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DEVELOPMENT AND VALIDATION OF UV-SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF AMBROXOL HCL AND LEVOCETRIZINE DI HCL IN BULK AND IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

On the basis of the experiments, it was concluded that the UV method developed for the determination of ambroxol hydrochloride and levo-cetrizine dihydrochloride was validated and found to be sensitive, accurate, precise and reliable for routine analysis. Simple sensitive and reproducible method was developed for simultaneous estimation of ambroxol hydrochloride and levo-cetrizine dihydrochloride in bulk and in pharmaceutical formulation. Developed method was successfully applied to the pharmaceutical formulation. After development of the method it was validated as per ICH guidelines, in terms of specificity, linearity, precision, accuracy. In the present investigation of UV analysis, the method simultaneous estimation by Simultaneous equation Method is employed for the assay of AMB and LCTZ Pharmaceutical formulation. The solvent selected for stock solution preparation was methanol : water (50:50) and required concentrations were prepared by using the same. The λ_{max} for detection of AMB and LCTZ were selected as 232nm and 246nm respectively.

KEYWORDS: Ambroxol Hydrochloride, Levo-Cetrizine Dihydrochloride, UV, ICH.

1. INTRODUCTION[1-3]

Quality Assurance is the branch of science which is the sum total of the organised arrangements made with the objective of ensuring that all materials are of the quality required for their intended use and that quality systems are maintained. The term "Quality" as applied to a drug product has been defined as the sum of all factors, which contribute directly or

indirectly to the safety, effectiveness and reliability of the product. These properties are built into drug product through research and during process by procedures collectively referred to as "Quality Control". Quality control guarantees within reasonable limits that products are: free of impurities, physically and chemically stable, contains the amount of active ingredients as stated on the label and provides optimal release of active ingredients when the product is administered.

1.1 Development of Analytical Method

Method development is a challenging and time consuming process requiring much experience, creativity, logical thinking and experimentation. With all the software and automated systems available today, method development is still very much a trial and error approach, expedited by a logical sequence of generic scouting runs and fine turning steps to achieve the requisite resolution and method performance. Drug analysis is the basis for the determination of the product. Very often, there is a time lag from the date of introduction of a drug in to the market to the date of its inclusion in pharmacopeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors.

1.3 Steps involved in method development

Documentation starts at the very beginning of the development process. A system for full documentation of development studies must be established. All data relating to these studies must be recorded in laboratory notebook or an electronic database.

1.11.2 Validation Parameters of Analytical Method[13, 14]

The validation parameters as per ICH guidelines and USP are:-

i) Linearity and Range

ii) Precision

iii) Specificity

iv) Accuracy

y) Limit of Detection

vi) Limit of Quantification

vii) Ruggednesas

viii) Robustness

ix) Solution Stability

x) System suitability

Furthermore revalidation may be necessary in the following circumstances:

- Changes in the synthesis of the drug substance
- Changes in the composition of the finished product
- Changes in the analytical procedure

The degree of revalidation required depends on the nature of the changes. Certain other changes may require validation as well.

1.14 DEGRADATION STUDIES

Forced degradation studies show the chemical behavior of the molecule which in turn helps in the development of formulation and package. A forced degradation study is an essential step in the design of a regulatory compliant stability program for both drug substances and products and formalized as a regulatory requirement in ICH Guideline Q1A in 1993. Forced degradation is a degradation of new drug substance and drug product at conditions more severe than accelerated conditions. It is required to demonstrate specificity of stability indicating methods and also provides an insight into degradation pathways and degradation products of the drug substance and helps in elucidation of the structure of the degradation products.

6. EXPERIMENTAL WORK

6.1 Solubility studies / Selection of solvent system

The pure drugs Ambroxol hydrochloride and Levocetrizine dihydrochloride were dissolved in different solvents such as water, ethanol and methanol as per literature survey. These solutions were scanned using UV Spectrophotometer. Out of these, water and methanol (50:50) gave spectra without noise, so it was selected as a solvent for the preparation of stock solution. Further dilutions were made with solvent in order to find the optimum conditions for Spectrophotometric estimation of Ambroxol hydrochloride and Levocetrizine dihydrochloride.

6.2 Preparation of standard stock solution (100µg/ml)

6.2.1 Ambroxol hydrochloride standard stock solution

Accurately weighed reference standard of AMB (10mg) was transferred to 100ml volumetric flask and dissolved in 50 ml solvent (Water, methanol 1:1) and sonicated for 5 min. then

volume was made up to the mark with same solvent; to obtain standard stock solution (100µg/ml) of drug. For the preparation of working standards, suitable aliquots of stock solution were pipetted out and volumes were made up to the mark with solvent (Water, methanol 1:1); so as to get required concentrations.

6.2.2 Levocetrizine dihydrochloride standard stock solution

Accurately weighed reference standard of LCTZ (10mg) was transferred to 100ml volumetric flask and dissolved in 50 ml solvent (Water, methanol 1:1) and sonicated for 5 min. then volumes was made up to the mark with same solvent to obtain standard stock solution (100µg/ml) of drug. For the preparation of working standards, suitable aliquots of stock solution were pipetted out and volumes were made up to the mark with solvent (Water, methanol 1:1); so as to get required concentrations.

6.3 Selection of Absorption Maxima (λmax)

An Absorption maxima was selected from spectra of the drugs **AMB & LCTZ** obtained by using UV-Vis spectrophotometer. Stock solutions (100µg/ml) of both the drugs were prepared in methanol and diluted to get 10µg/ml solutions with methanol. The individual UV spectrum was taken and overlaid.

6.3.1 Selection of analytical wavelength

Sample solutions were scanned over the wavelength range of 200 nm to 400 nm. λmax for AMB and LCTZ were found at 231 nm and 244 nm. Representative absorption spectra of AMB and LCTZ are shown in fig.

6.4 Analytical method validation of AMB and LCTZ

6.4.1 Preparation of calibration curve of AMB and LCTZ / linearity

By appropriate dilution of standard stock solution, different dilutions were prepared ranging from 5 µg/ml to 30 µg/ml for AMB and 5µg/ml to 30µg/ml for LCTZ. Absorbance of all the dilutions were plotted against the respective concentrations to obtain the calibration curve. The absorption overlain spectrum of AMB and LCTZ is shown in fig.

6.4.2 Precision

Precision is the measure of how close the data Values are to each other for a number of measurements under the same Analytical conditions. It is expressed as the % relative standard

deviation (RSD). The precision of an analytical method was studied by performing repeatability (intraday) and intermediate precision (interday).

6.4.3 Accuracy

Accuracy is the closeness of test results obtained to the true value. To check the accuracy of proposed method, recovery studies were carried out at 80%, 100% and 120% of the test conc. It was measured thrice for each concentration of the sample solution, as per ICH guidelines.

6.5 Analysis of Marketed Formulation

Ten syrup bottles were weighed accurately, average weight was determined and poured in calibrated measuring cylinder to check extractable volume. As per label claim syrup contain AMB 30mg/5ml and LCTZ 5mg/5ml, so 5ml syrup was pipetted out in 100 ml volumetric flask and dissolved in water: methanol (1:1) solution, sonicated for 10 min and filtered. Then different concentrations of syrup sample were prepared by serial dilution method and used for analysis.

6.6 Analytical Method Validation

The proposed method was validated by studying several ICH parameters such as linearity, precision, accuracy, repeatability, limit of detection (LOD) and limit of quantitation (LOQ) were checked as per ICH guidelines. The developed method was validated according to ICH guidelines.

1. Linearity
2. Precision
- Repeatability
- Inter day
- Intra day
3. Accuracy (recovery study)
4. Limit of detection (LOD)
5. Limit of quantitation (LOQ)
6. Specificity

6.6.1 Linearity

The linearity of an analytical procedure is its ability to produce a response, which is directly proportional to the concentration of analyte in the sample. Linearity is determined by six different concentration levels. For linearity study, six solutions at different concentrations (5-

30µg/ml) were prepared and the obtained data were used for the linearity calibration plot. By appropriate dilution of standard stock solution, different dilutions were prepared ranging from 5 µg/ml to 30 µg/ml for AMB and 5µg/ml to 30µg/ml for LCTZ. Absorbance of all the dilutions were plotted against the respective concentrations to obtain the linearity curve. The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of Ambroxol Hydrochloride and Levo cetirizine dihydrochloride.

