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Analytical Method Development and Validation of Rp-Hplc for Loratadine and Ambroxol Hydrochloride in Combined Tablet Dosage Form by Using Simultaneous Estimation Method

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ABSTRACT:
The purpose of this study was to develop and validate a simple, sensitive, accurate, and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method for simultaneous estimation of Loratadine and Ambroxol Hydrochloride in a Tablet dosage form. The chromatographic measurement was performed on a Phenomenex Luna C18 (2) (150 x 4.6 mm, 5µm) column, mobile phase containing 0.1% TFA: ACN in ratio of 52.5:47.5. The flow rate was 1 ml/min, and the detecting wavelength was 210 nm. In the concentration range of 4-6 µg/ml for Loratadine and for Ambroxol Hydrochloride 48-72 µg/ml showed a linear response of the suggested approach. The correlation coefficients (r^2 values) for Loratadine and Ambroxol Hydrochloride were 1 and the retention times were 3.07 for Loratadine and 1.69 for Ambroxol Hydrochloride respectively. The developed chromatographic technique was validated for specificity, linearity, precision, accuracy, LOD, and LOQ using ICH Q2(R1) criteria. The analysis results have been validated in accordance to ICH guidelines.

Keywords: Analytical Method Development, ICH Q2 (R1), Loratadine, Ambroxol Hydrochloride.

I. INTRODUCTION

In modern chemistry, high-performance liquid chromatography (HPLC) is a potent analytical instrument. It performs exceptionally well at locating, quantifying, and sorting the constituents of liquid-dissolved samples. Often used in the study of pharmaceutical products, high performance liquid chromatography (HPLC) is highly valued for its accuracy in quantitative and qualitative evaluations, greatly advancing the field of analytical chemistry.[(1)]The precision of HPLC stems from subtle component behaviors during partitioning, providing a reliable technique for examining a variety of samples in industries such as analytical chemistry and pharmaceuticals.[(2)]A compound having a lower affinity for the stationary phase moves faster and covers a greater distance in high-performance liquid chromatography, whereas a molecule with a higher affinity moves more slowly and covers a shorter distance. The efficient separation and analysis of sample components is made possible by this differential migration.[(3)]

Loratadine is a second-generation peripheral histamine H1-receptor blocker used to treat allergies. In structure, it is closely related to tricyclic antidepressants, such as imipramine, and is distantly related to the atypical antipsychotic quetiapine. Loratadine was discovered in 1981 and came to market in 1993 [(4)]. It is on the World Health Organization's List of Essential Medicines; It is available as a generic drug and is marketed for its nonsedating properties. There is a version combined with pseudoephedrine, a decongestant; known as pseudoephedrine/loratadine. Loratadine is a tricyclic antihistamine, which acts as a selective inverse agonist of peripheral histamine H1-receptors. Histamine is responsible for many features of allergic reactions.Ambroxol hydrochloride is N- desmethyl metabolite of bromhexine (alkaloid vasicine derivative obtained from plant Vasaka-Adhatodavasica) is a potent mucolytic agent, capable of inducing thin copious bronchial secretion thus facilitating expectoration.[(5-7)]Ambroxol hydrochloride facilitates expectoration of excessive secretions by virtue of mucolytic and mucokinetic action via depolymerization of long mucopolysaccharide chains which ultimately results in their fragmentation. Ambroxol hydrochloride also acts as tissue protective due to its inhibitory effect on release of destructive mediators and free oxygen radicals by phagocytosis.[(8-10)]

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II. MATERIALS AND METHODS:

1. Chromatographic Conditions:

a. Oven Temp: 30º C
b. Flow rate: 1 ml/min.
c. Mobile Phase: 0.1% Trifluro acetic acid : Acetonitrile (52.5:47.5, % v/v)

Preparation of 0.1% Trifluro acetic acid: In a 1000 ml beaker, take 1000 ml of HPLC grade water and add 1 ml of Trifluro acetic acid and mix well. Filter twice using 0.45µ membrane filter and degas for 15 min.

d. Runtime: 5 minutes
e. Injection Volume: 10µl
f. Wavelength: 210nm
g. Diluent: 0.1% Trifluro acetic acid : Acetonitrile (50 : 50, % v/v)
h. Column : Phenomenex Luna C18 (2) (150 x 4.6 mm, 5 µ)

2. Standard Preparation:

Loratadine Standard Stock Solution-I (LSSS-I):

Initially Prepare a Standard Stock Solution (LSSS-I) of by adding5 mg of Loratadine in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. Further 1.0 ml of above solution was transferred to 10 ml volumetric flask and diluted to 10 ml with diluent.(Conc. Of Loratadine= 50 µg/ml).

Ambroxol Hydrochloride Standard Stock Solution-I (ASSS-I):

Then prepare a Standard Stock Solution (ASSS-I) of Ambroxol Hydrochloride by adding 6 mg in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Ambroxol= 600 µg/ml).

Then add 1.0 ml of LSSS-I &1.0 ml ASSS-I in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Loratadine=5 µg/ml & Ambroxol Hydrochloride =60 µg/ml).

Drug Product Sample Preparation for Assay:

10 tablets were weighed and average weight was calculated. And tablets was crushed & mixed in mortar and pestle.

Powder weight equivalent to 0.5mg Loratadine and 6 mg of Ambroxol Hydrochloride was weighed into 10 ml volumetric flask & add 5 ml diluent, sonicated for 5 minutes and make the volume to 10 ml with diluent. (Conc. of Loratadine =50 µg/ml and Ambroxol Hydrochloride = 600µg/ml).

Further, pipette out 1.0 ml of above solution in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Loratadine = 5 µg/ml and Ambroxol Hydrochloride = 60 µg/ml).

Selection of Wavelength:

The sample was scanned from 200-400 nm with DAD detector. The Wavelength selected for analysis chosen was 210 nm on basis of appropriate intensity of both the peaks.

Specificity & Assay:

Individual samples of Loratadine of 5 µg/ml and Ambroxol Hydrochloride of 60 µg/ml were prepared and peaks were for identified from Retention Time.

Blank was injected to ensure there is no blank peak interfering with the main analyte peaks.

Assay was calculated by using following formula;

$$\% \textbf{ Assay} = \frac{\textbf{SampleArea}}{\textbf{StandardArea}} \textbf{x100}$$

Repeatability& System Suitability:

A single sample was prepared as described and 6 injections were made from same sample and checked for system suitability.

