A NOVEL ANALYTICAL APPROACH FOR SIMULTANEOUS ESTIMATION OF LEVOFLOXACIN AND AMBROXOL HCL BY HPLC-DAD METHOD WITH DEGRADATION STUDIES

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Abstract:

A simultaneous estimation of Levofloxacin and Ambroxol HCl by HPLC-DAD method with degradation studies was developed. It was carried out using Symmetry C8 (4.6 x 150mm, 5µm, Make: XTerra) column with mobile phase consists Phosphate Buffer (6.2): Methanol: ACN [30:30:40, v/v/v] with flow rate 1mL/min at 244nm. Levofloxacin and Ambroxol HCl were eluted at 2.235 and 3.787mins respectively. Validation was performed as per ICH guidelines, which shows linearity 10-50 µg/mL of Levofloxacin and 1.5-7.5µg/mL of Ambroxol HCl with R^2 was 0.999. Accuracy was obtained to be 99.24-101.6% for Levofloxacin and 98.20-99.84% for Ambroxol HCl respectively and precision which shows %RSD less than 2%. Stress studies were carried out which shows that Levofloxacin is maximum degraded in peroxide and alkaline degradation and moderately degraded in acidic and thermal degradation and Ambroxol HCl is maximum degraded in acidic, peroxide and thermal degradation and less degraded in alkaline degradation. This method was applicable for formulation development, routine analysis and stability of Levofloxacin and Ambroxol HCl.

Keywords: Levofloxacin, Ambroxol HCl, Stress studies, ICH guidelines.

INTRODUCTION

Levofloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It inhibits DNA gyrase, a type II topoisomerase, and topoisomerase IV, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division.[(1)]Chemically Levofloxacin is (2S)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo [7.3.1.0{5,13}]trideca-5(13),6,8,11-tetraene-11-carboxylic acid was shown in Figure 1.

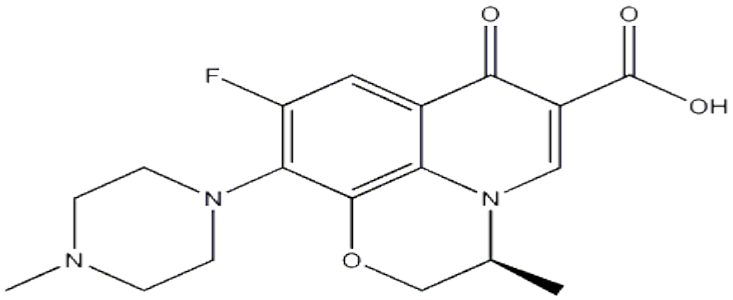

Figure 1. Chemical structure of Levofloxacin

Ambroxol HCl is a mucolytic agent which makes phlegm in the airways thinner and less sticky. It helps the cilia-tiny hairs that line respiratory tract-to transport the phlegm out of the lungs. [(2)] Chemically Ambroxol HCl is 4-[(2-amino-3, 5-dibromophenyl) methylamino] cyclohexan-1-ol; hydrochloride was shown in Figure 2.

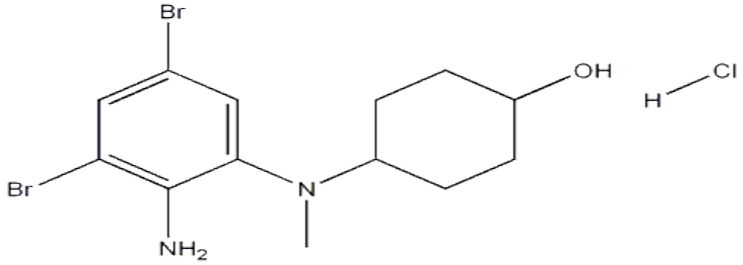

Figure 2. Chemical structure of Ambroxol HCl

Literature review revealed that there is a few analytical methods were developed either individual or combined with other drugs with maximum retention time and more consumption of mobile phase in the existing method. Such methods are as follows, HPLC method [(3-15)], UV Spectrophotometric method. [(16-19)] The objective of this work was to develop and validate a rapid method with forced degradation studies of Levofloxacin and Ambroxol HCl in bulk and pharmaceutical formulation by RP-HPLC as per ICH guidelines. [(20)] The basis for stability studies research is to present the information about the uniformity of a substance differs with an ecological variables such as temperature, humidity, light and permits the planned storage conditions, reanalysis phases and shelf life. [(21)]

MATERIALS AND METHODS

Chemicals:

Levofloxacin and Ambroxol HCl API were obtained from ASB Drugs, Hyderabad, India and Maps Labs Pvt. Ltd., India respectively. Sodium di-hydrogen orthophosphate, Sodium hydroxide, Water and Acetonitrile (Merck, HPLC-Grade) were utilized in the study.

