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

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LEVOFLOXACIN AND PHENYLPROPANOLAMINE IN PHARMACEUTICAL DOSAGE FORM

Pratima Kumari Jain J*, Anil Kumar S.M, Satish Kumar Shetty A, Manzoor Ahmed, Vijaya Krishna C Aradhya and Siddalinga Swamy M.S.
Department of Pharmaceutical Analysis, National College of Pharmacy (NCP), Shimoga - 577201, Karnataka, India.

KEYWORDS:

Levofloxacin, Phenylpropanolamine, RP – HPLC, Validation.

FOR CORRESPONDENCE:

Pratima Kumari Jain J*

ADDRESS:

Department of Pharmaceutical Analysis, National College of Pharmacy, Shimoga - 577201, Karnataka, India.

ABSTRACT

A simple, accurate, precise and rapid RP -HPLC method for the determination of Levofloxacin and Phenylpropanolamine was developed and validated as per ICH guidelines. Levofloxacin (LEVO) and Phenylpropanolamine (PPA) were separated by RP -HPLC using a Shimadzu C_{18} column (5µm, 250mm x 4.6mm i.d.) and isocratic elution with a flow rate 1 ml/min. Mixture of Phosphate buffer, Methanol and Acetonitrile in the ratio of (40:20:40 v/v), pH adjusted with orthophosphoric acid to 4.5 was used as mobile phase. The UV detection was done at 267nm. The retention time of Levofloxacin and Phenylpropanolamine was found to be 2.872 min and 6.349 min respectively. The linearity range found to be 4-20 µg/ml and 20-100 µg/ml with (r^2 = 0.9998, and r^2 = 0.9994) for Levofloxacin and Phenylpropanolamine respectively. The LOD and LOQ for Levofloxacin was found to be 0.2064 µg/ml and 0.6255 µg/ml respectively. The LOD and LOQ for Phenylpropanolamine was found to be 0.3059 µg/ml and 0.9270 µg/ml respectively. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision and Robustness.

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INTRODUCTION:

Levofloxacin [1-5] 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 [6-10] 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 [11-16]. Phenylpropanolamine alone has been estimated and simultaneous estimation in combination with other drugs has been reported [16-20]. 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 HPLC method for simultaneous estimation of titled drugs and extend it for their determination in formulations [11-20].

MATERIALS AND METHODS:

Instrument:

A high-performance liquid chromatography system (SHIMADZU Corporation, LC-20 AD), a Shimadzu SPD-20A UV/VIS detector was used for analysis. The data was recorded using Lab Solution Software.

Chemicals and Reagents:

Acetonitrile (HPLC grade) was procured from Merck Ltd, double distilled water (HPLC grade) was procured from Spectrochem PVT. Ltd. Mumbai, Potassium dihydrogen O-Phosphate. Methanol (HPLC grade) was procured from s d fine – chem Ltd, Marathon Icon, Lower Parel, Mumbai, All other chemical reagents were of analytical grade.

Drug Sample:

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

Preparation of mobile phase:

The mobile phase (1000 ml) was prepared by mixing of Phosphate Buffer, Methanol and Acetonitrile in the ratio of 40:20:40v/v pH adjusted to 4.5 with orthophosphoric acid. The mobile phase was sonicated for 20 min and then it was filtered through a 0.45 µm membrane filter paper.

Preparation of buffer solution:

6.804 g of Potassium dihydrogen phosphate was dissolved into 1000 ml of HPLC water (0.05M).

Preparation of standard stock solution:

100 mg each of LEVO and PPA were weighed separately and transferred in two different 100 ml volumetric flasks. Both the drugs were dissolved in 50 ml of mobile phase by sonication and then volume was made up to the mark with mobile phase to get a concentration of 1000 µg/ml of each component (stock A and A^1 solution).

From the above stock A and A^1 solution 10 ml of aliquot was pipetted out in a 100 ml volumetric flask and the volume was made up to the mark with mobile phase to get a concentration of 100 µg/ml of each component (stock B and B' solution).

Sample Preparation:

Twenty tablets were weighed and their average weight was determined. The tablets were crushed to fine powder and tablet powder equivalent to one tablet weight of 25 mg PPA was weighed which also contains 5 mg of LEVO and transferred to 100 ml volumetric flask, dissolved in 50 ml of mobile phase and the content was kept in sonicator for 15 min. The solution was filtered through 0.4 µm membrane filter paper. The finally the volume was made up to the mark with mobile phase to get a concentration of 250 µg/ml PPA and 50 µg/ml of LEVO and this solution was used as stock A^1 solution of the sample.

From the above stock A^1 solution, 40 ml of the aliquot was pipetted out and transferred to a 100 ml volumetric flask. The volume was made up to 100 ml with mobile phase to obtain a concentration of 100 µg/ml of PPA (stock B' solution of the sample) and which also contain 20 µg/ml of LEVO.