6.6.2 Precision

Precision is the measure of how close the data Values are to each other for a number of measurements under the same Analytical conditions. It is expressed as the % relative standard deviation (RSD). The precision of an analytical method was studied by performing repeatability (intraday) and intermediate precision (interday). Precision of the method was verified by using syrup stock solution, the repeatability indicates the performance of the UV instrument. Interday and intraday precision was determined by repeating the assay three times in same day for intraday precision and on different day for interday precision studies. The results of this analysis are shown in table.

Preparation of standard solution

As per label claim syrup contains AMB 30mg/5ml and LCTZ 5mg/5ml, so 5ml syrup was pipetted out in 100 ml volumetric flask and dissolved in water: methanol (1:1) solution, sonicated for 10 min and filtered. Then different concentrations of syrup sample were prepared by serial dilution method and used for precision analysis.

6.6.3 Accuracy (Recovery Study)

Accuracy is the closeness of test results obtained to the true value. To check the accuracy of proposed method, recovery studies were carried out at 80%, 100% and 120% of the test conc. As per ICH guidelines. To perform recovery study at 100% 5ml syrup pipette out in volumetric flask and to this standard 30mg AMB and 5mg LCTZ were added. The volume was made up to the mark with water: methanol (1:1) solution and further dilution with same solvent to obtain sample solution**.** Similarly, for 80% recovery study, 26mg AMB and 4mg LCTZ were added, and for 120% recovery study, 36mg AMB and 6mg LCTZ were added respectively. The result of recovery study along with its statistical validation is given in table.

6.6.4 & 6.6.5 Limit of detection (LOD) and Limit of quantitation (LOQ)

The LOD is calculated based on the standard deviation of the response and the slope. LOD = $3.3(\sigma/S)$ LOQ = $10(\sigma/S)$

Where, σ is the standard deviation And S is the slope of the Calibration curve.

6.6.6 Specificity

The specificity of the method was ascertained by analyzing standard drug and sample solutions of the marketed formulation. Both the results of standard drug and formulation were compared to indicate that there is no interference from the excipients in the formulation.

6.7 DEGRADATION STUDIES OF BULK DRUG

Forced degradation studies were carried out to provide evidence on how stability of drug varies under the influence of variety of environmental conditions like hydrolysis, oxidation, temperature etc. and to establish specific storage conditions. shelf-life and retest period.

6.7.1 Acid treatment

1 ml working standard solution of LCTZ (100 µg/ml) was mixed with 1 ml of 0.1 N HCl (methanolic) and 8 ml of Methanol. The mixture was refluxed for 2 hours. 3 µl of the resulting solution was applied to HPTLC. 1 ml working standard solution of AMB (600 µg/ml) was mixed with 1 ml of 0.1 N HCl (methanolic) and 8 ml of methanol. The mixture was refluxed for 2 hours. 3 µl of the resulting solution was applied to HPTLC.

6.7.2 Alkali treatment

1 ml working standard solution of LCTZ (100 µg/ml) was mixed with 1 ml of 0.1 N NaOH (methanolic) and 8 ml of Methanol. The mixture was refluxed for 2 hours. 3 µl of the resulting solution was applied to HPTLC. 1 ml working standard solution of AMB (600 µg/ml) was mixed with 1 ml of 0. 1 N 0. 1 N NaOH (methanolic) and 8 ml of methanol. The mixture was refluxed for 2 hours. 3 µl of the resulting solution was applied to HPTLC.

6.7.3 Oxidation

1 ml working standard solution of LCTZ (100 µg/ml) was mixed with 1 ml of 3% solution of H202 and 8 ml of methanol. The mixture was refluxed for 4 hours 3 µl of the resulting solution was applied to HPTLC. 1 ml working standard solution of AMB (600 µg/ml) was mixed with 1 ml of 3% solution of H202 and 8 ml of methanol. The mixture was refluxed for 4 hours. 3 µl of the resulting solution was applied to HPTLC.

6.7.4 Degradation under dry heat

Dry heat study was perfomed by keeping LCTZ and AMB in oven at 60°C for 6 hours. A sample was withdrawn at appropriate times, weighed and dissolved in methanol to get solution of 100 (µg/ml) and 600 (µg/ml). 3µl of the resulting solution was applied to HPTLC.

7. RESULTS AND DISCUSSION

7.1 Solubility studies / Selection of the solvent system

Solubility studies were carried out to find out a solvent in which the chosen drugs (ambroxol hydrochloride and levo-cetrizine dihydrochloride) were soluble. Various solvents e.g. water, ethanol, methanol were used for checking solubility of ambroxol hydrochloride and levo-cetrizine dihydrochloride. Out of these, water and methanol (50:50) gave spectra without noise, so it was selected as a solvent for the preparation of stock solution. Further dilutions were made with solvent in order to find the optimum conditions for Spectrophotometric estimation of Ambroxol hydrochloride and Levocetrizine dihydrochloride.

7.2 Selection of absorption maxima (λmax)

Absorption maxima was selected from spectra of the drugs **AMB & LCTZ** obtained by using UV-Vis spectrophotometer. Sample solutions were scanned over the wavelength range of 200 nm to 400 nm. λmax for AMB and LCTZ were found at 231 nm and 244 nm respectively.

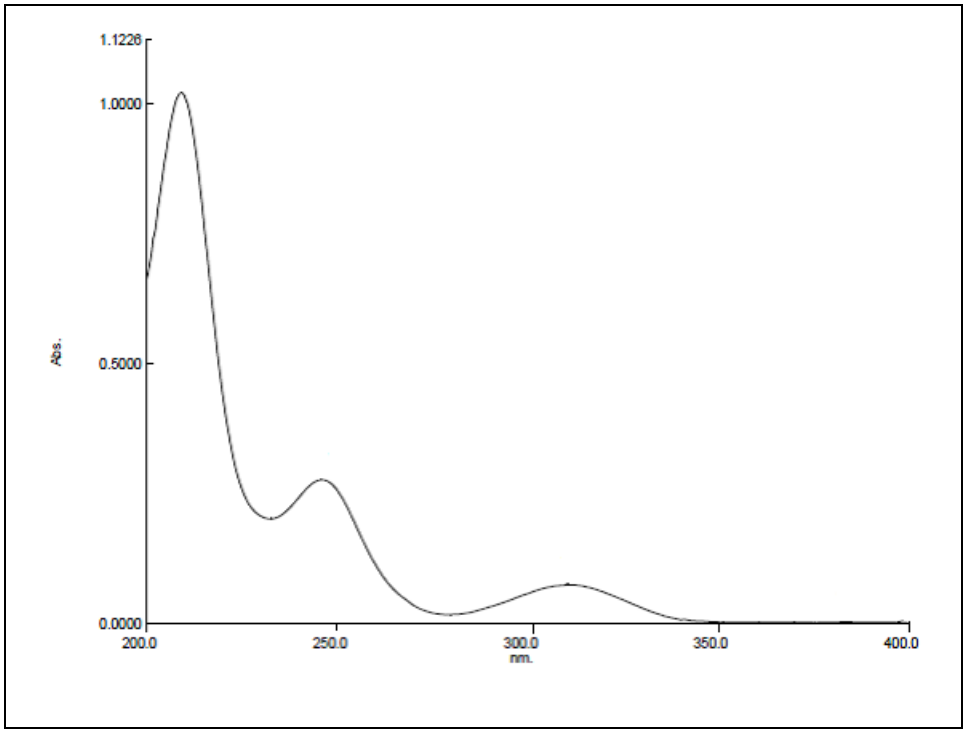

Fig: 8 Absorption spectra of AMB.