Instruments:

The separation done on Waters 515 HPLC system, attached with PDA 2998 Detector and Empower software; Symmetry C8 (4.6 x 150mm, 5µm, Make: XTerra) were used as stationary phase. All amounts were weighed in an electronic balance (Sartorius), pH Meter (Poloman) and Sonicator (Fast Clean) were utilized in the study.

Preparation of phosphate buffer and mobile phase:

2.5 milligrams of sodium di-hydrogen orthophosphate was exactly weighed and taken into a 1L vol. flask to it 900mL of HPLC grade water was added and then ultrasonication for degassing then final volume was made upto the mark with HPLC grade water and adjusted the P^H 6.2 with with sodium hydroxide. The above phosphate buffer solution (30%), Methanol HPLC grade (30%) and Acetonitrile HPLC grade (40%) were mixed and degassed in an ultrasonicator about 15mins and then filtered through 0.45µ filter under vacuum filtration.

Standard and sample solution preparation:

Both standard and sample solution of 30µg/mL of Levofloxacin and 4.5µg/mL of Ambroxol HCl were prepared from standard and sample stock solution for this study.

RESULTS

Assay Methodology:

From the above standard and sample solution, 20µL were injected inside HPLC and % assay were calculated and results was revealed in Table 1 and Figure 3 and 4.

Table 1: Reports of Assay studies

Serial No.	Name of drug	Labelled amount (mg)	Observed amount (mg)	Average %Assay (n=6)	SD	%RSD
1.	Levofloxacin	500	491.05	98.21	0.89	0.91
2.	Ambroxol HCl	75	75.52	100.7	1.5	1.5

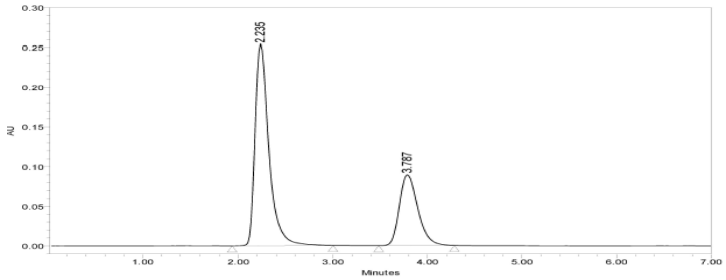

Figure 3. Chromatogram for standard Levofloxacin and Ambroxol HCl

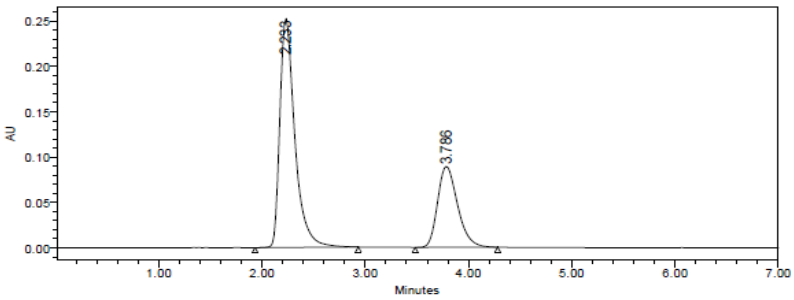

Figure 4. Chromatogram for sample Levofloxacin and Ambroxol HCl

Analytical method validation:

System Suitability:

It is important for assuring the performance quality of chromatographic system. So, system suitability studies were performed and the results were revealed in Table 2.

Table 2: Results of System suitability studies

Parameter	Levofloxacin	Ambroxol HCl
Theoretical plate number	2575	3259
Retention time	2.235min	3.787min
Tailing factor	1.55	1.29
Resolution		5.01
LOD (µg/mL)	0.03	0.002
LOQ (µg/mL)	0.09	0.005

Linearity:

A concentration range of 10-50 µg/mL of Levofloxacin and 1.5-7.5µg/mL of Ambroxol HCl was prepared and were injected inside HPLC system and the results were shown in Figure 5 and 6.

