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RESEARCH ARTICLE

Analytical Method Development, Validation and Simultaneous Estimation of Guaifenesin, Levocetirizine Hydrochloride, and Ambroxol Hydrochloride in Syrup Dosage form by RP-HPLC

Konagala Sravanthi, Bhauvaneswara R. C.*, Priyanka M. V., Anusha M., Kalyani T.

Department of Pharmaceutical Analysis, Sesachala College of Pharmacy, Puttur, India.

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ABSTRACT

In the present study a validated stability indicating RP-HPLC method for quantification of GFS, LCZ and ABX was established. Compared with the reported methods, this method represents the first report about a stability indicating method for the determination of GFS, LCZ and ABX. With the proposed method a satisfactory separation of three drugs from the degradation products and impurities, extended linear range and rapid analysis time were carried out. A high recovery of GFS, LCZ and ABX in formulation was achieved. The proposed method ensured a precise and accurate determination of GFS, LCZ and ABX in formulation. No interference from the excipients was noticed.

KEYWORDS

Guaifenesin, Levocetirizine hydrochloride, Ambroxol hydrochloride, RP HPLC, Validation

INTRODUCTION

Pharmaceutical Analysis plays a very vital role in the quality assurance and quality control of bulk drugs and their medications. Pharmaceutical analysis is a focused branch of analytical chemistry[1] involves separating, identifying and formative the relative amounts of components in a sample of material. It is anxious with the chemical characterization of matter both quantitative and qualitative. High-performance liquid chromatography (HPLC) is the fastest growing analytical technique for analysis of drugs. Its effortlessness, high specificity and broad range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids. Levocetirizine dihydrochloride produces selective inhibition of peripheral histamine (H1) receptors. It has an affinity for H1 receptors approximately 2-fold higher than racemic cetirizine. Levocetirizine also inhibits wheal and flare reactions in children and adults. Ambroxol hydrohloride is a Sodium channel blocker and mucolytic agent with antioxidant, anti-viral and anti-inflammatory properties. Inhibits tetrodotoxin (TTX)-resistant channels more potently than TTX-sensitive subtypes.

Guaifenesin act as an irritant to gastric vagal receptors, and recruit efferent parasympathetic reflexes that cause glandular exocytosis of a less viscous mucus mixture. Expectorant and widely used in the treatment of cough

Each and every day number of diseases is being diagnosed. Cold and cough are commonly occurring infection diseases. So, various pharmaceutical organizations are working to develop new drug molecules and new combinations of drugs for better treatment. This is the reason for a greater competition in the pharmaceutical sector, and the future scenario is

***Address for Correspondence:**
Bhauvaneswara Rao,
Department of Pharmaceutical analysis,
Sesachala College of Pharmacy, Puttur, India.
E-Mail Id: bhuvaneshwararao151@gmail.com

likely to be the same. The scope of developing and validating a method is to ensure a suitable strategy for evaluation of a particular analyte which is more specific, accurate and precise.

The main focus is drawn to achieve improvement in the manufacturing and analytical conditions and making proper amendments in the standard operating procedures being followed.

The present study aims to develop simple, precise and accurate methods for the determination of Guaifenesin, Levocetirizine, Ambroxol and its force degradation studies by RP-HPLC in formulation.

MATERIAL AND METHODS

Materials Used

Guaifenesin, Levocetirizine HCl and Ambroxol HCl are procured as a gift samples from Bio Leo Labs, Hyderabad (India).

The other solvents like HPLC Solvents, Methanol, Water, Acetonitrile and chemicals and reagents like Ammonium phosphate, H_2O_2, NaOH, HCl used in the study are obtained from Merck, Mumbai (India).

Optimized Chromatographic Conditions

Selection of Wavelength

Wavelength for detection was selected by obtaining absorption spectra of Guaifenesin (GFS), Levocetirizine (LCZ) and Ambroxol (ABX) in Methanol by using double beam UV-VIS spectrophotometer in the range of 200-400nm.

Selection of Mode of Separation

As the drug was polar in nature, RP-HPLC method was preferred.

Selection of Mobile Phase

A number of trials were made to find out the ideal solvent system (mobile phase) for eluting the drugs. The mobile phase containing methanol: water and Buffer: Acetonitrile in different ratios (60:15, 70:15, 85:15,) was tried. Better peak resolution with less tailing was obtained with the ratio of Buffer: acetonitrile (HPLC grade) (60:40).

Selection of Diluents

HPLC grade of mobile phase [Buffer: Acetonitrile (60:40)] which was used as diluents for further dilution of standard stock solution.

Preparation of Mobile Phase, Standard and Sample Solutions

Mobile Phase

A mixture of 600 ml of Buffer, 400ml of Acetonitrile (HPLC grade). The mobile phase was sonicated for 10min to remove gases.

Buffer Preparation

Weigh accurately about 1.32gm of ammonium phosphate was weighed and dissolved in small amount of milli-Q water and make upto the volume of 1000ml sonicate to dissolve it completely. The buffer was filtered through 0.45mc filters to remove all fine particles and gases.

Standard Stock Solution Preparation

Weigh and transfer 50 mg of GFS working standard 2.5 mg of LCZ working standard and 15 mg of ABX working standard into 50 ml volumetric flask, add 25 ml of diluent and sonicate to dissolve and dilute to volume with diluents.

Standard Preparation

Transfer 10 ml of standard stock solution into 100 ml volumetric flask and dilute to volume with diluents.

Sample Preparation

Transfer liquid sample quantitatively equivalent to 50 mg of GFS and 2.5 mg of LCZ and 15 mg of ABX in to 50 ml volumetric flask add 25 ml of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through 0.45µ filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

TRIAL and Error Methods

Trial -1

Chromatographic Conditions

Column : Zodiac C18 (4.6 x 100mm, 5 mm)

Detector : 236nm

Flow rate : 1ml/min

Injection volume : 20µl

Run time : 10min

Mobile Phase : Water: Acetonitrile: Methanol (50: 25:25)

Trial - 2

Chromatographic Conditions

Column : Inertsil ODS C18, 100 X 4.6 mm, 5µ.

Mobile phase: Buffer (0.01 N Ammonium phosphates): Acetonitrile (55: 35)

Flow rate : 1.0 ml /min

Wavelength: 236 nm

Injection volume: 20 µl

Run time: 10 min

Trial - 3

Chromatographic Conditions

Column: Inertsil ODS C18, 100 X 4.6 mm, 5µ.

Mobile phase: Buffer (0.01 N Ammonium phosphate)

Acetonitrile: 60:40

Flow rate: 1.0 ml /min

Wavelength: 236 nm

Column temperature: 30°C

Injection volume: 20 µL

Run time: 13 min

Analytical Method Optimisation

The present study is to develop a new reverse phase liquid chromatographic method for simultaneous determination of GFS, LCZ and ABX in syrup dosage form.

Optimized Chromatographic Parameters

Optimized Chromatographic Conditions	
Mode of separation	Isocratic elution
Mobile phase	Buffer (0.01 N Ammonium phosphate): Acetonitrile (60:40)
Column	Inertsil ODS C18, 100 X 4.6 mm, 5µ.
Flow rate	1 ml/ min
Detection Wavelength	236 nm
Injection volume	20 µl
Column oven temperature	30^0C
Run time	13 min
Diluent	Mobile phase
Needle wash	Water: Acetonitrile 90:10 (v/v

Simultaneous Estimation of Drugs in Syrup Solution

Standard Stock Solution Preparation

Weigh and transfer 50 mg of GFS working standard 2.5 mg of LCZ working standard and 15 mg of ABX working standard into 50 ml volumetric flask, add 25 ml of diluent and sonicate to dissolve and dilute to volume with diluent.

Standard Preparation

Transfer 10 ml of standard stock solution into 100 ml volumetric flask and dilute to volume with diluent. Inject 20 ml of the standard solution into the chromatographic system and measure the area for the GFS, LCZ and ABX peaks and calculate the %Assay by using the assay formula.

Sample Preparation

Transfer liquid sample quantitatively equivalent to 50 mg of GFS and 2.5 mg of LCZ and 15 mg of ABX in to 50 ml volumetric flask add 25 ml of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through 0.45µ filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

Procedure

Inject 20 µL of blank solution, placebo solution, Standard solution, Disregard peaks due to blank and placebo if any.