Appropriate aliquots were pipetted out from the sample stock B' solution (100 µg/ml) in to a series of 10 ml volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range of 4, 8, 12, 16 and 20 µg/ml of LEVO and 20, 40, 60, 80 and 100 µg/ml of PPA.

A 20 µl volume of each sample mixture was injected in to the sample injector of HPLC system and their chromatograms were recorded under the same chromatographic conditions as described earlier. The area of each peak was determined at 267 nm and the amount of drug present in the sample mixture was determined. Under this chromatographic condition Retention of LEVO was found to be 2.872 min (fig.3) and PPA was found to be 6.349 min (fig.4).

RESULTS AND DISCUSSION

The developed method for determination of Levofloxacin and Phenylpropanolamine was further validated by using the following parameters:

Linearity:

Linearity was established by least square regression analysis of the calibration curve. The constructed calibration curve was linear over the concentration range of 4-20 µg/ml for LEVO and 20-100 µg/ml for PPA respectively. Peak area of LEVO and PPA were plotted versus their respective concentration and linear regression analysis was performed on the resultant curves (fig.6 & fig.7). The regression equation was found to be y = 29909x - 1904.8 (r^2 = 0.9998) for LEVO and y = 20622x – 11810 (r^2 = 0.9994) for PPA. Summary of Validation Parameters by developed method shown in Table 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 formulation at levels of 80%, 100% and 120% of the test concentration. The results are tabulated in Table 2.

Precision

The Intraday and Interday precisions of the proposed HPLC method was 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 of LEVO and PPA and reported in terms of relative standard deviation (RSD). Statistical validation data for Intraday and Interday precision methods as shown in Table 3 and Table 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 calibration curve.

The LOD and LOQ for Levofloxacin was found to be 0.2064 µg/ml and 0.6255 µg/ml respectively. The LOD and LOQ for Phenylpropanolamine was found to be 0.3059 µg/ml and 0.9270 µg/ml respectively.

Robustness

The evaluation of robustness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of analysis with respect to deliberate variations in method parameters. The solution containing 8 µg/ml of LEVO and 40 µg/ml of PPA was injected into sample injector of HPLC three times under different parameters like deliberate variations in flow rate and wavelength. The Robustness results for variation in flow rate (ml/min) and wavelengths as shown in table 5 and table 6.

Ruggedness

The evaluation of ruggedness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of analysis with respect to deliberate variations in analyst or instrument. The solution containing 8 µg/ml of LEVO and 40 µg/ml of PPA was injected into sample injector of HPLC three times by different analysts. Ruggedness result for variations in Analyst as shown in table 7.

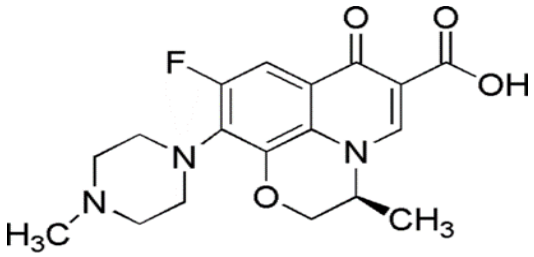

Fig 1 : Chemical structure of Levofloxacin.

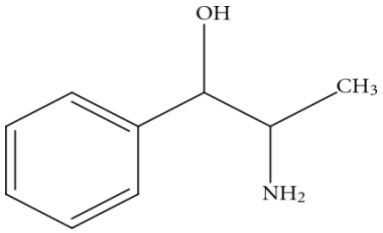

Fig 2 : Chemical structure of Phenylpropanolamine.

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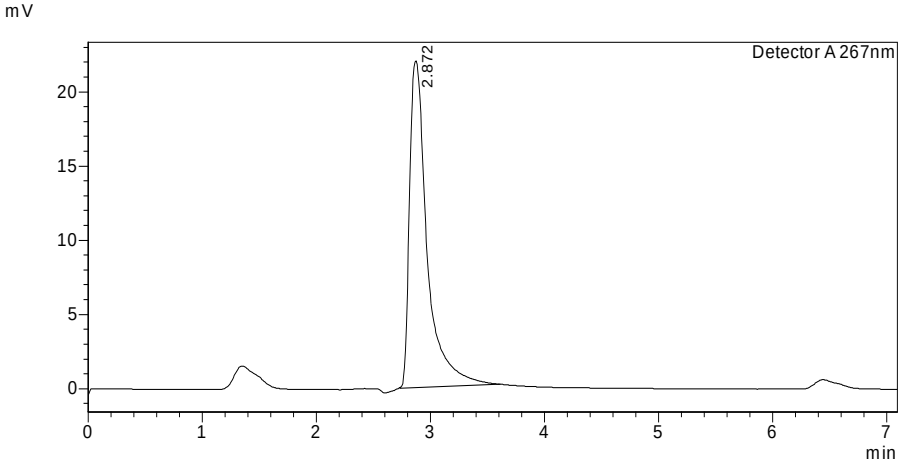

Fig. 3: Chromatogram Showing Retention Time of Levofloxacin.

