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RESEARCH ARTICLE

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AMBROXOL HYDROCHLORIDE AND LORATADINE IN TABLET DOSAGE FORM BY RP-UHPLC

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Abstract

Analytical method for the combination of Ambroxol and Loratadine by UHPLC is not reported till date. Hence UHPLC method has been developed and validated for the estimation of Ambroxol hydrochloride and Loratadine in tablet dosage form. The separation was carried out using a UHPLC column Waters C_{18} column (50 mm x 4.6 mm, 3.5µ) and further elution was optimized using degassed mobile phase containing a mixture of 300 volumes of Methanol, 300 volumes of phosphate buffer pH 6.0 and 400 volumes Acetonitrile. The retention time for Ambroxol Hydrochloride and Loratadine was found to be 1.96 min and 3.41 min respectively. The validation results were found to be linear in the concentration range of 300-900µg/ml for Ambroxol Hydrochloride and 25-75µg/ml for Loratadine with corresponding correlation co-efficient of 0.9996 and 0.9994 respectively. The percentage recovery was found to be 99.55% for Loratadine and 99.12% for Ambroxol Hydrochloride. The amount of drug in the formulation was found to meet with the label claim and the percentage assay was 59.737 mg (99.56%) for Ambroxol Hydrochloride and 4.9574 mg (99.15 %) for Loratadine. The results showed that this proposed UHPLC method to be specific, accurate, precise, linear, and rugged for determination of Ambroxol hydrochloride and Loratadine in pharmaceutical dosage form, the validation of method was carried out according to ICH guidelines.

Introduction:-

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 [1-3]. 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[4-6].Loratadine is a second-generation peripheral histamine H_1-receptor blocker used to treat allergies. In structure, it

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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 H_1-receptors. Histamine is responsible for many features of allergic reactions.

Though High performance liquid chromatography (HPLC) is a well established reliable technique used in controlling the quality and consistency of active pharmaceutical ingredients (APIs) and dosage forms, it is often a slow technique because of complexity of the samples. Ultra high performance liquid chromatography (UHPLC) is a new category of elution technique in which the mobile phase is forced through the column at high speed. Owing to its speed and sensitivity, this technique is gaining significance in recent years for pharmaceutical studies the quantitative determination of Ambroxol hydrochloride and Loratadine was performed by RP-UHPLC. This method was successfully validated according to the International conference Harmonization (ICH) guidelines.

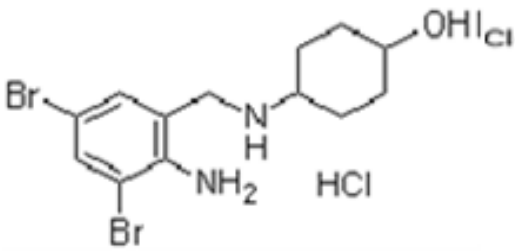

Fig.1:- Ambroxol Hydrochloride

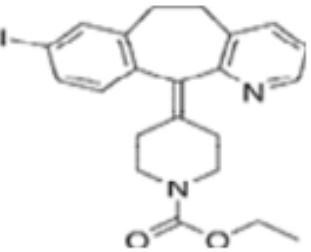

Fig.2:- Loratadine.

Materials and methods:-

Chemicals and Solvents:-

Acetonitrile, Water, and Potassium dihydrogen orthophosphate, Methanol used were of HPLC grade and purchased from Rankem. The reference samples of Ambroxol Hydrochloride (API) and Loratadine (API) were obtained as gift samples from New Jersey Life Care Pharma Pondicherry. Pharmaceutical formulation was obtained from Indian market.

Instrument and Chromatographic Conditions:-

Analysis was carried out using UHPLC (AGILENT 1220 infinity LC) with binary pump and variable wavelength UV-VISIBLE detector. Data was analyzed by using Open lab Chemstation software. Waters C_{18} column (50 mm x 4.6 mm, 3.5µ) was used for the separation. The optimized degassed mobile phase consisting of 300 volumes of Buffer (Prepared by dissolving 2.7218gm of potassium dihydrogen orthophosphate in 1000 ml Volumetric flask, dissolve it and dilute to 1000 ml with HPLC water and the pH was adjusted to 6.0 with Sodium hydroxide), 300 volumes of Methanol and 400 volumes of Acetonitrile and the mobile phase was filtered through 0.45µm membrane filter using vacuum pump prior to use. Isocratic elution technique was employed and the detection was carried out at 247 nm. The injection volume was 2µl at a flow rate of 0.6 ml/min. Methanol and Mobile phase was used as diluents.

