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Simultaneous Estimation of Loratadine and Ambroxol Hydrochloride from Tablet Dosage Form by HPLC Method

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Madhusudan T. Bachute[1], S.V.Shanbhag*[2]

1. Department of Chemistry, KBP Mahavidyalaya, Pandharpur, Solapur University, Solapur, MS, 413304,India.

2. Department of Chemistry, R & D Centre, Bharathiar University, Coimbatore, TN, 641046, India .

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ABSTRACT

A simple, precise, accurate and rapid RP-HPLC method has been developed for simultaneous estimation of Ambroxol Hydrochloride and Loratadine in tablets dosage form. The method was carried out on a C18 column (25cm x4.6 mm x 5µm) with a mobile phase consisting of Water: Acetonitrile: Glacial Acetic acid in 65:30:05 ratio. The flow rate was adjusted to 1.2 ml/minute and detection was carried out at 254 nm. The retention time obtained for Ambroxol Hydrochloride and Loratadine was 2.12 and 11.54 minutes respectively. The calibration areas were linear in the concentration range of 40-70 µg/ml for Ambroxol Hydrochloride and 8-13 µg/ml for Loratadine. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantification, solution stability. The proposed method can be used for simultaneous estimation of these two drugs in tablet dosage form.

INTRODUCTION

Loratadine is chemically (Fig.1) Ethyl4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidine carboxylate with a potent antihistaminic activity used in the treatment of urticaria and allergic rhinitis.

Ambroxol Hydrochloride is chemically (Fig.2) 1({[2-4 Amino-3, 5 dibromophenyl] methyl} amino) cyclohexanol, monohydrochloride, which is a semi-synthetic derivative of vasicine obtained from the Indian shrub "Adhatoda Vasica". It is an expectorant and mucolytic agent which is used in the treatment of Bronchial asthma and Chronic Bronchitis. Ambroxol Hydrochloride has also been reported to show a cough suppressing and anti-inflammatory properties. Recently the inhibition of nitric oxide-dependent activities of soluble *guanylate cyclase* was suggested as one of the molecular mechanisms of the therapeutic action of Ambroxol Hydrochloride, also used in pulmonary alveolar proteinosis in pulmonary distress and infant respiratory distress syndrome.

A literature survey showed that very few analytical methods have been reported for the estimation of Loratadine and Ambroxol Hydrochloride individually and in combination with other drugs using UV-VIS spectrophotometer, liquid chromatography, LC-MS, RP-LC, Capillary electrophoresis, HPLC with potentiometric detection etc.

Fixed combination containing Ambroxol Hydrochloride (60mg) and Loratadine (5mg) is available in the tablet dosage form in the market. Only one method was available for estimation but there was a lot of scope for improvement. So efforts were taken to make available simultaneously evaluating, optimized, simple and cost-effective HPLC method for estimation of Loratadine and Ambroxol Hydrochloride in tablet dosage form as per guidelines of International Conference on Harmonisation (ICH) [9].

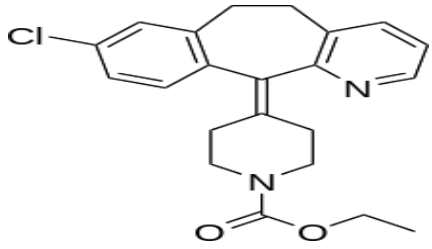
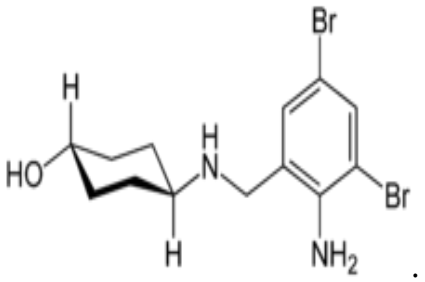


Fig. 1 Loratadine



HCL

Fig. 2 Ambroxol Hydrochloride

EXPERIMENTAL

Acetonitrile, HPLC grade was procured from Merck (India) Limited. Glacial acetic acid was procured from S.D Fine Chemicals, India. Water HPLC grade was obtained from Milli-Q-RO water purification system. The reference standard of Ambroxol Hydrochloride and Loratadine were procured from Wallace Pharmaceuticals Pvt. Ltd, Goa, India.

