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# Method development and validation for the simultaneous estimation of ambroxol Hcl and loratadine in a pharmaceutical formulation by RP-HPLC method

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## ABSTRACT

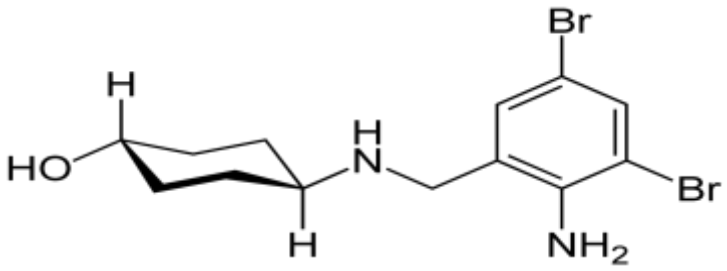
An isocratic Simultaneous estimation by RP-HPLC Method were developed and validated for the quantification of Ambroxol HCl and Loratadine in tablet dosage form. Quantification was achieved by using a reversed-phase C18 column (INERTSIL Column , 5µ, 250 mm × 4.6 mm) at ambient temperature with mobile phase consisting of Phosphate Buffer buffer (30mM) pH: 4.5: Acetonitrile : Methanol (30: 50:20). The flow rate was 1.0 ml/min. Measurements were made at a wavelength of 220nm. The average retention time for Ambroxol HCl and Loratadine were found to be 2.450 min and 4.367. The proposed method was validated for selectivity, precision, linearity and accuracy. The assay methods were found to be linear from 72-168 µg/ml for Ambroxol HCl and 6 to 14 µg/ml for Loratadine. All validation parameters were within the acceptable range. The developed method was successfully applied to estimate the amount of Ambroxol HCl and Loratadine in tablet dosage form.

**Keywords:** Ambroxol HCl, Loratadine, RP-HPLC method, INERTSIL Column, Methanol, Acetonitrile, KH2PO4, Ortho phosphoric acid and Validation.

## Ambroxol

### Description[3]

Ambroxol is a secretolytic agent used in the treatment of respiratory diseases associated with viscid or excessive mucus. It is the active ingredient of Mucosolvan, Lasolvan or Mucoangin. The substance is a mucoactive drug with several properties including secretolytic and secretomotoric actions that restore the physiological clearance mechanisms of the respiratory tract which play an important role in the body's natural defence mechanisms. It stimulates synthesis and release of surfactant by type II pneumocytes. Surfactants acts as an anti-glue factor by reducing the adhesion of mucus to the bronchial wall, in improving its transport and in providing protection against infection and irritating agents.




**Structure of Ambroxol [4]**

**IUPAC name[4]:** trans-4-(2-Amino-3,5-dibrombenzylamino)-cyclohexanol
**Molecular formula:** $C_{13}H_{18}Br_2N_2O$
**Molecular weight:** 378.10g/mol

## Mechanism of action

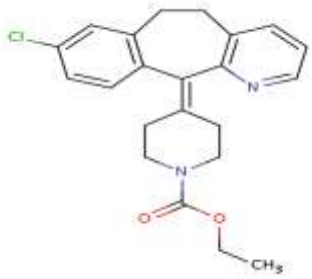
Ambroxol is a mucolytic agent. Excessive Nitric oxide (NO) is associated with inflammatory and some other disturbances of airways function. NO enhances the activation of soluble guanylate cyclase and cGMP accumulation. Ambroxol has been shown to inhibit the NO-dependent activation of soluble guanylate cyclase. It is also possible that the inhibition of NO-dependent activation of soluble guanylate cyclase can suppress the excessive mucus secretion, therefore it lowers the phlegm viscosity and improves the mucociliary transport of bronchial secretions. [15]

## Indication

Ambroxol is indicated as "secretolytic therapy in broncho pulmonary diseases associated with abnormal mucus secretion and impaired mucus transport. It promotes mucus clearance, facilitates expectoration and eases productive cough, allowing patients to breathe freely and deeply. [3]

## Loratadine [5]

Loratadine is a derivative of azatadine and a second-generation histamine H1 receptor antagonist used in the treatment of allergic rhinitis and urticaria. Unlike most classical antihistamines (histamine H1 antagonists) it lacks central nervous system depressing effects such as drowsiness




