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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LEVOFLOXACIN HEMIHYDRATE AND AMBROXOL HYDROCHLORIDE IN THE PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A new simple, rapid, specific, accurate and precise Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method has been developed for the simultaneous estimation of Levofloxacin hemihydrate and Ambroxol hydrochloride in the combined pharmaceutical dosage form. The chromatographic separation for Levofloxacin hemihydrate and Ambroxol hydrochloride was achieved with mobile phase containing 0.1% Triethyl amine (pH adjusted to 5 with Orthophosphoric acid) and Acetonitrile (50:50 % v/v), Universil C18 column (150 × 4.6mm i.d, particle size of 5µ) at room temperature and UV detection at 250 nm. The compounds were eluted in the isocratic mode at a flow rate of 0.6ml/min. The retention times of Levofloxacin hemihydrate was at 4.8±0.059 min and Ambroxol hydrochloride was at 5.7±0.090 min. The above method was validated in terms of linearity, accuracy, precision, LOD, LOQ in accordance with ICH guideline.

KEYWORDS: Levofloxacin hemihydrate, Ambroxol hydrochloride, simultaneous equation method, RP-HPLC, Validation.

INTRODUCTION

Levofloxacin hemihydrates is broad spectrum antibiotic of flouroquinoline drug class and levo isomer of oflaxacin. Levofloxacin alone or in combination with other antibiotics used to treat certain infection including pneumonia, urinary tract infections and abdominal infections. Chemically it is (2S)-7-fluoro-2-methyl-(4-methylpiperazin- 1-yl)-10-oxo-4- oxa-1-azatricyclo [{5,13}] trideca-5(13),6,8,11- tetraene-11-carboxylic acid (Figure 1).

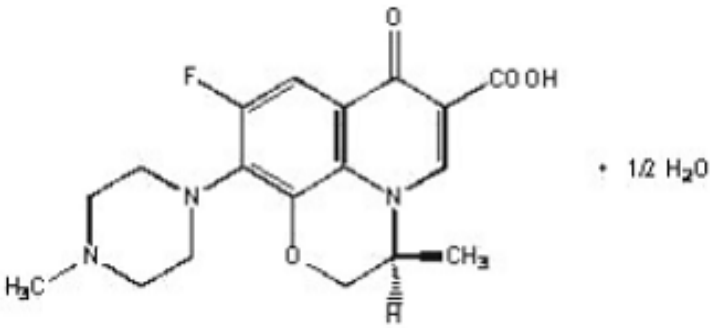

Figure 1: Structure of Levofloxacillin hemihydrate.

Ambroxol hydrochloride is a mucoactive drugwith several properties like scleretolytic amd sclerotomotoric actions that restore the physiological clearance from the respiratory tract. Chemically it is Cyclohexanol, 4-(N-(2-amino-3,5-Dibromobenzyl)amino)-,hydrochloride (Figure 2).

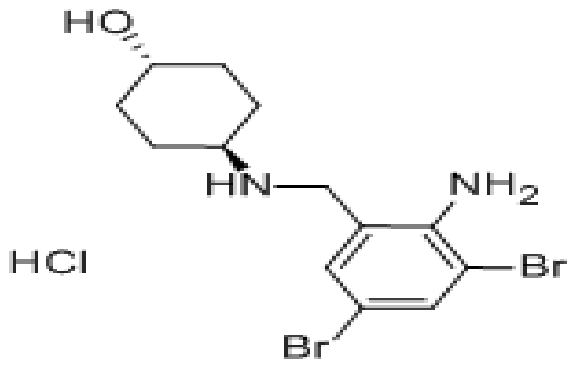

Figure 2: Structure of Ambroxol hydrochloride.

Both the drugs are marketed as combined dose tablet formulation in the ratio of 500:75mg LEV: AMB. Literature survey revealed that there are few methods reported for the simultaneous estimation of these drugs, individually or with other drugs using UV-spectrophotometry,[1-4] RP-HPLC[5-7]. Hence present study aim to developing a precise, linear, simple, rapid, validated and cost effective. RP HPLC method for the simultaneous estimation of these drugs in combined dosage forms.

MATERIALS AND METHODS

Chemicals

Analytically pure samples of LEV and AMB were obtained as gift samples from Akums labs (Mumbai). Tablets of brand "L-Cin A" having combination of LEV (500mg) and AMB (75mg) were purchased from local pharmacy. Acetonitrile (HPLC grade), Triethyl amine were obtained from Merck Specialities Pvt Ltd, Mumbai. Water (HPLC grade) was obtained from milli Q system.