Inject 20 µl of the standard and sample solution into the chromatographic system and measure the area for the GFS, LCZ and ABX peaks and calculate the %Assay by using the assay formula.

Calculation

Spl area: Sample Peak area

Std area: Standard Peak area

Std. Dil. Fac: Standard Dilute Factor

Spl. Dil. Fac: Sample Dilute Factor

Avg. Wt: Average weight

L.C: Lable claim

Potency of Std: Potency of Standard

Method Validation

The method was validated according to the ICH guidelines. Characteristics were addressed: linearity, accuracy, precision, and specificity, limits of detection and quantization, robustness.

System Suitability

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated and the parameters like tailing factor, retention time, theoretical plates per unit, resolution factor are determined.

Procedure

Standard solutions were prepared as per the test method and inject six times into the chromatographic system and results are tabulated in table 4-6.

Acceptance Criteria

- The column efficiency is not less than 2000 theoretical plates.
- The tailing factor for the analyte peak is not more than 2.0.
- The relative standard deviation for the replicate injections more than 2.0%.

Specificity

For determining Specificity of the method, a syrup dosage form was analyzed. Inject standard solution, placebo and sample solutions and the resulted chromatograms are as shown in figure 1-11, results are tabulated in table 6. Chromatograms were examined to determine if compounds of interest co-eluted with each other or with any additional excipient peaks. Injections of the marketed product revealed the absence of interferences with the elution of the drug and these results demonstrate that there was no interference from other materials in the syrup formulation therefore, confirm the specificity of the method.

Acceptance Criteria

There should not be any peak in the blank and Placebo solution run at the retention time corresponding to GFS, LCZ and ABX as in standard run.

Precision

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample. Precision of analytical method is usually expressed as the standard deviation and relative standard deviation.

Procedure

Method Precision

Preparation of Precision Solution

Dilute to 10 ml of standard stock solution, with 100 ml of diluents, Prepare six solutions and inject each solution. Measure the area for the GFS, LCZ and ABX peaks and calculate the %RSD for the area of six replicate injections and results are tabulated in table 7.

% RSD Formula

(σ/μ)*100

Where σ is standard deviation, μ is mean.

Acceptance Criteria

The % of RSD of areas from six preparations should not be more than 2.0**%**

System Precision

Preparation of Precision Solution

Dilute to 10 ml of standard stock solution with 100 ml of diluent. Inject 20 ml of the above solution six times measure the area for the GFS, LCZ and ABX peaks and calculate the %RSD for the area of six replicate injections and results are tabulated in table 8.

% RSD Formula

(σ /μ)*100

Where σ is standard deviation, μ is mean.

Acceptance Criteria

The % of RSD of areas from six injections should not be more than 2.0**%**

Intraday and Inter Day Precision

Intra-day precision was determined by analysis of the standard solutions three times on the same day. Inter-day precision was assessed by analysis of the standard solutions on three different days area of drug peaks and percentage RSD were calculated and results are tabulated in table 9-10.

Linearity

The linearity of calibration curves (peak area Vs concentration) in pure solution was checked over the concentration ranges of about 25- 150μg/ml for GFS, 1.25-15μg/ml for LCZ and 7.5 - 45 μg/ml for ABX. The total eluting time was less than 13min.The regression line relating standard concentrations of drug using regression analysis, the calibration curves were linear in the studied range and equations of the regression analysis were obtained = 14032x+0.0573, r^2 =0.999 for GFS, 23106x+0.0266,r^2 =0.999 for LCZ and y =23083x+0.0525, r^2=0.999 for ABX.

The mean ± standard deviation (SD) for the slope, intercept and correlation coefficient of standard curves (N=3) were calculated. The represented data was shown in tables 11-15 and figure 12-14.

Accuracy

Accuracy of the method was determined by recovery experiments. To the formulation, the reference standards of the drug were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage relative standard deviation of the recovery for GFS, LCZ and ABX were calculated and shown in table 16 and figure 15-17.

Assay

Standard Preparation

Transfer 10 ml of standard stock solution in to 100 ml volumetric flask and make up to volume with diluent.

Sample Preparation

Transfer liquid sample quantitatively equivalent to 50 mg of GFS and 2.5 mg of LCZ and 15 mg of ABX in to 50 ml volumetric flask add 25 ml of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through 0.45μ filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

Procedure

Inject 20 μL of blank solution, Standard solution and Sample Solution record the chromatogram. And calculate percentage of assay. The assay procedure was made triplicate and weight of sample taken for assay was calculated. The percentage of drug found in formulation, mean and standard deviation in formulation were calculated and shown in table 17 and figure 18.

Robustness

It is a measure of ability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the method was evaluated by analyzing the system suitability standards and evaluating system suitability parameter data after varying the HPLC pump flow rate (±0.1%) and temperature variations. None of the alterations caused a significant change in peak area R.S.D., USP tailing factor and theoretical plates. Results were presented in table 18. Inject 20µl of the blank solution and the standard solution for Three times and analysed using varied flow rates (0.9ml, 1.1 ml) along with method flow rate and calculate the %RSD for the area of five replicate injections and results are tabulated in table 19.

Forced Degradation Studies

Degradation studies were carried out as per ICH guidelines. The objective of the study was to find out the degradation products, which in turn help in the establishment of degradation pathways and the intrinsic stability of drug molecule. In order to check the selectivity of the proposed method, degradation studies were carried out by using acidic, basic, neutral, oxidative, photo and thermal conditions.

The stress conditions were as follows

Acid Degradation Studies

Acid decomposition studies were carried out in 1N HCl at Room temperature for a period of 6 hrs.

Procedure

Transfer 10 ml of standard stock solution into 100 ml volumetric flask and dilute to volume with 1N HCl at Room temperature for a period of 6 hrs and injected into HPLC system and the resulted chromatographs are as shown in Figure 15, Blank (sample solution without drug) was used as control was injected to determine interference of the mobile phase. From the peak areas in the chromatogram of stress degraded samples. The percentage content of degraded drug was determined.

Alkaline Degradation Studies

Alkaline decomposition studies were carried in 1N NaOH at room temperature for a period of 6 hrs. .

Procedure

Transfer 10 ml of standard stock solution into 100 ml volumetric flask and dilute to volume with 1N NaOH at Room temperature for a period of 6 hrs and injected into HPLC system and the resulted chromatographs are as shown in Figure 16, Blank (sample solution without drug) was used as control was injected to determine interference of the mobile phase. From the peak areas in the chromatogram of stress degraded samples. The percentage content of degraded drug was determined.

Oxidative Degradation Studies

The oxidative stress studies were done in 1% H2O2 at room temperature for a period of 6 hrs.

Procedure

Transfer 10 ml of standard stock solution into 100 ml volumetric flask and dilute to volume with 1% H2O2 at Room temperature for a period of 6 hrs and injected into HPLC system and the resulted chromatographs are as shown in Figure 17, Blank (sample solution without drug) was used as control was injected to determine interference of the mobile phase. From the peak areas in the chromatogram of stress degraded samples. The percentage content of degraded drug was determined. The results are tabulated and shown in table 20.

Thermal Degradation Studies

Thermal degradation studies for drug solution were conducted by exposing the samples at 60^0C for 6 h in hot air oven.

Procedure

Place a sufficient amount of GFS, LCZ and ABX in hot air oven at 100c for a period of 3 hrs. Weigh and transfer 50 mg of GFS working standard 2.5 mg of LCZ working standard and 15 mg of ABX working standard into 50 ml volumetric flask, add 25 ml of diluents and

sonicate to dissolve and dilute to volume with diluents.

Transfer 10 ml of above solution into 100 ml volumetric flask and dilute to volume with diluent and kept in hot air oven at 60°c for a period of 6 hrs and injected into HPLC system and the resulted chromatographs are as shown in Figure 18, Blank (sample solution without drug) was used as control was injected to determine interference of the mobile phase. From the peak areas in the chromatogram of stress degraded samples. The percentage content of degraded drug was determined. The results are tabulated and shown in table 21.

Photolytic Degradation Studies

Photo stability degradation studies of drug solutions were carried out in a photo stability chamber by exposing to UV light.

Procedure

Transfer 10 ml of standard stock solution into 100 ml volumetric flask and dilute to volume with diluent and these solutions were exposed to UV light in Stability chamber for a period of 6 hrs and injected into HPLC system and the resulted chromatographs are as shown in Figure 19, Blank (sample solution without drug) was used as control was injected to determine interference of the mobile phase. From the peak areas in the chromatogram of stress degraded samples. The percentage content of degraded drug was determined.