**Structure of Loratidine**

**IUPAC name:** ethyl 4-{13-chloro-4-azatricyclo[9.4.0.0^{3,8}] pentadeca-1(11),3,5,7,12,14-hexaen-2-ylidene}piperidine-1-carboxylate
**Molecular formula:** $C_{22}H_{23}ClN_2O_2$
**Molecular weight:** 382.883 g/mol

## Category

- Anti-Allergic Agents
- Antipruritics
- Histamine H1 Antagonists, Non-Sedating
- Histamine Antagonists

### Mechanism of action

Loratadine competes with free histamine and exhibits specific, selective peripheral $H_1$ antagonistic activity. These blocks the action of endogenous histamine, which subsequently leads to temporary relief of the negative symptoms (eg. nasal congestion, watery eyes) brought on by histamine. Loratadine has low affinity for cholinergic receptors and does not exhibit any appreciable alpha-adrenergic blocking activity in-vitro. Loratadine also appears to suppress the release of histamine and leukotrienes from animal mast cells, and the release of leukotrienes from human lung fragments, although the clinical importance of this is unknown.

### Pharmacodynamics

Loratadine is a long acting second generation antihistamine that is similar in structure to cyproheptadine and azatadine. The pharmacology of loratadine is similar to other antihistamines, but unlike other $H_1$-blockers, loratidine is shown to exhibit competitive, specific, and selective antagonism of $H_1$ receptors. The exact mechanism of this interaction is unknown, but disposition of the drug suggests that loratadine's prolonged antagonism of histamine may be due to the drug's slow dissociation from the receptor or the formation of the active metabolite, desloratadine. Loratadine does not penetrate the CNS effectively and has a low affinity for CNS $H_1$-receptors.

### Indication

A self-medication that is used alone or in combination with pseudoephedrine sulfate for the symptomatic relief of seasonal allergic rhinitis. Also used for the symptomatic relief of pruritus, erythema, and urticaria associated with chronic idiopathic urticaria in patients (not for children under 6 unless directed by a clinician).

## EXPERIMENTAL

### Equipments

The chromatographic technique performed on a Shimadzu LC20-AT Liquid chromatography with SPD-20A prominence UV-visible detector and Spinchrom software, reversed phase C18 column (INERTSIL 5µ, 250 mm × 4.6 mm) as stationary phase. Thermo electron corporation double beam UV-visible spectrophotometer (vision pro-software) ,Ultrasonic cleaner, Shimadzu analytical balance AY-220,Vaccum micro filtration unit with 0.45µ membrane filter was used in the study.

### Materials

Pharmaceutically pure sample of AMBROXOL HCL and LORATADINE were obtained as gift samples from Chandra Labs, Prashanthinagar, Kukatpally, and Hyderabad, India. The purity of the drug was evaluated by obtaining its melting point and ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drug was used without further purification. HPLC-grade Acetonitrile was from standard reagents pvt ltd. $KH_2PO_4$ (AR grade) was from Merck. A tablet formulation of AMBROXOL HCL and LORATADINE (60 mg and 5 mg label claims) were procured from local market (IROVEL-H, SUN PHARMACEUTICALS, India).

### Chromatographic conditions

The sample separation was achieved on a C18 (5 µm, 25 cm X 4.6 mm i.d.) INERTSIL column, aided by mobile phase mixture of Phosphate Buffer buffer(30mM) pH: 4.5: Acetonitrile : Methanol (30: 50:20), that was filtered and degassed prior to use, at a flow rate of 1ml/min. Injection volume is 20 µl and detected at 220 nm at ambient temperatures.

### Preparation of mobile phase

### Buffer Preparation

Weigh accurately about 4.08 gms of $KH_2PO_4$ and dissolve with 500ml of HPLC Grade water than make up to 1000 ml with HPLC grade water then adjust the pH: 4.5 with ortho phosphoric acid or sodium hydroxide.

### Mobile phase

Then add 30 volumes of buffer, 50 volumes of Acetonitrile and 20 volumes of Methanol sonicated for 15 min and filtered through a 0.45 µ membrane filter.

