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SIMULTANEOUS ESTIMATION OF AMBROXOL HYDROCHLORIDE AND LEVOFLOXACIN HEMIHYDRATE USING ABSORBANCE RATIO METHOD

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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 tablet dosage forms. Absorbance ratio method was used. From the overlay spectra of ABH and LFH in distilled water at concentration of 10 µg /mL each, two wavelengths 244.6 nm (λ max of ABH) and 218.6 nm (isoabsorptive point) were selected for estimation of both drugs. ABH and LFH solution individually follows the Beer-Lambert's law over concentration range 3 - 7 µg/mL and 5 – 25 µg/mL respectively. Different analytical parameters such as linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), recovery were determined as per ICH guidelines. The recovery values between prescribed limit of 99-100% 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, Absorbance ratio method, etc.

INTRODUCTION

Levofloxacin hemihydrate (LVF) (Fig.1)chemically, [(-)(s)-9-fluro-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) chemically, 4-[(2-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. 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.

MATERIALS AND METHODS

Instrumentation

UV experimentation was performed on Shimadzu 1800 UV-visible spectrophotometer, equipped with photo diode array (PDA) detector, with 1 cm quartz cell. Chromatographic experimentations were performed using Systronics HPLC system equipped with 8600 HPLC pump and dual wavelength UV-Vis detector, data acquisition and processing was performed using Chemitochrom automation system software. The methods were conducted using an isocratic reverse phase techniques. The mobile phase was prepared freshly filtered through 0.45 µm membrane filter (Millipore, USA) and sonicated for 30 min before used in order to degas the mobile phase. A RP-Presnosphere C_{18} column (250 x 4.6mm, 5 µm) KNAVER, Germany was used for analysis.

Chemicals and Reagents

The bulk drugs of Ambroxol hydrochloride (ABH) and Levofloxacin hemihydrate (LFH) was procured from the Wintech Pharmaceutical, Nashik (Maharashtra). All solvents and reagents used were of HPLC and analytical grade, respectively. HPLC grade methanol, acetonitrile and water were obtained from Qualigens, Mumbai. Acetic acid, ammonium hydroxide and ammonium chloride were obtained from Merck Labs Ltd. Tablets of ABH (75 mg) and LFH (500 mg) in combined dosage form of brand name LIVBEST-AM, (Piramal Healthcare), were purchased from local pharmacy.

Method

Preparation of Standard Solution

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.

Absorbance ratio method

For simultaneous determination the wavelengths selected were 218.6 nm and 244.6 nm, absorbance maxima of Levofloxacin hemihydrates and Ambroxol hydrochloride 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:

$$Cx = \frac{Qm - Qy}{Qx - Qy} \times \frac{A}{ax_1}$$

$$Cy = \frac{Qm - Qx}{Qy - Qx} \times \frac{A}{ay_1}$$

Where,

Cx and Cy are concentrations of ABH and LFH respectively

Qx is the ratio of absorptivity of ABH at λ_1 and λ_2

Qy is the ratio of absorptivity of LFH at λ_1 and λ_2

Qm is the ratio of absorbance of Std.lab mixture at λ_1 and λ_2

A is the absorbance of Std.lab mixture at isoabsorptive point

ax_1 and ay_1 are the absorptivity value of ABH and LFH

Spectrophotometric Conditions

Validation of the method

The method is validated with respect to linearity, accuracy, intraday and interday precision, ruggedness, 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 5-25µg/ml for Levofloxacin and 3-7µg/ml for Ambroxol hydrochloride and the correlation coefficient was found to be 0.999 for both the drugs.

Precision

Precision is determined by intra-day and inter-day precision. Intra-day precision was determined by analyzing the ABH (4, 5, 6 µg/mL) and LFH (26.4, 33, 39.6 µg/mL) for three times in the same day. Inter-day precision was determined by analyzing the ABH (4, 5, 6 µg/mL) and LFH (26.4, 33, 39.6 µg/mL) daily for three days.

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 × σ/S

LOQ = 10 × σ/S

Where, **σ**=the standard deviation of the response and S=slope of the calibration curve

Accuracy (Recovery Study)

A known amount of standard solution of pure drug (ABH and LFH) was added to preanalysed tablet solution in three different levels. These solutions were subjected for analysis.

The absorbances were recorded and the % recoveries were calculated using formula;

% Recovery = [A - B/ C] X 100

Where,

A = Total amount of drug estimated.

B = Amount of drug found on preanalysed basis.