Preparation of standard solution:-

Weighed accurately about 250 mg of Loratadine and 600 mg of Ambroxol separately in two individual clean and dry 100ml volumetric flask then methanol was added to dissolve completely and made upto the volume with methanol. Further Pipette out 1 ml of Loratadine solution and 5 ml of Ambroxol Solution into same clean and dry 50 ml volumetric flask diluted to the mark with mobile phase. (To get Concentration of 50mcg/ml for Loratadine and 600 mcg for Ambroxol).

Preparation of Sample solution:-

Weighed accurately 10 tablets, crush in mortar and pestle and transferred weight equivalent to one tablet (marketed formulation), in clean dry 100 ml volumetric flask then added 15 ml of methanol, dissolved by sonicating it in ultrasonic water bath for 30 minutes, and then added mobile phase to dissolve well and made upto the mark with mobile phase.

Optimized chromatographic conditions:-

Instrument used	:	Agilent 1220 UHPLC with Auto sampler and UV detector
Mobile Phase	:	Buffer : Methanol : Acetonitrile (300 : 300 : 400)
Temperature	:	30°C
Column	:	Waters RP 18 (4.6 x 50 mm, 3.5 µm)
Buffer	:	2.7218 gm of potassium dihydrogen orthophosphate in 1000 ml water pH adjusted with sodium hydroxide
pH	:	6.0
Flow rate	:	0.6 ml/min
Wavelength	:	247 nm
Injection Volume	:	2 µl
Run Time	:	5 min

Method validation:-

Validation is a means to prove that an equipment or process actually performs as per design or requirement. This is achieved by measuring any attribute that is possible to quantify. Method was validated according to ICH guidelines.

System Suitability:-

The System suitability/System Precision of an analytical procedure expresses the suitability of chromatographic conditions and an evident that the system is giving a precise value. System suitability was carried out and the results were shown in Table-01 (Figure1, 2).

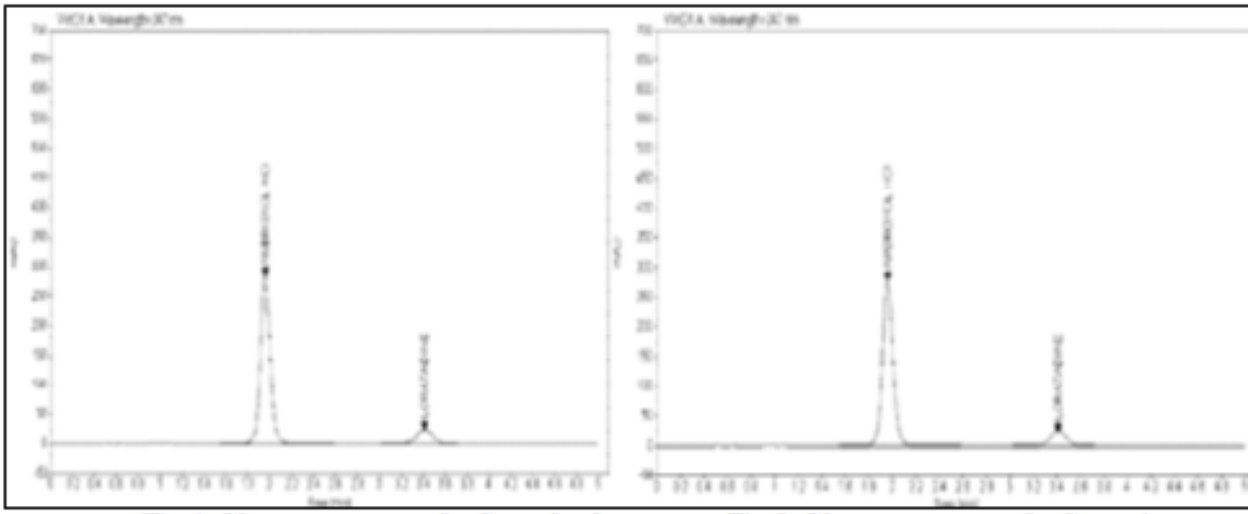

Fig 1 Chromatogram for Standard

Fig 2 Chromatogram for Sample

Table 1:- Results of System Suitability.