### Analysis of formulation

### Preparation of standard solution

A 120 mg of standard Ambroxol HCl and 10 mg Loratadine ware weighed and transferred to 100 ml of volumetric flask and dissolved in mobile phase. The flask was shaken and volume was made up to mark with mobile phase to give a primary stock solution containing 1200 µg/ml Ambroxol HCl and 100 µg/ml of Loratadine. From the above solution 5ml of solution is pipette out into a 50 ml volumetric flask and volume was made up to mark with mobile phase to give a solution containing 1200 µg/ml Ambroxol HCl and 100 µg/ml of Loratadine.

### Preparation of sample solution (Tablet Formulation)

For the estimation of the drug in tablet formulation twenty tablets were weighed and their average weight was determined. The tablets were then finely powdered. Appropriate quantity equivalent to 120mg Ambroxol HCl and 10 mg Loratadine ware accurately weighed and The powder was transferred to 100 ml volumetric flask and shaken vigorously with mobile phase and sonicated for 15 min and volume made up to the mark with mobile phase. The solution was shaken vigorously and filtered by using whatmann filter no.41. from the above filtered clear solution 5ml of sample pipetted out into a 50 ml volumetric flask volume made up to the mark with mobile phase to give a solution containing 120µg/ml Ambroxol HCl and 10µg/ml of Loratadine.

## RESULTS AND DISCUSSIONS

### Determination Of Working Wavelength(λmax)

10 mg of the Ambroxol HCl standard drug is taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 0.1ml is pipette out into 10 ml volumetric flask and made upto the mark with the methanol to give a concentration of 10 µg/ml. The above prepared solution is scanned in uv between 200-400 nm using methanol as blank. The λmax was found to be 212nm

10 mg of the Loratadine standard drug is taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 0.1ml is pipette out into 10 ml volumetric flask and made upto the mark with the methanol to give a concentration of 10 µg/ml . The above prepared solution is scanned in uv between 200-400 nm using methanol as blank. The λmax was found to be 291nm.

The Isosbestic Point of Ambroxol HCl and Loratadine were found to be 220nm. The U.V Graph shown in Figure: 1

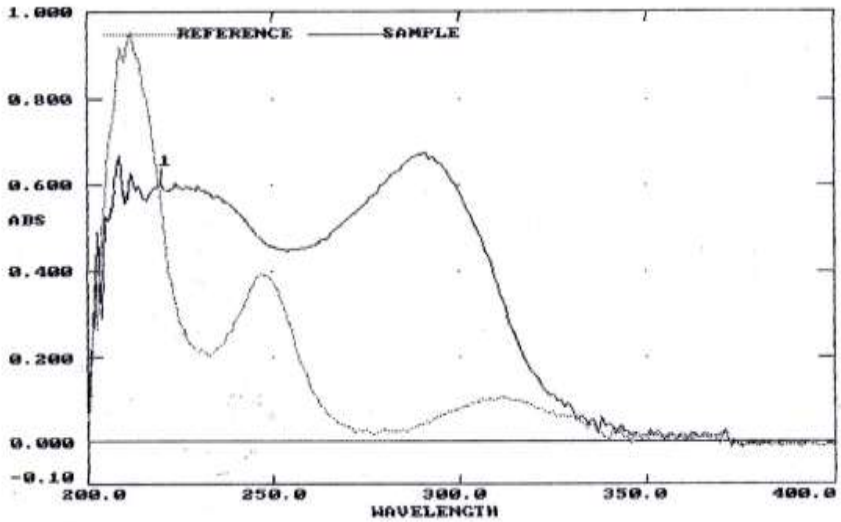



**Figure: 1 U.V Graph of Ambroxol HCl and Loratadine**

After several initial trails with mixtures of methanol, water, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of Phosphate Buffer buffer(30mM) pH: 4.5: Acetonitrile : Methanol (30: 50:20) brought sharp and well resolved peaks. The chromatogram was shown in Figure-2**.**