Degradation by Day Light

Photo stability degradation studies of drug solutions were carried out by exposing the drug solutions to day light.

Procedure

Transfer 10 ml of standard stock solution into 100 ml volumetric flask and dilute to volume with diluents and these solutions were exposed to day light for a period of 6 hrs and injected into HPLC system and the resulted chromatographs are as shown in Figure 20, Blank (sample solution without drug) was used as control was injected to determine interference of the mobile phase. From the peak areas in the chromatogram of stress degraded samples. The percentage content of degraded drug was determined. The results are tabulated and shown in table 24.

Acid Degradation Studies

When Guaifenesin, Levocetirizine and Ambroxol drug solutions were exposed to 1N HC at room temperature for 6h there was 6%, 5% and 5.4 degradation was observed for Guaifenesin, Levocetirizine and Ambroxol and there was no impurities are observed.

Alkaline Degradation Studies

When the three drugs were exposed to 1N NaOH at room temperature for 6h there was 5.2%, 5.3% and 5.3% degradation was observed for Guaifenesin, Levocetirizine and Ambroxol and there was no impurities are observed**.**

Oxidative Degradation Studies

When the three drugs were exposed to 1% H2o2 at room temperature for 6h there was 91.1%, 14.70% degradation was observed for Levocetirizine and Ambroxol and there was two impurities are observed at retention time of 2.3 and 2.8, and there was no degradation observed for Guaifenesin.

Table 1: Oxidative degradation

S.NO	Degradants	Retention time	Peak area
1	1	2.3	50064
2	2	2.8	5561

Thermal Degradation Studies

When the powder drugs were exposed to heat in a hot air oven at 60°c for 6h there was 0.30%, 0.20%, and 0.30% degradation was observed for Guaifenesin, Levocetirizine and Ambroxol and there was one impurities were observed at retention time of 4.2 with the area of 3429.

Photolytic Degradation by UV light

When the three drugs solutions were exposed to uv rays in uv chamber at room temperature for 6h there was 6.2%, 1.4% and 2.15% degradation was observed for Guaifenesin, Levocetirizine and

Ambroxol and there was no impurities are observed.

Table 2: Thermal Degradation

S.NO	Degradants	Retention time	Peak area
1	1	4.2	3429

Photolytic Degradation by Day Light

When the three drugs solutions were exposed to day light for 6h there was 0.20%, 0.60% and 0.46% degradation was observed for Guaifenesin, Levocetirizine and Ambroxol and there was one impurities is observed at retention time of 1.5 with the area of 1411.

Table 3: Photolytic degradation

S.NO	Degradants	Retention time	Peak area
1	1	1.5	1411

RESULTS AND DISCUSSION

Selection of Wavelength

The complete spectrum of GFS, LCZ and ABX and their Isobestic in UV range is shown in the Figures. From the figure concluded that GFS, LCZ and ABX have maximum absorption at λmax of 274 nm, 248 nm, 230 nm and Isobestic point from the overlay spectrum shows at λmax of 236 nm Figure 4. So all detections are performed at 236 nm.

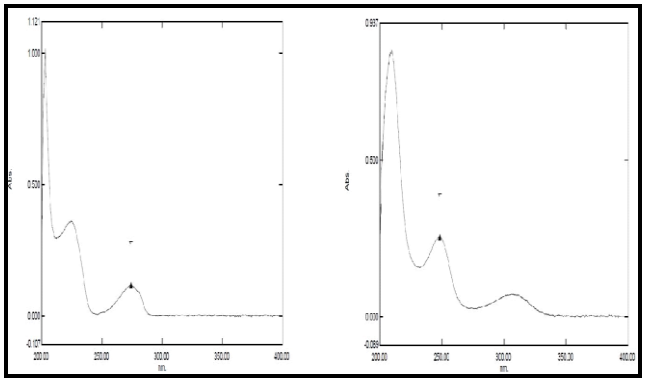

Figure 1 & 2: UV spectrum of Guaifenesin, 274nm and UV spectrum of Levocetirizine, 248 nm

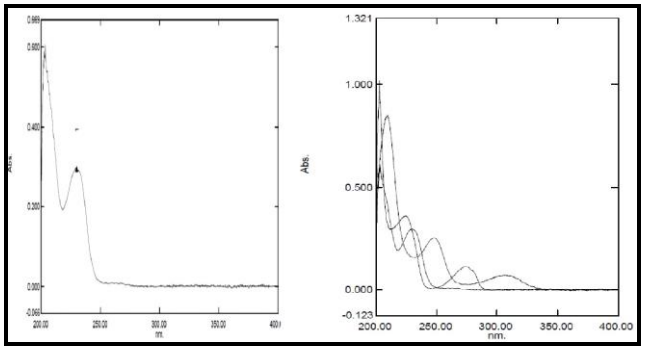

Figure 3 & 4: UV spectrum of Ambroxol, 230 nm and UV overlay Spectrum, 236 nm.

Analytical Method-Optimisation

The present study is to develop a new reverse phase liquid chromatographic method for simultaneous determination of Guaifenesin, Levocetirizine, Ambroxol in syrup dosage form.

Trial -1

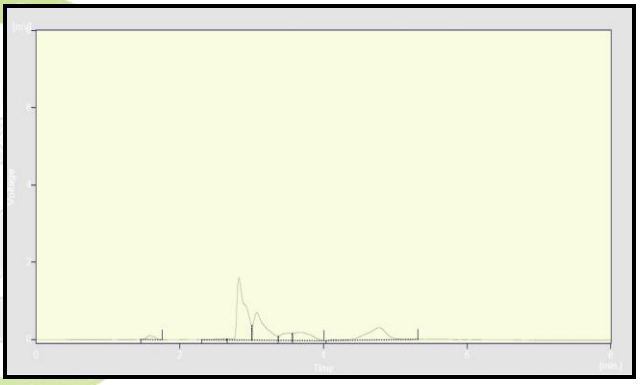

Figure 5: Chromatogram of trial 1

Results of Trial-1: Peak shape was not good, Due to asymmetry in peaks and longer RT's another trial is made with change in mobile phase.

Trial-2

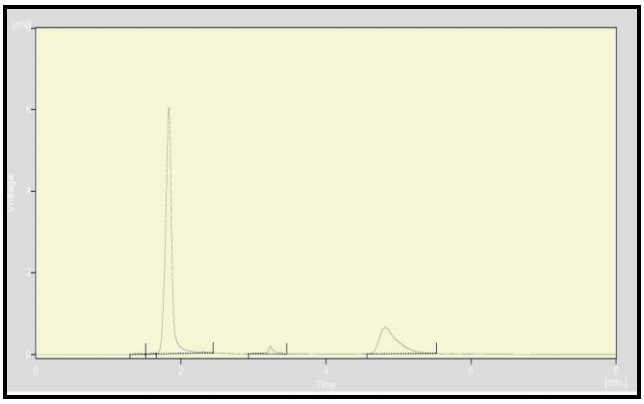

Figure 6: Chromatogram of trial 2

Results of Trial-2: Peak shape was good but second and third compound have more tailing factor so another trail was made.

Trial-3

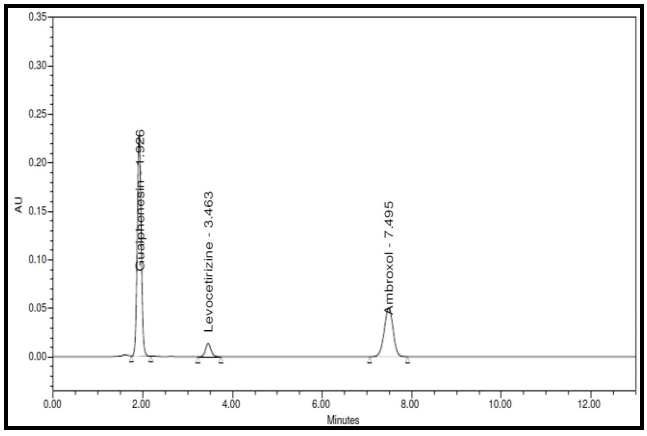

Figure 7: Chromatogram of trial 3

Results of Trial-3: RT's were observed at 1.926 (Guaifenesin), 3.463 (Levocetirizine) and 7.496 (Ambroxol). The peaks are sharply resolved with less tailing and hence the trial-3 method is optimized for analysis.