Chromatographic separation was performed on HPLC system with following details:

System : Thermo Fischer Ultimate 3000

Column: C18 (250 x 4.6 mm x 5 µm) Cosmosil

Electronic Balance: LCGC

Column: Thermostat column compartment

Sonicator: Spectra-Physics

Detector: DAD-300 Diode Array Detector

pH Meter: Digisun AS220/X

Software: Chromeleon: **Injector:** Autosampler

Chromatographic conditions

A Cosmosil C18 (250 mm X 4.6 mm X 5µm) column was used at ambient temperature. Mixed 650 ml of water, 300ml of Acetonitrile and 50 ml of Glacial Acetic Acid to make 1000ml of the mobile phase. This was filtered through glass fiber filter (0.45 µ). This was degassed. The flow rate was maintained at 1.2 ml /minute. The elution was observed at 254 nm. Some trials were carried out w. r. t change in the ratio of constituents of the mobile phase like 50:40:10/50: 50:00/ 45:50:05/70:25:5 etc. of Water: Acetonitrile: Glacial Acetic Acid. Injection volume and runtime were 20µl and 20 minutes respectively. In the ratio of 65:30:05 retention time for Ambroxol Hydrochloride and Loratadine observed to be 2.12 minutes and 11.54 minutes respectively. The two peaks were well resolved with good, sharp shape and symmetry were obtained.

Preparation of Mobile Phase

Mixed 650ml of HPLC grade water, 300ml of Acetonitrile and 50ml of Glacial Acetic Acid. The solution was filtered and degassed.

Preparation of standard stock solution

A. Ambroxol Hydrochloride standard solution (Solution "A"): Weighed accurately about 120mg of Ambroxol Hydrochloride working standard and transferred to a 100ml volumetric flask. Added 50ml of mobile phase and dissolved it completely. Made up the volume with additional mobile phase. Mixed well.

B. Loratadine standard solution (Solution 'B'): Weighed accurately about 50mg of Loratadine Working standard and transferred to the a100ml volumetric flask. Added 50ml of mobile phase and dissolved completely. Made up the volume with additional mobile phase and mixed well.

C. Standard solution: Pipette out 10ml of standard solution (Solution B) and 50 ml of Ambroxol Hydrochloride standard solution (Solution A) to a 100ml volumetric flask. Added 50ml of mobile phase and mixed for 15 minutes. Made up the volume with additional mobile phase and mixed. Filtered through glass fiber filter paper.

D. Preparation of sample solution: Weighed powdered 20 tablets. Weighed accurately powder equivalent to 60 mg of Ambroxol Hydrochloride and 5 mg of Loratadine and transferred to a 100ml volumetric flask. Diluted to the mark with mobile phase and mixed well. Filtered through glass fiber filter paper.

Analysis of a Marketed formulation

To determine the content of Ambroxol hydrochloride and Loratadine in the conventional tablet (Brand name: Pulmolor /Marketed product), Label claim: 60 mg Ambroxol Hydrochloride and 5 mg Loratadine per tablet. Twenty tablets were weighed. Their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 60 mg of Ambroxol Hydrochloride and 5 mg of Loratadine was transferred into a 100 ml volumetric flask containing 70 ml diluent, sonicated for 30 minutes and diluted up to 100 ml with diluent. Taken 2.5 ml from above solution in 25 ml volumetric flask and made up the volume to 25 ml with diluent and made concentrations of 60 µg of Ambroxol Hydrochloride

and 5 µg of Loratadine respectively. A 20- µL volume of the sample solution was injected into HPLC system under the conditions described above.

RESULTS AND DISCUSSION

Method Development

1) Solubility: To arrive at the right choice of Mobile Phase and diluents solubility of each compound was checked in all HPLC compatible solvents. Since the target of Method Development was to estimate two compounds simultaneously, it was necessary to find a common solvent or diluents in which all compounds will have satisfactory solubility. Additionally, the selected diluents should be capable to extract both compounds from tablets dosage form. A detailed and thorough suitability study narrowed down to Water, Acetonitrile and Glacial Acetic Acid in the ratio of 65:30:05.

2) Selection of UV detection wavelength: A detailed review of UV spectrum of two compounds suggested that 254nm is the most suitable wavelength, which can be employed for detecting all these components.

3) Selection of working pH range for mobile phase: Since two compounds are present in the sample matrix, pH of mobile phase plays very vital role in separation. pKa values of all these two compounds were studied to select proper pH of the mobile phase. From the study, the conclusion was acidic pH would be the better choice for separation of two actives.

