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Quantitative Determination of Levofloxacin and Ambroxol hydrochloride in Pharmaceutical Dosage Form by Reversed-Phase High Performance Liquid Chromatography

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Abstract

The objective of this present work was to develop and validate analytical method for quantitative determination of Levofloxacin and Ambroxol hydrochloride in a new tablet formulation. Chromatographic separation of the two drugs were analyzed on a Hypersil BDS C18 column (25cm X 4.6mm, 5μm). The mobile phase constituted of Buffer: Acetonitirile: Methanol (650:250:100) with triethylamine and pH adjusted to 5.2 with dilute orthophosphoric acid was delivered at the flow rate 1.0 mL min^{-1}. Detection was performed at 220 nm. Separation was completed within 10 min. Calibration curves were linear with coefficient correlation between 0.99 to 1.0 over a concentration range of 7 to 22 μg mL^{-1} of Levofloxacin and 50 to 150 μg/mL for Ambroxol hydrochloride respectively. The relative standard deviation (R.S.D) was found <2.0%.

Keywords: Levofloxacin; Ambroxol hydrochloride; Reversed-phase; HPLC; Combination Tablets.

1. Introduction

Levofloxacin and Ambroxol hydrochloride fixed dose combination tablet contains Levofloxacin hemihydrate and Ambroxol hydrochloride (as sustained release). Levofloxacin is a fluoroquinolone antiinfective, is the optically active L-isomer of ofloxacin. Levofloxacin is effective against gram positive and gram negative bacteria [1-3]. Levofloxacin inhibits bacteria type II topoisomerases, topoisomerases IV and DNA gyrase. Levofloxacin like other

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fluoroquinolone, inhibits the A subunits of DNA gyrase; two subunit encoded by gyrA gene. This result in strand breakage on a bacterial chromosome, supercoiling and resealing, DNA replication and transcription is inhibited.

Ambroxol hydrochloride is a mucolytic expectorant. It is a metabolite of bromhexine and acts to reduce the viscosity of tenacious mucous secretions via fragmentation of long mucopolysaccharide chains.

Fixed dose combination of Levofloxacin and Ambroxol hydrochloride are indicated for the treatment and relief of symptoms of both upper and lower respiratory tract infections. Ambroxol hydrochloride also enhances penetration power of antibiotic.

Some analytical methods for quantitative determination of fluoroquinolones in pharmaceutical formulations are described in literature like capillary electrophoresis; UV spectrophotometry and high-performance liquid chromatography (HPLC) [4-6].

Methods available for the determination of Ambroxol hydrochloride include capillary electrophoresis [7-9] and, spectrophotometry [10], gas chromatography [11,12] and L.C. with potentiometric detection [13], MS detection [14] and UV detection [15-18]. However no references have been found for quantitative determination of Levofloxacin and Ambroxol hydrochloride in pharmaceutical preparations. The major advantage of the proposed method is that Levofloxacine and Ambroxol hydrochloride can be determined on a single chromatographic system with the same detection wavelength.

2. Material And Methods

2.1. Chemicals and Materials

Hetero drugs supplied Levofloxacin hemihydrate and Vent Petro Chem and Pharma supplied Ambroxol hydrochloride. Acetonitrile and Potassium dihydrogen orthophosphate (Spectrochem and E-Merck Limited).

2.2. Instrumentation:

Shimadzu 2010C integrated high performance liquid chromatographic system was used for this experiment. Shimadzu 2010C system equipped with quaternary gradient pump, 2010C UV-VIS detector, 2010C Column Oven and 2010C programmable auto sampler controlled by

CLASS-VP software. The Hypersil BDS C18 (250X4.6 mm), 5µm was used as a stationary phase. HPLC conditions are given in Table 1.