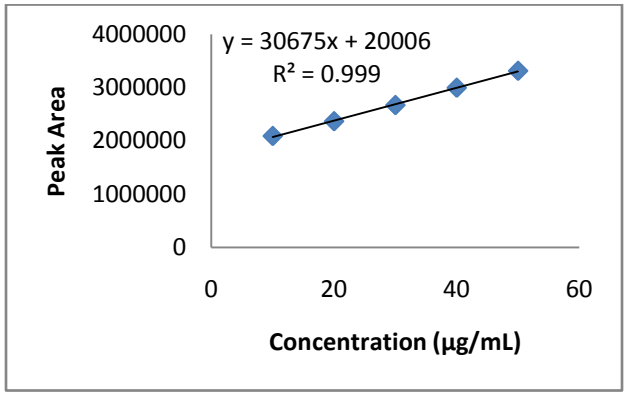

Figure 5. Linearity of Levofloxacin

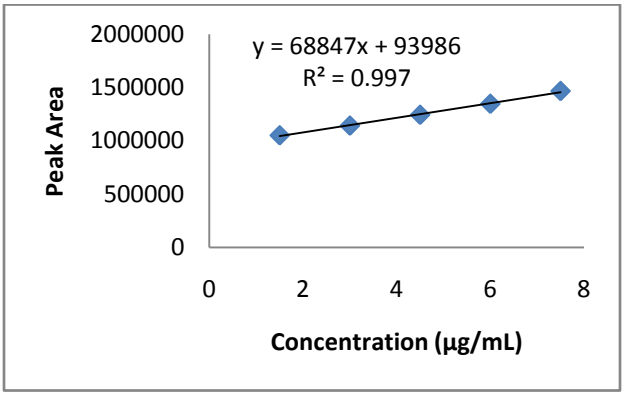

Figure 6. Linearity of Ambroxol HCl

Accuracy:

It expresses the closeness of agreement between the conventional true value and the value found. 50%, 100% and 150% concentration level were chosen three times for accuracy studies by standard addition method. % recovery and RSD were calculated and was shown in Table 3and 4.

Table 3: Recovery Studies of Levofloxacin

%Concentration (at specification Level)	Amount Added (mg)	Amount Found (mg)	Average % Recovery (n=3)	SEM	% RSD
50%	5	5.08	101.6	0.02	0.71
100%	10	9.97	99.73	0.06	1.12
150%	15	14.89	99.24	0.12	1.42

Table 4: Recovery Studies of Ambroxol HCl

%Concentration (at specification Level)	Amount Added (mg)	Amount Found (mg)	Average % Recovery (n=3)	SEM	% RSD
50%	5	4.91	98.20	0.04	1.54
100%	10	9.93	99.30	0.04	0.73
150%	15	14.98	99.84	0.05	0.64

Precision:

It is defined as the degree of agreement between individual test outcomes acquired when the method is applied to multiple sampling of a homogenous sample.

(a) System precision:

Standard solution of 30µg/mL of Levofloxacin and 4.5µg/mL of Ambroxol HCl were injected six times inside HPLC and %RSD were calculated and revealed in Table 5.

Table 5: System Precision of Levofloxacin and Ambroxol HCl

Injection/concentration (30µg/mL)	**Levofloxacin Peak Area**	**Injection/concentration (4.5µg/mL)**	**Ambroxol HCl Peak Area**
Injection-1	2552781	Injection-1	1195510
Injection-2	2557015	Injection-2	1197944
Injection-3	2568778	Injection-3	1202450
Injection-4	2568596	Injection-4	1201753
Injection-5	2571432	Injection-5	1203149
Injection-6	2571439	Injection-6	1203151
Average	2565007	**Average**	1200660
Standard Deviation	8038.6	**Standard Deviation**	3184.2
%RSD	0.31	**%RSD**	0.27

(b) Method precision:

Homogeneous sample solution of 30µg/mL of Levofloxacin and 4.5µg/mL of Ambroxol HCl were injected six times inside HPLC and % Assay with SD and %RSD were calculated and revealed in Table 6.

Table 6: Method Precision of Levofloxacin and Ambroxol HCl

Injection/concentration (30µg/mL)	**Levofloxacin % Assay**	**Injection/concentration (4.5µg/mL)**	**Ambroxol HCl % Assay**
Injection-1	99.44	Injection-1	99.37
Injection-2	99.59	Injection-2	99.57
Injection-3	100.03	Injection-3	99.13
Injection-4	100.03	Injection-4	99.07
Injection-5	100.13	Injection-5	99.19
Injection-6	100.13	Injection-6	99.19
Average	99.89	**Average**	99.25
SD	0.3	**SD**	0.2
%RSD	0.3	**%RSD**	0.2

Intermediate Precision:

It was also called as ruggedness and performed on different analyst and day. Standard solution of 30µg/mL of Levofloxacin and 4.5µg/mL of Ambroxol HCl were injected six times inside HPLC and %RSD were calculated and revealed in Table 7.

Table 7: Intermediate Precision of Levofloxacin and Ambroxol HCl

Injection/concentration (30µg/mL)	**Levofloxacin Peak Area**	**Injection/concentration (4.5µg/mL)**	**Ambroxol HCl Peak Area**
Injection-1	2549013	Injection-1	1195510
Injection-2	2551573	Injection-2	1197944
Injection-3	2559450	Injection-3	1194129
Injection-4	2568596	Injection-4	1198196
Injection-5	2570195	Injection-5	1197842
Injection-6	2570193	Injection-6	1197841
Average	2561503	**Average**	1196910
Standard Deviation	9593.72	**Standard Deviation**	1682.40
%RSD	0.37	**%RSD**	0.14

Robustness:

Standard solution of 30µg/mL of Levofloxacin and 4.5µg/mL of Ambroxol HCl were injected six times inside HPLC by varying flow rate at ±0.1mL/min and organic composition of mobile phase from ±2, %v/v and the robustness result were revealed in Table 8 and 9.