System suitability parameters are as below:

1. Retention Time,
2. Theoretical plates,
3. Asymmetry (Tailing factor),
4. Resolution.

Linearity & Range:

5 samples of varying concentrations ranging from 80-120% were made.

The concentrations are given below

% Level	Loratadine Conc. (µg/ml)	Ambroxol Conc. (µg/ml)
80	4.0	48
90	4.5	54
100	5.0	60
110	5.5	66
120	6.0	72

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The sample preparations are given as below;

X ml of Loratadine and Y ml of Ambroxol Hydrochloride were added to 10 ml diluent to make up the concentrations given above:

X ml of LSSS-I	Y ml of ASSS-I	Diluted to
0.8	0.8	10 ml
0.9	0.9	10 ml
1.0	1.0	10 ml
1.1	1.1	10 ml
1.2	1.2	10 ml

Accuracy:
Samples were prepared of 80%, 100% and 120% concentration by spiking the same amount of concentration given above in table for both Loratadine and Ambroxol Hydrochloride.Samples were injected in triplicate to calculate % RSD. % recovery was also calculated.

LOD/ LOQ:
Was calculated for both drugs by using ANOVA technique.
Formula:

$$\text{LOD} = \frac{3.3 \times \text{Std. ErrorofIntercept}}{\text{CoefficientsofXVariable 1}}$$

$$\text{LOQ} = \frac{10 \times \text{Std. ErrorofIntercept}}{\text{CoefficientsofXVariable 1}}$$

Robustness:
The Robustness was performed by changing the column temperature by ± 2˚C.Each Sample was injected % Assay was calculated at each condition was calculated.

Condition	Increased	Normal	Decreased
Column Oven Temperature	32˚C	30˚C	28˚C

Intra & Inter-day Precision:
Single mixture working standard and drug product samples were prepared and injected twice in a day at different time intervals to evaluate intra-day precision.Same mixture working standard and drug product samples were analyzed on second day to evaluate the inter-day precision.% Assay and % RSD was calculated at each interval and stability of solutions was estimated.[11-13]

III. RESULT AND DISCUSSION:

i) Selection of analytical wavelength:
Selection of Wavelength:
The sample was scanned from 200-400 nm with DAD detector. The Wavelength selected for analysis chosen was 210 nm on basis of appropriate intensity of both the peaks.

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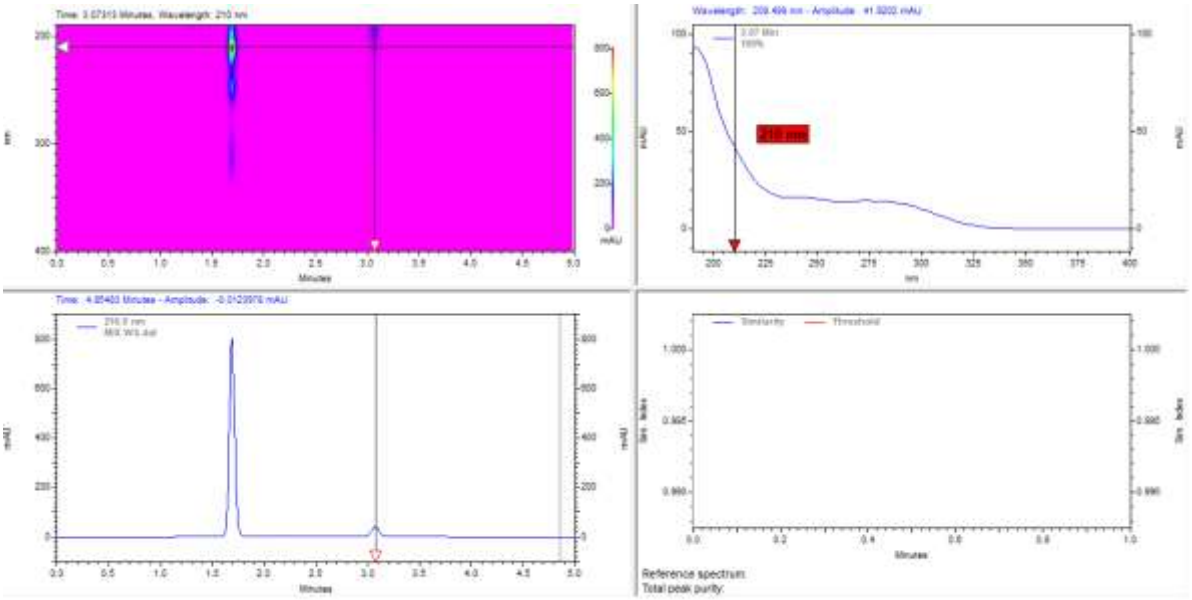

Figure 1: Spectrum of Loratadine and Ambroxol hydrochloride between 200-400nm in mobile phase.

Loratadine RT 3.07 min and Ambroxol hydrochloride RT 1.69 min show the maximum absorbance at 210 nm. Hence, HPLC analysis was carried out at 210 nm. (Figure. 1)

ii) Optimization of Chromatographic Conditions for Loratadine and Ambroxol hydrochloride:

The column was saturated with the mobile phase. Standard solution of Loratadine and Ambroxol hydrochloride was injected to get the chromatogram. The retention times for the two drugs were found to be:

Drug name:
Retention time.
Loratadine
3.07 ± 0.5 min
Ambroxol hydrochloride 1.69 ± 0.5 min

Table 1. Details of Various trial of mobile phase for mixture containing of Loratadine and Ambroxol hydrochloride.

Sr. No.	MP	Ratio	Diluent	Wavelength	Ambroxolhydrochloride			Loratadine		
					RT	Asymmetry	TP	RT	Asymmetry	TP
1	0.1% TFA : ACN	50-50	50 0.1% TFA - 50 ACN	250 nm	1.61	1.02	5142	7.61	0.84	10523
2	0.1% TFA : ACN	55-45	50 0.1% TFA - 50 ACN	210 nm	1.78	1.01	5408	3.59	1.00	9010
3	**0.1% TFA : ACN**	**52.5-47.5**	**50 0.1% TFA - 50 ACN**	**210 nm**	**1.69**	**1.05**	**5175**	**3.07**	**0.99**	**8515**

Final Method: Phenomenex Luna C18 (2) (150 x 4.6 mm, 5µm) column, at 1 ml/min flow rate, detection wavelength is 210 nm, mobile phase containing **0.1% TFA : ACN** in ratio of **52.5:47.5.**

Different mobile phases like **0.1% TFA: ACN** in ratio of **52.5:47.5** were used with different ratio and analyzed for best resolution of peaks in chromatogram.