4) Selection of Column: In a reverse phase chromatographic method development, selection of proper column is one of the key factors of Method Development. In reverse phase, chromatographic separation wide range of columns like C8, C18, Cyano, Phenyl etc. of different make are available, which can be used for separation. Extensive literature survey revealed that in general 150 or 250 mm columns having diameter 4.6 mm and particle size 5 µm have been used for method development. Trials were taken on various columns and came to the conclusion for the C18 column with 4.6 mm diameter with 5um particle size, which was finalized.

Final Method optimization:

Final optimization was done to fix the remaining method parameters like flow rate of the mobile phase, column oven temperature, a concentration of each compound in standard and

sample preparation. Effect of each individual parameter on separation was studied. Typical chromatogram showing separations between two compounds are shown in Fig.5.

Method Validation

The developed method was validated as per ICH (International Conference on Harmonization) guidelines with respect to System suitability, Precision; Specificity, Linearity, Accuracy, Limit of Detection, Limit of quantification, Ruggedness, and Robustness.

Specificity:

Specificity is the ability of Analytical Method to identify and quantify the compounds of interest, without any interface in the presence of impurities or degradants which are likely to be present. Interferences may be either from blank or from placebo with the retention times of Ambroxol Hydrochloride and Loratadine. Identification of Ambroxol Hydrochloride, Loratadine from sample solution was done by comparing retention time of the standard solution of individual components. Peak purity of both active in sample solution was checked to confirm uniformity of all these peaks using Photo Diode Array Detector (PDA). Compliance with the method of the requirement for blank interference, identification, and peak purity tests indicate that method is specific.

Linearity:

Linearity shows the proportionate response of analyte against the concentration of the analyte. The linearity of the method was estimated by using five concentrations of each compound within the 80% to 120% range of working concentration. For linearity experiments, 42.29 mg to 63.29 mg for Ambroxol Hydrochloride and 8.7 mg to 13.0 mg for Loratadine was used. Linearity curves for Loratadine and Ambroxol Hydrochloride are as shown in Fig. 3 & Fig.4 respectively.

Linearity- Loratadine
80% 8.6956 µg/ml
90% 9.7423 µg/ml
100% 10.8541 µg/ml
110% 11.8557 µg/ml
120% 13.0441 µg/ml
Linearity- Ambroxol HCl
80% 42.2963 µg/ml
90% 47.3341 µg/ml
100% 52.7023 µg/ml
110% 57.4036 µg/ml
120% 63.2970 µg/ml

