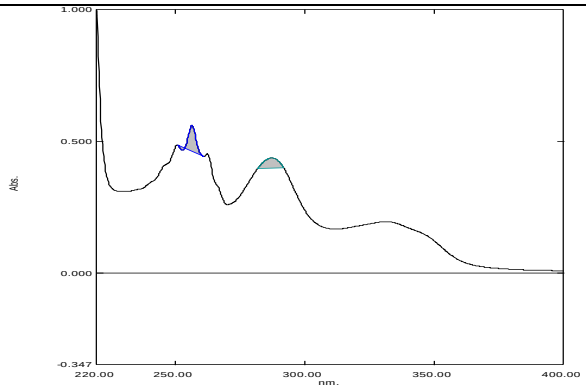

Fig 4: Calibration curve for LEVO between 282-292nm by Area under curve method

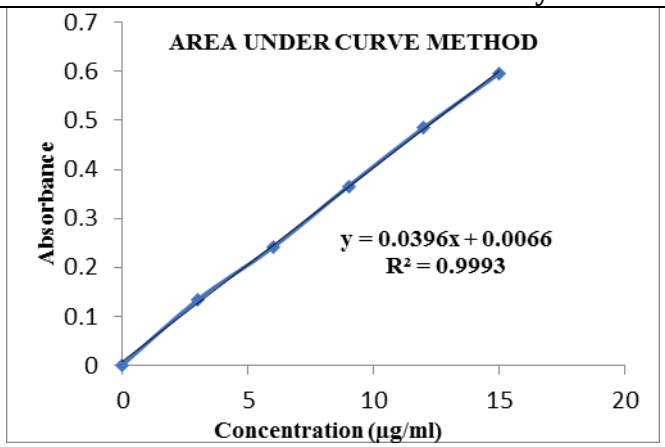

Fig 5: Calibration curve for PPA between 251-261nm by Area under curve method.