INJECTION No	Ambroxol Area	Loratadine Area
1	1766.12	227.40
2	1774.93	228.55
3	1790.78	230.93
4	1798.79	231.44
5	1802.30	231.90
Average:	1786.58	230.04
SD :	15.56	1.96
%RSD :	0.871	0.853

Specificity:-

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present and also to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample. It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample. Results were shown in Table- 02

Table 2:- Results of Specificity.

Name of solution	Retention time (min)
Blank	No peaks
Ambroxol hydrochloride	1.96
Loratadine	3.41

Accuracy and precision:-

The accuracy of an analytical procedure express the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Assessed using 9 determinations over 3 concentrations over specified range by preparing the Solutions of 80 %, 100 % and 120 % sample solutions and Inject the standard solution and different concentrations of sample solution (80 %, 100 %, and 120 %).Calculate the amount found and amount added for Ambroxol and Loratadine and calculate the individual recovery and mean recovery values. The % Recovery for each Level should be between 98.0 to 102.0 % and the results are given in Table 03, 04

Table 3:- Results of Ambroxol Accuracy and Precision

AMBROXOL HYDROCHLORIDE		ACCURACY		PRECISION		
S no.	Sample ID	mg / Cap	Assay percentage	Average at individual concentration levels	SD at individual concentration levels	% CV at individual concentration Levels
	Low level - 01	59.747	99.58	99.4	0.13	0.13
	Low level - 02	59.593	99.32			
	Low level - 03	59.668	99.45			
	Middle level - 01	59.628	99.38	99.31	0.23	0.23
	Middle level - 02	59.694	99.49			
	Middle level - 03	59.430	99.05			
	High level - 01	59.472	99.12	99.35	0.24	0.24
	High level - 02	59.740	99.57			
	High level - 03	59.691	99.48			
	Overall Average:	59.629	99.38	99.35	0.06	0.20
	Overall SD:	0.112	0.187			
	Overall % CV:	0.189	0.189			

Table 4:- Results of Loratadine Accuracy and Precision.

LORATADINE		ACCURACY		PRECISION		
S.no.	Sample ID	mg / Cap	Assay percentage	Average at individual concentration levels	SD at individual concentration levels	% CV at individual concentration Levels
1	Low level - 01	4.978	99.56	99.70	0.20	0.20
2	Low level - 02	4.988	99.75			
3	Low level - 03	4.998	99.96			
4	Middle level - 01	4.995	99.90	99.91	0.02	0.02
5	Middle level - 02	4.995	99.90			
6	Middle level - 03	4.997	99.94			
7	High level 01	4.966	99.32	99.61	0.28	0.28
8	High level 02	4.993	99.87			
9	High level 03	4.982	99.64			
	Overall Average:	4.988	99.76	99.76	0.13	0.17
	Overall SD:	0.011	0.217			
	Overall % CV:	0.217	0.217			

Intermediate precision/ruggedness:-

Carried out by injecting the solutions prepared on different days and the results are given in Table-05 which shows that the method is rugged

Table 5:- Results of Ruggedness.

S.No	Change in Days	Assay (in %)		% RSD		System Suitability			
						Theoretical Plates		Asymmetry (Tailing	
		AMB	LOR	AMB	LOR	AMB	LOR	AMB	LOR
1.	Day 01	99.52	99.40	0.23	0.25	2364.16	2935.80	1.07	0.93
2.	Day 02	99.41	99.25	0.22	0.30	2457.17	3230.73	1.18	1.05

Linearity and range:-

Linearity of detector response was established by measuring the peak area of a series of solution at varied concentration ranging from 25 mcg/ml to 75 mcg/ml for Loratadine and 300mcg/ml to 900 mcg/ml for Ambroxol Hydrochloride and the results are shown in Table 06 and the plots are given in Fig 3 and Fig 4

Table 6:- Results of Linearity and range.