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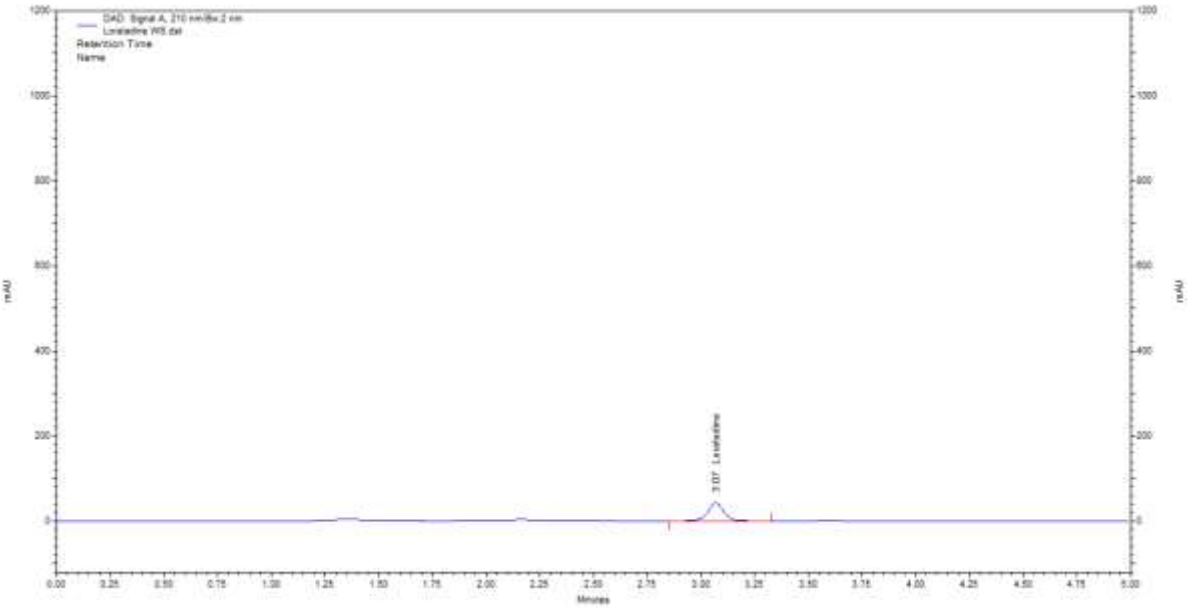

Figure 2: Chromatogram of Standard Loratadine.

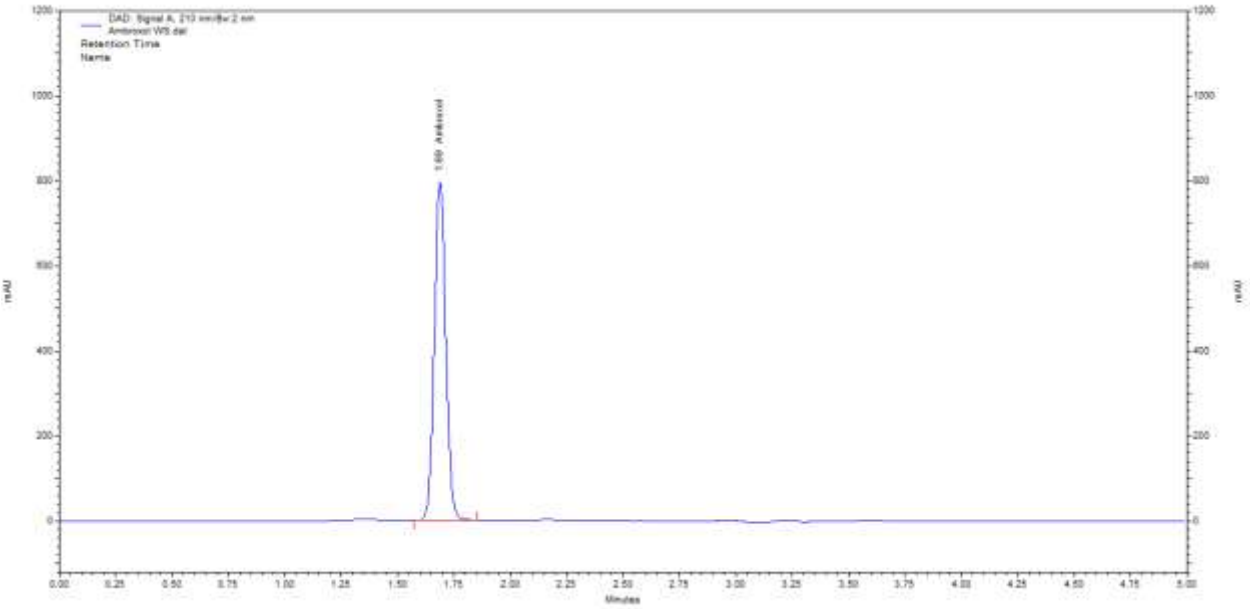

Figure 3: Chromatogram of Standard Ambroxol hydrochloride.

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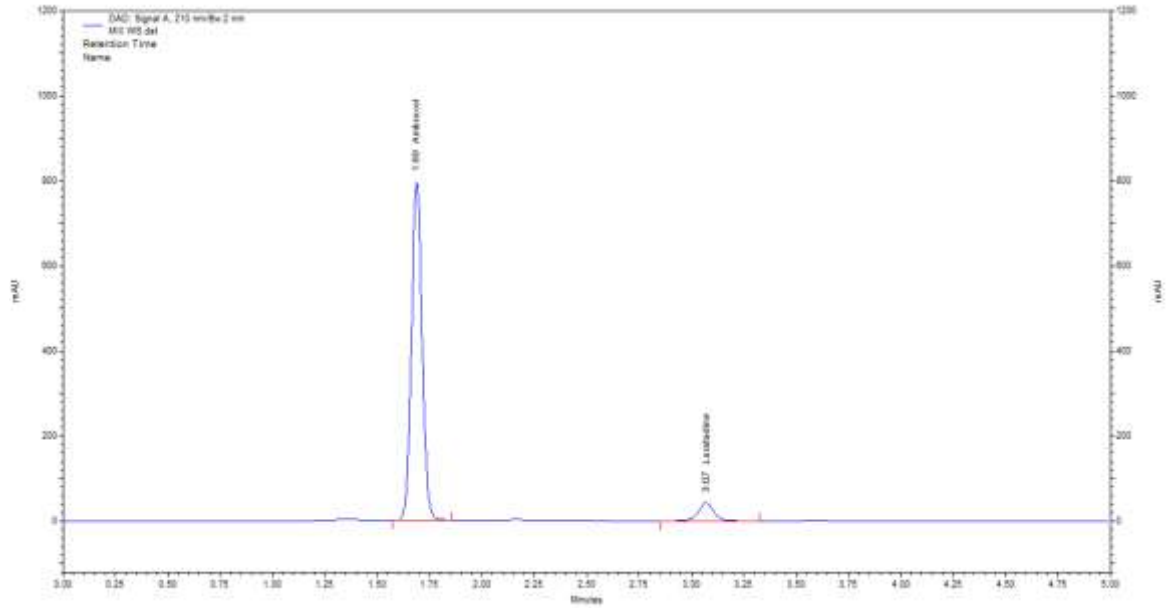

Figure 4: Chromatogram of Standard Mixture of Loratadine and Ambroxol hydrochloride in optimized chromatographic conditions.