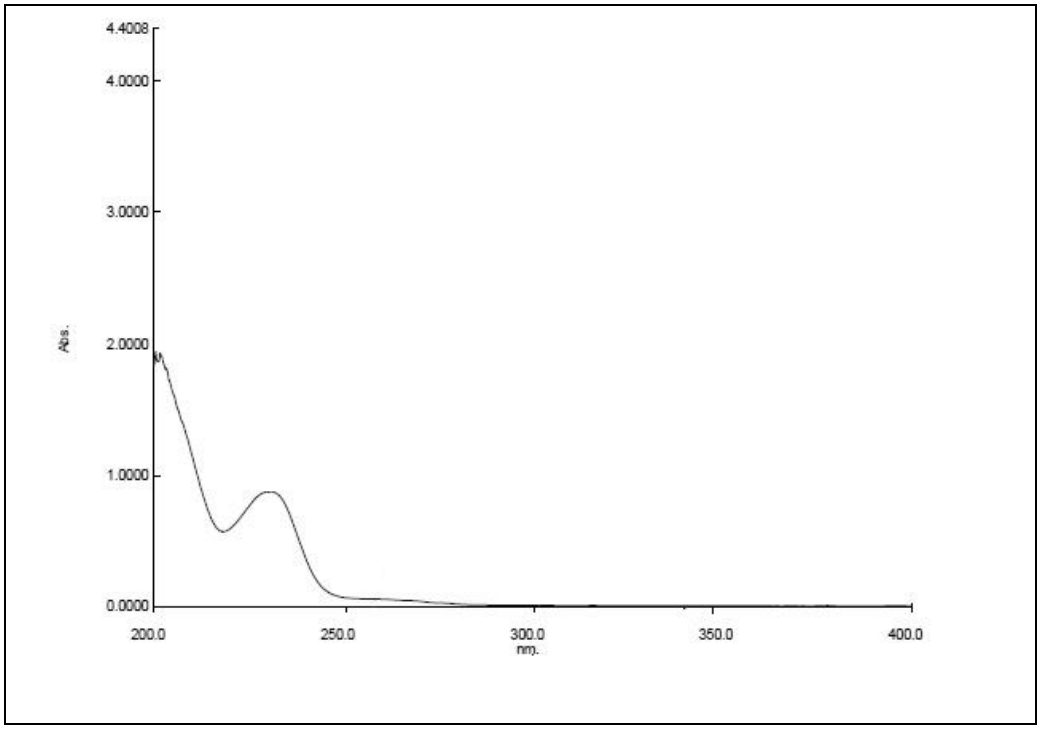

Fig: 9 Absorption Spectra of LCTZ.

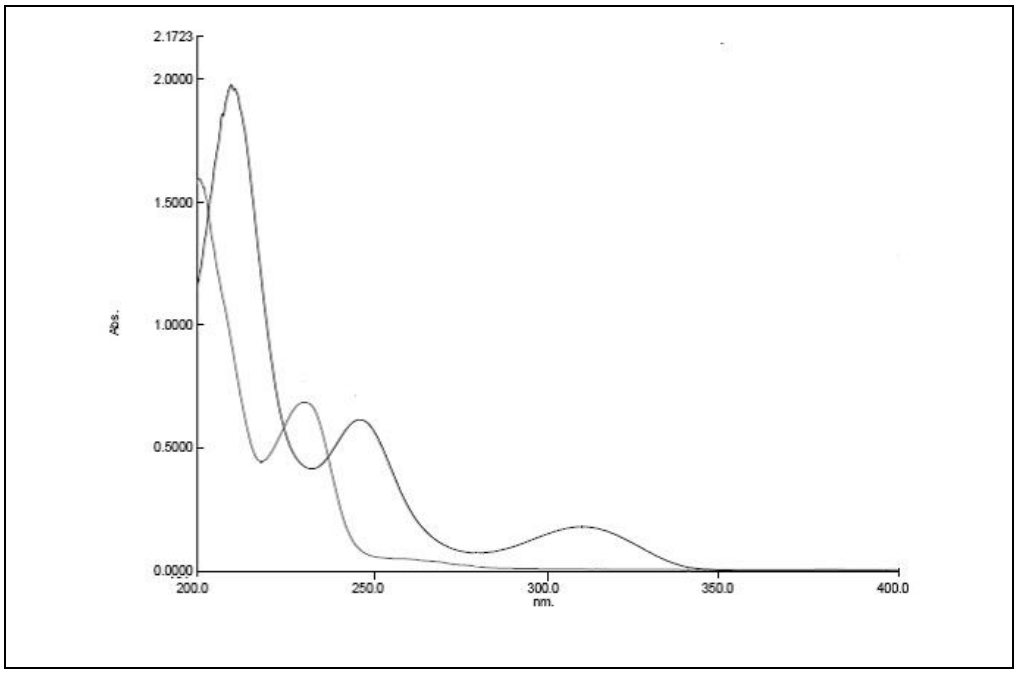

Fig: 10 overlay spectra of AMB & LCTZ.

7.3 Analytical method validation of AMB and LCTZ

7.3.1 Calibration curve of AMB and LCTZ / linearity: Ambroxol hydrochloride and levo-cetrizine dihyrochloride showed good correlation coefficient linearity. The calibration curves were found to be linear over concentration range of 5, 10, 15, 20, 25, 30 µg/ml for both

drugs. The results of linearity study are given in table. Beer's lambert law was obeyed in the concentration range of 5-30 µg/ml.

Table: 6 Linearity Study of AMB.

Sr. No.	Concentration(µg/ml)	Absorbance	Regression Data
1	5	0.180	m = 0.029 c = 0.029 r^2 = 0.9997
2	10	0.327	
3	15	0.470	
4	20	0.632	
5	25	0.767	
6	30	0.924	

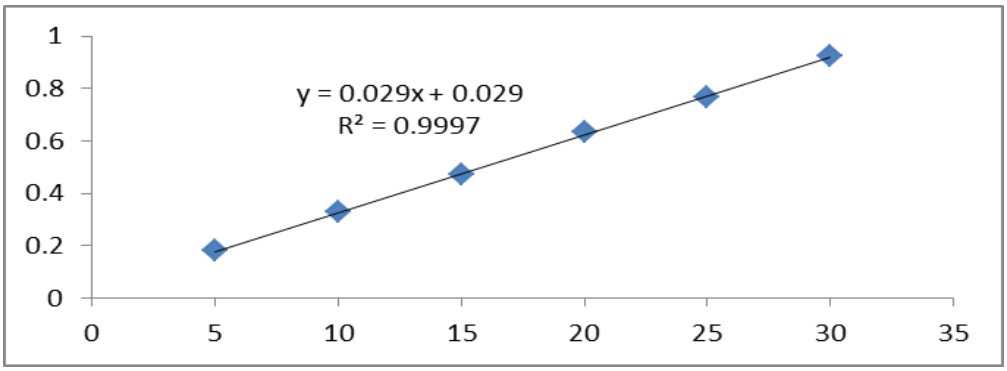

Fig: 11 Calibration curve of AMB.

Table: 7 Linearity Study of LCTZ.

Sr. No.	Concentration(µg/ml)	Absorbance	Regression Data
1	5	0.201	m = 0.036 c = 0.025 r^2 = 0.9998
2	10	0.392	
3	15	0.565	
4	20	0.745	
5	25	0.932	
6	30	1.102	

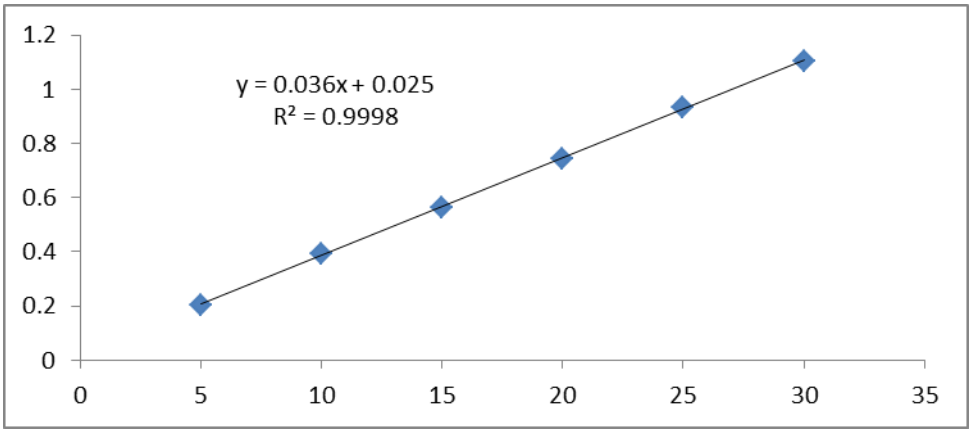

Fig: 12 Calibration Curve of LCTZ.