Table 8: Variations in flow rate

Sample Name	Change in flow rate (mL/min)	Retention Time (min) (n=6)	Average area of six injections	SD	%RSD
	0.9	2.348	2449988	9195	0.38
Levofloxacin	1.1	1.982	2158634	3401	1.58
Ambroxol	0.9	3.376	1035808	11057	1.07
HCl	1.1	3.357	1001357	17742	1.77

Table 9: Variations in organic composition of mobile phase

Sample Name	Change in organic composition of mobile phase	Retention Time (min) (n=6)	Average area of six injections	SD	%RSD
	32:29:39,%v/v	2.348	2449954	9948	0.41
Levofloxacin	28:31:41,%v/v	2.148	2383827	11695	0.49
Ambroxol	32:29:39,%v/v	3.783	1035180	11063	1.16
HCl	28:31:41,%v/v	3.785	1001968	17729	1.16

Stress studies:

A stress study was performed to assess the stability and specificity of the method. A concentration of 30µg/mL of Levofloxacin and 4.5µg/mL of Ambroxol HCl were chosen for the stress studies. 0.1 M HCl was taken for acid decomposition stress study and alkaline degradation was carried out using 0.1 M NaOH and both acid and base influenced drug samples were refluxed at 80ºC for 30 mins. After cooling the solutions was neutralized and diluted with mobile phase. For Hydrogen peroxide stress testing, diluted hydrogen peroxide of 0.3 to 3% can be used at 40°C. Drug solutions were also exposed to thermal degradation and the percentage drug recovered and the drug decomposed were calculated and revealed in Table 10.

Table 10: Forced degradation of Levofloxacin and Ambroxol HCl

S. No	Stress conditions	%Drug recovered		%Drug decomposed	
		Levofloxacin	Ambroxol HCl	Levofloxacin	Ambroxol HCl
1.	Acidic degradation	93.6	89.05	6.4	10.95
2.	Alkaline degradation	89	99.6	11	0.4
3.	Thermal degradation	96.9	80	3.1	20
4.	Peroxide degradation	84.3	89.4	15.7	10.6

DISCUSSION

Several mobile phase compositions were tried for the optimization of the method and a better resolution and symmetry peak of Levofloxacin and Ambroxol HCl was achieved using Phosphate Buffer (6.2): Methanol: ACN [30:30:40, v/v/v] mobile phase with a flow rate of 1mL/min at 244nm. Levofloxacin and Ambroxol HCl was eluted at 2.235 and 3.787mins respectively. Validation was performed as per ICH guidelines, which shows linearity 10-50 µg/mL of Levofloxacin and 1.5-7.5µg/mL of Ambroxol HCl with R^2 was 0.999. Accuracy was obtained to be 99.24-101.6% for Levofloxacin and 98.20-99.84% for Ambroxol HCl respectively

and precision which shows %RSD less than 2% and the LOD value of Levofloxacin was 0.03 µg/mL and Ambroxol HCl was 0.09 µg/mL and LOQ value of Levofloxacin was 0.002 µg/mL and Ambroxol HCl was 0.005 µg/mL. Robustness studies were successfully carried out and found to be < 2% RSD which indicates that the proposed method is robust. Stress studies were carried out which shows that Levofloxacin is maximum degraded in peroxide and alkaline degradation and moderately degraded in acidic and thermal degradation and Ambroxol HCl is maximum degraded in acidic, peroxide and thermal degradation and less degraded in alkaline degradation. The results and discussion reports revealed that the developed method is unambiguous and valid for the estimation of Levofloxacin and Ambroxol HCl by RP-HPLC and were applicable for formulation development, routine analysis and stability of Levofloxacin and Ambroxol HCl.

CONCLUSION

RP-HPLC method for Levofloxacin and Ambroxol HCl in bulk and mixed dosage form were developed and validated in accordance to ICH guidelines. Levofloxacin and Ambroxol HCl with correlation coefficient 0.999 were achieved with better linearity. Accuracy of the drug was attained in the range of 98-102% within the acceptance criteria. Precision were successfully carried out and found to be < 2% RSD which indicates that the proposed method is precise. The developed method is unambiguous and valid for estimation of Levofloxacin and Ambroxol HCl by RP-HPLC and also provides information about stability of drugs under stress conditions.

Compliance and Ethical Standards

Ethical Approval: NA

Funding details: NA

Conflict of interest: No

Informed consent: NA

Author's Contribution:

Each author contributed to the conception, design and execution of the study and also agreed to submit to the current journal.

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