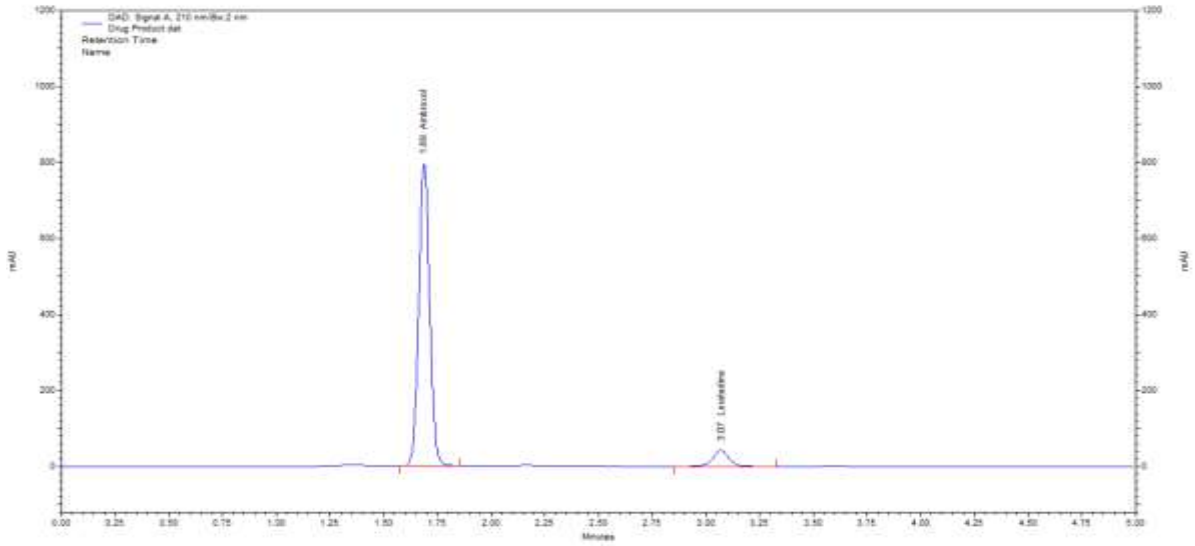

Figure 5: Chromatogram of Sample of Loratadine and Ambroxol hydrochloride in optimized chromatographic conditions

iii) Analysis of tablet formulation:-

Table 2 Analysis of Marketed formulation

Sample	Ambroxol hydrochloride.			Loratadine		
	RT	Area	% Assay	RT	Area	% Assay
Blank	-	-	-	-	-	-
Ambroxol hydrochloride.	1.69	5852145	-	-	-	-
Loratadine	-	-	-	3.07	458546	-

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MIX WS	1.69	5886869	-	3.07	451169	-
Drug Product	1.69	5862241	**99.58**	3.07	447520	**99.19**

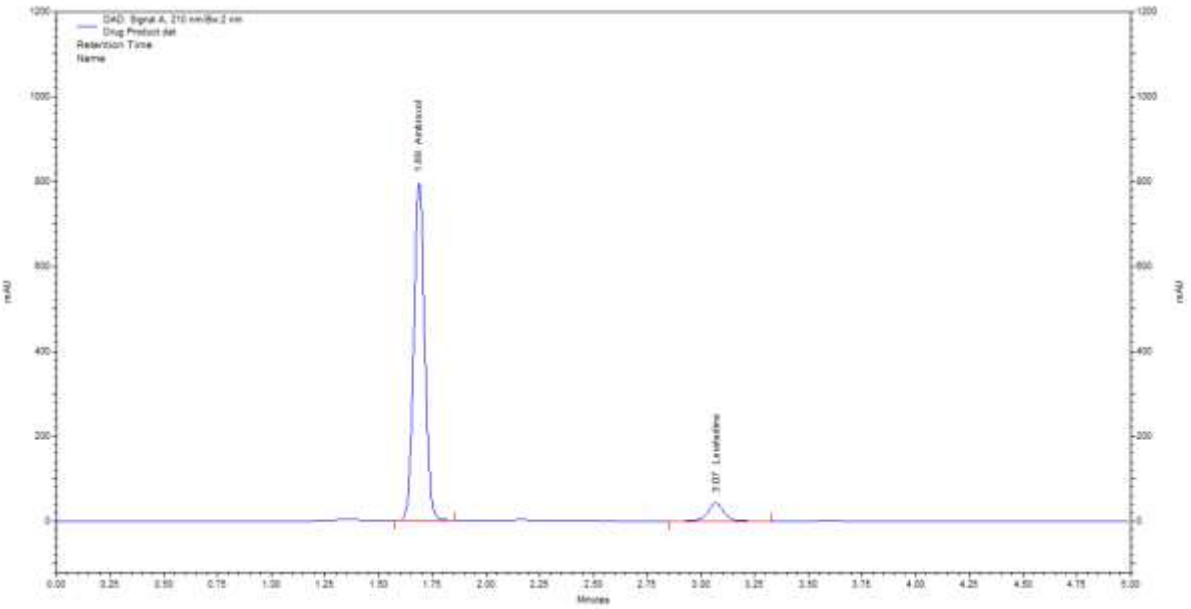

Figure 6 Chromatogram of Loratadine and Ambroxol hydrochloride in tablet formulation.

Amount of drug present in the marketed formulation was calculated using RP-HPLC. Amount of Loratadine and Ambroxol hydrochloride was found to be 99.19 &99.58 % respectively. This method can be employed for routine analysis of Loratadine and Ambroxol hydrochloride. The result of assay of marketed formulation is given in Table 1.