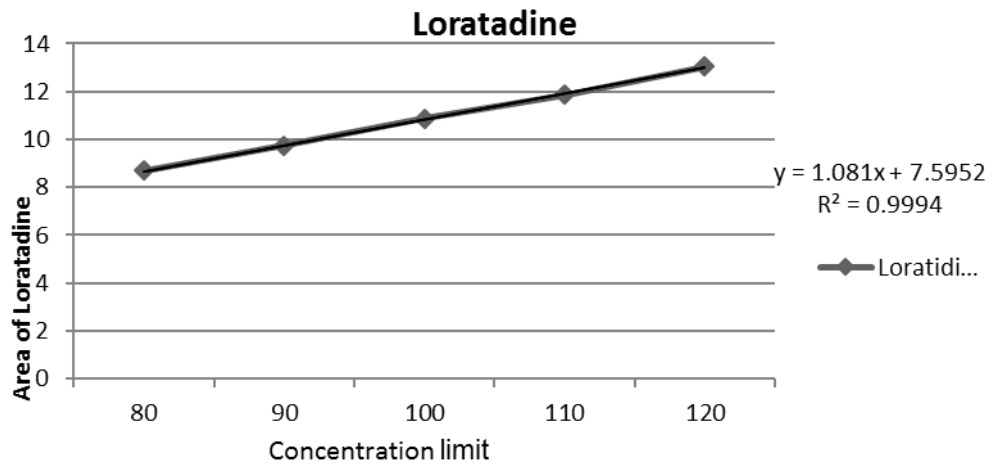


Fig 3. Linearity curve for Loratadine

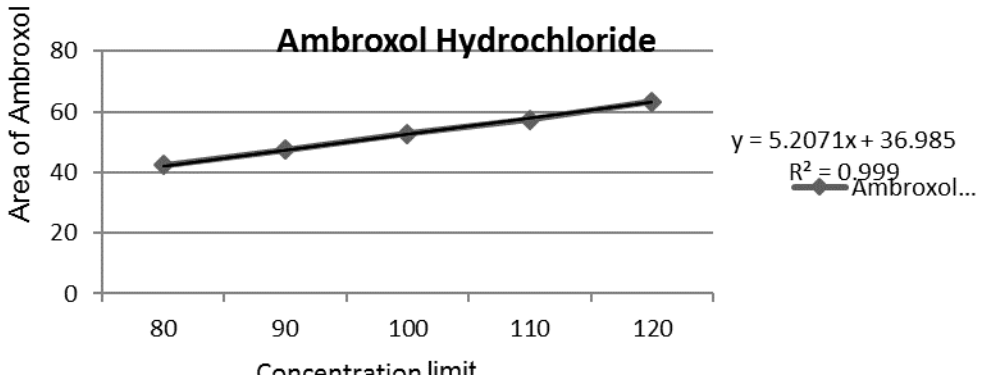


Fig 4. Linearity curve for Ambroxol HCl

Accuracy:

The accuracy of a method is the closeness of observed values obtained using the method to the true value. Estimation of recovery by standard addition is a sound approach to demonstrate the accuracy of the method. During recovery experiment, the known amount of reference standard of each compound was spiked into the placebo of the sample at three different levels i.e.80%, 100% and 120% of sample concentration and prepared three samples of each level. These spiked samples along with one control sample were analyzed. The experimental value of each compound obtained for each level was calculated and compared with the actual added amount of respective component. Mean accuracy in percentage was calculated for all the three levels.

Precision:

The precision of the method was demonstrated by repeatability (Intraassay Precision) and intermediate Precision (Inter-assay).Six different sample solutions of same concentration were prepared from the same uniform sample and analyzed against working standard solution. Assay values for each component were calculated and relative standard deviation (RSD) of assay values were evaluated. Very low RSD values indicate a closeness of the results. Percent RSD of assay values from six samples were less than 1.0% for each compound indicates that the method is precise or repeatable.

Ruggedness

The intermediate precision was evaluated by preparing six different sample solution of the same concentration as prepared in method precision and analyzed on different days. Percent cumulative RSD of assay results for twelve samples were done. Six samples for method precision and six for intermediate precision were calculated. Percent RSD of assay values of each compound from twelve samples were less than 1.0%. The closeness of assay results and percent RSD values demonstrated that the method is rugged.