Table: 8 Linear regression data for calibration curve of AMB and LCTZ.

Name of the drug	Linearity range µg/ml	r^2	Slope	Intercept
AMB	5 - 30	0.9997	0.029	0.029
LCTZ	5 - 30	0.9998	0.036	0.025

7.3.2 Precision

The intra-day and inter-day precision study of Ambroxol hydrochloride and levo-cetrizine dihydrochloride was carried out. The concentrations used were 10 µg/ml for both. Precision was expressed as %RSD. The results are given in the following tables.

Table 9: Intra-day and inter-day precision for AMB.

INTRA-DAY PRECISION			INTER-DAY PRECISION		
MEAN	SD	%RSD	MEAN	SD	%RSD
100.02%	0.3813	0.3510	100.04%	0.3031	0.3029

***conc. Of Ambroxol hydrochloride used for intra-day and inter-day precision was 10 µg/ml.*

Table 10: intra-day and inter-day precision for LEVC.

INTRA-DAY PRECISION			INTER-DAY PRECISION		
MEAN	SD	%RSD	MEAN	SD	%RSD
99.40%	0.1345	0.1353	99.56%	0.0799	0.080

***conc. Of levo-cetrizine dihydrochloride used for intra-day and inter-day precision was 10 µg/ml.*

7.3.3 Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were performed at three different levels (80%, 100% and 120%) of test concentration as per ICH guidelines.

Table 11: recovery studies for AMB.

LEVEL	%mean recovery	MEAN	SD	RSD
80%	100.08			
100%	99.64	99.88	0.170	0.1689
120%	99.94			

Table 12: recovery studies for LEVC.

LEVEL	%mean recovery	MEAN	SD	RSD
80%	99.44			
100%	99.56	99.66	0.0732	0.089
120%	100.01			

7.4 Analysis of marketed formulation

For the present work the syrup solution contained AMB 30mg/5ml and LCTZ 5mg/5ml respectively.

7.5 Analytical Method Validation

7.5.1 Linearity

Table: 13 Analysis of Syrup Formulation.

Sr. No.	Label claim (mg/5ml)		Amount found (mg/5ml)		% of Label claim	
	AMB	LCTZ	AMB	LCTZ	AMB	LCTZ
1	30	5	30.21	4.97	100.70	99.57
2	30	5	30.10	4.96	100.34	99.35
3	30	5	29.95	4.96	99.84	99.28
4	30	5	30.20	4.97	100.68	99.54
5	30	5	29.93	4.96	99.78	99.25
6	30	5	30.14	4.97	100.47	99.42

Table: 14 Statistical validation analysis of syrup formulation.

Name of the drug	Mean*	SD	%RSD
AMB	100.30	0.4040	0.4027
LCTZ	99.40	0.1328	0.1336

* Indicates average of six determinations.

7.5.2 Precision

The intra-day and inter-day precision study of Ambroxol hydrochloride and levo-cetrizine dihydrochloride was carried out by estimating the corresponding responses six times on the same day and two times on two different days. The solutions of concentrations 30 µg/ml and 5 µg/ml for Ambroxol hydrochloride and levo-cetrizine dihydrochloride respectively were used. Precision was expressed as % R.S.D.

7.5.2.1 Repeatability data

The repeatability of sample application and measurement of absorbance were express in term of %RSD and found to be less than 2%. The results of precision studies are given in following tables.

Table: 15 Repeatability Data of AMB and LCTZ.

Sr. No.	Concentration (µg/ml)		Absorbance		% Recovery	
	AMB	LCTZ	AMB	LCTZ	AMB	LCTZ
1	30	5	1.012	0.885	100.58	99.72
2	30	5	1.013	0.887	100.64	99.78
3	30	5	1.009	0.882	99.84	99.58
4	30	5	1.014	0.888	100.72	99.85
5	30	5	1.012	0.884	100.57	99.70
6	30	5	1.007	0.880	99.77	99.42

Table: 16 Statistical validation of repeatability data.

Name of the drug	Mean*	SD	%RSD
AMB	1.0111	0.0026	0.2571
LCTZ	0.8843	0.0030	0.3392

* Indicates average of six determinations.

7.5.2.2 Intraday Precision

Table: 17 Intra-day Precision data of marketed formulation.

Sr. No.	Interval of	Concentration (µg/ml)		% Recovery	
	Time	AMB	LCTZ	AMB	LCTZ
I		30.10	4.96	100.34	99.35
II	Intra-day	29.95	4.95	99.84	99.28
III		30.20	4.97	100.68	99.54

Table: 18 Statistical validation of intra-day precision data.

Name of the drug	Mean*	SD	%RSD
AMB	100.28	0.4225	0.4213
LCTZ	99.40	0.1345	0.1353

* Indicates average of three determinations.

7.6.2.3 Inter-day Precision

Table: 19 Inter-day Precision Data.

Sr. No.	Interval of	Concentration (µg/ml)		% Recovery	
	Time	AMB	LCTZ	AMB	LCTZ
I		30.14	4.98	100.45	99.79
II	Inter-day	30.09	4.98	100.32	99.72
III		29.96	4.98	99.87	99.63

Table: 20 Statistical validation of inter-day precision data.

Name of the drug	Mean*	SD	%RSD
AMB	100.21	0.3043	0.3036
LCTZ	99.71	0.0802	0.0804

* Indicates average of three determinations.

7.5.3 Accuracy (Recovery study)

To ascertain the accuracy of the proposed methods, recovery studies were performed at three different levels (80%, 100% and 120%) of test concentration as per ICH guidelines. The result of recovery studies along with its statistical validation are given in table.

Table: 21 Recovery study data.

Level of Recovery	Amount present (mg/5ml)		Added concentration (mg)		Amount recovered (mg)		% Recovery	
	AMB	LCTZ	AMB	LCTZ	AMB	LCTZ	AMB	LCTZ
	30	5	24	4	54.31	8.97	100.58	99.74
80%	30	5	24	4	54.20	8.95	100.38	99.52
	30	5	24	4	54.38	8.98	100.72	99.82
	30	5	30	5	60.28	9.96	100.47	99.68
100%	30	5	30	5	60.32	9.97	100.54	99.70
	30	5	30	5	60.22	9.95	100.38	99.54
	30	5	36	6	66.31	10.96	100.48	99.69
120%	30	5	36	6	66.36	10.97	100.56	99.72
	30	5	36	6	66.24	10.95	100.37	99.58

Table: 22 Statistical validation of recovery study data.

Level of Recovery	% Mean recovery		S.D.		% R.S.D.	
	AMB	LCTZ	AMB	LCTZ	AMB	LCTZ
80%	100.56	99.70	0.1708	0.1553	0.1698	0.1557
100%	100.46	99.64	0.0802	0.0871	0.0798	0.0874
120%	100.47	99.67	0.0953	0.0737	0.0948	0.0951

7.5.4 & 7.5.5 Limit of Detection and Limit of Quantitation

The LOD values were found 3.1645 and 3.0891 µg/ml for Ambroxol hydrochloride and levo-cetrizine dihydrochloride respectively. The LOQ values were found 9.5896 and 9.3611 µg/ml respectively. The results of LOD & LOQ are given in table.

Table: 23 LOD values of AMB & LCTZ.

Name of the drug	LOD µg/ml
AMB	3.1645
LCTZ	3.0891

Table: 24 LOQ values of AMB & LCTZ.