Table 1. HPLC Conditions

Column	Hypersil BDS C18 (250X4.6 mm), 5µm
Detector	220 nm
Injection volume	20 µL
Flow rate	1.0 mL/min
Temperature	30˚ C
Run time	10 min
Mobile phase	Buffer: Acetonitrile: Methanol (650:250:100)

2.3. Buffer preparation

Weigh 6.8 g potassium dihydrogen orthophosphate in to 1000 mL of Milli Q water and mix.

2.4. Standard preparation

Standard stock solutions were prepared in methanol and further for second dilution, dilute it with water to make final concentration Levofloxacin 500 µg and Ambroxol hydrochloride 15 µg respectively.

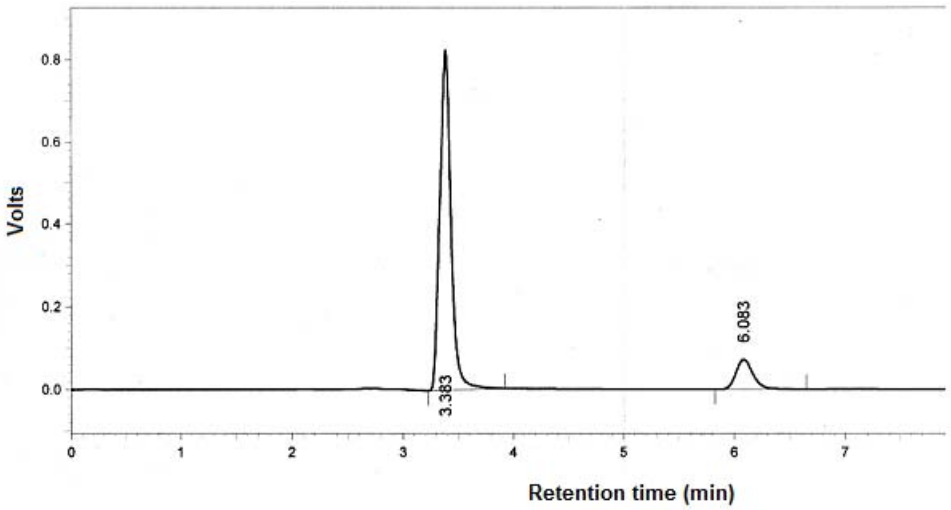
2.5. Sample preparation

Weigh accurately tablets powdered equivalent to about 500 mg of Levofloxacin and 75 mg of Ambroxol hydrochloride in to 250-mL volumetric flask. Add about 100-ml methanol and sonicate it for 30 minute to dissolve. Filtered it through 0.45 µ HVLP nylon filter and made further dilution 5.0 mL to 100.0 mL with water and mix.

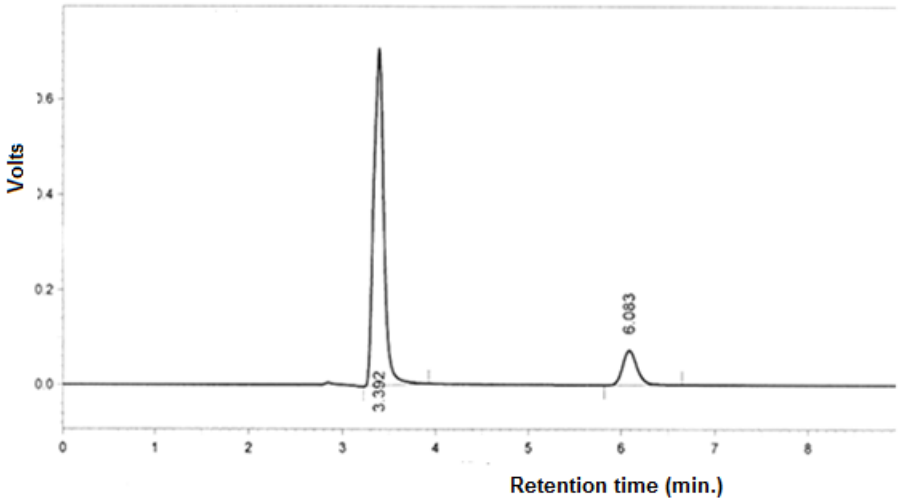
3. Results And Discussion

The detection wavelength of 220 nm was chosen in order to achieve a good sensitivity for quantitative determination of Levofloxacin and Ambroxol hydrochloride in tablet dosage. The