Optimized Chromatographic Conditions

Mode of separation	Isocratic elution
Mobile phase	Buffer (0.01 N Ammonium phosphate): Acetonitrile(60:40)
Column	Inertsil ODS C18, 100 X 4.6 mm, 5µ.
Flow rate	1 ml/ min
Detection Wavelength	236 nm
Injection volume	20 µl
Column oven temperature	30^0c
Run time	13 min
Diluent	Mobile phase
Needle wash	Water: Acetonitrile 90:10 (v/v)

System Suitability

System suitability parameters such as resolution, number of theoretical plates were calculated and the results are presented in table. The resolution value of more than 2 indicates satisfactory results in quantitative work and the high resolution value obtained indicates the complete separation of the drugs. The no. of theoretical plates was high indicating the efficient performance of the column.

Specificity

The specificity of the method was confirmed due to the absence of any peak in the blank and Placebo by injecting the placebo, standard and formulation and the results are tabulated below.

Acceptance Criteria

% RSD for area responses of six standard injections should not be more than 2.0.

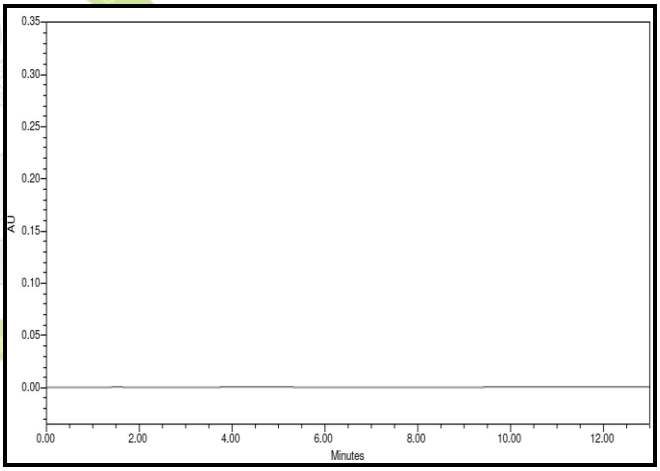

Figure 8: Chromatogram of blank

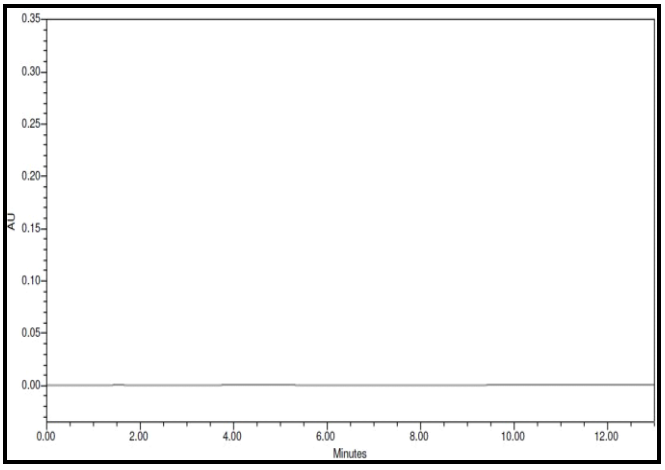

Figure 9: Chromatogram of placebo

Acceptance Criteria

There should not be any peak in the blank and Placebo solution run at the retention time corresponding to GFS, LCZ and ABX as in standard run.

Precision

Method Precision

The precision of the method was determined by replicate injections of sample solution. The % R.S.D of Area and retention time is present within the acceptance criteria of 2%. The data is reported in table 22. Thus the proposed method was found to be high degree of precision and reproducibility.

Table 4: System suitability Data of GFS, LCZ and ABX

Drug	Conc. (µg/ml)	Retention time	RSD	Peak area	RSD
Guaifenesin	100	1.926*	0.065	1400585*	0.095
Levocetirizine	5	3.451*	0.101	115501*	0.165
Ambroxol	30	7.46*	0.105	691216*	0.105

*Avg. of Six determinations

Table 5: System suitability parameters for GFS, LCZ and ABX

Sl . No	Parameter	Values obtained			Acceptance criteria
		Guaifenesin	Levocetirizine	Ambroxol	
1	USP Plate Count	2277.05	3862.20	6855.85	>2000
2	USP Tailing Factor	1.03	1.11	0.98	≤ 2.0
3	USP Resolution	--	7.81	13.56	>1.5

Table 6: Blank and Placebo interference

S. No	Sample Name	Guaifenesin		Levocetirizine		Ambroxol	
		RT	Area	RT	Area	RT	Area
1	Blank	-	-	-	-	-	-
2	Mixed-Standard	1.922	14404188	3.451	125645	7.465	698344
3	Placebo	-	-	-	-	-	-
4	Sample	1.926	1408898	3.488	115442	7.488	697755

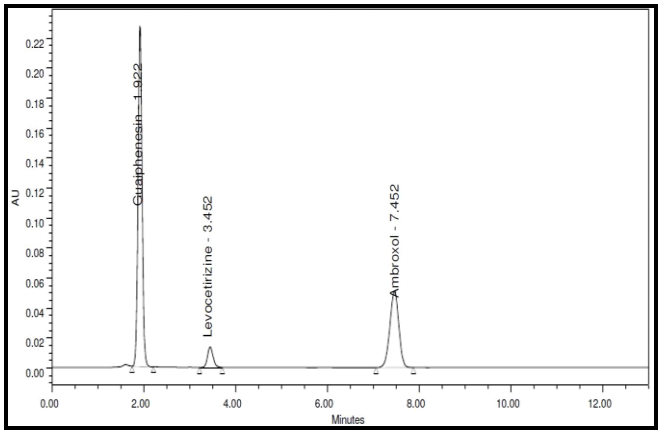

Figure 10: Chromatogram of standard

System Precision

The precision of the system was determined by replicate injections of mixed standard solution. The % R.S.D of Area, retention time is present within the Acceptance criteria of 2%. The data is reported in table 23.

Acceptance Criteria

% Relative standard deviation (%RSD) for the areas of Guaifenesin, Levocetirizine and Ambroxol from the standard chromatograms should not be more than 2.0.

Table 7: Method Precision for GFS, LCZ and ABX

Sl. No	Guaifenesin		Levocetirizine		Ambroxol	
	RT	Area	RT	Area	RT	Area
1	1.925	1401522	3.452	115622	7.465	694245
2	1.924	1402244	3.455	115544	7.466	692335
3	1.926	1403322	3.452	114875	7.455	698887
4	1.922	1410022	3.453	115645	7.471	692645
5	1.925	1404090	3.455	115675	7.472	692478
6	1.921	1401144	3.454	115435	7.477	691595
Avg	1.924	1403724	3.454	115466	7.468	693698
Std Dev	0.002	3275.85	0.001	302.12	0.008	2687.10
RSD	0.101	0.233	0.040	0.262	0.102	0.387

Table 8: System Precision for GFS, LCZ and ABX

S. No	Guaifenesin		Levocetirizine		Ambroxol	
	RT	Area	RT	Area	RT	Area
1	1.926	1400585	3.450	115501	7.462	691216
2	1.923	1403245	3.45	115545	7.464	692418
3	1.924	1401712	3.452	115178	7.469	691285
4	1.924	1403920	3.453	115729	7.471	692504
5	1.926	1404090	3.456	115651	7.479	692362
6	1.924	1402359	3.456	115428	7.479	690976
Avg	1.925	1402652	3.453	115505	7.471	691794
Std Dev	0.001	1361.56	0.003	192.86	0.007	704.04
RSD	0.064	0.097	0.079	0.167	0.097	0.102

Intraday Precision

Intra-day precision was determined by analysis of the solutions three times on the same day.

Area of drug peaks and percentage RSD were calculated. The data is reported in table 24.

Inter day Precision

Inter-day precision was assessed by analysis of the solutions on three different days.

Area of drug peaks and percentage RSD were calculated.

Acceptance Criteria

% Relative standard deviation (%RSD) for the areas of GFS, LCZ and ABX from the standard chromatograms should not be more than 2.0

Linearity

For the linearity 20µl of each of working standard solutions of Guaifenesin (25-150µg/mL), Levocetirizine (1.25-7.5µg/mL) and Ambroxol (7-45µg/mL) injected in to HPLC system and the results obtained are tabulated below.