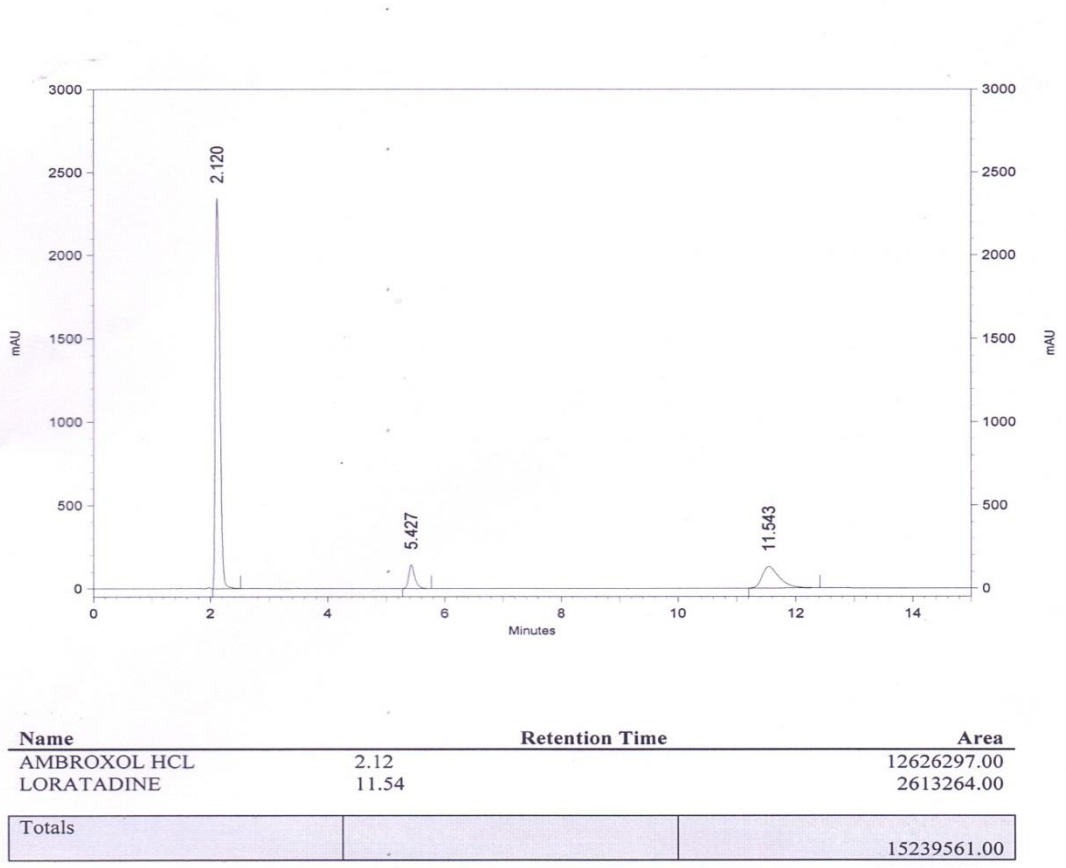
Robustness

Robustness is a validation parameter, which shows an ability of an analytical method to remain unaffected by slight but deliberate changes in method parameters. Robustness was demonstrated by making slight changes in parameters like flow rate (±5%), column temperature (± 2°C) and mobile phase composition (± 5%). Robustness study demonstrates that by making slight but deliberate changes in method parameters, the method remains unchanged and gives consistent results. Results of original conditions and altered conditions are comparable.

Solution Stability:

The Solution Stability of sample solution was evaluated by comparison of assay value of freshly prepared samples at room temperature for 24 hours. Standard solution and sample solution were prepared as mentioned in chromatographic conditions. The sample solution was analyzed and assay value was calculated against the standard solution. Both the solutions were kept at room temperature for 24 hours were reanalyzed against the freshly prepared

standard solution and assay values were compared. Assay value of stored samples was compared with initial assay value. Difference between these two assays was less than 2.0% for both active. The study demonstrated that sample solutions were stable up to 24 Hours.



Name	Retention Time	Area
AMBROXOL HCL	2.12	12626297.00
LORATADINE	11.54	2613264.00
Totals		15239561.00

Fig. 5 HPLC Chromatogram showing retention time of Ambroxol HCl and Loratadine

Sr. No.	Name	Ret.Time (detected)	Asymmetry (EP)	Resolution (EP)	The.Plates (EP)
1	Ambroxol Hcl	2.12	1.29	40.17	9276
2	Loratadine	11.54	1.29	N.A	15232

Table No.1. Precision of Ambroxol HCl and Loratadine

Sr. No.	Ambroxol Hydrochloride standard solution	Ambroxol Hydrochloride test solution	Loratadine standard solution	Loratadine test solution
1	12679839.0	12626297.0	2438483.0	2613264.0
2	12362613.0	12646715.0	2419163.0	2615747.0
3	12271203.0	12689661.0	2416706.0	2632258.0
4	12319535.0	12618778.0	2443130.0	2624952.0
5	12385948.0	12594918.0	2450641.0	2631571.0
Average	**12403827.6**	**12635273.8**	**2433624.6**	**2623558.4**
RSD in %	**1.29**	**0.28**	**0.62**	**0.33**

Table No.2. The accuracy of Ambroxol HCl and Loratadine.

Sr. No.	Conc. in %	% w/w Recovery Ambroxol HCl	% w/w Recovery Loratadine	Average recovery % Ambroxol HCl	Average recovery % Loratadine	Mean recovery in % Ambroxol HCl	Mean recovery in % Loratadine
1	80 %	81.59	80.31	101.99	100.38	101.46%	100.05%
2	100 %	101.75	99.42	101.75	99.42		
3	120 %	120.77	120.42	100.64	100.35		

Table No.3. Robustness of Ambroxol HCl and Loratadine.

Change in instrument				
No. of Injections	**Standard area of Ambroxol HCl**	**Test area of Ambroxol HCl**	**Standard area of**	**Test area of Loratadine**
1	204.7	205.6	21.8	23.3
2	204.4	205.3	21.7	23.4
3	204.6	205.3	21.8	23.3
4	204.8	205.4	21.8	23.4
5	204.7	205.3	21.8	23.3
Average	**204.7**	205.4	**21.8**	**23.4**
RSD %	**0.1242**	**0.0524**	**0.0801**	**0.0436**
Assay of 1) Ambroxol HCl= 60.199 mg 2) Loratadine= 5.29 mg				
Change in extraction time from 15 minutes to 20 minutes				
No of Injections	**Standard area of**	**Test area of Ambroxol HCl**	**Standard area of Loratadine**	**Test area of Loratadine**
1	12329910.0	12592363.0	2489979.0	2657431.0
2	12289347.0	12475184.0	2491848.0	2623256.0
Average	**12309628.5**	**12533773.5**	**2490913.5**	**2640343.5**
RSD %	**0.23**	**0.66**	**0.05**	**0.92**
Assay of 1) Ambroxol HCl= 61.07 mg : 2) Loratadine= 5.22 mg				

Table No.4. The ruggedness of Ambroxol HCl and Loratadine.