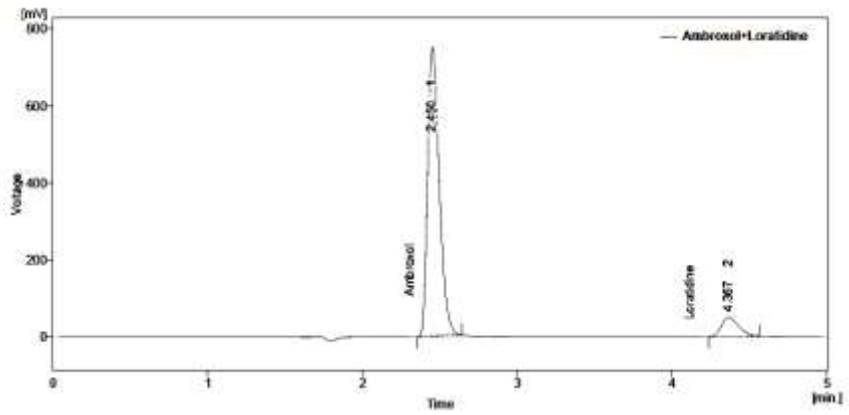



**Figure: 2 Chromatogram of Ambroxol HCl and Loratadine**

## METHOD VALIDATION

### Linearity

Linearity was studied by analyzing five standard solutions covering the range of 72-168µg/ml for Ambroxol HCl and 6 to 14µg/ml for Loratadine of the drug. From the primary stock solution 0.6ml,0.8ml,1.0ml,1.2ml,1.4 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 72µg/mL ,96µg/mL, 120 µg/mL ,144µg/mL and 168 µg/mL of Ambroxol HCl and 6µg/mL, 8µg/mL 10 µg/mL,12µg/mL ,14µg/mL of Loratadine .

Calibration curve with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

### Method precision (repeatability)

The precision of the instrument was checked by repeated injections and measurement of peak areas and retention times of solutions (n = 6) for, 120 µg/ml of Ambroxol HCl and 10 µg/ml of Loratadine without changing the parameter of the proposed chromatographic method.

### Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (2) and (3), respectively.

$$LOD = 3.3\ \delta/S \quad \text{……. (3)}$$

$$LOQ = 10\ \delta/S \quad \text{…….. (4)}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

### Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Ambroxol HCl and Loratadine by the standard addition method. Known amounts of standard solutions of Ambroxol HCl and Loratadine were added at 10% concentration to pre quantified sample solutions of Ambroxol HCl (120,144,168/ml) and Loratadine (10, 12, 14µg/ml) (Figure No.5.1 and 5.2). The amount of Ambroxol HCl and Loratadine recovered was estimated by using the following formulas.

$$\%\ Recovery = \frac{amount\ found}{Amount\ added} \times 100$$

$$Amount\ Found(mcg/ml) = \frac{Mean\ test\ area}{Mean\ standard\ area} \times Standard\ concentration$$

### Specificity

In an assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay results are unaffected by the presence of these extraneous materials. There should be no interference of the diluents at retention time of drug substances.

### Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in (Table no.4)

### Ruggedness

The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The %RSD assay values between two analysts was calculated i.e.,(limit <2%).

This indicates the method was rugged. The results were shown in Table no.5.

## DISCUSSION

In RP HPLC method, the primary requirement for developing a method for analysis is that the using different solvents and buffers and columns to get better retention time and theoretical plates, and better cost effective and time saving method than the previously developed methods. . The Isobestic Point of Ambroxol HCl and Loratadine were found to be 220nm (Figure No: 2.3) by scanning in UV region. The chromatographic method was optimized with mobile phase consisting of $KH_2PO_4$ Buffer (30mM): Acetonitrile: Methanol (30:50:20) and C18 INERTSIL column. All the validation parameters were studied at a the wavelength 220nm. Accuracy was determined by calculating the recovery (Table No.3) and the results were in acceptable range (limit 98-102%). The method was successfully used to determine the amount of Ambroxol HCl and Loratadine present in the Tablet. The results obtained were in good agreement with the corresponding labeled amount (Table No.3). The method was linear in the concentration range of 72 to 168µg/ml for Ambroxol HCl and 6 to 14µg/ml for Loratadine (Figure no.1, Table No.1). Precision was calculated (% RSD) for the drug (Table No.7 Robustness and ruggedness results were in acceptable range (Table No.4 and Table No.5).Summary of all validation parameters for method is given in Table No.8. By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis Ambroxol HCl and Loratadine in tablet dosage form.