Instrumentation and Chromatographic Conditions

HPLC method development and validation was done on a Shimadzu Liquid Chromatograph equipped with pump (LC 20 AD) and UV detector (LC 20 A) and LC solotions software. Stationary phase used was Universil C18 ODS 250mm x 4.6mm, 5µm.

Preparation of Mobile Phase

Acetonitrile: 0.1% Triethyl amine buffer pH 5 (50:50 v/v) was preparedand filtered through 0.45 µm membrane filter.

Preparation of standard stock solution

The standard stock solution 1mg/ml of LEV and AMB were prepared separately by dissolving 100mg of each drug in 100ml mobile phase. From the standard stock solution, mixed standard solution was prepared to contain 100 µg/ml of LEV and 100 µg/ml of AMB.

Preparation of sample solution

Twenty tablets were weighed and powder equivalent to 10 mg of Levofloxacin hemihydrate and 1.5 mg of Ambroxol hydrochloride was weighed and transferred into 100 ml volumetric flask. 8.5 mg of pure Ambroxol was added. It is extracted with mobile phase. The volumetric flask was sonicated for 20 minutes to affect the complete dissolution of the drugs and the solution was made up to the volume with mobile phase and filtered. 6ml solution was pipette and transferred to 100ml volumetric flask made volume upto mark with mobile phase to obtain concentration of 6 µg/ml of LEV and 6 µg/ml of AMB.

Validation of HPLC method

The method was validated for the parameters like linearity, precision, accuracy, LOD and LOQ.

Linearity studies were done by constructing calibration curves of LEV and AMB standard solutions with concentrations ranging from 2-16 µg/ml each and correlation coefficients determined.

To study accuracy of the method, recovery studies were done carried out by addition of standard drug solution to sample at three different levels 50%, 100%, 150% of the test concentration and the contents were reanalyzed by the proposed method.

Precision of the method was checked in respect to repeatability and ruggedness. The %RSD was determined by six replicate injections of same standard solution on the same day under same experimental conditions. Ruggedness was assessed by injecting the same standard on different days. % RSD was determined in order to assess the ruggedness of the method.

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined by injecting progressively low concentrations of the standard solutions.

RESULTS AND DISCUSSION

Method Development

The solutions of LEV and AMB were injectedinto the HPLC system and run in different solvent systems as mobile phases. Finally Acetonitrile: TEA buffer pH 5 (50:50%v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for both LEV and AMB. Representative chromatogram of mixed standard of LEV and AMB is shown in Figure 3.

From the standard stock solution further dilutions were done using mobile phase and scanned over the range of 200-400nm and the spectra were overlain. It was observed that at 250nm both LEV and AMB showed considerable absorbance and therefore selected as detection wavelength. Overlain spectra of both druge are shown in Figure 4.

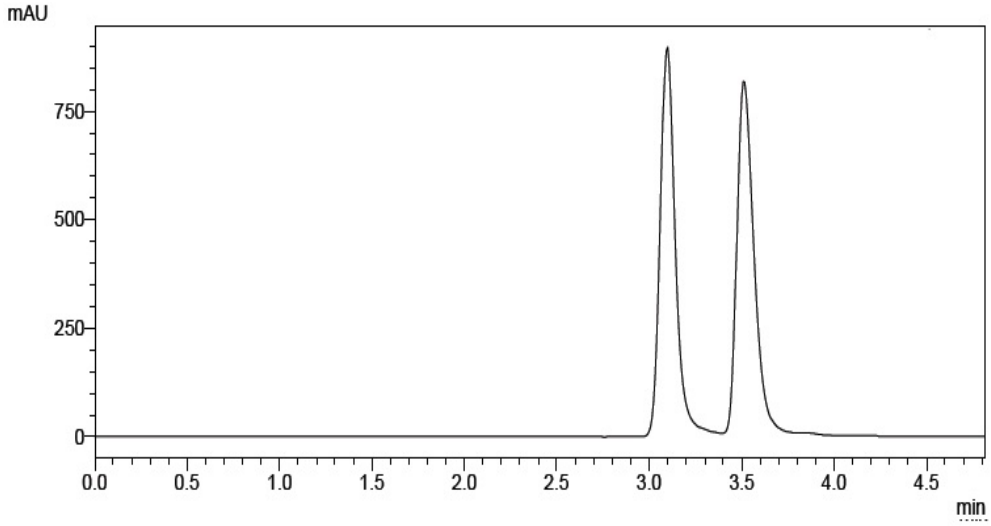

Figure 3: Chromatogram o Standard solution of LEV and AMB.