S.No	Concentration(µg/ml)		Area	
	AMB	LOR	AMB	LOR
1	300	25	897.43	111.82
2	450	37.5	1332.01	169.95
3	600	50	1805.89	233.01
4	750	62.5	2249.53	286.50
5	900	75	2741.56	350.65

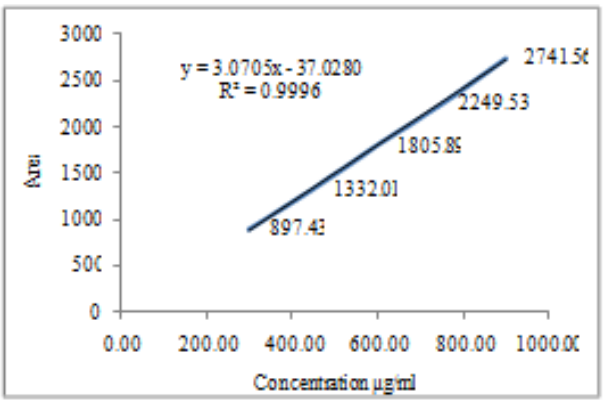

Fig 3 Linearity for Ambroxol Hydrochloride

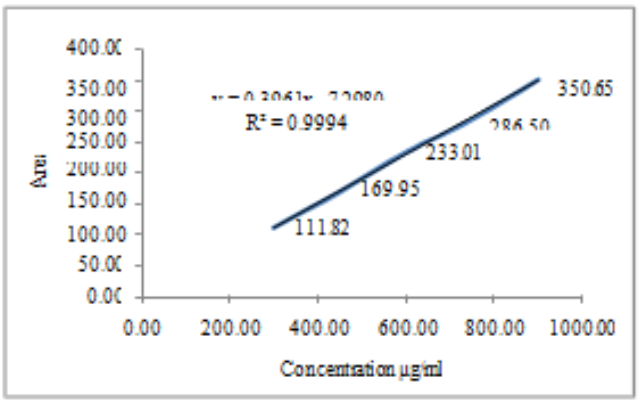

Fig 4 Linearity for Loratadine

Robustness:-

Results from the robustness studies were used to determine the final method operating parameters to ensure validity of analytical method. Robustness shows reliability of the analysis with respect to deliberate variations in method parameters which include changes in Flow rate, Mobile phase ratio, Wavelength, pH of the buffer and the results are given in Tables 07-10.

Table 7:- Results of Change in flow rate.

S.No	Change in Flow rate	Assay (in %)		% RSD		System Suitability			
						Theoretical Plates		Asymmetry (Tailing)	
		AMB	LOR	AMB	LOR	AMB	LOR	AMB	LOR
1.	Less 0.4 ml	99.36	99.20	0.49	0.66	2671.60	3636.80	1.27	1.13
2.	Actual 0.6 ml	99.56	99.15	0.707	0.271	2371.25	2858.31	1.08	0.93
3.	More 0.8 ml	99.24	99.34	0.38	0.19	2714.76	3177.93	1.07	1.12

Table 8:- Results of Change in Mobile Phase ratio

S.No	Change in Mobile phase ratio	Assay (in %)		% RSD		System Suitability			
						Theoretical Plates		Asymmetry (Tailing	
		AMB	LOR	AMB	LOR	AMB	LOR	AMB	LOR
1.	10% Increase in Buffer	99.25	99.14	0.14	0.46	2671.60	3636.80	1.27	1.13
2.	Actual Mobile phase	99.56	99.15	0.707	0.271	2371.25	2858.31	1.08	0.93
3.	10 %Increase in Acetonitrile	99.35	99.20	0.38	0.19	2714.76	3177.93	1.07	1.12

Table 9:- Results of Change in Wavelength

S.No	Change in Wavelength (± 2nm)	Assay (in %)		% RSD		System Suitability			
						Theoretical Plates		Asymmetry (Tailing	
		AMB	LOR	AMB	LOR	AMB	LOR	AMB	LOR
1.	Less 245 nm	99.62	99.42	0.14	0.25	2213.12	2673.60	0.99	0.95
2.	Actual 247 nm	99.56	99.15	0.707	0.271	2371.25	2858.31	1.08	0.93
3.	More 249 nm	99.17	99.32	0.52	0.41	2196.25	2659.67	1.02	0.95

Table 10:- Results of Change in pH of Mobile Phase.

S.No	Change in pH of Mobile phase	Assay (in %)		% RSD		System Suitability			
						Theoretical Plates		Asymmetry (Tailing	
		AMB	LOR	AMB	LOR	AMB	LOR	AMB	LOR
1.	pH 5.8	99.43	99.18	0.87	0.64	2234.87	3074.12	1.08	0.99
2.	Actual pH 6.0	99.56	99.15	0.707	0.271	2371.25	2858.31	1.08	0.93
3.	pH 6.2	99.22	99.21	0.39	0.62	2316.46	3089.31	1.15	1.05

Solution stability:

Solutions of Ambroxol and Loratadine tablets were prepared and stored at 25°C and the solutions were analyzed after 8hours and 16 hours against freshly prepared standard solutions and solutions were found to be stable and the results are given in table-11

Table 11:- Results of Solution stability.