VALIDATION OF RP-HPLC METHODE:

I. Linearity:

Different concentration of solution prepared for Linearity of both Loratadine and Ambroxol hydrochloride are shown in (Table 3 and Table 4) calibration curves are shown in Figure 7 & 8 respectively.

Table 3 Linearity dilutions for Loratadine.

Loratadine		
% Level	**Conc. (µg/ml)**	**Area**
80	4	360303
90	4.5	406505
100	5	451169
110	5.5	496849
120	6	541663

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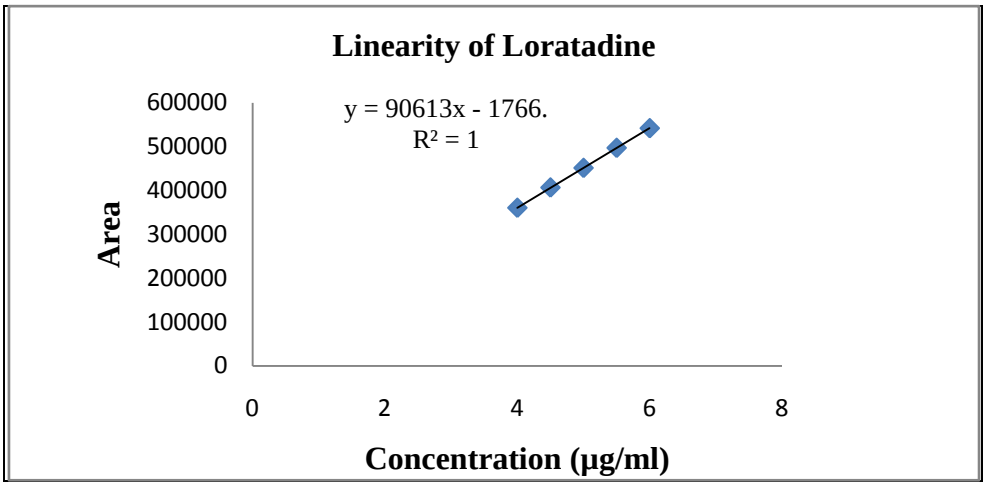

Figure 7 Calibration curve of Loratadine

Table 4 Linearity dilutions for Ambroxol hydrochloride.

Ambroxol hydrochloride		
% Level	**Conc. (µg/ml)**	**Area**
80	48	4711880
90	54	5300843
100	60	5886869
110	66	6464593
120	72	7040942

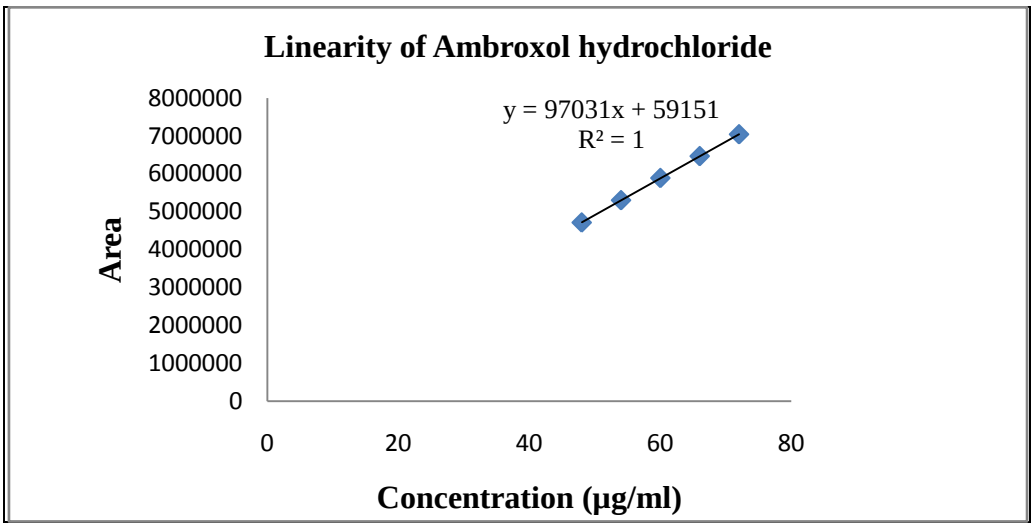

Figure 8 Calibration curve of Ambroxol hydrochloride.

According to ICH guideline linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of an analyte Linearity was studied by plotting a graph of area v/s concentration. A series standard solution of Loratadine and Ambroxol hydrochloridewere prepared in the concentration range of 4 µg/ml to 6 µg/mL and 48 µg/mL to 72 µg/mL respectively

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with linearity range 80-120% for both the drug and is shown in Table 3 and 4.

II. Precision:

The Precision study of Loratadine and Ambroxol hydrochlorideare shown **Table 7.15** respectively.

Table 5 Precision of Loratadine and Ambroxol hydrochloride.

Intra Day precision					
Day 1	**Sample ID**	**Ambroxol hydrochloride**		**Loratadine**	
		Area	**Assay**	**Area**	**Assay**
Morning	**WS**	5886869	-	451169	-
	DP	5862241	99.58	447520	99.19
Evening	**WS**	5871045	-	449574	-
	DP	5844147	99.54	445478	99.09
Inter Day precision					
Day	**Sample ID**	**Ambroxol hydrochloride**		**Loratadine**	
		Area	**Assay**	**Area**	**Assay**
Day 2	**WS**	5845175	-	441785	-
	DP	5812142	99.43	437511	99.03
		% RSD	0.08	**% RSD**	0.08

The accuracy of an analytical process used to determine intra-day and inter-day variation. The percentage relative standard deviation (RSD) for inter-day precision was 0.08 % for Ambroxol hydrochloride and 0.08 % for Loratadine. The obtained findings are less than 2% suggests a high level of precision.

III. Accuracy:

The accuracy study of Loratadine and Ambroxol hydrochloride are shown inTable 6 and 7respectively.

Table 6. Accuracy Study of Loratadine.

% Level	**Reps**	**Spiked Conc. (µg/ml)**	**Area**	**Amount Recovered (µg/ml)**	**% Recovery**	**AVG**	**STDEV**	**RSD**
80%	Rep 1	3.988	360303	3.98	99.78	99.92	0.13	0.13
	Rep 2	3.988	361214	3.99	100.03			
	Rep 3	3.988	360957	3.99	99.96			
100%	Rep 1	4.985	451169	4.98	99.95	100.22	0.23	0.23
	Rep 2	4.985	452814	5.00	100.32			
	Rep 3	4.985	453102	5.00	100.38			
120%	Rep 1	5.982	541663	5.98	100.00	99.99	0.06	0.06
	Rep 2	5.982	541254	5.98	99.92			
	Rep 3	5.982	541875	5.98	100.04			

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Table 7. Accuracy Study of Ambroxol hydrochloride.