Table 9: Intraday precision of GFS, LCZ and ABX

Sl .No.	Drug	Conc. (µg/ ml)	Peak area mean ± S.D	%RSD
1	Guaifenesin	100	1401841 ±1335	0.10
	Levocetirizine	5	115406 ±200	0.16
	Ambroxol	30	691638.66 ±674	0.10
2	Guaifenesin	100	1405085 ±4521	0.32
	Levocetirizine	5	115585 ±130	0.11
	Ambroxol	30	692239 ±564	0.08
3	Guaifenesin	100	1401847.33 ±1335.15	0.10
	Levocetirizine	5	115521±22867	0.22
	Ambroxol	30	691342±118.92	0.01

Table 10: Inter day precision of GFS, LCZ and ABX

Sl .No.	Drug	Conc. (µg/ ml)	Peak area mean ± S.D	%RSD
1	Guaifenesin	100	1401847.33 ±1335.15	0.10
	Levocetirizine	5	115408 ±200.39	0.17
	Ambroxol	30	691639 ±674.93	0.10
2	Guaifenesin	100	1412366.33 ±1627.04	0.13
	Levocetirizine	5	115501 ±225.67	0.20
	Ambroxol	30	691342 ±118.92	0.01
3	Guaifenesin	100	1413287 ±1335.15	0.12
	Levocetirizine	5	115521 ±22867	0.22
	Ambroxol	30	691509.33 ±34050	0.05

Table 11: Linearity of Guaifenesin

S. No.	%Concentration (µg/ ml)	Peak Area
1	25	349614
2	50	700389
3	75	1054228
4	100	1403474
5	125	1753303
6	150	2104934

Table 12: Analytical performance parameters of Guaifenesin

Linear regression Analysis	Guaifenesin conc. in µg/ml vs. peak area
Correlation coefficient (r^2)	0.999
Slope (m)	14039.5
y- intercept (c)	801.8

Table 13: Linearity of Levocitirizine

S. No.	%Concentration (µg/ ml)	Peak Area
1	1.250	29105
2	2.500	58097
3	3.750	87177
4	5.000	115674
5	6.250	144371
6	7.500	172830

Table 14: Analytical performance parameters of Levocetirizine

Linear regression Analysis	Levocetirizine conc. in µg/ml vs. peak area
Correlation coefficient (r^2)	0.9989
Slope (m)	22993
y- intercept (c)	614.6

Table 15: Linearity of Ambroxol

S. No.	%Concentration (µg/ ml)	Peak Area
1	7.500	172058
2	15.000	344803
3	22.500	519828
4	30.000	692894
5	37.500	866108
6	45.000	1038474

Table 16: Analytical performance parameters of Ambroxol

Linear regression Analysis	Ambroxol conc. in µg/ml vs. peak area
Correlation coefficient (r^2)	0.999
Slope (m)	23120.2
y- intercept (c)	1211.9

Acceptance Criteria

Correlation coefficient should not be less than 0.99.

Accuracy

Accuracy studies are performed at 80%, 100%, and 120% by spiking method and the results are given below.

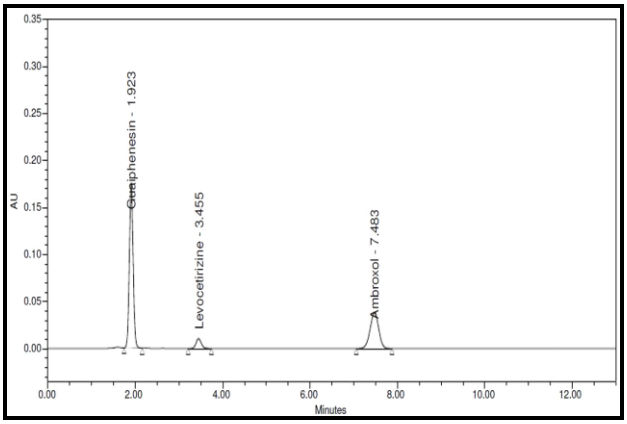

Figure 11: Chromatogram of Accuracy in 80% level

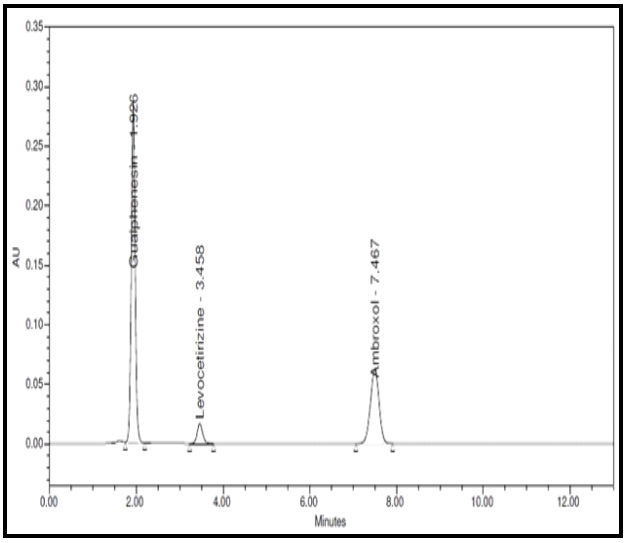

Figure 12: Chromatogram of Accuracy in 100% level

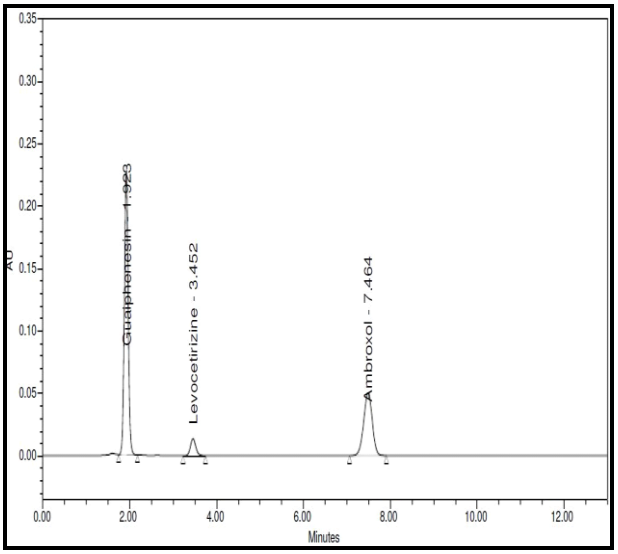

Figure 13: Chromatogram of Accuracy in 120% level

Table 17: Recovery Report of GFS, LCZ and ABX

Inj. Sample	Spike Level	Average	Amount recovered	% recovered	Mean recover	Acceptance Criteria
Guaifenesin	80%	1127230	79.45 µg	99.31%	99.54%	98-102%
	100%	1403218*	98.90 µg	98.90%		
	120 %	1709936*	120.51µg	100.43%		
Levocetirizine	80%	91299*	73.34 µg	99.17%	100.14 %	98-102%
	100%	115043*	99.97 µg	99.97%		
	120 %	138525*	120.37µg	100.31%		
Ambroxol	80%	549568*	79.37 µg	99.22%	100.12%	98-102%
	100%	693205*	100.12µg	100.12%		
	120 %	837486*	120.96µg	100.80%		

*Indicates average of three determinations.

Table 18: Assay report of GFS, LCZ and ABX

S.NO	Formulation Levoriv plus (Syrup)	Labelled claim (mg)	Peak area mean ± SD	Amount found (mg)	Assay	% RSD
1	Guaifenesin	50	1418955±4352.94	49.76	99.52	0.31
2	Levocetirizine	2.5	115516.5±1421	2.46	98.42	1.23
3	Ambroxol	15	697530±1407.1	14.96	99.75	0.20

Acceptance Criteria

Percentage recovery in all the cases should be between 98.0 and 102.0 %. The above result serves as a good index of the accuracy and reproducibility of the proposed method.

Assay

Assay was performed by injecting the sample solution (pharmaceutical dosage form) chromatogram was obtained and the results are tabulated below.

Acceptance Criteria

The %Assay content of the sample should not be less than 97.0and should not be more than 103.0 and the obtained results will be within the limits.

Robustness

The robustness of test method is demonstrated by carrying out method variation like flow (0.9ml and 1.1 ml/min) and column oven temperature variations etc. The results are mentioned below.