S.No	Solution Stability	Assay (in %)		% RSD		System Suitability			
						Theoretical Plates		Asymmetry (Tailing	
		AMB	LOR	AMB	LOR	AMB	LOR	AMB	LOR
1.	After 8 hrs	99.43	99.18	0.87	0.64	2234.87	3074.12	1.08	0.95
2.	Initial	99.56	99.15	0.707	0.271	2371.25	2858.31	1.08	0.93
3.	After 16 hrs	99.22	99.21	0.39	0.62	2316.46	3089.31	1.15	1.05

Recovery studies:-

The accuracy of the method is determined by recovery studies. The recovery was performed by adding known quantities of standard. (Sample is spiked with 10%, 20% and 30% with respect to label claim) and the results was given in Table-12 and Table-13

Table 12:- Results of Ambroxol Hydrochloride Recovery Studies.

S.No	Sample ID	Calculated content (mg/ml of drug)	Actual assay (mg/ml of drug	Recovered amount (mg/ml of drug)	Actual amoun added (mg/ml of drug)	Recovery percentag (%)
1.	Recovery - Spiked with 10%	65.6138	59.6354	5.9784	6.0324	99.11
2.	Recovery - Spiked with 20%	71.6015	59.6354	11.9661	12.0647	99.18
3.	Recovery - Spiked with 30%	77.5615	59.6354	17.9265	18.0971	99.06
					Average	99.12
					SD	0.06
					%RSD	0.06

Table 13:- Results of Loratadine Recovery Studies

S.No	Sample ID	Calculated content (mg/ml of drug)	Actual assay (mg/ml of drug	Recoverec amount (mg/ml of drug)	Actual amount added (mg/ml of drug)	Recovery percentage (in %)
1.	Recovery - Spiked with 10%	5.4855	4.9880	0.4975	0.4992	99.66
2.	Recovery - Spiked with 20%	5.9848	4.9880	0.9968	0.9984	99.84
3.	Recovery - Spiked with 30%	6.4729	4.9880	1.4849	1.4977	99.14
					Average	99.55
					SD	0.36
					%RSD	0.36

Detection limit and quantitation limit:-

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of quantification (LOQ) is defined as the lowest concentration that can be quantified reliably with a specified level of accuracy and precision.

For this study six replicates of the analyte at lowest concentration were measured and quantified and signal to noise ratio was calculated as shown in the Table-14

Table 14:- Results of Detection Limit and Quantitation Limit.

DETECTION LIMIT					
S.No	Drug Name	Concentration (µg/ml)	Baseline noise (Height in µV)	Signal Obtained (Height in µV)	S/N Ratio
1.	AMB	4.291	0.6	1.832	3.05
2.	LOR	4.249	0.6	1.815	3.03
QUANTITATION LIMIT					
1.	AMB	14.303	0.6	6.110	10.18
2.	LOR	14.164	0.6	6.052	10.09

Assay:-

Estimation of Ambroxol Hydrochloride and Loratadine tablet dosage forms by the developed UHPLC method was carried out. The assay procedure was performed and the assay percentage was calculated.

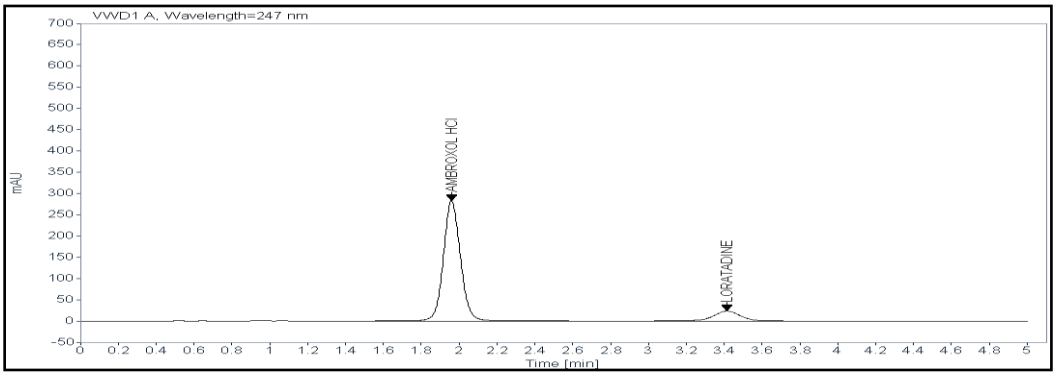

Fig 5:- Chromatogram for Standard for Assay.