Name of the drug	LOQ µg/ml
AMB	9.5896
LCTZ	9.3611

7.5.6 Specificity

The specificity of the method was ascertained by analyzing standard drug and sample solutions of syrup formulation. It was observed that peaks were obtained for ambroxol hydrochloride and levo-cetrizine dihydrochloride under optimized conditions. It shows no difference from the excipients and impurities. The retention time of Ambroxol hydrochloride and levo-cetrizine dihydrochloride was compared with that of the standard drug.

7.6 DEGRADATION STUDIES OF BULK DRUG

7.6.1 Acid treatment

11.33% degradation was observed for LCTZ with degradation peak at Rf 0.14 and 12.56% degradation was observed for AMB with degradation peak at Rf 0.61.

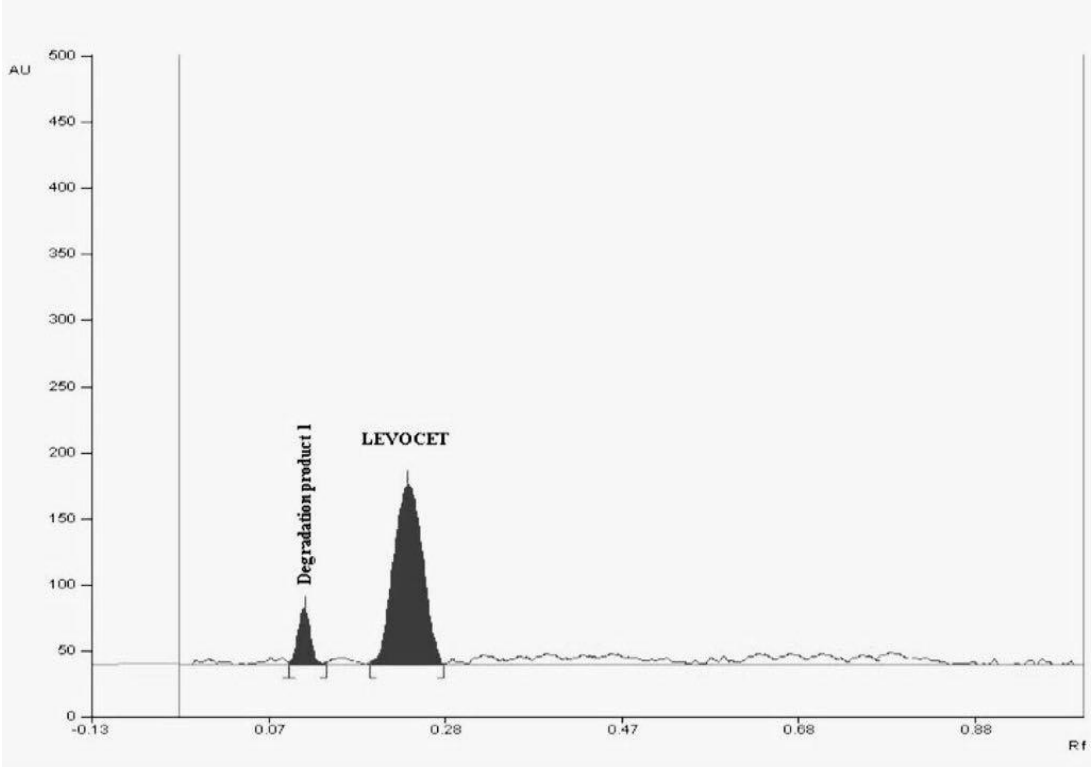

Fig 13: Densitogram after acid treatment of Levocetrizine.

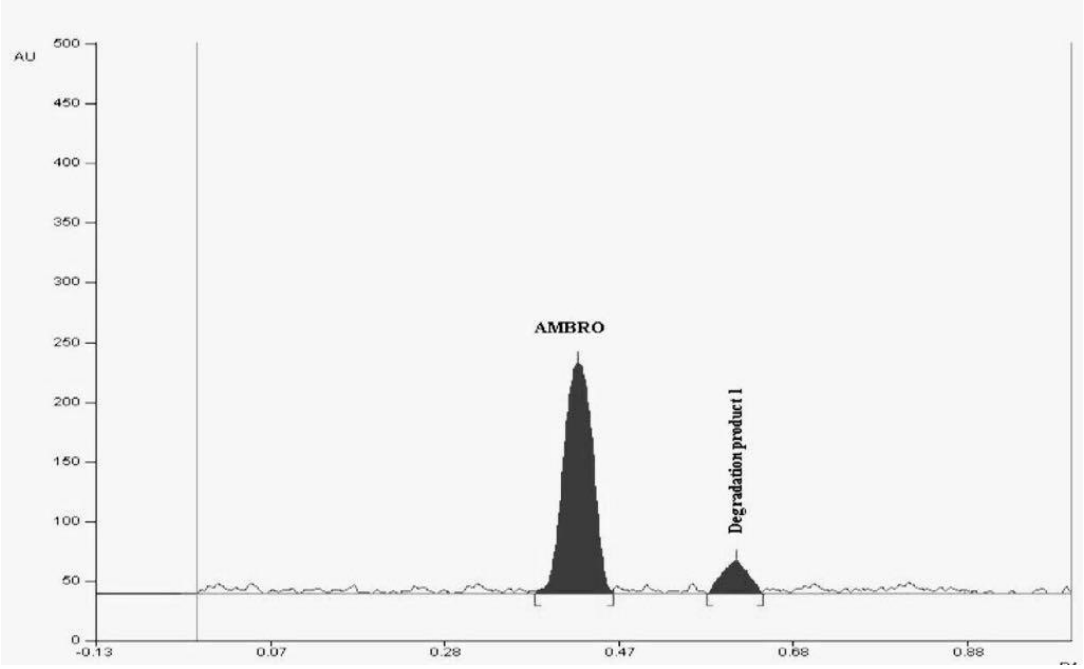

Fig 14: Densitogram after acid treatment of Ambroxol.

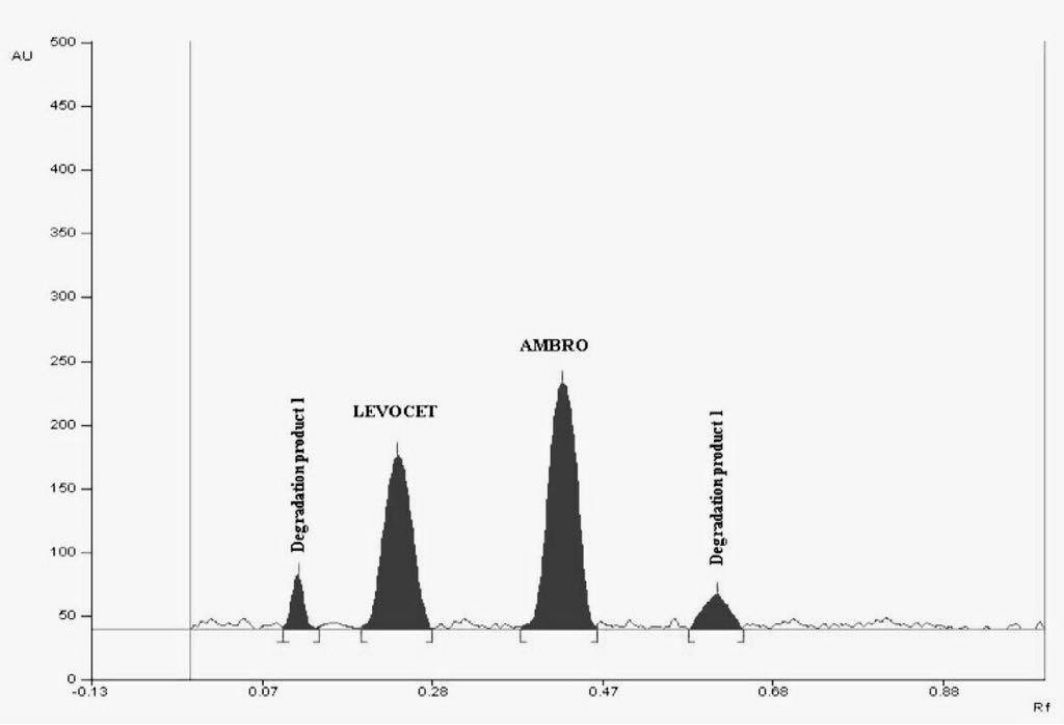

Fig 15: Densitogram after acid treatment of Mixture.