C = Amount of Pure drug added.

Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions.

Assay

In order to see the feasibility of proposed method for simultaneous estimation of ambroxol hydrochloride and levofloxacin hemihydrate in marketed pharmaceutical formulations, twenty tablets were weighed accurately and finely powdered. The powder equivalent to ABH (75mg) and LFH (500mg) was accurately weighed and transferred to 100mL volumetric flask containing distilled water (50mL). Then the content was shaken for 30 min. and volume was made up to mark using distilled water. The above solution was filtered through whatman filter paper no.1. This solution was again filtered through 0.45µ millipore membrane filter. This tablet solution was further diluted with mobile phase to obtain mixed sample solutions having concentration of ABH (7.5 µg/mL) and LFH (50 µg/mL).

The amount of each drug estimated in marketed pharmaceutical formulation was calculated using following formula;

% Label claim =	$\frac{At}{As}$	X	$\frac{Ds}{Dt}$	X	$\frac{Ws}{Wt}$	X	$\frac{A}{Lc}$	X	100

Where,

At = AUC for sample solution

As = AUC for standard solution

Ds = Dilution of standard

Dt = Dilution of sample
Ws = Weight of standard (mg)
Wt = Weight of sample (mg)
A = Average weight
Lc = Label claim

RESULT AND DISCUSSION

Absorbance ratio method

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of LEVO and AMB. From the overlay spectra of ABH and LFH in distilled water (Fig. 1) at concentration of 10 µg /mL each, two wavelengths 244.6 nm (λ max of ABH) and 218.6 nm (isoabsorptive point) were selected for estimation of both drugs by absorption ratio method. The relation between concentration and absorbance for individual drug was studied. ABH and LFH solution individually follows the Beer-Lambert's law over concentration range 3 - 7 µg/mL and 5 – 25 µg/mL respectively. (Fig. 2, 3) The absorbances of both the drugs were found to be satisfactorily additive at selected wavelength. (Table No. 1,2) The E (1%, 1cm) values for both the drugs were determined at the selected wavelengths. (Table No. 3) On the basis of the above studies, absorption ratio method was evolved. The proposed method extended for the estimation of drugs in marketed tablet formulation. (Table No. 4)

LOD and LOQ

The LOD and LOQ of ABH and LFH found to be (0.060 µg/mL), (0.17 µg/mL) and for (0.18 µg/mL), (0.35 µg/mL), respectively.

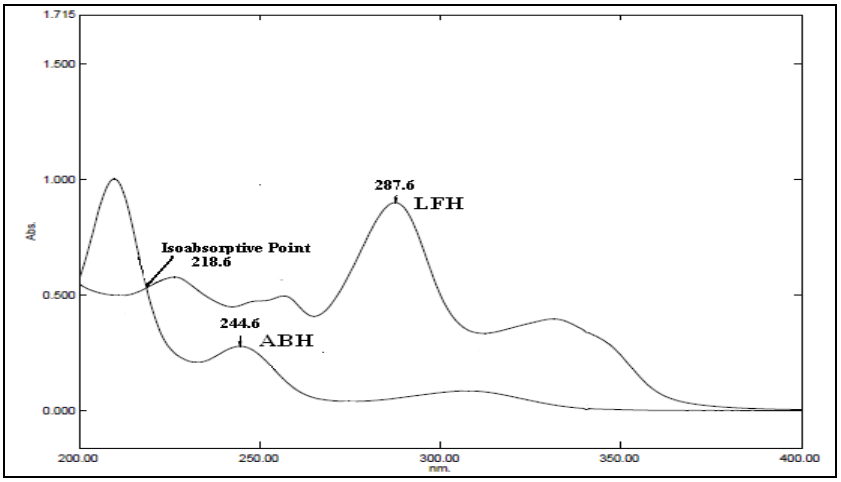

Fig. 1: Overlay Spectra of ABH and LFH.