Table 19: Robustness results of GFS, LCZ and ABX

S. No	Parameter	Modification	Retention time			Tailing		
			GFS	LCZ	ABX	GFS	LCZ	ABX
1	Flow rate	0.9 ml/min	2.233	4.029	8.740	1.03	1.13	0.98
		1.0 ml/min	1.926	3.450	7.462	1.01	1.15	0.98
		1.1 ml/min	1.693	3.035	6.526	1.02	1.11	0.95
					RSD	0.98	1.77	1.79
2	Column oven temperature	25^0c	1.935	3.446	8.341	1.02	1.12	0.98
		30^0c	1.926	3.450	7.462	1.01	1.15	0.98
		35^0c	1.910	3.483	6.672	0.98	1.11	0.95
					RSD	2	1.85	1,79

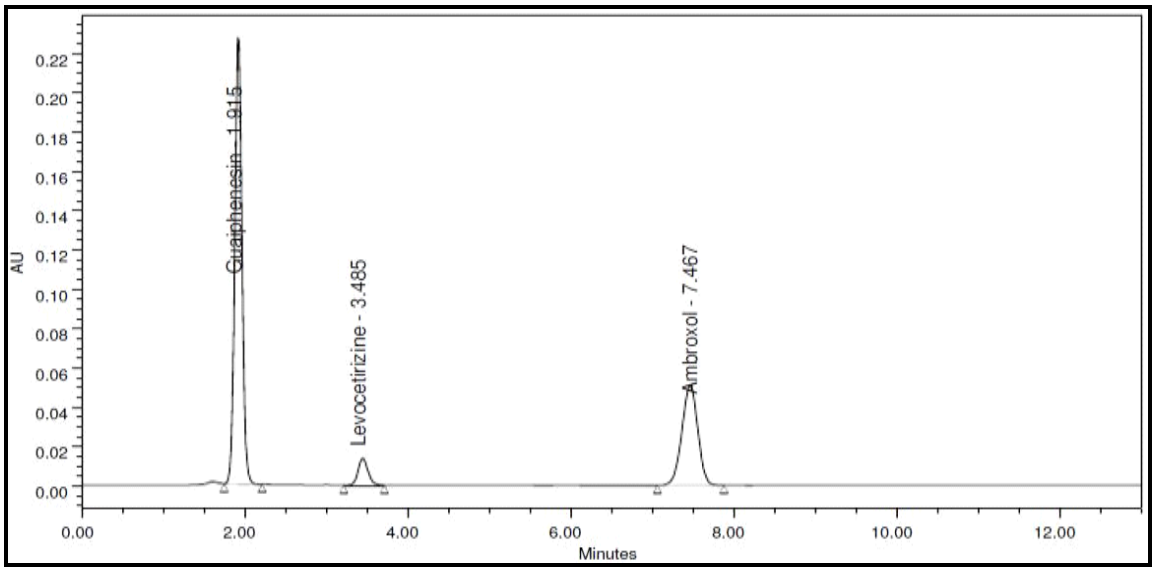

Figure 14: Sample chromatogram for Assay

Results for Forced Degradation Studies

HPLC studies on the drugs under different stress conditions indicated the following behavior.

Acid Degradation Studies

When GFS, LCZ and ABX drug solutions were exposed to 1N HCl at room temperature for 6h there was 5.07%, 39.89% and 37.8% degradation was observed for GFS, LCZ and ABX and there was no impurities are observed.

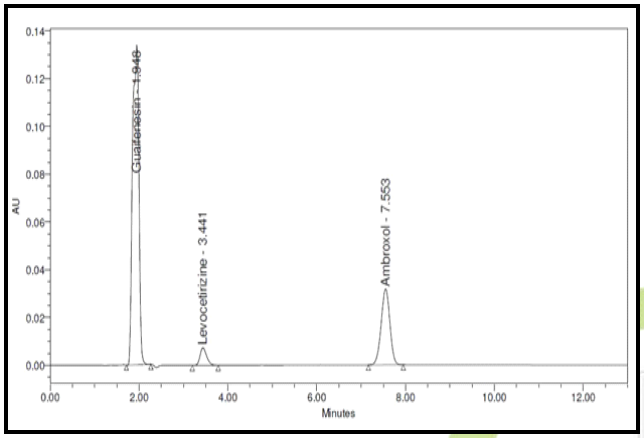

Figure 15: Chromatogram of Acid degradation

Alkaline Degradation Studies

When the three drugs were exposed to 1N NaOH at room temperature for 6h there was 5.2%, 40.1% and 37.7% degradation was observed for GFS, LCZ and ABX and there was no impurities are observed**.**

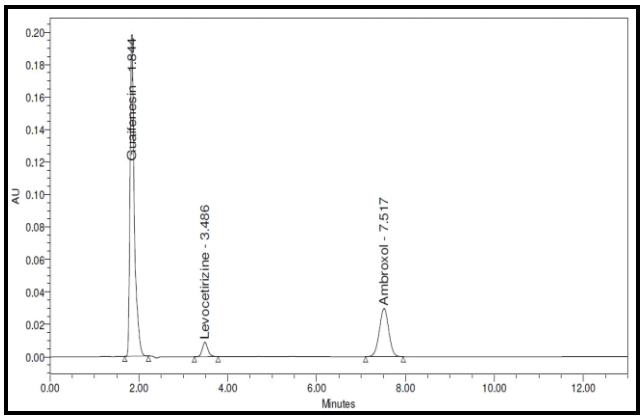

Figure 16: Chromatogram of Alkaline degradation

Oxidative Degradation Studies

When the three drugs were exposed to 1% H_2O_2 at room temperature for 3h there was 45%, 22% degradation was observed for LCZ and ABX and there was two impurities are observed at retention time of 2.3 and 2.8, and there was no degradation observed for GFS.

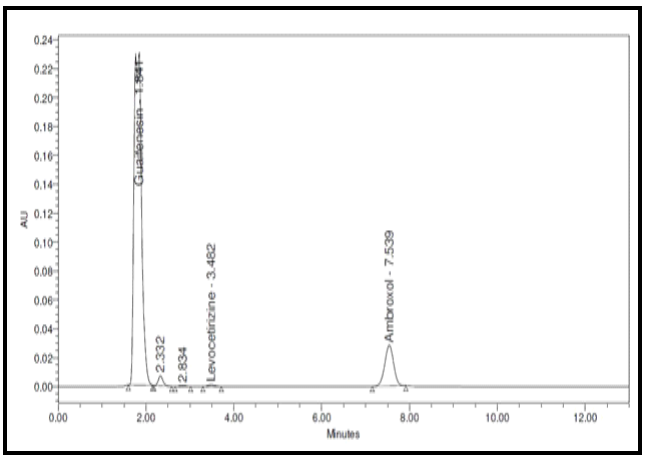

Figure 17: Chromatogram of oxidative degradation

Table 20: Oxidative degradation

S.NO	Degradants	Retention time	Peak area
1	1	2.3	50064
2	2	2.8	5561

Thermal Degradation Studies

When the powder drugs were exposed to heat in a hot air oven at 100^{o}c for 3h there was 36.72%, 34.4% degradation was observed for LCZ and ABX and there was one impurity is observed at retention time of 4.2, and there was no degradation observed for GFS.

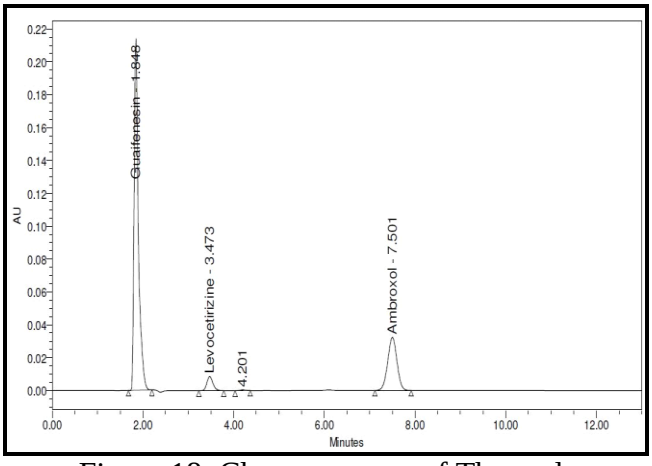

Figure 18: Chromatogram of Thermal degradation

Table 21: Thermal Degradation

S.NO	Degradants	Retention time	Peak area
1	1	4.2	3429

Photolytic Degradation by UV light

When the three drugs solutions were exposed to UV rays in uv chamber at room temperature for 6h there was 1.86%, 37.5% and 35.6% degradation was observed for GFS, LCZ and ABX and there was no impurities are observed.