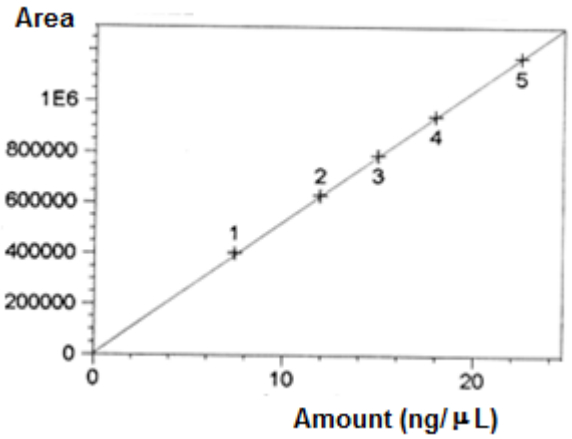
mobile phase consisting of Buffer: Acetonitirile: methanol (650:250:100) with triethylamine added (pH 5.2) with orthophosphoric acid offered a good separation at ambient temperature under these conditions using a flow rate of 1.0 mL min^{-1} and a runtime of 10 min, Levofloxacin elutes at first and then Ambroxol hydrochlorides shown in the chromatogram, Fig. 1(a), (b) and (c), which illustrate the separation of both active ingredients in this system. The isocratic program throughout HPLC method was adopted to analyze both components in a single run. The proposed method is simple and donot involve laborious time-consuming sample preparation.



(a)



(b)



(c)

Fig.1 (a) Chromatogram of the Sample solution (b) Chromatogram of the Standard solution (c) Calibration curve of Ambroxol Hydrochloride

3.1. System suitability and system precision:

System suitability and system precision was daily performed during entire validation of this method. The results of system suitability and system precision were presented in Table 2.

Table 2. System suitability and system precision

Compound	Retention time (Mean ± SEM)	n	k'	R	T	α
Levofloxacin	4.196 ± 0.0055	7163.85	0.1998	-	1.356	-
Ambroxol	5.614 ± 0.0182	8359.87	0.6036	6.38	1.316	3.0

n= Theoretical plates
k'= Capacity Factor
R= Resolution
T= Asymetry
α = Selectivity

3.2. Linearity and calibration curve:

The plot of peak area response against concentration is shown in Fig. 1 (C) and Fig. 2 The plot is linear over the concentration range of 7 to 22 µg mL^{-1} and 50 to 150 µg mL^{-1} for levofloxacin and Ambroxol hydrochloride respectively. Linearity of the calibration curve was

determined by weighed (1/c) least square regression analysis. The correlation coefficient was found to be 0.99 to 1.00. A linear relationship was found for all components. The results of linearity, limit of detection and limit of quantification were presented in Table 3.

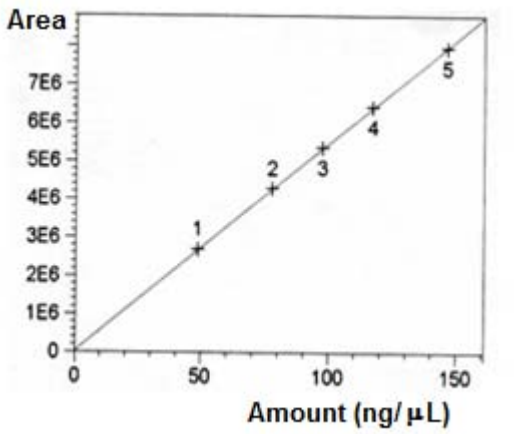


Fig.2 Calibration curve of Levofloxacin

Table 3. Characteristics of the analytical method derived from the standard calibration curve