7.6.2 Alkali treatment

12.70% degradation was observed for LCTZ with no degradation peak and 14.31% degradation was observed for AMB with degradation peaks at Rf 0.54, 0.58.

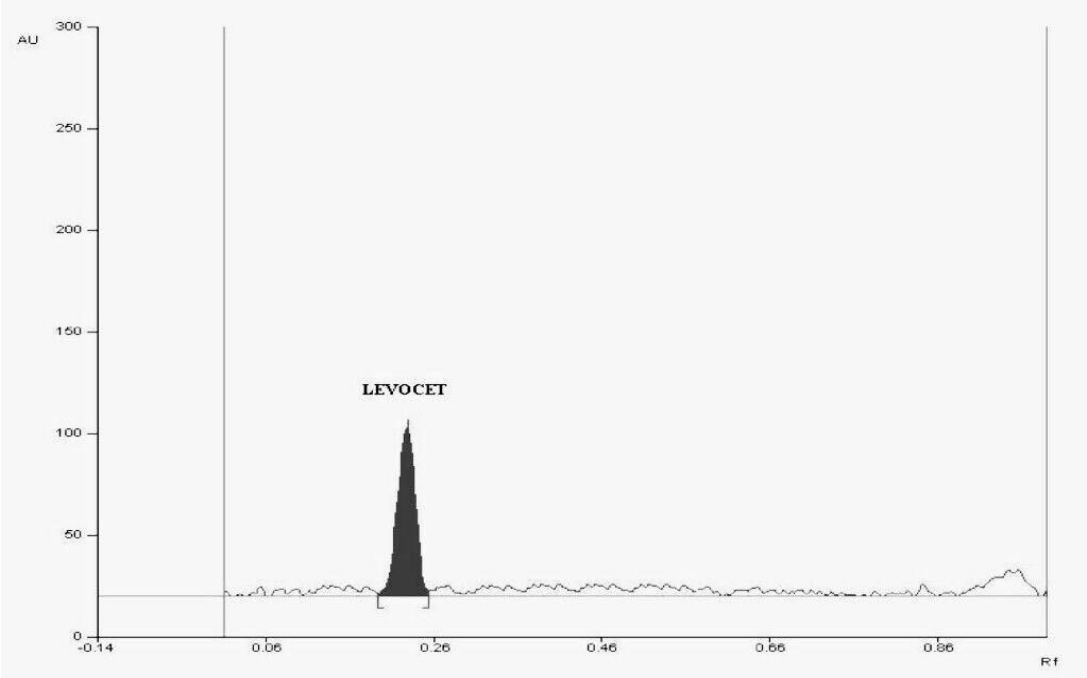

Fig 16: Densitogram after alkali treatment of Levocetrizine.

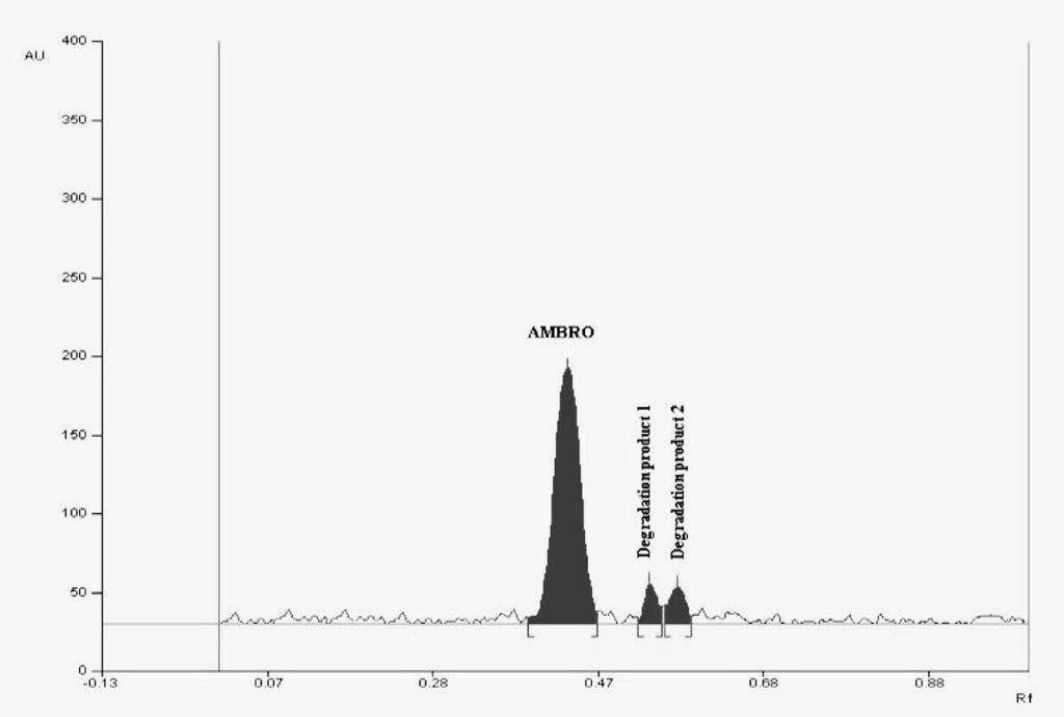

Fig 17: Densitogram after alkali treatment of Ambroxol.

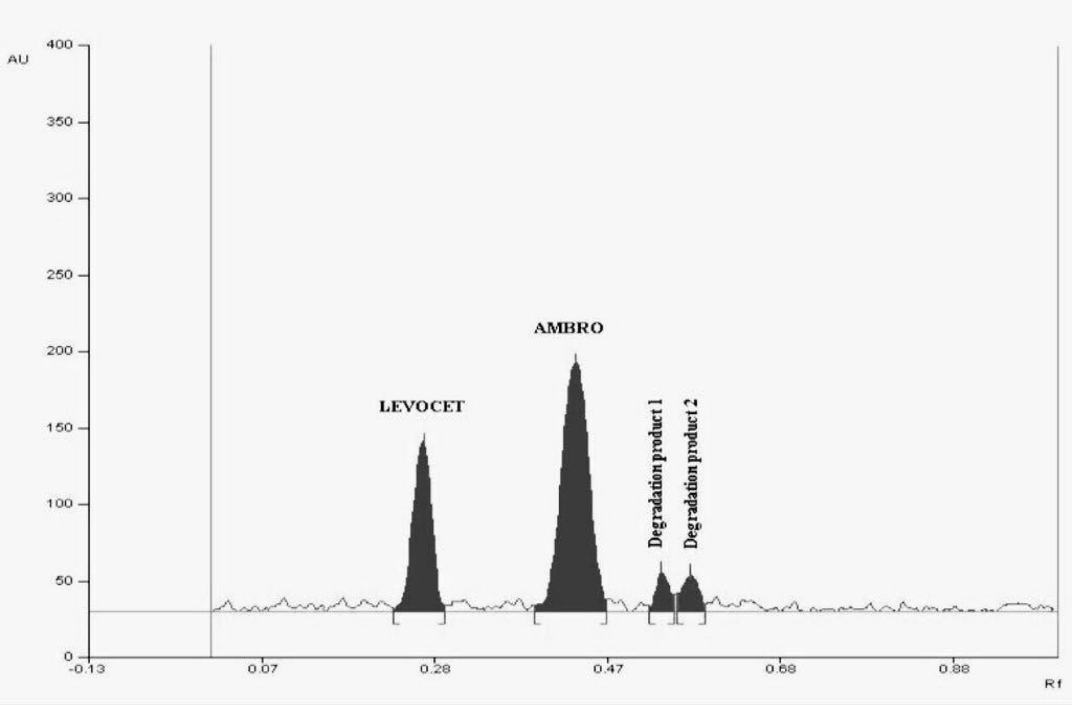

Fig 18: Densitogram after alkali treatment of Mixture.