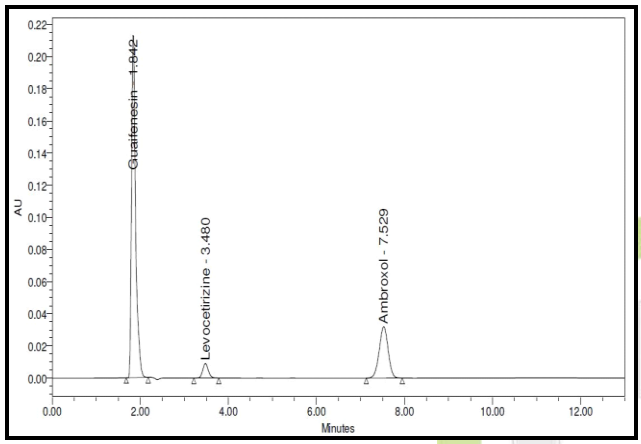

Figure 19: Chromatogram of Degradation by UV light

Photolytic Degradation by Day Light

When the three drugs solutions were exposed to day light for 6h there was 36.72%, 34% degradation was observed for LCZ and ABX and there was one impurities is observed at retention time of 1.5 with the area of 1411, And there was no degradation observed for GFS.

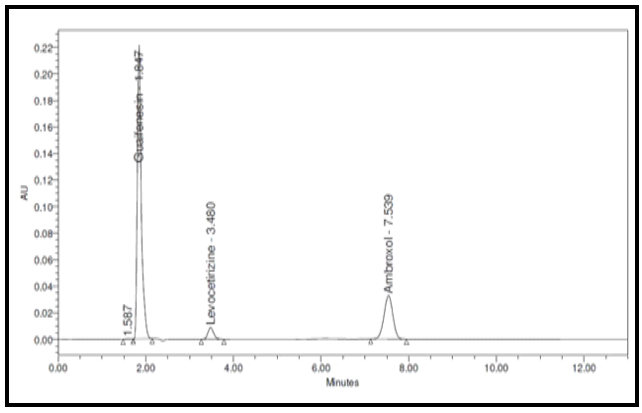

Figure 20: Chromatogram of Degradation by day light

Table 22: Photolytic degradation by day light

S.NO	Degradants	Retention time	Peak area
1	1	1.5	1411

Table 23: Forced degradation studies

Condition	Exposure	Result (% degradation)		
		GFS	LCZ	ABX
Acid degradation	1N HCl at Room temp for 6h	5.07	39.89	37.8
Alkaline degradation	IN NaOH at Room temp for 6hrs	5.2	40.1	37.7
Oxidative degradation	1% H_2O_2 at Room temp for 6h.	stable	45	22
Thermal degradation	Drug powder at 100^0c for 3h	stable	36.7	34.4
Photolytic degradation in UV chamber	Drug solution to UV light for 6 hrs	1.86	37.55	35.6
Photolytic degradation in UV chamber	Drug solution to day light for 6hrs	stable	36.4	34

Table 24: Specificity

S.NO	Sample Name	Guaifenesin		Levocetirizine		Ambroxol	
		RT	Area	RT	Area	RT	Area
1	Guaifenesin Std	1.931	1197904	-	-	-	-
2	Levocetirizine Std	-	-	3.446	59580	-	-
3	Ambroxol Std	-	-	-	-	7.456	394556
4	Mixed Std	1.922	14404188	3.451	125645	7.465	698344
5	Placebo	-	-	-	-	-	-
6	Sample	1.926	1408898	3.488	115442	-	-
7	Blank	-	-	-	-	-	-
8	Mixed Std	1.922	14404188	3.451	125645	7.465	698344
9	Placebo	-	-	-	-	-	-
10	Sample	1.926	1408898	3.488	115442	7.488	697755

Table 25: Retention time

Sample ID	Retention Time	Interference		
		Guaifenesin	Levocetirizine	Ambroxol
Blank	No peaks observed at retention time of principle peaks.	Nil	Nil	Nil
Placebo	No peaks observed at retention time of principle peaks.	Nil	Nil	Nil

Table 26: Results for system precision

S. No	Name	Guaifenesin		Levocetirizine		Ambroxol	
		RT	Area	RT	Area	RT	Area
1	System Precision-1	1.926	1400585	3.450	115501	7.462	691216
2	System Precision-2	1.923	1403245	3.45	115545	7.464	692418
3	System Precision-3	1.924	1401712	3.452	115178	7.469	691285
4	System Precision-4	1.924	1403920	3.453	115729	7.471	692504
5	System Precision-5	1.926	1404090	3.456	115651	7.479	692362
Avg		1.925	1402652	3.453	115505	7.471	691794
Std		0.001	1361.56	0.003	192.86	0.007	704.04
RSD		0.064	0.097	0.079	0.167	0.097	0.102

Table 27: Results for Method precision

S. No	Name	Guaiphenes		Levocetirizine		Ambroxol	
		RT	Area	RT	Area	RT	Area
1	Method Precision-1	1.925	1401522	3.452	115622	7.465	694245
2	Method Precision-2	1.924	1402244	3.455	115544	7.466	692335
3	Method Precision-3	1.926	1403322	3.452	114875	7.455	698887
4	Method Precision-4	1.922	1410022	3.453	115645	7.471	692645
5	Method Precision-5	1.925	1404090	3.455	115675	7.472	692478
6	Method Precision-6	1.921	1401144	3.454	115435	7.477	691595
Avg		1.924	1403724	3.454	115466	7.468	693698
Std Dev		0.002	3275.85	0.001	302.12	0.008	2687.10
RSD		0.101	0.233	0.040	0.262	0.102	0.387

Table 28: Results for Inter day precision

S No	Name	Guaifenesin		Levocetirizine		Ambroxol	
		RT	Area	RT	Area	RT	Area
1	Ruggedness-Day-1-1	1.926	1400585	3.450	115501	7.462	691216
2	Ruggedness-Day-1-2	1.923	1403245	3.45	115545	7.464	692418
3	Ruggedness-Day-1-3	1.924	1401712	3.452	115178	7.469	691285
4	Ruggedness-Day-2-1	1.925	1411442	3.457	115412	7.465	691321
5	Ruggedness-Day-2-2	1.924	1414245	3.458	115334	7.466	691422
6	Ruggedness-Day-2-3	1.928	1411412	3.455	115758	7.468	691285
7	Ruggedness-Day 3	1.924	1414245	3.458	115334	7.466	691422
8	Ruggedness-Day 3	1.928	1411412	3.455	115758	7.468	691285
9	Ruggedness-Day 3	1.925	1411442	3.457	115412	7.465	691321

Table 29: Peak area

Sr. No	Drug	Conc. (µg/ml)	Peak area mean ± S.D		%RSD
1	Guaifenesin	100	1401847.33 ±1335.15		0.10
	Levocetirizine	2.5	115408	±200.39	0.17
	Ambroxol	30	691639	±674.93	0.10
2	Guaifenesin	100	1412366.33 ±1627.04		0.13
	Levocetirizine	2.5	115501	±225.67	0.20
	Ambroxol	30	691342	±118.92	0.01
3	Guaifenesin	100	1413287 ±1335.15		0.12
	Levocetirizine	2.5	115521 ±22867		0.22
	Ambroxol	30	691509.33 ±34050		0.05

Inter day

Guaifenesin working standard 2.5 mg of Levocetirizine working standard and 15 mg of Ambroxol

Table 30: Results for Accuracy

Accuracy-80-1	1.925	1115221	3.455	91122	7.465	549133
Accuracy-80-2	1.923	1141245	3.450	91357	7.466	549825
Accuracy-80-3	1.928	1125224	3.452	91417	7.469	549745
Avg	**1.925**	**1127230**	**3.452**	**91299**	**7.467**	**549568**
Amt Recovered		**79.45**		**79.34**		**79.37**
%Recovery		**99.31**		**99.17**		**99.22**
Accuracy-100-1	1.926	1401866	3.445	114405	7.466	692212
Accuracy-100-2	1.923	1404245	3.452	115445	7.464	693318
Accuracy-100-3	1.924	1403544	3.458	115278	7.461	694085
Avg	1.924	**1403218**	**3.452**	**115043**	**7.464**	**693205**
Amt Recovered		**98.90**		**99.97**		**100.12**
%Recovery		**98.90**		**99.97**		**100.12**
Accuracy-120-1	1.925	1703345	3.454	140145	7.462	831122
Accuracy- 120-2	1.926	1715311	3.458	135366	7.467	841101
Accuracy-100-3	1.921	1711153	3.452	140065	7.466	840235
Avg	**1.924**	**1709936**	**3.455**	**138525**	**7.465**	**837486**
Amt Recovered		**120.51**		**120.37**		**120.96**
%Recovery		**100.43**		**100.31**		**100.80**