Chemist A				
Sr. No.	**Standard area of Ambroxol HCl**	**Standard area of Loratadine**	**Test area of Ambroxol HCl**	**Test area of Loratadine**
1	12162833.0	2425711.0	12403158.0	2591978.0
2	12107572.0	2413717.0	12451316.0	2618706.0
3	12096949.0	2421877.0	12481790.0	2614289.0
4	12093289.0	2415082.0	12522946.0	2623726.0
Average	**12115160.8**	**2419096.8**	**12464802.5**	**2612174.8**
RSD %	**0.27**	**0.23**	**0.41**	**0.54**
Assay: Ambroxol Hydrochloride: 61.7 mg ,Loratadine: 5.32 mg				
Chemist B				
Sr. No.	**Standard area of Ambroxol HCl**	**Standard area of Loratadine**	**Test area of Ambroxol HCl**	**Test area of Loratadine**
1	12187796.0	2455559.0	12505255.0	2625844.0
2	12231273.0	2447572.0	12503534.0	2620099.0
3	12291623.0	2454607.0	12595529.0	2625737.0
4	12231704.0	2453642.0	12521483.0	2604181.0
Average	**12235599.0**	**2452845.0**	**12531450.3**	**2618965.3**
RSD %	**0.35**	**0.15**	**0.35**	**0.39**
Assay: Ambroxol Hydrochloride: 61.43 mg ,Loratadine: 5.26 mg				

Table No.5. Method validation of assay results of Ambroxol HCl and Loratadine.

Validation Parameter	Acceptance Criteria	observation		
Specificity	Analyte Ambroxol Hydrochloride and Loratadine chromatographic peak should be specific, pure and distinct from each other	**Content**	Ambroxol Hydrochloride	Loratadine
		Retention time	About 2.1 min	About 11.5 min
		No apparent interference observed between the peaks, Ambroxol Hydrochloride and Loratadine peak is selectively separated from formulation matrix.		
Precision	The relative standard deviation should not be more than 2% for test solution and standard solution.	**Content**	**RSD**	
		Ambroxol Hydrochloride	Std =1.29% :Test =0.28%	
		Loratadine	Std =0.62% :Test =0.33%	
		It is observed that the precision of five replicates injections of homogeneous test solution assay for Ambroxol Hydrochloride and Loratadine & its standard solution results are within specified RSD limit.		
Accuracy	Recovery should be 98% to 102% with respect to the added percentage	**Content**	**Average Mean recovery**	
		Ambroxol Hydrochloride	101.46%	
		Loratadine	100.05 %	
Linearity	The test results with respect to test concentration should be linear and co-relation coefficient should not be less than 0.998.	**Content**	**Correlation coefficient**	**Slope**
		Ambroxol Hydrochloride	0.9998	1.021
		Loratadine	0.9996	0.9965
Ruggedness	The analytical	**Content**	**Chemist A**	**Chemist B**

	result should be reproducible	Ambroxol Hydrochloride	61.7 mg	61.43 mg
		Loratadine	5.32 mg	5.26 mg
Robustness	The analytical result should be reproducible	**Content**	**Change in instrument**	
		Ambroxol Hydrochloride	60.199 mg/tab	
		Loratadine	5.29 mg /Tab	
		Content	**Change in extraction time 15 mins to 20 mins**	
		Ambroxol Hydrochloride	61.07 mg	
		Loratadine	5.22 mg	

CONCLUSION

The developed method is accurate, simple, rapid and selective for the simultaneous estimation of Ambroxol Hydrochloride and Loratadine in tablet dosage form. The sample preparation is simple, analysis time is short and elution is by an isocratic method. The retention time of Ambroxol HCl and Loratadine are found to be 2.12 & 11.54 minutes respectively. The excipients of this commercial sample analyzed did not interfere in the analysis, which proves excellent specificity of the method for these drugs analysis. Hence the proposed method can be conveniently adopted for the routine quality control analysis for this combination in tablet dosage form.

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