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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF LEVOFLOXACIN HEMIHYDRATE AND AMBROXOL HYDROCHLORIDE IN THEIR COMBINED DOSAGE FORM

SHIVANI CHANDA, SIREESHA D., Dr. V. V. L. N PRASAD, Dr. PRAKASH. V. DIWAN
Department of Pharmaceutical Analysis and Quality Assurance, Lalitha College of Pharmacy,
Hyderabad, Andhra Pradesh, India.

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Abstract: A simple, specific, accurate, precise and economical spectrophotometric method has been developed for the estimation of Levofloxacin (LVF) and Ambroxol (AMB) in pharmaceutical dosage forms. Simultaneous equation method was used. The absorption maxima of the drugs were found to be 288 nm and 244 nm for Levofloxacin and Ambroxol respectively. Levofloxacin and Ambroxol obeyed Beer's law in the concentration range of 2-16 μ g/ml and 1-50 μ g/ml respectively. Different analytical parameters such as linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) were determined as per ICH guidelines. The % assay for commercial formulation was found to be in the range of 99-101%. The recovery values between prescribed limit of 98-102% shows that the method is free from interference of excipients present in formulation. Hence, the developed method can be used quality control analysis for routine.

Keywords: Levofloxacin hemihydrate, Ambroxol hydrochloride, UV spectrophotometric method, Simultaneous equation method



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Corresponding Author: Ms. SHIVANI CHANDA

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INTRODUCTION

Levofloxacin hemihydrate (LVF) (Fig.1) chemically, $[(S)-9\text{-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid}]$ is an optically L isomer of ofloxacin. It is a broad spectrum fluoroquinolone class of antibacterial agent and effective against many gram positive and gram negative bacteria. It is a potent inhibitor of bacterial DNA gyrase enzyme (topoisomerase II & IV), which is necessary for negative super coiling of DNA prior to replication.

Ambroxol hydrochloride (AMB) (Fig.2) chemically, $4-[(2\text{-amino-3,5-dibromophenyl)-methyl]-amino]$ cyclohexanol hydrochloride is a mucolytic expectorant and used to reduce the viscosity of mucous secretions.

A fixed dose combination of Levofloxacin hemihydrate (LVF) and Ambroxol hydrochloride (AMB) is available for the treatment of upper and lower respiratory tract infections.

Literature survey reveals that several methods have been developed for the quantitative determination of LVF in formulations as well as in plasma and urine. These include capillary electrophoresis and UV spectrophotometry, HPLC, simultaneous HPTLC method with ornidazole and flow injection analysis.

It has been reported that Levofloxacin and Ambroxol hydrochloride have been

estimated by capillary electrophoresis, spectrophotometry, gas chromatography and liquid chromatography either as single component or in combination with other drugs.

Simultaneous reverse phase high performance liquid chromatographic method for determination of LVF and AMB in pharmaceutical formulations has been also reported, which is quite expensive.

This work is aimed to investigate the utility of UV spectrophotometric method for the simultaneous determination of LVF and AMB in pharmaceutical preparations. The method developed is accurate, precise and is simple and cost effective assay for these compounds in mixtures.

STRUCTURE:

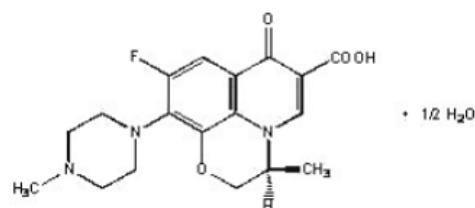


Figure 1: Chemical Structure of Levofloxacin hemihydrates



Figure2: Chemical structure of Ambroxol hydrochloride

MATERIALS AND METHODS

Instrumentation

SHIMADZU double beam UV/Visible spectrophotometer (Model: 1700) with 2 nm spectral bandwidth was employed for all spectrophotometric measurement. A 10mm matched quartz cell and Borosil glass wares were used for the study. Calibrated electronic single pan balance Sartorius CP 225 D, pH Meter (LABINDIA), Enertech Fast Clean Ultrasonicator were also used during the analysis.

Chemicals & Reagents: Analytically pure Levofloxacin hemihydrate and Ambroxol hydrochloride were obtained as gift samples from M/s Akums Drugs & Pharmaceuticals Ltd., Haridwar (India). Commercial tablet formulations were purchased from the local market. The pharmaceutical dosage form used in the study was L-Cin A (label claim LEVO 500 mg, AMB 75 mg) manufactured in India by Hetero Labs Limited. All chemicals and reagents used were of Analytical Grade, obtained from Merck.