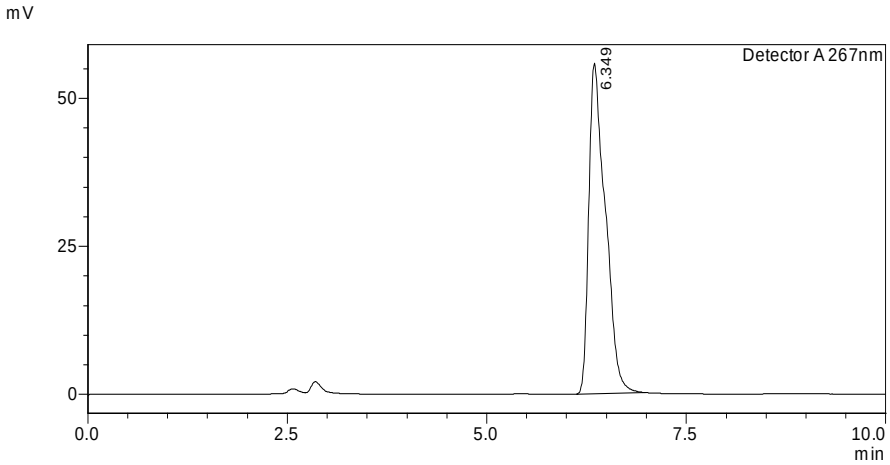

Fig. 4: Chromatogram Showing Retention Time of Phenylpropanolamine.

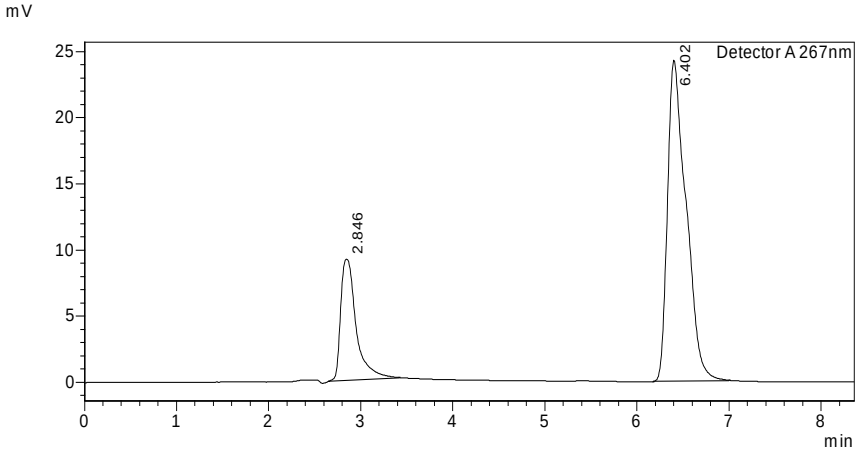

Fig. 5: Chromatogram Showing Retention Time of Levofloxacin and Phenylpropanolamine.

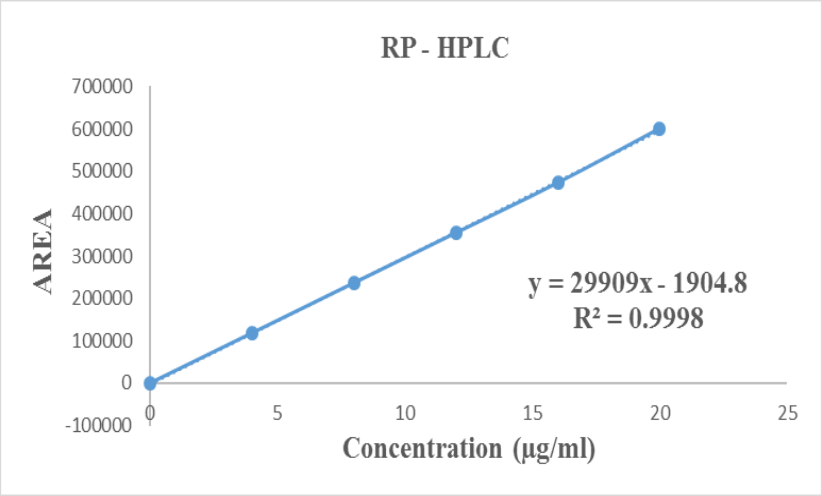

Fig. 6: Calibration Curve of LEVO at 267 nm by RP – HPLC Method.