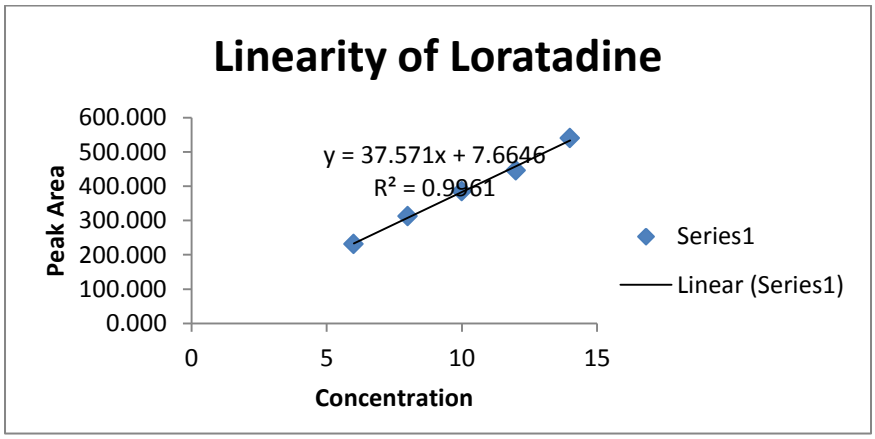
**Table No: 1**

| Concentration (µg/ml ) | Peak Area |
|---|---|
| 72 | 2488.713 |
| 96 | 3252.121 |
| 120 | 3941.042 |
| 144 | 4690.891 |
| 168 | 5454.513 |

**Table No: 1.1**

| Concentration (µg/ml ) | Peak Area |
|---|---|
| 6 | 231.895 |
| 8 | 312.713 |
| 10 | 385.139 |
| 12 | 446.353 |
| 14 | 540.788 |

**Figure No.1&1.1: Linearity (calibration) curve of Ambroxol HCl and Loratadine**



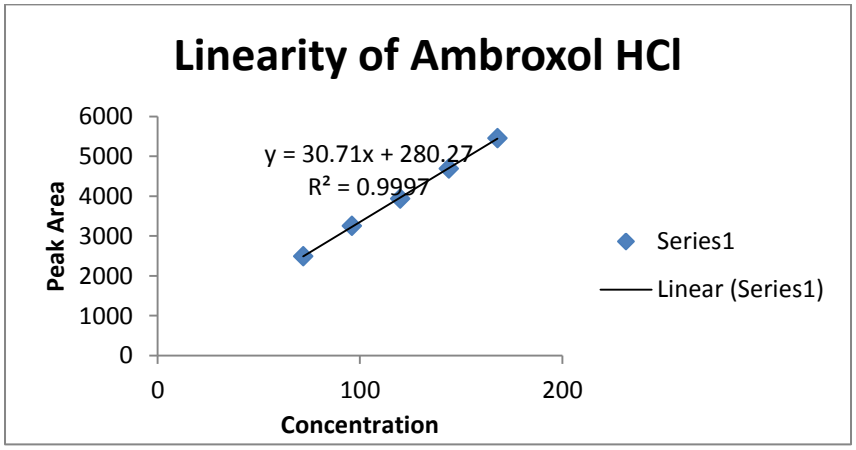




**Table no.2: LOD and LOQ values Calculated from calibration curve**

| | Ambroxol HCl | | Loratadine | |
|---|---|---|---|---|
| | mcg | Area | mcg | Area |
| **LOD** | 4.078 | 125.24 | 0.28 | 10.46 |
| **LOQ** | 12.36 | 379.53 | 0.84 | 31.69 |

**Table No.3: Recovery data**

| LEVEL | S.No | Amount of Sample taken (%) | Amount of Standard Spiked (mcg) | %Recovery of Ambroxol HCl | %Recovery of Loratadine |
|---|---|---|---|---|---|
| **I.** | **1** | **80** | **24** | **98.23%** | **100.74%** |
| | **2** | **80** | **24** | | |
| | **3** | **80** | **24** | | |
| **II.** | **1** | **100** | **24** | **99.36%** | **98.43%** |
| | **2** | **100** | **24** | | |
| | **3** | **100** | **24** | | |
| **III.** | **1** | **120** | **24** | **100.08%** | **100.82%** |
| | **2** | **120** | **24** | | |
| | **3** | **120** | **24** | | |

**Table No.4: Results of Robustness study**

**Ambroxol HCl**

| Parameter | Rt | Tailing factor | Theoretical plates |
|---|---|---|---|
| Flow Rate(1.0ml) | 2.460 | 1.667 | 4464 |
| 0.8ml | 2.653 | 1.667 | 5193 |
| 1.2ml | 2.283 | 1.750 | 4513 |
| Wave Length220nm±2 | | | |
| 218nm | 2.460 | 1.611 | 4828 |
| 222nm | 2.460 | 1.611 | 4828 |