% Level	Reps	Spiked Conc. (µg/ml)	Area	Amount Recovered (µg/ml)	% Recovery	AVG	STDEV	RSD
80%	Rep 1	47.856	4711880	47.98	100.26	100.12	0.14	0.14
	Rep 2	47.856	4705314	47.91	100.12			
	Rep 3	47.856	4698545	47.84	99.97			
100%	Rep 1	59.82	5886869	59.94	100.21	100.00	0.19	0.19
	Rep 2	59.82	5872456	59.80	99.96			
	Rep 3	59.82	5865215	59.72	99.84			
120%	Rep 1	71.784	7040942	71.70	99.88	100.34	0.41	0.41
	Rep 2	71.784	7094568	72.24	100.64			
	Rep 3	71.784	7086210	72.16	100.52			

The method's accuracy defines how close the method's results are to the true value. The results of the accuracy testing revealed that the technique is accurate within acceptable ranges. When the % RSD for Vildagliptin and Pioglitazone is calculated, all of the results are within acceptable bounds. A maximum RSD of 2.0% indicated acceptable accuracy within the range. The results are shown in Table 6 and 7.

According to the Accuracy research, the percent recovery of Loratadine is 99.92-100.22 % and Ambroxol hydrochloride is 100-100.34 %, both of which are within the ICH standards.[14-15]

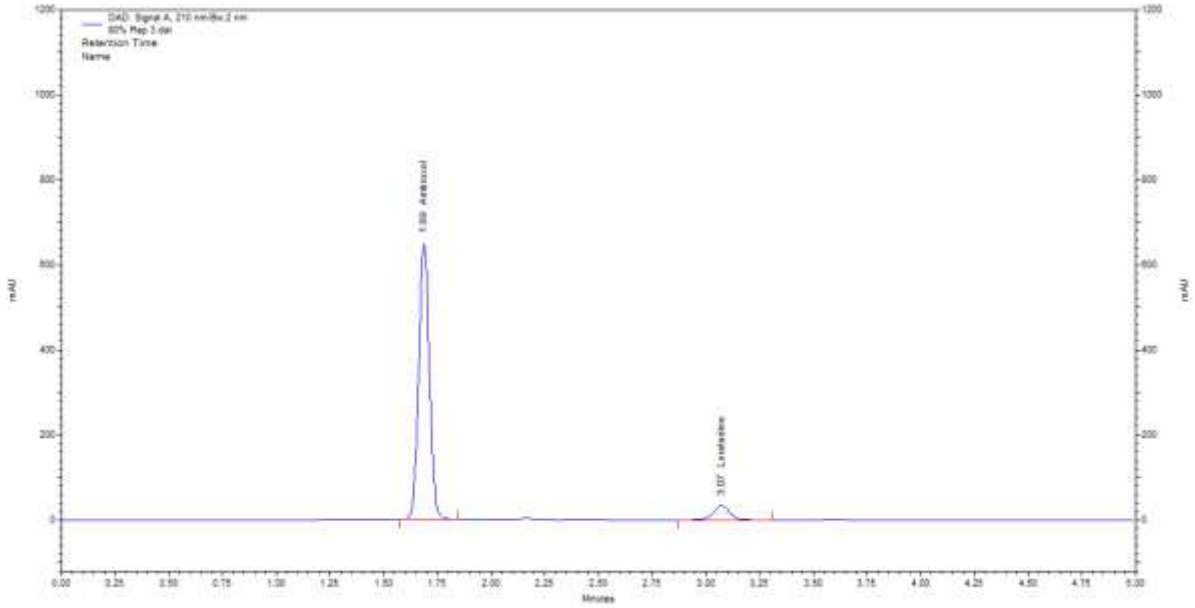

Figure 9 Chromatogram of Accuracy at 80%

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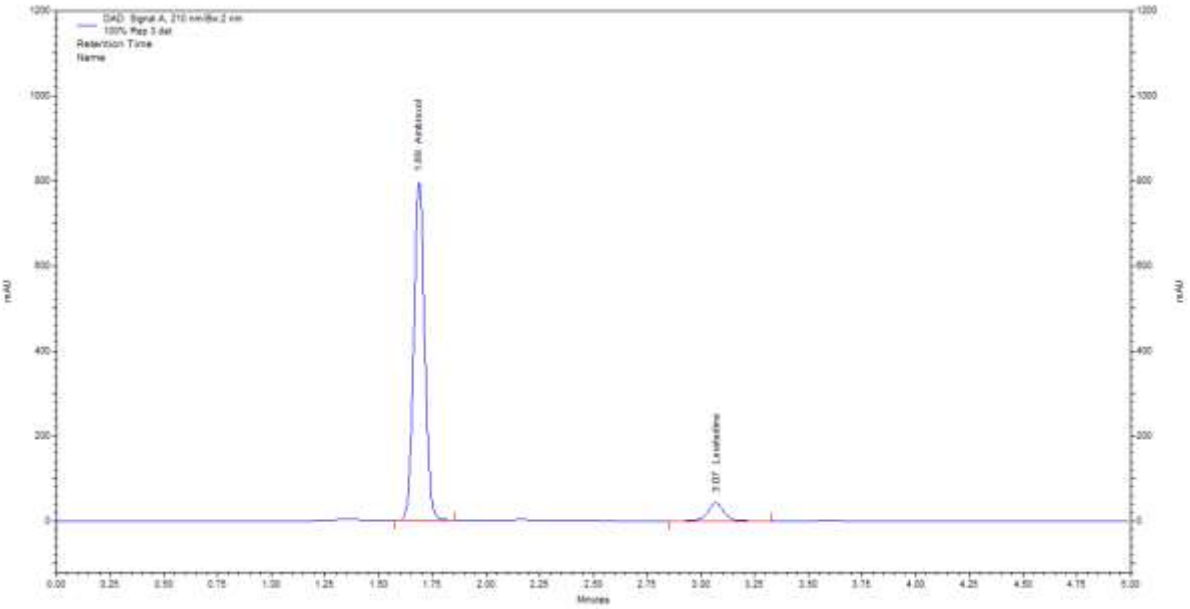