METHOD

Preparation of Standard Solutions

A 10 mg of standard LEVO and AMB were weighed and transferred to 100 ml separate volumetric flasks and dissolved in distilled water. The solutions were sonicated to dissolve the drugs and were made upto mark with distilled water to give solutions containing 100µg/ml each of LEVO and AMB.

1. SIMULTANEOUS EQUATION METHOD

For simultaneous determination (vierodt's method) the wavelengths selected were 288 nm and 244 nm, absorbance maxima of Levofloxacin hemihydrates and Ambroxol hydrochloride respectively. The absorbance of Levofloxacin hemihydrate and Ambroxol hydrochloride were measured at 288 and 244 nm respectively. The absorbtivities of both the drugs at both the wavelengths were determined. The content of both ingredient in the marketed formulation were obtained by using following equations:

$$C_x = \frac{A_{2y1} - A_{1y2}}{a_{x2} - a_{y1} - a_{x1}a_{y2}}$$

$$C_y = \frac{A_{1x2} - A_{2x1}}{a_{x2}a_{y1} - a_{x1}a_{y2}}$$

Where,

A₁ = Absorbance of the diluted sample at 288 nm

A₂ = Absorbance of the diluted sample at 244 nm

a_{x1} = Absorptivity of Levofloxacin hemihydrate at 288 nm

a_{x2} = Absorptivity of Levofloxacin hemihydrate at 244 nm

a_{y1} = Absorptivity of Ambroxol hydrochloride at 288 nm

a_{y2} = Absorptivity of Levofloxacin hemihydrate at 244 nm

C_x = Concentration of Levofloxacin hemihydrate

C_y = Concentration of Ambroxol hydrochloride

SPECTROPHOTOMETRIC CONDITION

VALIDATION OF THE METHOD

The method is validated with respect to linearity, accuracy, intraday and interday precision, limit of detection (LOD) and limit of quantification (LOQ), in accordance to ICH guidelines.

Linearity

For both drugs, appropriate dilutions of standard stock solutions were analysed as per the developed method. Calibration curve was plotted in the concentration range of 2-16 μ g/ml for Levofloxacin and 1-50 μ g/ml for Ambroxol hydrochloride and the correlation coefficient was found to be 0.999 for both the drugs.

Precision

Precision of the method was confirmed by interday and intraday analysis i.e. the

analysis of formulation was repeated three successive days. The amount of drugs was determined and % RSD also calculated.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived using the following equations designated by International Conference on Harmonisation (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 study)

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. To an equivalent quantity of formulation powder, a known quantity of standard Levofloxacin hemihydrate and Ambroxol hydrochloride added at 50%, 100% and 150% level and the contents were re-analysed by the proposed method. The %recovery was calculated.

Assay

It was tested by analysis of commercially available marketed formulation. Twenty tablets were weighed accurately and powdered. Standard addition method was used. A quantity of tablet powder equivalent 10 mg of Levofloxacin

hemihydrate was transferred to 100 ml volumetric flask containing 80 ml of distilled water. An accurately weighed 8.5 mg of pure Ambroxol hydrochloride powder was added. The volume was made up to 100 ml with distilled water. With the addition of pure AMB, the ratio of LVF to AMB in samples was brought to 1:1. The tablet sample solution was filtered through Whatmann filter paper. From the 100 µg/ml of sample stock solution take 6 ml of solution and diluted up to the mark in 100 ml volumetric flask. So the final solution was made which contains 6 µg/ml Levofloxacin hemihydrate and 6 µg/ml of Ambroxol hydrochloride both.

RESULTS AND DISCUSSION

SIMULTANEOUS EQUATION METHOD

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of LEVO and AMB. For simultaneous determination method, wavelengths selected for analysis were 288 nm for LEVO and 244 nm for AMB. The optimized method was applied for marketed formulation and the % label claim for LEVO and AMB was found to be 100.08 and 100.2. The method is accurate and

precise and can be used for routine pharmaceutical analysis.

The data for linearity, precision, accuracy, LOD, LOQ is represented in the table 1.1 and 1.2. Recovery studies were carried out by standard addition method to check the accuracy of the developed methods and to study the interference of formulation additives (Table 1.3). The validated method was successfully applied for the determination of tablet mixture of LEVO and AMB. The results are given in Table 1.4, indicate that the amount of drug in tablet samples met with requirements.