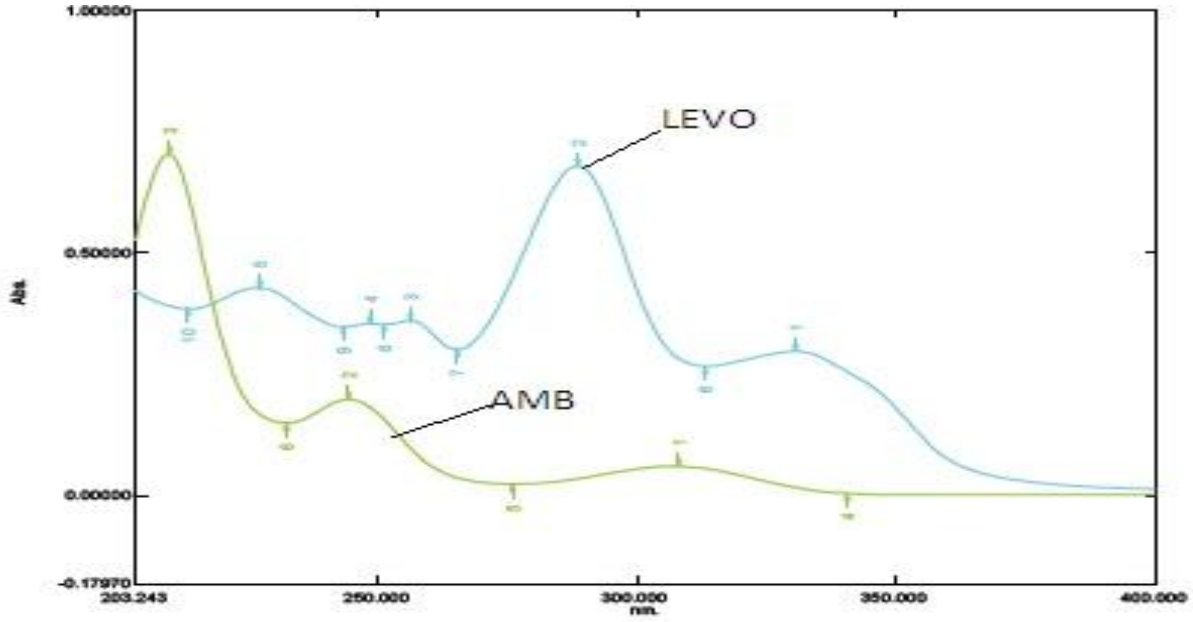

Figure 4: Overlain spectra of LEV and AMB.

Method validation

The method was validated as per ICH guidelines[8].

Linearity: the linear relationship was observed between the peak area and concentration over the range of 2-16 µg/ml for both the drugs. The method was proven to be linear in the above range as the correlation coefficient was 0.998 for both the drugs. Correlation coefficient, y-intercept, slope of regression line are shown in the Figure 5 and 6.

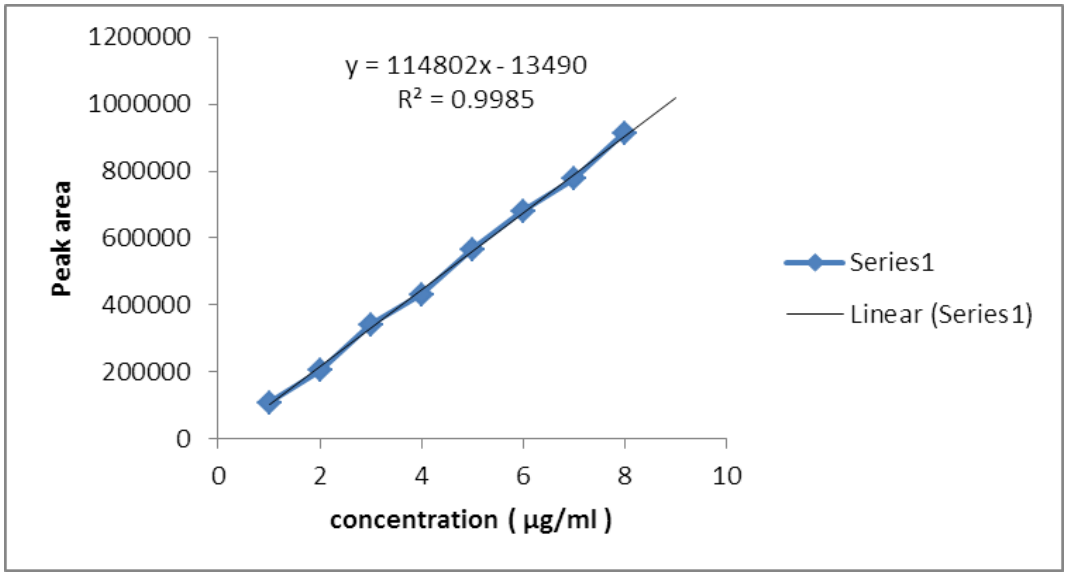

Figure 5: Calibration curve of LEV.