Figure 10 Chromatogram of Accuracy at 100%

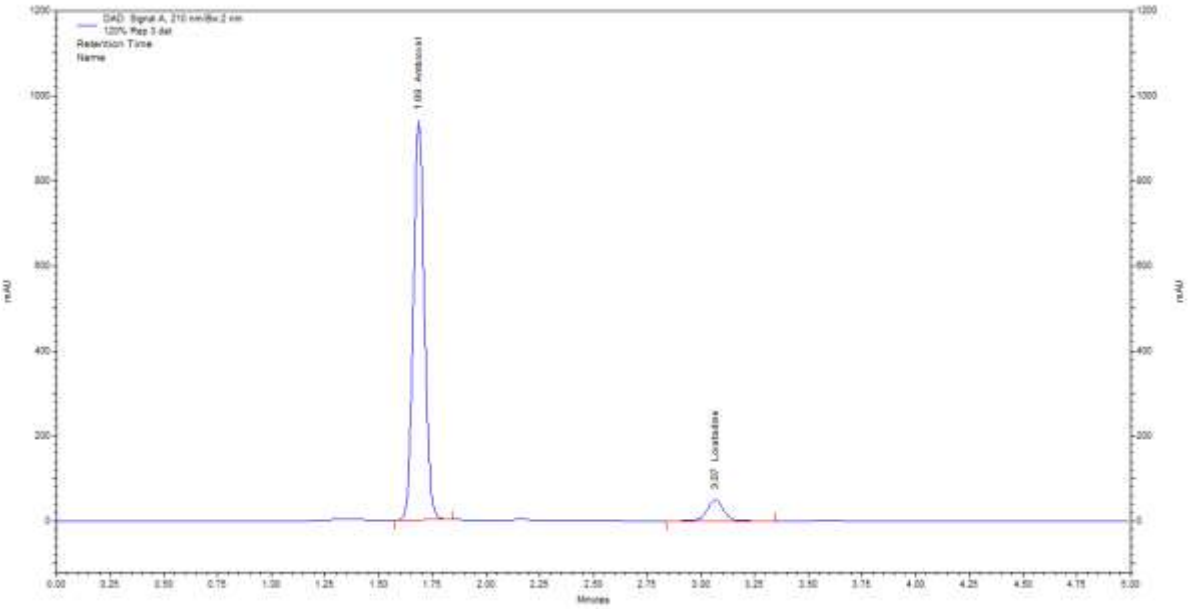

Figure 11 Chromatogram of Accuracy at 120%

iv. Limit of Detection (LOD) and Limit of Quantification (LOQ):

Table 8. The LOD and LOQ of Loratadine and Ambroxol hydrochloride.

Sr.No	Name of drug	LOD (µg/mL)	LOQ (µg/mL)
1.	Loratadine	0.05	0.15
2.	Ambroxol hydrochloride	0.57	1.73

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iv. System suitability:
System suitability data of Loratadine and Ambroxol hydrochloride given in below Table 9 and 10.

Table 9 System suitability parameter of Loratadine.

	Loratadine			
Sample ID	**RT**	**Asymmetry**	**Theoretical Plates**	**Resolution**
100% Rep 1	3.07	0.97	8552	12.26
100% Rep 2	3.07	0.99	8501	12.26
100% Rep 3	3.07	0.98	8544	12.26
100% Rep 4	3.07	1.01	8625	12.26
100% Rep 5	3.07	0.98	8419	12.26
100% Rep 6	3.07	1.02	8564	12.26
AVG	3.07			
STDEV	0.00			
RSD	0.00			

Table 10 System suitability parameter of Ambroxol hydrochloride.

Ambroxol hydrochloride				
Reps	**RT**	**Asymmetry**	**Theoretical Plates**	**Resolution**
100% Rep 1	1.69	1.11	5237	0.00
100% Rep 2	1.69	1.12	5187	0.00
100% Rep 3	1.69	1.08	5322	0.00
100% Rep 4	1.69	1.08	5025	0.00
100% Rep 5	1.69	1.04	5199	0.00
100% Rep 6	1.69	1.09	5254	0.00
AVG	1.69			
STDEV	0.00			
RSD	0.00			

The system, method, and column performance were validated by testing system suitability features. Six times, a standard solution of Loratadine and Ambroxol hydrochloridewas injected into the system, and the system's suitable features were evaluated. Results are shown inTable 9 and 10.

V. Robustness: Robustness data of Loratadine and Ambroxol hydrochloridegiven in below

Table 11 Robustness parameter of Loratadine and Ambroxol hydrochloride.

Column Oven Temp Change							
Condition	**Sample**	**Ambroxol**			**Loratadine**		
		RT	**Area**	**Assay**	**RT**	**Area**	**Assay**
28℃	**WS**	1.69	5841405	-	3.07	450525	-
	DP	1.69	5812414	99.50	3.07	446526	99.11
30℃	**WS**	1.69	5886869	-	3.07	451169	-
	DP	1.69	5862241	99.58	3.07	447520	99.19
32℃	**WS**	1.69	5836545	-	3.07	450625	-

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	DP	1.69	5802054	99.41	3.07	446921	99.18
	% RSD		0.08			0.10	