CONCLUSION

The optimized simultaneous determination method provides simple, specific, precise, accurate, economical and reproducible quantitative analysis for simultaneous determination of LEVO and AMB in combined tablet dosage form. The method was validated as per ICH guidelines in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ). The method can be used for routine analysis of LEVO and AMB in combined dosage form.

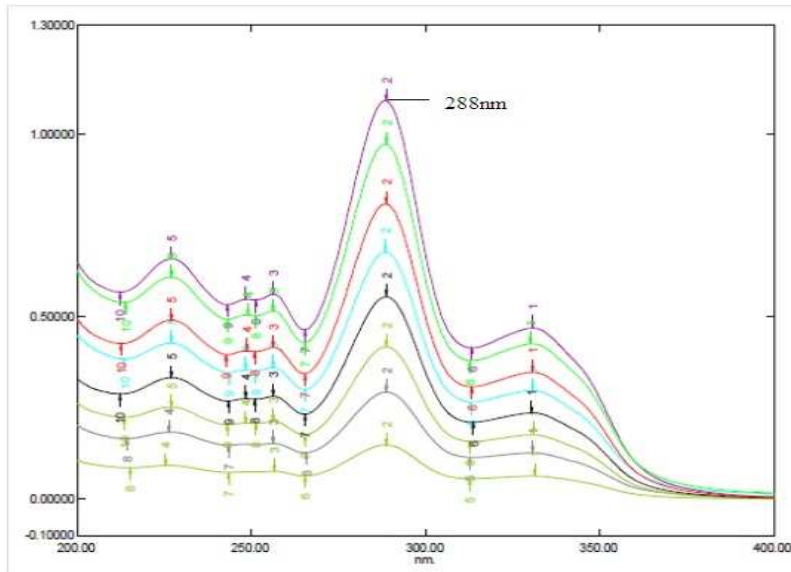


Figure 3: Overlay spectra of LEVO at 288 nm

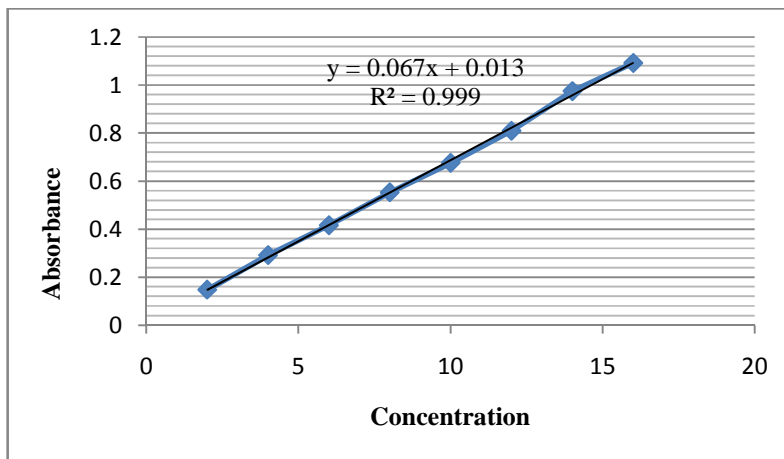


Figure 4: Calibration curve of Levofloxacin hemihydrate at 288 nm

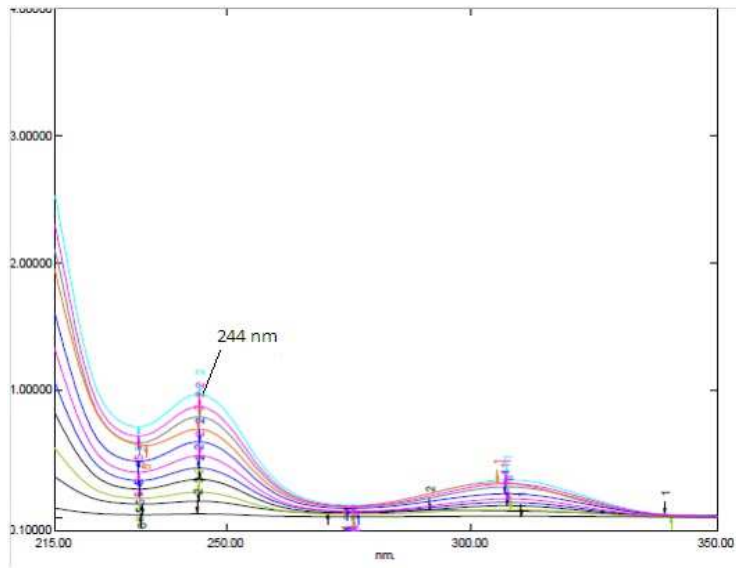


Figure 5: Overlay spectra of AMB at 244 nm

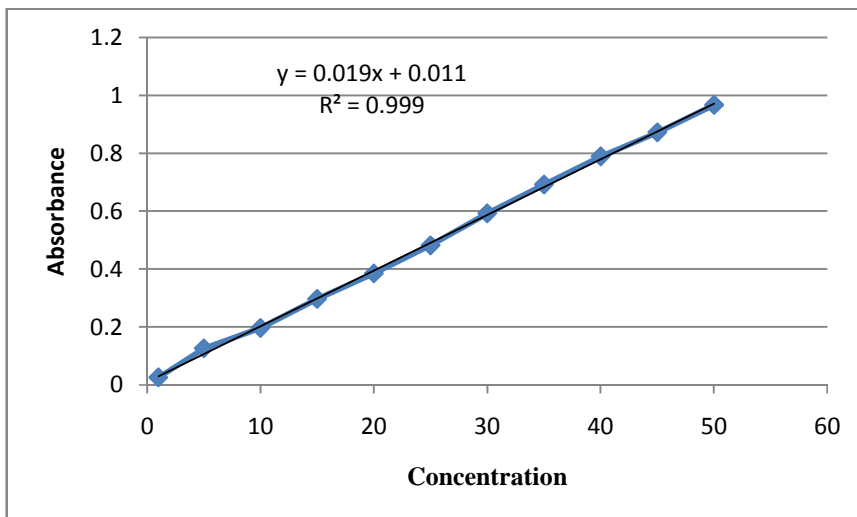


Figure 6: Calibration curve of AMB at 244 nm

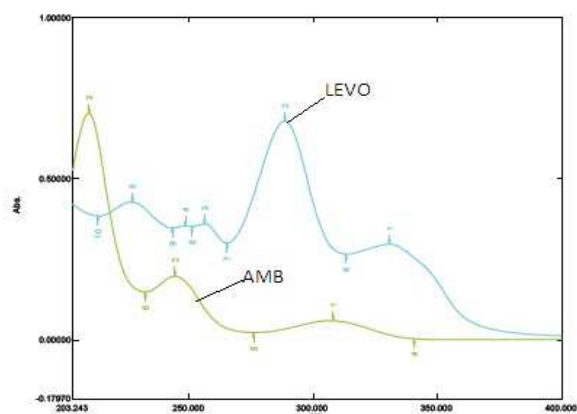


Figure 7: Overlay spectra of LEVO (10µg/ml) and AMB (10µg/ml)

Table 1.1

Summary of validation parameters of Simultaneous Method

parameters	LEVO	AMB
	288 nm	244 nm
Precision(%RSD)		
Intra-day(n=3)	0.7	0.20