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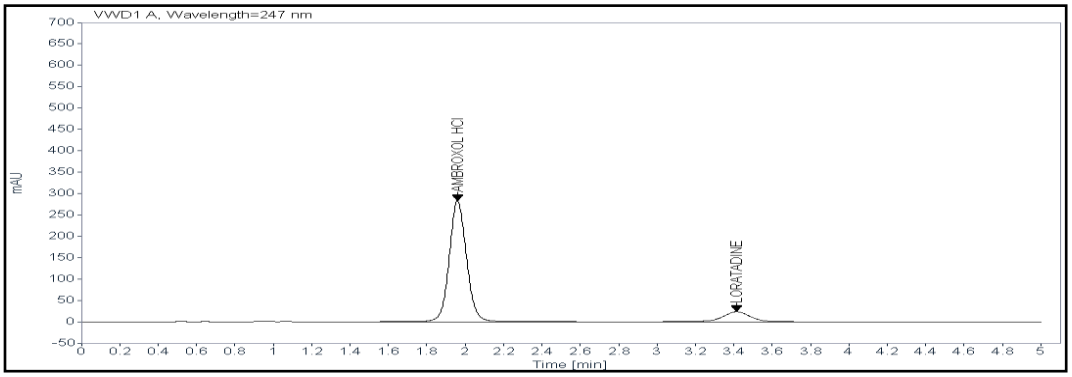

Fig 6:- Chromatogram for Sample for Assay

Table 15:- Summary Of Assay Results.

Description: White Colour, Circular Shaped Slightly biconvex, Film coated Tablet having embossing of "AM" on one side and other side is plain.			
Assay:			
Each Film coated Table contains:	**Labelled Claim**	**Results**	**Limit**
Ambroxol Hydrochloride IP	60 mg	59.737 mg (99.56%)	98 % to 102%
Loratadine USP	5 mg	4.9574 mg (99.15 %	

Results and Discussion:-

The developed UHPLC method was applied for the estimation of Ambroxol Hydrochloride and Loratadine. The chromatographic separation was achieved on Waters C_{18} (50 mm x 4.6 mm, 3.5µ) at a detection wavelength of 247 nm. The method has been optimized after studying the effect of various parameters on the elution of Ambroxol Hydrochloride and Loratadine. The mobile phase was chosen after several trials in various proportions and at different pH. The studies suggested that a mobile consisting of 300 volumes of methanol and 300 volumes of buffer pH 6.0 and 400 volumes of Acetonitrile gave good peak shape with high resolution and less retention time for Ambroxol Hydrochloride and Loratadine. The drugs exhibits linearity in concentration range of 25 mcg/ml to75mcg/ml with correlation coefficient 0.9994 for Loratadine and 300 mcg/ml to 900 mcg/ml with correlation coefficient of 0.9996 for Ambroxol Hydrochloride at 247 nm. The Percentage recovery was found to be 99.12 % for Ambroxol Hydrochloride and 99.55 % for Loratadine. The validation is carried out as per ICH Guidelines. The developed method was found to be precise.

The simplicity of this method was low cost, rapid technique and its high specificity. For the separation of Ambroxol Hydrochloride and Loratadine in the presence of various excipients, this UHPLC method with UV detection on a Waters C18 column was suitable for the quantification of Ambroxol Hydrochloride and Loratadine in pharmaceutical dosage forms.

Conclusion:-

UHPLC is an advanced technique of HPLC and generally used for separation and determination of components in pharmaceutical dosage forms and APIs. The Proposed UHPLC method is less expensive, less time consuming, specific accurate precise which is reproducible. This technique is used for the estimation of Ambroxol Hydrochloride and Loratadine. Compared to RP-HPLC this method gives linear results with good peak shape with high resolution at low injection volume with less retention time. The proposed method can be used for routine analysis for simultaneous estimation of Ambroxol hydrochloride and Loratadine.

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