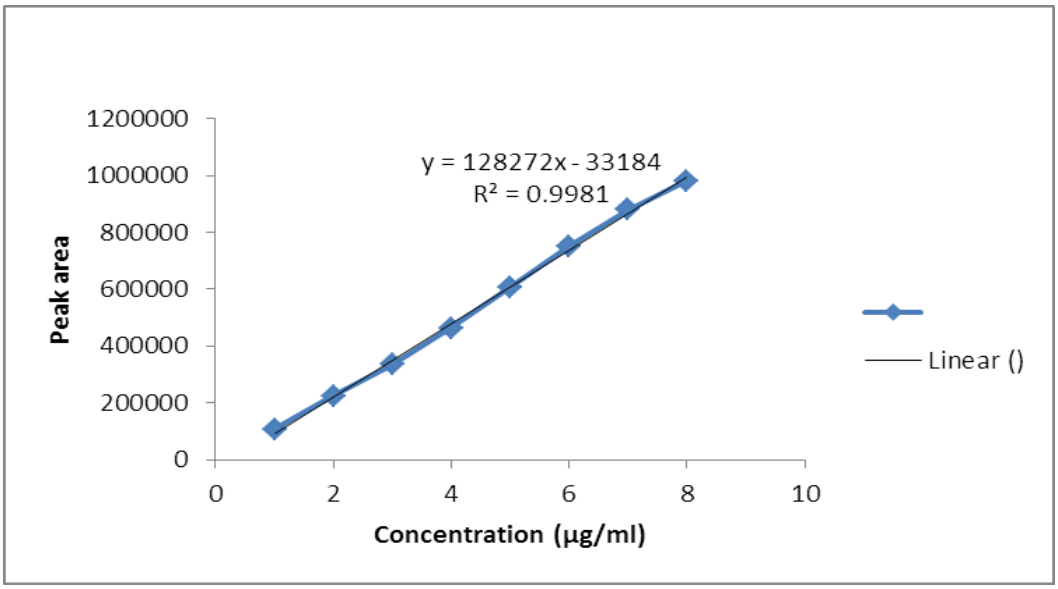

Figure 6: Calibration curve of AMB.

Accuracy: accuracy is generally assessed by analyzing the samples with the known concentration and comparing the measured value with the true value. The measured values were obtained by conducting recovery studies. % recoveries are shown in Table No. 1

Precision: the method was found to be precise as %RSD values for both repeatability and ruggedness were less than 2. The results of precision are given in Table No. 2 and 3.

LOD was determined to be 0.15µg/ ml and 0.18µg/ ml for LEV and AMB respectively.
LOQ for LEV and AMB was found to be 0.48µg/ ml and 0.59µg/ ml respectively.

Analysis of marketed formulation: the proposed method was evaluated in the assay of tablet formulation containing LEV and AMB. %assay were found to be 99.4% and 102% FOR LEV and AMB respectively, shown in Table No.4

Table No. 1: Accuracy of LEV and AMB.

Drugs	Amount taken µg /ml	Amount added µg/ ml	Total amount found µg/ ml	% Recovery
Levofloxacin	4	2	5.9	98.3
		4	7.9	98.7
		6	10.1	101
	6	3	9.1	101.1
		6	12.08	100.6
		9	14.9	99.3
Ambroxol	4	2	5.9	98.3
		4	7.9	98.7
		6	10.1	101
	6	3	9.1	101.1
		6	11.9	99.1
		9	15.1	100.6

Table No. 2: Repeatability of LEV and AMB.

Drug	Concentration (µg/ml)	% RSD
Levofloxacin	4	0.3
	6	0.5
Ambroxol	4	0.2
	6	0.13

Table No. 3: Riggedness of LEV and AMB.

Drug	Concentration (µg/ml)	% RSD
Levofloxacin	4	0.4
	6	0.3
Ambroxol	4	0.4
	6	0.2

Table No. 4: Analysis of marketed formulation.

Drugs	Labeled amount, mg/ tablet	Amount found, mg/ tablet	% Label claim
Levofloxacin	500	497	99.4
Ambroxol	75	77	102

CONCLUSION

The method described enables the quantification of LEV and AMB in combined tablet dosage form. The validation data demonstrates good precision and accuracy which proves to be reliable of the proposed method. Hence, this RP HPLC method can be used for simultaneous estimation of both components in solid oral dosage form.

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