Compound n=(5)	LOD co-efficient	LOQ regression µg/mL	Linearity regression	Correlation µg/mL	Residual std. µg/mL	Slope of range
Levolfoxacin	2.1	7	7 to 22	0.99998	21787.133	54523.383
Ambroxol	0.6	1	50 to 150	0.99996	4382.175	52202.072

LOD= Limit of detection
LOQ= Limit of quantification

3.3. Specificity

There was no interference from sample placebo and peak purity of Levofloxacin and Ambroxol hydrochlorides were 0.99998 and 0.99974, respectively. It showed that developed analytical method was specific for the analysis of Levofloxacin and Ambroxol hydrochloride in tablet dosage form.

3.4. Standard and sample solution stability

Standard and sample solution stability was evaluated at room temperature for 18 h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 18 h at room temperature.

3.5. Method precision

The precision of the method was established by carrying out the analysis of the analyte (n=6) using the proposed method. The low value of standard deviation showed that the method was precise. The results obtained were presented in Table 4.

Table 4. Method precision

Compound (n=6)	Concentration µg/mL	Retention time Mean ± SEM	% Assay Mean ±SEM (n=6)	% RSD of Assay (n=6)
Levofloxacin	500	3.83 ± 0.00	104.9 ± 0.396	0.9
Ambroxol	15	6.08 ± 0.00	95.6 ±0.680	1.7

3.6. Method accuracy

To ensure the reliability and accuracy of the method recovery studies were carried out at three different levels. The results of recovery studies were presented in Table 5.

Table 5. Method accuracy

	Level	Drug Added (mg)	Drug recovered (mg)	% Assay (Mean ± SEM) (n=3)	% RSD of Assay (n=3)
Levofloxacin	50%	249.63	255.28	99.2 ± 0.352	0.6
	100%	500.20	483.07	99.5 ± 0.166	0.3
	150%	749.30	717.14	98.6 ± 0.290	0.5
Ambroxol	50%	37.66	38.13	101.5 ± 0.260	0.4
	100%	74.68	75.25	100.8 ± 0.393	0.7
	150%	112.34	112.97	100.5 ± 0.831	1.3

3.7. Method robustness

Robustness of the method was determined by small deliberate changes in flow rate, mobile phase ratio and column oven temperature. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust. The results of robustness were presented in Table 6.

Table 6. Method robustness (% RSD) in Normal and Changed condition (n=5)

Compound	Condition	Change	% RSD
Levofloxacin	Temperature	Normal	0.16
		-5°C	0.40
		+5°C	0.20
	pH	Normal	0.16
		-0.2 unit	0.13
		+0.2 unit	0.03
	Flow Rate	Normal	0.16
		-10%	0.08
		+10%	0.17
	Mobile phase ratio	Normal	0.16
		-2%	0.12
		+2%	0.17
Ambroxol	Temperature	Normal	0.42
		-5°C	0.15
		+5°C	0.08
	pH	Normal	0.42
		-0.2 unit	0.26
		+0.2 unit	0.23
	Flow Rate	Normal	0.42
		-10%	0.26
		+10%	0.23
	Mobile phase ratio	Normal	0.42
		-2%	0.13
		+2%	0.13

3.8. Method Ruggedness

Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results of ruggedness were presented in Table 7.

Table 7 Method ruggedness

Compound	% Assay (n=6)	% RSD of Assay Mean ± SEM (n=6)
Day 1	Analyst-1, Instrument-1 & Column-1	
Levofloxacin	104.9 ± 0.396	0.9
Ambroxol	95.6 ± 0.680	1.7
Day 2	Analyst-2, Instrument-2 & Column-2	
Levofloxacin	105.1 ± 0.162	1.1
Ambroxol	94.1 ± 0.553	0.4

4. Conclusion

The method described enables to the quantification of Levofloxacin and Ambroxol hydrochloride. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility. Hence, this HPLC method can be used for routine drug analysis.

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