7.6.3 Oxidation

13.91% degradation was observed for LCTZ with degradation peak at Rf 0.35 and 16.23% degradation was observed for AMB with degradation peaks at 0.65, 0.72.

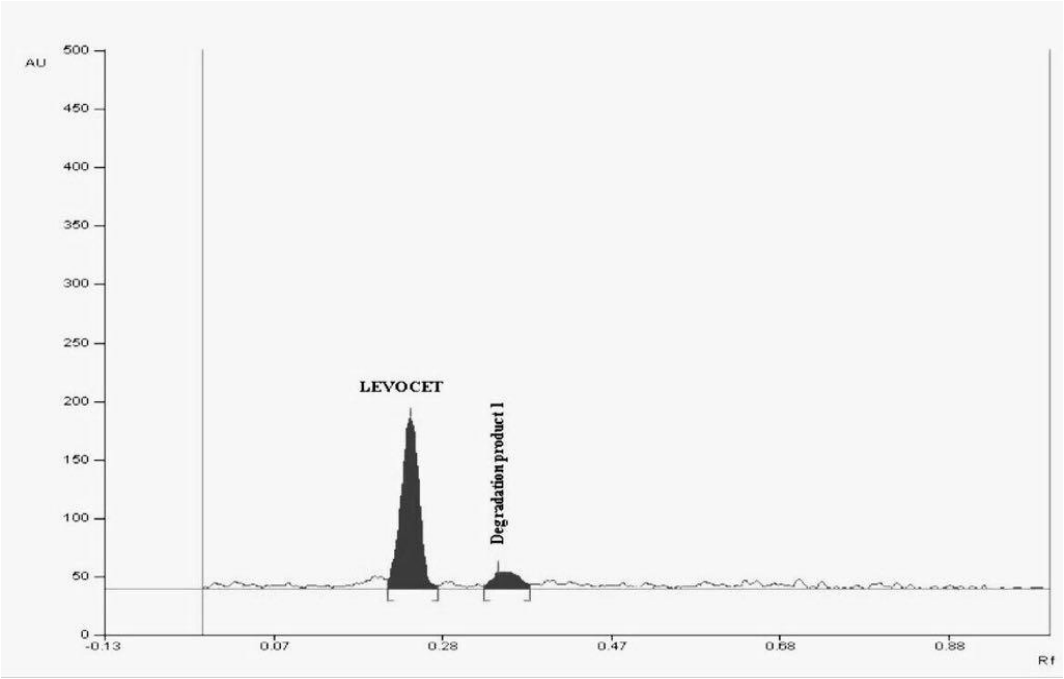

Fig 19: Densitogram after Oxidative degradation of Levocetrizine.

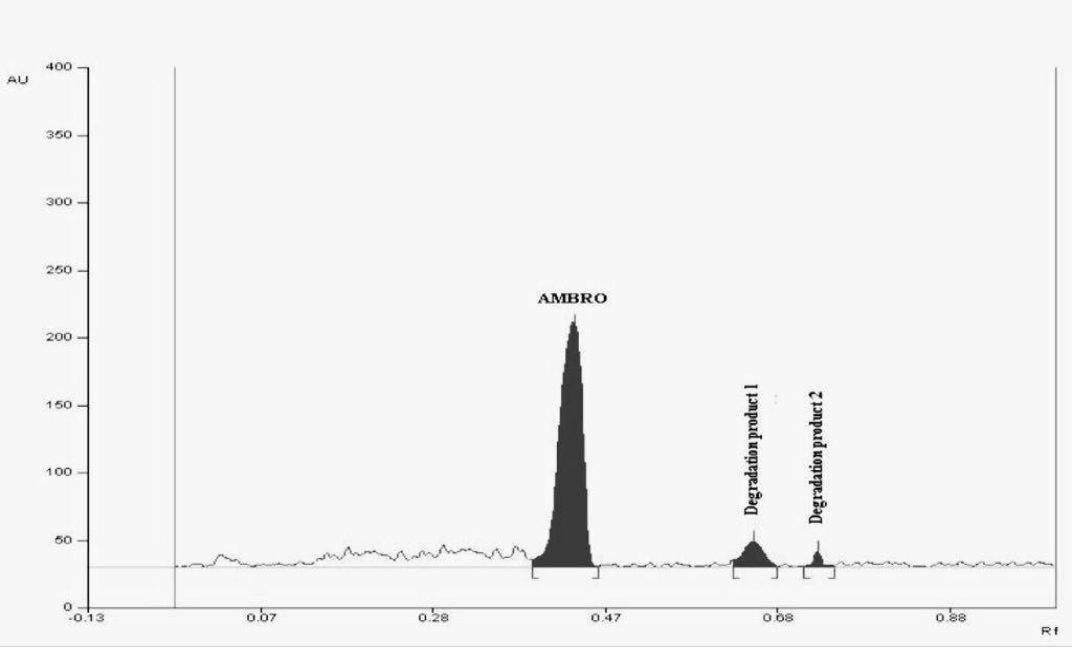

Fig 20: Densitogram after Oxidative degradation of Ambroxol.

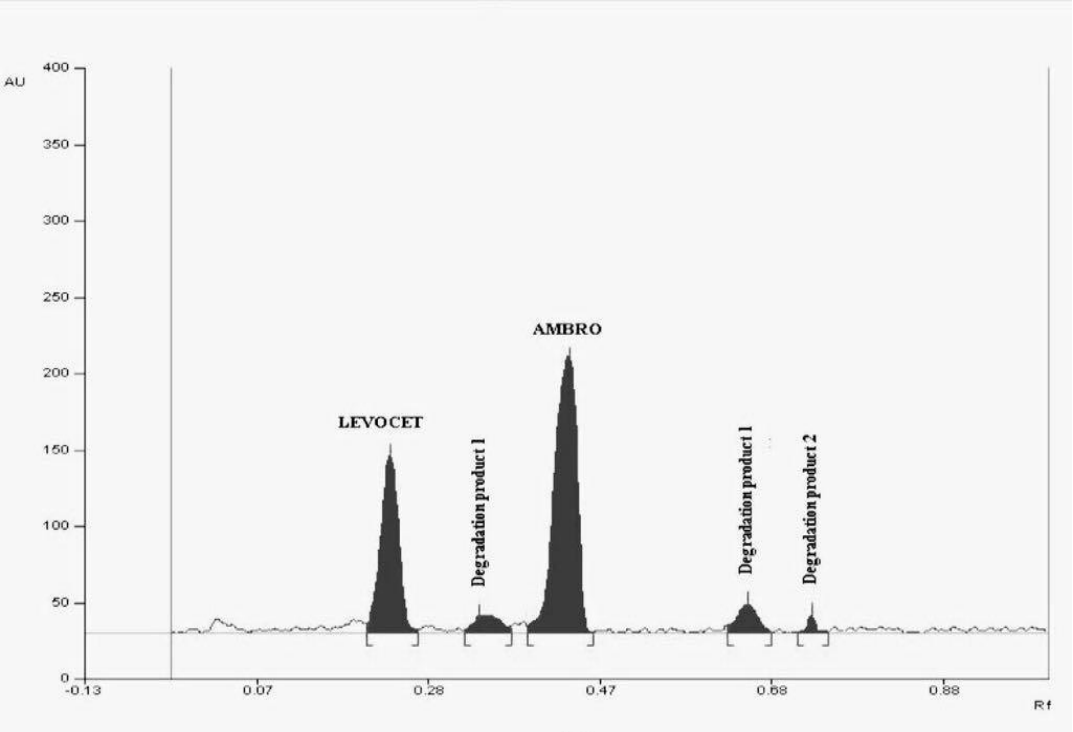

Fig 21: Densitogram after Oxidative degradation of Mixture.

7.6.4 Degradation under dry heat

7.20% degradation was observed for LCTZ with no degradation peak and 15.11% degradation was observed for AMB with degradation peak at Rf 0.73.