Inter-day(n=3)	1.25	1.27
LOD(µg/ml)	0.214	0.6
LOQ((µg/ml)	0.649	1.82
Recovery %	98.8-100.6	99.8-101.1

Table 1.2

Statistical Data LEVO and AMB by Simultaneous Method

Parameters	LEVO		AMB	
Analytical Wavelength	288 nm	244 nm	288 nm	244 nm
Range	2-16µg/ml	2-16µg/ml	1-50µg/ml	1-50µg/ml
Slope	0.0673	0.0335	.0029	0.0192
Intercept	0.0138	0.0063	0.0067	0.9991
Regression Coefficient(r ²)	0.9991	0.9964	.999	0.9991

Table 1.3

Accuracy Data for LEVO and AMB by Simultaneous Method

% Level	Amount of Drug		Amount added		Amount Recovered		% Recovery	
	LEVO ($\mu\text{g/ml}$)	AMB ($\mu\text{g/ml}$)	LEVO ($\mu\text{g/ml}$)	AMB ($\mu\text{g/ml}$)	LEVO ($\mu\text{g/ml}$)	AMB ($\mu\text{g/ml}$)	% LEVO	% AMB
50	6	6	3	3	8.9	9.1	98.8	101.1
100	6	6	6	6	11.9	12.09	99.16	100.75
150	6	6	9	9	15.1	14.98	100.6	99.8

Table 1.4

Assay Results of Marketed Formulation

Table	Drug	Labeled claim(mg)	Amount Found(mg)	% label claim
L-cin A	Levofloxacin hemihydrate	500	500.4	100.08
	Ambroxol hydrochloride	75	75.2	100.2

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