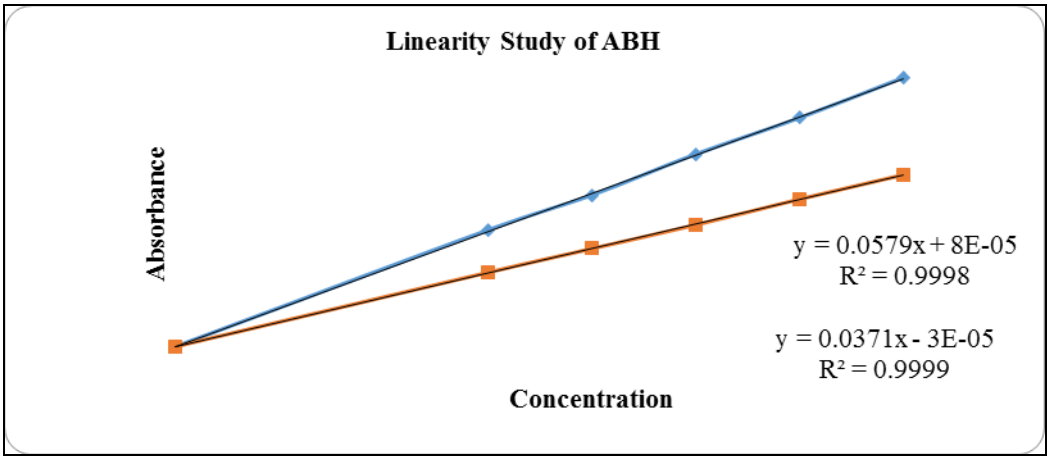

Fig.2: Calibration Curve of ABH at 218.6 nm and 244.6 nm.

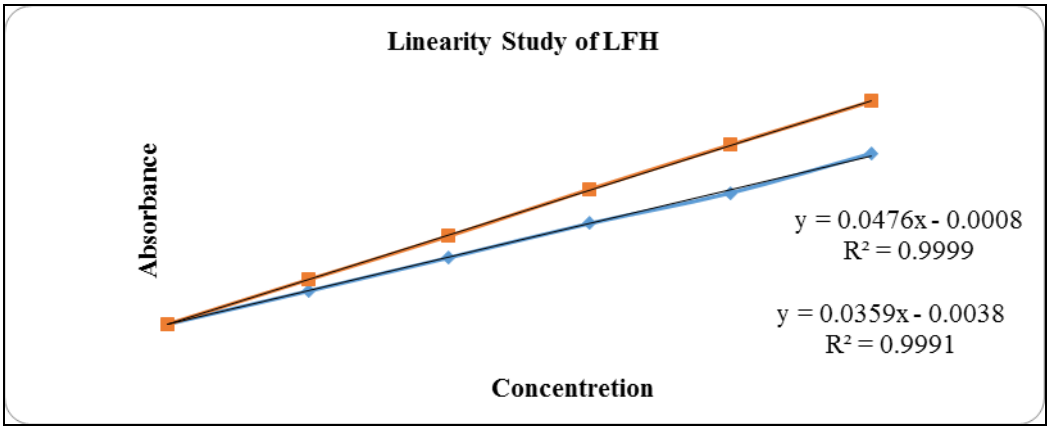

Fig.3: Calibration Curve of LFH at 218.6 and 244.6 nm.

Table No. 1: Additivity Study.

Sr. No.	Conc. of ABH (µg/mL)	Conc. of LFH (µg/mL)	Abs. of ABH at 218.6 nm	Abs. of LFH at 218.6 nm	Theoretical Absorbance of Mixture	Abs. of Mixture at 218.6 nm
			A_1	A_2	$(A_1 + A_2)$	
1	3	5	0.176	0.176	0.352	0.354
2	4	10	0.229	0.354	0.583	0.584
3	5	15	0.291	0.536	0.827	0.829
4	6	20	0.346	0.698	1.044	1.046
5	7	25	0.407	0.908	1.315	1.313

Table No. 2: Additivity Study.

Sr. No.	Conc. of ABH (µg/mL)	Conc. of LFH (µg/mL)	Abs. of ABH at 244.6 nm	Abs. of LFH at 244.6 nm	Theoretical Absorbance of Mixture	Abs. of Mixture at 244.6 nm
			A_1	A_2	$(A_1 + A_2)$	
1	3	5	0.112	0.237	0.349	0.352
2	4	10	0.148	0.474	0.622	0.624
3	5	15	0.184	0.711	0.895	0.893
4	6	20	0.223	0.948	1.171	1.172
5	7	25	0.260	1.185	1.445	1.448

Table No. 3: E (1%, 1cm) values for ABH and LFH.