Table 31: Results for ruggedness: System Precision

S No	Name	Guaifenesin		Levocetirizine		Ambroxol	
		RT	Area	RT	Area	RT	Area
1	Ruggedness-Day-1-1	1.926	1400585	3.450	115501	7.462	691216
2	Ruggedness-Day-1-2	1.923	1403245	3.458	115545	7.464	692418
3	Ruggedness-Day-1-3	1.924	1401712	3.452	115178	7.469	691285
4	Ruggedness-Day-1-4	1.924	1403920	3.453	115729	7.471	692504
5	Ruggedness-Day-1-5	1.926	1404090	3.456	115651	7.479	692362
6	Ruggedness-Day-1-6	1.924	1402359	3.449	115428	7.482	690976
Avg		1.925	1402652	3.453	115505	7.471	691794
Std Dev		0.001	1361.56	0.003	192.86	0.008	704.04
RSD		0.064	0.097	0.100	0.167	0.107	0.102

Table 32: Ruggedness

S No	Name	Guaifenesin		Levocetirizine		Ambroxol	
		RT	Area	RT	Area	RT	Area
1	Ruggedness-Day-2-1	1.925	1411442	3.457	115412	7.465	691321
2	Ruggedness-Day-2-2	1.924	1414245	3.458	115334	7.466	691422
3	Ruggedness-Day-2-3	1.928	1411412	3.455	115758	7.468	691285
4	Ruggedness-Day-2-4	1.928	1412928	3.454	115662	7.488	691442
5	Ruggedness-Day-2-5	1.927	1414192	3.457	115452	7.495	691422
6	Ruggedness-Day-2-6	1.927	1412045	3.458	115645	7.485	691451
Avg		1.927	1412711	3.457	115544	7.478	691391
Std Dev		0.002	1291.12	0.002	167.28	0.013	69.65
RSD		0.085	0.091	0.048	0.145	0.174	0.010

Table 33: Mean ruggedness

S No	Name	Guaifenesin		Levocetirizine		Ambroxol	
		RT	Area	RT	Area	RT	Area
1	Ruggedness-Day-1-1	1.926	1400585	3.450	115501	7.462	691216
2	Ruggedness-Day-1-2	1.923	1403245	3.45	115545	7.464	692418
3	Ruggedness-Day-1-3	1.924	1401712	3.452	115178	7.469	691285
4	Ruggedness-Day-1-4	1.924	1403920	3.453	115729	7.471	692504
5	Ruggedness-Day-1-5	1.926	1404090	3.456	115651	7.479	692362
6	Ruggedness-Day-1-6	1.924	1402359	3.456	115428	7.479	690976
7	Ruggedness-Day-2-1	1.925	1411442	3.457	115412	7.465	691321
8	Ruggedness-Day-2-2	1.924	1414245	3.458	115334	7.466	691422
9	Ruggedness-Day-2-3	1.928	1411412	3.455	115758	7.468	691285
10	Ruggedness-Day-2-4	1.928	1412928	3.454	115662	7.488	691442
11	Ruggedness-Day-2-5	1.927	1414192	3.457	115452	7.495	691422
12	Ruggedness-Day-2-6	1.927	1412045	3.458	115645	7.485	691451
Avg		**1.926**	**1407681**	**3.455**	**115525**	**7.474**	**691592**
Std Dev		**0.002**	**5403.24**	**0.003**	**173.29**	**0.011**	**521.35**
RSD		**0.09**	**0.3838**	**0.083**	**0.150**	**0.144**	**0.075**

Robustness

Table 34: Results for robustness

S No	Parameter	Guaifenesin		Levocetirizine		Ambroxol	
		RT	Area	RT	Area	RT	Area
1	Standard	1.926	1400585	3.450	115501	7.462	691216
2	Robust-Flow-1	2.233	1643566	4.029	135358	8.740	809631
3	Robust-Flow-2	1.693	1229271	3.035	101619	6.526	606878
4	Robust-Temp-1	1.935	1406690	3.446	115818	8.341	693517
5	RobustTemp-2	1.910	1404880	3.483	116449	6.672	693433

Assay

Table 35: Results for assay

Standard	Guaifenesin		Levocetirizine		Ambroxol	
	RT	Area	RT	Area	RT	Area
Standard- 1	1.922	1411552	3.452	115451	7.452	693345
Standard- 2	1.928	1418245	3.465	117541	7.474	694545
Average	**1.925**	**1414899**	**3.459**	**116496**	**7.463**	**693945**

Method development

The developed method has an advantage for the estimation of Guaifenesin, Levocetirizine and Ambroxol in RP-HPLC. The following tables give the results of the method development quantitation and validation parameters.

Fixed Chromatographic Condition

Table 36: Fixed Chromatographic Condition

Mode of separation	Isocratic elution
Mobile phase	Buffer (0.01 N Ammonium phosphate): Acetonitrile(60:40)
Column	Inertsil ODS C18, 100 X 4.6 mm, 5µ.
Flow rate	1 ml/ min
Detection Wavelength	236 nm
Injection volume	20 ml
Column oven temperature	30^0c
Run time	13 min
Diluent	Mobile phase
Needle wash	Water: Acetonitrile 90:10 (v/v)

Method Validation

The method was validated according to the ICH guidelines and results are tabulated in table

Table 37: Summary results of method validation for GLC, LCZ and ABX

S. No	Parameter	Requirement	Results			Acceptance criteria
			GFS	LCZ	ABX	
1.		RT	1.926	3.451	7.46	
2.	System suitability	Tailing factor	1.03	1.11	0.98	NMT 2
3.		Resolution		7.81	13.56	>1.5
4.		Plate count	2277	3862	6855	NLT 2000
5.	Accuracy	Assay value	99.52%	98.42%	99.75%	98-102%
6.		% Recovery	99.54%	100.14%	100.12%	.
7.	Precision	Method % RSD	0.233	0.262	0.387	NMT 2%
		System % RSD	0.097	0.167	0.102	NMT 2%
8.	Specificity	No interference	Pass	Pass	pass	No interference
8.	Linearity	Correlation coefficient	0.999	0.9989	0.999	NLT 0.999
9.	Range	Concentration	25-150µg/ ml	1.25-7.5 µg/ml	7.5-45µg/ml	Nil
10.	Robustness	F.R Variation % RSD	0.98	1.77	1.79	NMT 2%
		TEMP Variation % RSD	2	1.85	1.79	

Forced Degradation Studies

Degradation studies were carried out as per ICH guidelines. By subjecting to acidic, basic, oxidative, photo and thermal conditions and summary of results tabulated below.

Table 38: Summary of forced degradation results

S.No	Condition	Exposure	Result (% degradation)		
			GFS	LCZ	ABX
1	Acid degradation	1N HCl at Room temp.	5.07	39.89	37.8
2	Alkaline degradation	1N NaOH at Room temp.	5.2	40.1	37.7
3	Oxidative degradation	1% H_2O_2 at Room temp.	Stable	45	22
4	Thermal degradation	Drug powder at 100^0c	Stable	36.7	34.4
5	Photolytic Degradation in UV chamber	Drug solution to UV light	1.86	37.55	35.6
6	Photolytic Degradation in day light	Drug solution to day light	Stable	36.4 7	34

CONCLUSION

A validated stability indicating RP-HPLC method for quantification of GFS, LCZ and ABX was established. Compared with the reported methods, this method represents the first report about a stability indicating method for the determination of GFS, LCZ and ABX. With the proposed method a satisfactory separation of three drugs from the degradation products and impurities, extended linear range and rapid analysis time were carried out. A high recovery of GFS, LCZ and ABX in formulation was achieved. The proposed method ensured a precise and accurate determination of GFS, LCZ and ABX in formulation. No interference from the excipients was noticed.

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