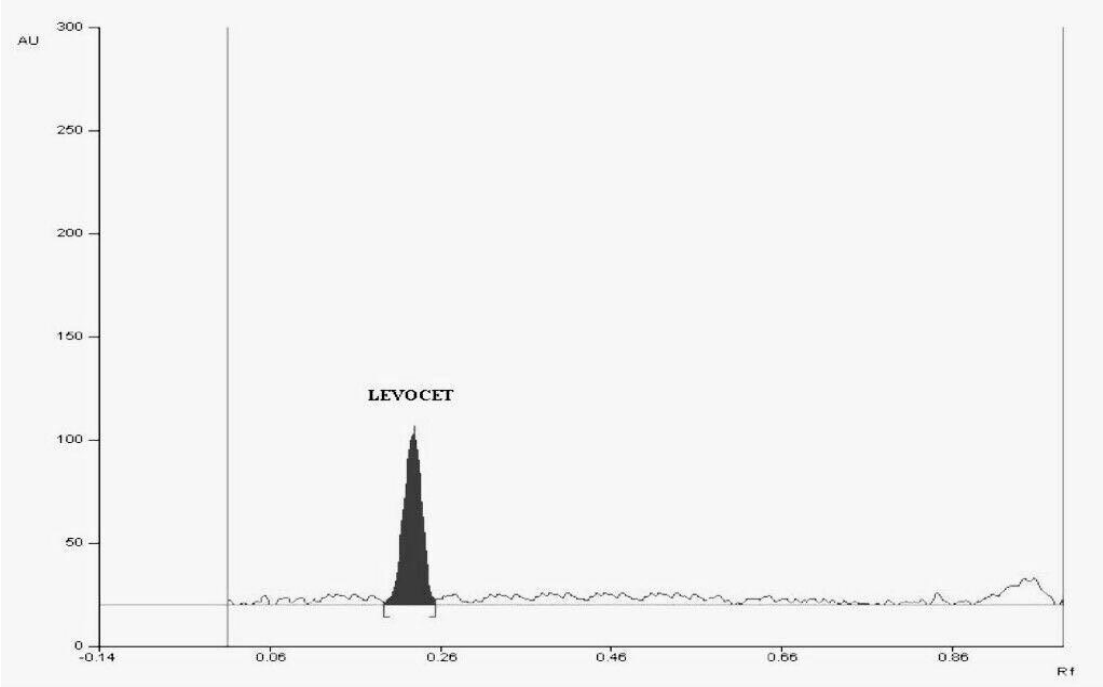

Fig 22: Densitogram after Dry heat degradation of Levocetrizine.

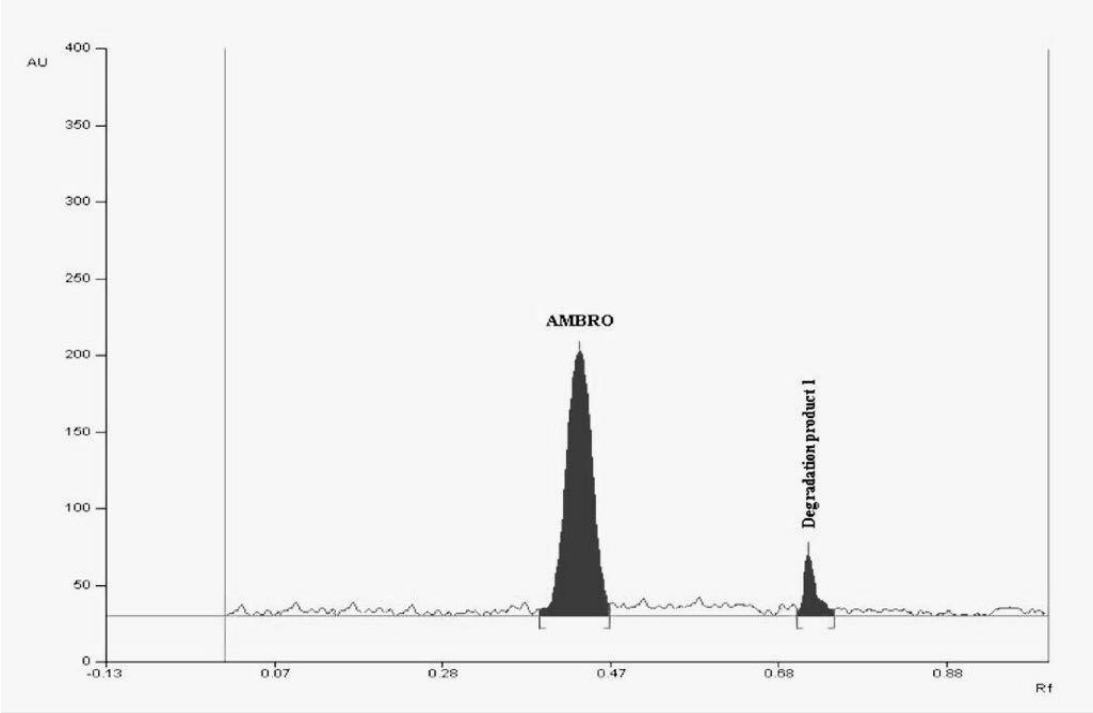

Fig 23: Densitogram after Dry heat degradation of Ambroxol.

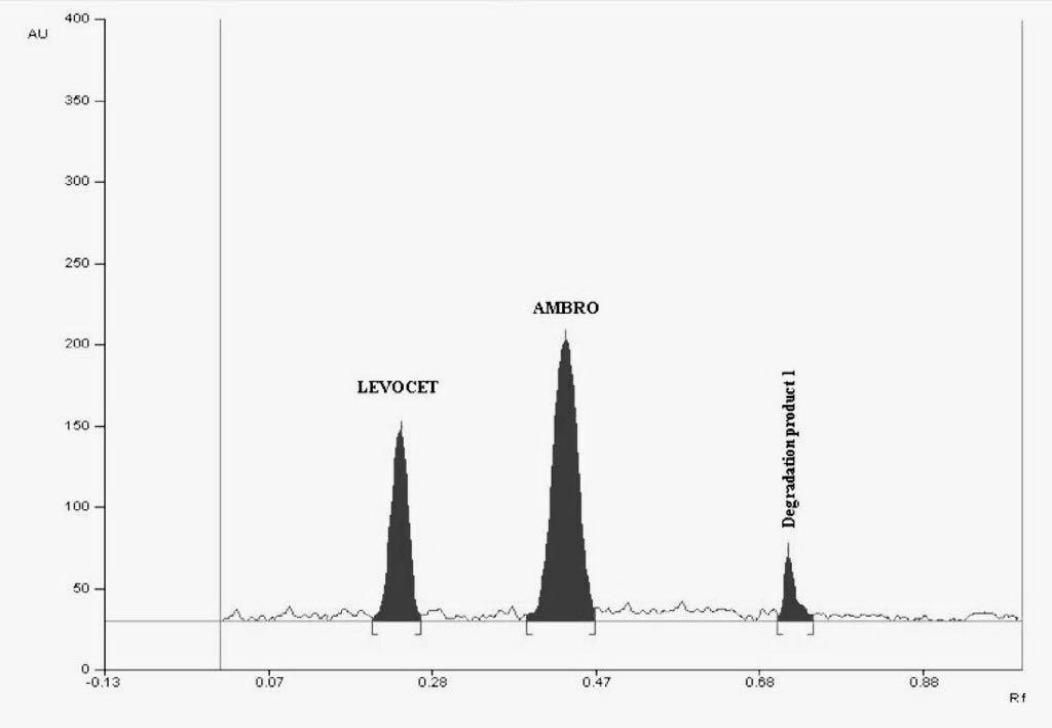

Fig 24: Densitogram after Dry heat degradation of mixture.

Table: 25 Summary of degradation studies of AMB AND LCTZ.

Stress condition/duration/state	LCTZ degradation	AMB degradation
Acidic/0.1 N HCl/reflux 2hrs	**11.33%**	**12.56%**
Alkaline/0.1 N NaOH/reflux 2hrs	**12.70%**	**14.31%**
Oxidative/3% H_2O_2/reflux 4hrs	**13.91%**	**16.23%**
Dry heat/60degree/ 6hrs	**7.20%**	**15.11%**

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