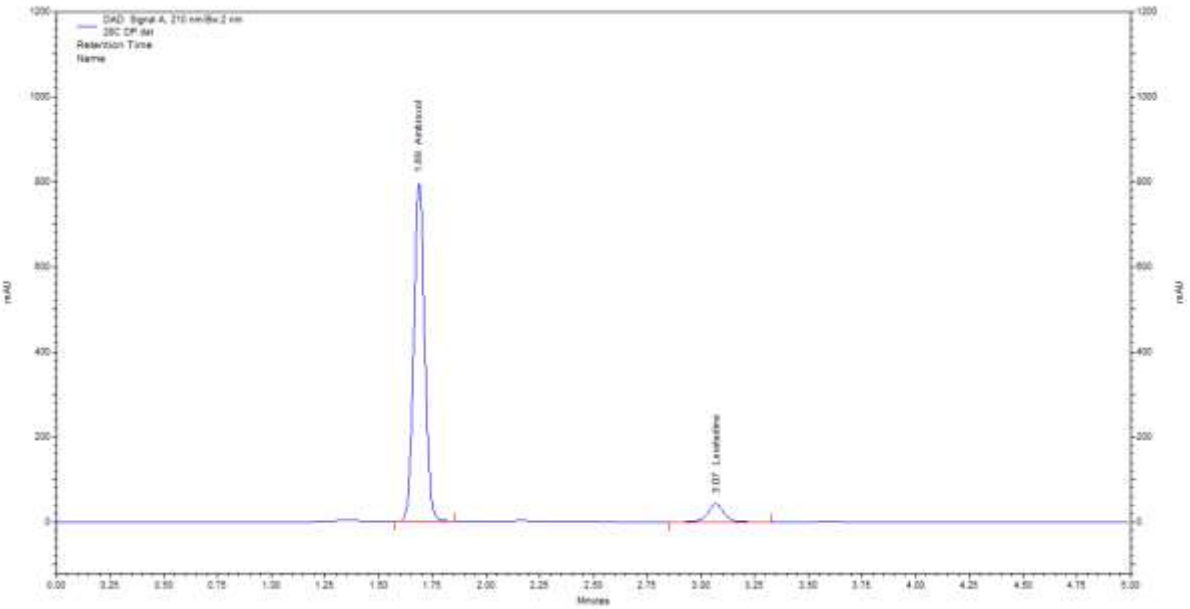

Figure 12 Chromatogram of Column Oven Temperature at 28 ° C

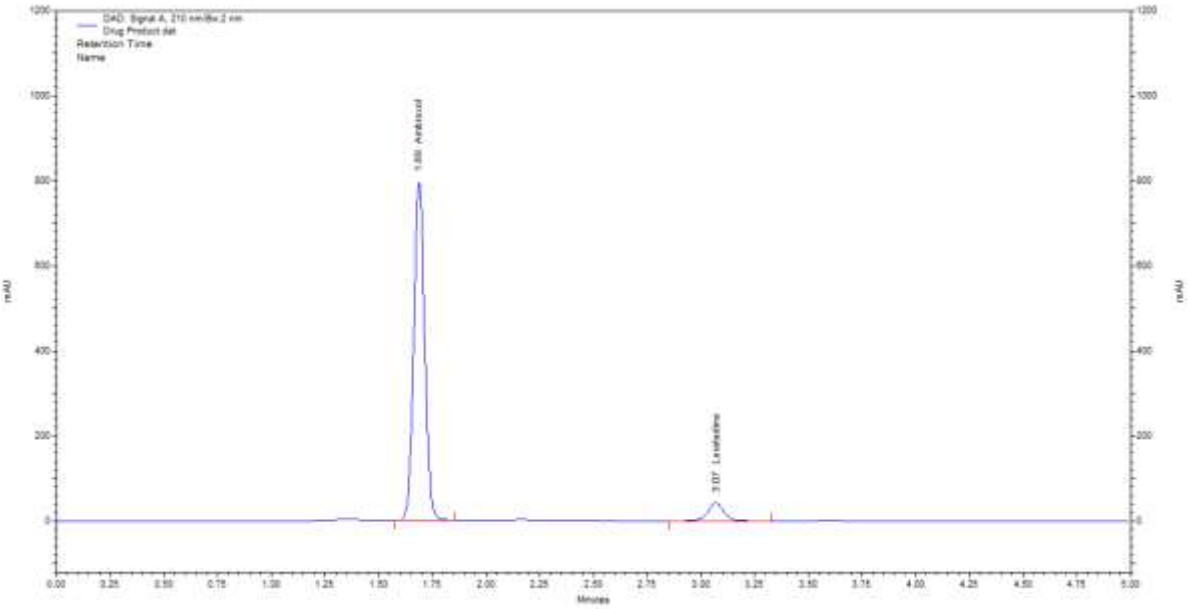

Figure 13 Chromatogram of Column Oven Temperature at 30 ° C

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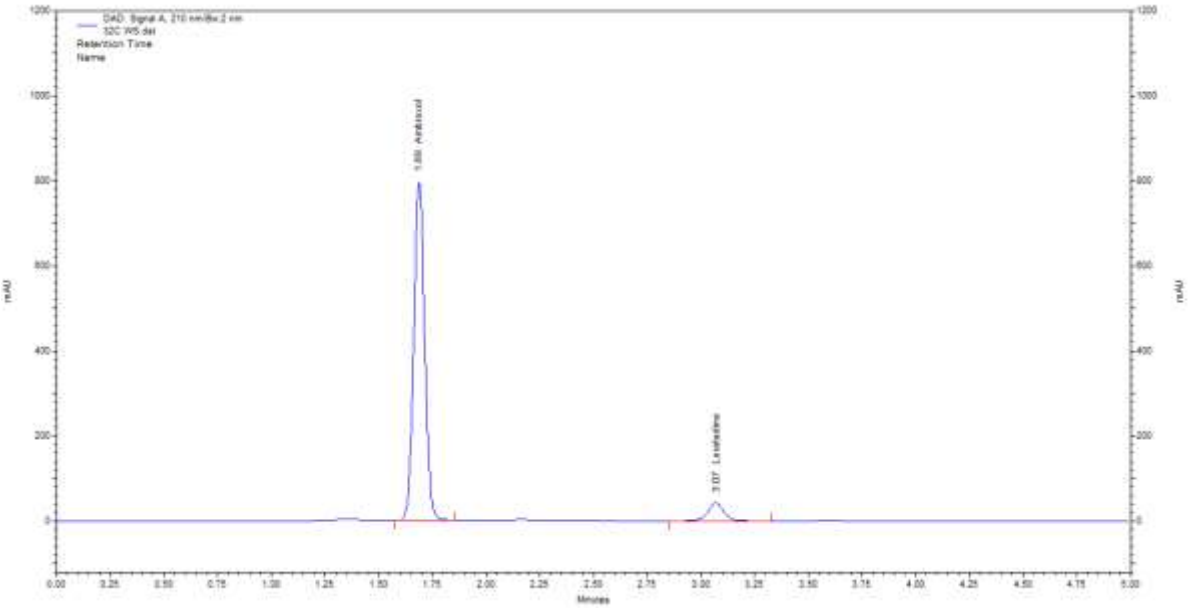

Figure 14 Chromatogram of Column Oven Temperature at 32 º C

Robustness was investigated using various deliberate alterations in chromatographic settings, such as changes in column Condition like 28˚C, 30˚C and 32˚C. RSD was shown to be less than 2% in the Loratadine and Ambroxol hydrochloride robustness studies. As a result, it is strong and adheres to ICH criteria. Results are shown in Table 11.

IV. CONCLUSION:

The developed and validated RP-HPLC method makes it simple and rapid to determine the quantitative amounts of Loratadine and Ambroxol hydrochloride from their formulations. According to ICH guidelines, all validation parameters were verified to be within the allowed ranges. Regardless of the excipients employed, it was found that the suggested procedure was simple, exact, accurate, robust, and particular for the medications of interest. It can be used for the routine analysis of the sold formulations.

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