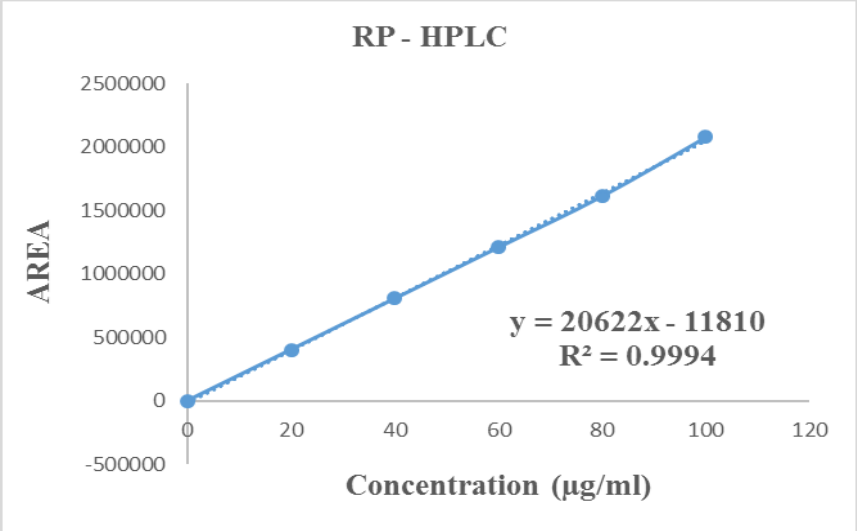

Fig. 7: Calibration Curve of PPA at 267 nm by RP – HPLC Method

Table no. 1: Summary of Validation Parameters by Developed Methods.

Parameters	LEVO	PPA
Linearity Range (µg/ml)	4-20	20-100
Slope	29909x	20622x
Intercept	1904.8	11810
Regression Coefficient (r^2)	0.9998	0.9994
Limit of Detection (µg/ml)	0.2064	0.3059
Limit of Quantification (µg/ml)	0.6255	0.9270
Retention time (min)	2.872	6.349
Tailing factor	1.612	1.391
Resolution factor	11.155	
Theoretical plate	2138	4470

Table no. 2: Statistical Validation Data for Accuracy determination.

Level of % Recovery	Components	Amount present (µg/ml)	Amount of standard drug added (µg)	Total amount recovered (µg)	% Recovery	RSD
80%	LEVO	8	6.4	14.39	99.86	0.0695
	PPA	40	32	71.99	99.97	0.0138
100%	LEVO	8	8	15.99	99.87	0.0625
	PPA	40	40	79.99	99.97	0.0125
120%	LEVO	8	9.6	17.59	99.88	0.0568
	PPA	40	48	87.99	99.97	0.0113

Table no. 3: Statistical Validation Data for Intra-day Precision.

Component	Mean*	Standard Deviation*	Relative Standard Deviation*	Standard Error*
LEVO	99.72	0.3104	0.3112	0.1267
PPA	99.95	0.0707	0.0707	0.0288

***n=6**

Table no. 4: Statistical Validation Data for Inter-day Precision.

Components	Mean*	Standard Deviation*	Relative Standard Deviation*	Standard Error*
LEVO	99.73	0.3178	0.3187	0.1297
PPA	99.94	0.0615	0.0615	0.0251

***n=6**

Table no. 5: Robustness Results for Variation in Flow Rate (ml/min).

Method Parameter	Level	Retention Time		Tailing factor	
Flow Rate (ml/min)		**LEVO**	**PPA**	**LEVO**	**PPA**
0.9	-1	2.889	6.856	1.628	1.403
1.0	0	2.872	6.349	1.612	1.391
1.1	+1	2.865	6.335	1.605	1.389

Table no. 6: Robustness result for variations in Wavelength (nm).

Method Parameter	Level	Retention Time		Tailing factor	
Wavelength (nm)		**LEVO**	**PPA**	**LEVO**	**PPA**
265	-2	2.882	6.368	1.615	1.393
267	0	2.872	6.349	1.612	1.391
269	+2	2.859	6.339	1.606	1.389

Table no. 7: Ruggedness result for variations in Analyst.

Method Parameter	Retention Time		Tailing factor	
Analysts	**LEVO**	**PPA**	**LEVO**	**PPA**
Analysts 01	2.872	6.349	1.612	1.391
Analysts 02	2.879	6.355	1.620	1.384

CONCLUSION

An accurate and precise HPLC method with UV detection for the simultaneous estimation of Levofloxacin and Phenylpropanolamine was developed and validated for quality control analysis in combined tablets. It is convenient for laboratory quality control of tablet dosage forms containing both substances.

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