**Loratadine**

| Parameter | Rt | Tailing factor | Theoretical plates |
|---|---|---|---|
| Flow Rate(1.0ml) | 4.253 | 1.571 | 6589 |
| 0.8ml | 4.540 | 1.556 | 7507 |
| 1.2ml | 4.013 | 1.200 | 10243 |
| Wave Length220nm±2 | | | |
| 218nm | 4.200 | 1.519 | 7180 |
| 222nm | 4.183 | 1.538 | 7123 |

**Table No.5: Results of Ruggedness**

| | | %Assay | %RSD |
|---|---|---|---|
| Analyst-1 | Loratadine | 98.63 | 0.45% |
| Analyst-2 | | 99.23 | |
| Analyst-1 | Ambroxol HCl | 99.23 | 1.62% |
| Analyst-2 | | 96.98 | |

**Table No.6: Assay Results**

| AMBROXOL HCL | | | LORATADINE | | |
|---|---|---|---|---|---|
| Standard Area | 1 | 3945.931 | | 378.411 | |
| | 2 | 3934.148 | | 367.951 | |
| | 3 | 3941.042 | | 375.523 | |
| | 4 | 3925.782 | | 350.591 | |
| | 5 | 3941.191 | | 384.450 | |
| | Average | 3937.619 | Average | 371.3852 | |
| Sample area | 1 | 3933.444 | | 372.761 | |
| | 2 | 3930.759 | | 371.408 | |
| | 3 | 3936.783 | | 370.373 | |
| | 4 | 3920.484 | | 341.451 | |
| | 5 | 3908.165 | | 370.382 | |
| | Average | 3925.927 | Average | 365.275 | |
| Tablet average weight | | 110 | mg | 110 | mg |
| Standard weight | | 120 | mg | 10 | mg |
| Sample weight | | 220 | mg | 220 | mg |
| Label amount | | 60 | mg | 5 | mg |
| std.purity | | 99.2 | | 99.3 | % |
| Cal. | | 59.34 | mg | 4.88 | mg |
| | %Assay | 98.91 | % | 97.67 | % |

**Table No.7: Method Precision (Repeatability)**

| | AMBROXOL HCL | | LORATADINE | |
|---|---|---|---|---|
| **S.No.** | **Rt** | **Area** | **Rt** | **Area** |
| 1 | 2.46 | 3984.852 | 4.253 | 399.316 |
| 2 | 2.460 | 3983.028 | 4.243 | 396.285 |
| 3 | 2.460 | 3986.756 | 4.227 | 397.666 |
| 4 | 2.457 | 3983.864 | 4.210 | 390.391 |
| 5 | 2.46 | 3955.742 | 4.300 | 397.802 |
| 6 | 2.450 | 3915.924 | 4.367 | 385.427 |
| avg | 2.4578 | 3968.361 | 4.267 | 394.481 |
| stdev | 0.0040 | 28.195 | 0.058 | 5.413 |
| %RSD | 0.16 | 0.71 | 1.35 | 1.37 |

**Table No.8: Validation parameters of evaluated method**

| S.No | Parameter | Limit | Value Obtained |
|---|---|---|---|
| 1. | **ACCURACY(%Recovery)** | 98-102% | 98.23 to 100.08% (Ambroxol HCl)<br>98.43 to100.82% (Loratadine) |
| 2. | **Linearity concentrations Range(µg/mL)**<br>**Regression coefficient (R2 value)** | NLT 0.99% | 72 to 168µg/ml (Ambroxol HCl)<br>$R^2$=0.999<br>and 6 to 12 µg/ml(Loratadine)<br>$R^2$=0.996 |
| 3. | **Method precision(Repeatability) (%RSD, n = 6)** | NMT 1%(For Rt) NMT 2%(For Area) | %RSD of Rt=0.57% and %RSD of Area 0.62% (Ambroxol HCl)<br>%RSD of Rt=0.52% and %RSD of Area 1.48% (Loratadine) |
| 4. | **Robustness** | Should meets with system suitability | Complies |
| 5. | **Ruggedness (%RSD analyst to analyst variation)** | NMT2% | Complies |

## CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of AMBROXOL HCL and LORATADINE was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories.

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