Sr. No.	at 218.6 nm (λ_1)		at 244.6 nm (λ_2)	
	ABH	LFH	ABH	LFH
1	584.28	359.09	370	474.24
2	582.85	359.30	368.57	474.02
3	584.28	359.30	370	474.24
4	581.42	359.09	367.14	473.80
5	582.85	359.30	368.57	474.45
Mean	**583.13 (ax_1)**	**359.21(ay_1)**	**368.85 (ax_2)**	**474.15 (ay_2)**
SD	1.196	1.190	0.115	0.247
% RSD	0.205	0.331	0.032	0.052

Table No. 4: Estimation of ABH and LFH in Marketed Formulation.

Tablet- LIVBEST-AM			Average Weight of Tablet- 942.66 mg.				
Sr. No.	Quantity of Tablet Powder Taken (mg)	Absorbance of Mixture at 218.6 nm	Absorbance of Mixture at 244.6 nm	Amount of Drug Estimated (mg)		Percentage of Drug Estimated	
				ABH	LFH	ABH	LFH
1	942.63	2.263	2.682	74.80	499.15	99. 53	99.77
2	942.60	2.262	2.682	74.71	499.31	99.48	99.82
3	942.67	2.262	2.683	74.89	500.47	99.72	100.08

4	942. 72	2.264	2.682	74.63	499.63	99.34	99.86
5	942.70	2.261	2.683	74.67	500.62	99.49	100.06
Mean						**99.51**	**99.91**
SD						**0.1366**	**0.1425**
% RSD						**0.1372**	**0.1426**

Table No. 5: Precision Study of ABH and LFH.

Sr. No.	Drug	Conc. [µg/mL]	Intraday ± SD (n = 3)		Inteday ± SD (n = 3)	
1.	ABH	4	3.89 ± 0.0372	0.9562	3.71 ± 0.0217	0.5849
		5	4.93 ± 0.0156	0.3164	4.87 ± 0.0372	0.7638
		6	5.83 ± 0.0256	0.4391	5.92 ± 0.0290	0.4898
2.	LFH	26.4	26.8 ± 0.0452	0.1686	26.5 ± 0.0173	0.0652
		33	32.78±0.0148	0.0451	32.83±0.0315	0.0959
		39.6	39.3 ± 0.0237	0.0603	39.8 ± 0.0247	0.0620

Table No. 6: Ruggedness Study of ABH and LFH.

	ABH 7.5 µg/mL		LFH 50 µg/mL	
	Amount Found in µg/mL mean ± S.D. (n = 3)	% RSD	Amount Found in µg/mL mean ± S.D. (n = 3)	% RSD
Analyst-I	7.62 ± 0.0549	0.7204	50.59 ± 0.0426	0.0842
Analyst-II	7.49 ± 0.0289	0.3858	50.75 ± 0.0365	0.0719
Day-I	7.60 ± 0.0293	0.0824	50.54 ± 0.0953	0.1885
Day-II	7.54 ± 0.0749	0.3855	50.83 ± 0.0118	0.0232

Table No. 7: Recovery Study of ABH and LFH.

Tablet- LIVBEST-AM			Average Weight of Tablet- 942.66 mg.					
Sr. No.	Quantity Tablet Powder Taken (mg)	%	Amount of Pure Drug Added (mg)		Total Amount of Drug Recovered (mg) ± S.D. (n = 3)		% of Drug Recovered (n = 3)	
			ABH	LFH	ABH	LFH	ABH	LFH
1.	942.63	80	45.12	300.05	119.71± 0.016	799.37± 0.044	99.75	99.91
2.	942.67	100	75.08	500.14	149.63± 0.065	998.45± 1.472	99.55	99.83
3.	942.61	120	105.1	700.03	179.84± 0.067	1197.01± 0.092	99.80	99.74
Mean							**99.70**	**99.82**
SD							**0.132**	**0.085**
% RSD							**0.132**	**0.085**

CONCLUSION

Extensive literature survey reveled that various methods were reported for the estimation of Ambroxol hydrochloride and Levofloxacin hemihydrate individually but yet not a single method is reported for the combined dosage form. Hence present research work was undertaken to Develop and Validate UV-Spectrophotometric Method for the Simultaneous Estimation of Ambroxol hydrochloride and Levofloxacin hemihydrate in tablet dosage form.

From the studies it can be concluded that, the analysis of combined tablet dosage formulation containing Ambroxol hydrochloride and Levofloxacin hemihydrate can be successfully performed by the UV-spectrophotometric method. Use of distilled water for every dilution made the method more economic. The developed methods is simple, accurate, precise, reproducible, economical and rapid also. It may be adopted for routine control analysis of these two drugs in combined dosage form. Further it would be interesting to apply these methods for biological samples containing these drugs.

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