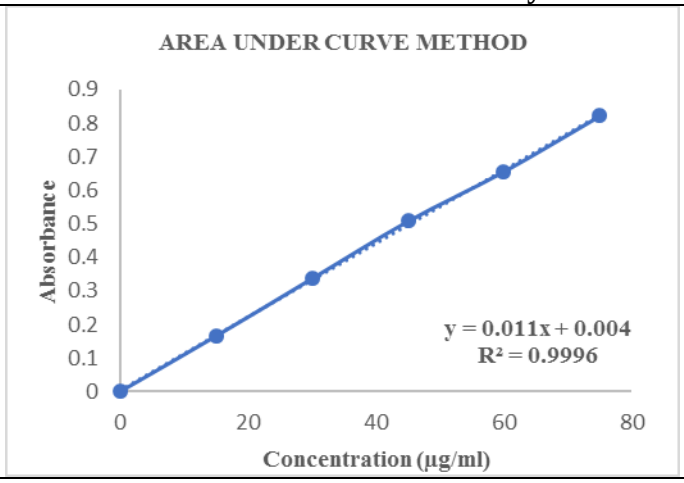

Fig 6: Isoabsorptive point of LEVO and PPA at 267nm

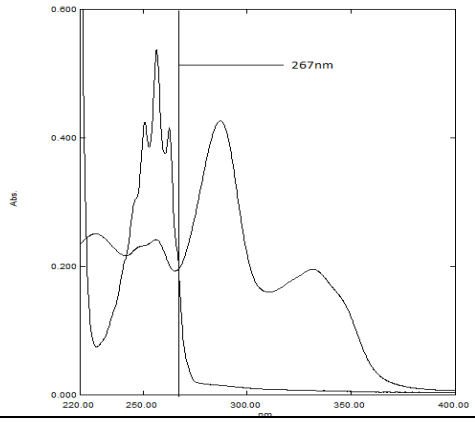

Fig 7: Calibration curve of PPA at 267nm by Q-Absorption ratio method

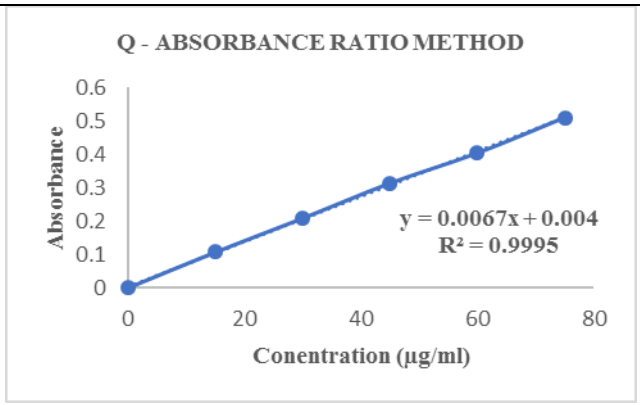

Fig 8: Calibration curve of LEVO at 287nm by Q-Absorption ratio method

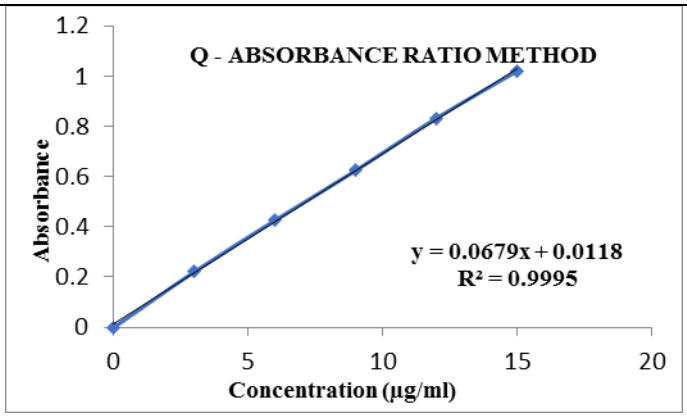

Table 1: Summary of Validation Parameters by Developed Methods.

Parameter	Method A		Method B	
	LEVO	PPA	LEVO	PPA
Wavelength (nm)	282-292	251-261	287	267
Linearity Range (µg/ml)	3-15	15-75	3-15	15-75
Regression equation (y = a + bc)	y = 0.0396x + 0.0066	y = 0.011x + 0.004	y = 0.0679x + 0.0118	y = 0.0067x + 0.004
Slope (b)	0.0396x	0.011x	0.0679x	0.0067x
Intercept (a)	0.0066	0.004	0.0118	0.004
Correlation Coefficient (r^2)	0.9993	0.9996	0.9995	0.9995
LOD (µg/ml)	0.1559	0.5612	0.1505	0.7249
LOQ (µg/ml)	0.4724	1.7007	0.4563	2.1969

Table 2: Statistical Validation Data for Accuracy Determination.

Level of % Recovery	Components	Amount present (µg/ml)	Amount of Standard drug added (µg)	Method A			Method B		
				Total amount recovered (µg)	% Recovery	RSD	Total amount recovered (µg)	% Recovery	RSD
80%	LEVO	6	4.8	10.79	99.96	0.1928	10.77	99.72	0.0928
	PPA	30	24	53.99	99.93	0.0466	53.97	99.92	0.0185
100%	LEVO	6	6	12.02	100.02	0.1734	12.04	100.08	0.2497
	PPA	30	30	60.02	100.01	0.0346	60.02	100.01	0.0509
120%	LEVO	6	7.2	13.11	99.59	0.2671	13.17	99.74	0.1160
	PPA	30	36	65.97	99.95	0.0151	65.99	99.98	0.0381

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

Components	Method A		Method B	
	LEVO	PPA	LEVO	PPA
Mean	99.66	99.92	99.66	99.92
Standard Deviation	0.4714	0.0828	0.4713	0.0720
Relative Standard Deviation	0.4729	0.0828	0.4728	0.0720
Standard Error	0.1924	0.0338	0.1924	0.0294

n*= 3

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

Components	Method A		Method B	
	LEVO	PPA	LEVO	PPA
Mean	99.65	99.92	99.66	99.92
Standard Deviation	0.4542	0.0866	0.4722	0.0720
Relative Standard Deviation	0.4558	0.0866	0.4738	0.0720
Standard Error	0.1855	0.0353	0.1928	0.0294

n^*=3

CONCULSION:

The developed Area under curve and Q- Absorbance ratio 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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