



RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF AMBROXOL MONTELUKAST AND LEVOCETIRIZINE BY USING SOLID DOSAGE FORM

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ABSTRACT

A simple and selective LC method is described for the determination of Ambroxol, Montelukast and Levocetirizine dosage forms. Chromatographic separation was achieved on a C_{18} column using mobile phase consisting of a mixture of Phosphate buffer pH: 4 Acetonitrile (55:45v/v), with detection of 275nm. Linearity was observed in the range 40-120 $\mu\text{g/ml}$ for Ambroxol ($r^2 = 0.999$) 20-60 $\mu\text{g/ml}$ for Montelukast ($r^2 = 0.999$) & 5-15 $\mu\text{g/ml}$ for Levocetirizine ($r^2 = 0.999$) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

KEYWORDS: Ambroxol, Montelukast, levocetirizine, RP-HPLC, validation.

INTRODUCTION

High Performance Liquid Chromatography (HPLC) is a technique for separation, identification and quantification of components in a mixture.^[1] It is especially suitable for compounds which are not easily volatilized, thermally unstable and have high molecular weights.^[2-4] Detection techniques are employed for detection and quantification of the eluted components.^[2] The aim of the study was to develop new Reverse Phase (RP)-HPLC method for the simultaneous estimation of Ambroxol, Montelukast and

Levocetirizine in pharmaceutical dosage forms.^[5-6] The main objective of the study was to develop a new RP-HPLC method for validation of AMBROXOL, MONTELUKAST AND LEVOCETIRIZINE in solid dosage forms and also to validate the drug using different solvents, retention time and other parameters. Validation was done as per ICH guidelines for the determination Ambroxol, Montelukast and Levocetirizine in pharmaceutical dosage forms.

MATERIALS AND METHODS

Table 1.1 Instruments used.

UV-Visible Spectrophotometer	Nicolet evolution 100
HPLC	Shimadzu (LC 20 AT VP)
HPLC	Agilent 1200 series
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	INERTSIL column, C18(150x4.6 ID) 5 μm

Table 1.2: Reagents used.

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Dihydrogen ortho Phosphate	AR Grade
Acetonitrile	HPLC Grade
Ammonium acetate	AR Grade
Tetra Hydro Furan	AR Grade

Table 1.3: Drug used.

Ambroxol, Montelukast and Levocetirizine	Gift Samples from an industry
Lezest-M (75+5+10mg) label claims	Obtained from local pharmacy

Mobile Phase

A mixture of 55 volumes of Phosphate Buffer and 45 volumes of Acetonitrile were prepared. The mobile phase was sonicated for 10min to remove gases.

Preparation of buffer

Weigh 6.8gm of potassium dihydrogen phosphate in 1000ml of water.

Solubility Studies

These studies are carried out at 25 °C.

AMBROXOL

Soluble in methanol and insoluble in water.

MONTELUKAST

Soluble in ethanol, methanol, ethyl acetate, and acetone.

LEVOCETIRIZINE

soluble in Methanol and slightly soluble in water. Insoluble in ethanol.

Determination of Working Wavelength (λ_{max})

In simultaneous estimation of three drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate three drugs accurately.

Preparation of standard stock solution of Ambroxol

10 mg of Ambroxol was weighed and transferred in to 25ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 80 µg /ml of solution by diluting 2 ml to 10ml with methanol.

Preparation of standard stock solution of Montelukast

10 mg of Montelukast was weighed in to 25ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 40 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of Levocetirizine

10 mg of Levocetirizine was weighed in to 25ml volumetric flask and dissolved in Methanol and then

dilute up to the mark with methanol and prepare 10µg/ml of solution by diluting 0.25 ml to 10ml with methanol.

METHOD DEVELOPMENT OF AMBROXOL, MONTELUKAST AND LEVOCETIRIZINE**Trial – 1.****Chromatographic conditions**

Mobile phase : Triethyl amine + ACN+Methanol
pH : 5.0
Ratio : 20:30:50
Column : BDS (250×4.6mm)5
Wavelength : 275nm
Flow rate : 1ml/min

Preparation of mixed standard solution

Weigh accurately 16 mg of Ambroxol and 10mg of Montelukast and 5mg of Levocetirizine in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 160µg/ml of Ambroxol and 100µg/ml of Montelukast and 50µg/ml of Levocetirizine is prepared by diluting 5.3ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Trial- 2**Chromatographic conditions**

Mobile phase : Water: ACN
Ratio : 50:50
Column : Zodiac C18 Column (250×4.6 ×5µ)
Wavelength : 275nm
Flow rate : 1.2 ml/min

Preparation of mixed standard solution

Weigh accurately 80mg of Ambroxol and 40mg of Montelukast and 10mg of Levocetirizine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 80µg/ml of Ambroxol and 40µg/ml of Montelukast and 10µg/ml of Levocetirizine is prepared by (pipette out 2ml from Ambroxol and 1ml from Montelukast and 0.25 ml from Levocetirizine). diluting 3.25ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Trial- 3.**Chromatographic conditions**

Mobile phase : Ammonium acetate: ACN
 Ratio : 30:70
 Column : Zodiac, C18 (250×4.6× 5μ)
 Wavelength : 275nm
 Flow rate : 1ml/min

Preparation of mixed standard solution

Weigh accurately 80mg of Ambroxol and 40mg of Montelukast and 10mg of Levocetirizine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 80μg/ml of Ambroxol and 40μg/ml of Montelukast and 10μg/ml of Levocetirizine is prepared by (pipette out 2ml from Ambroxol and 1ml from Montelukast and 0.25 ml from Levocetirizine). diluting 3.25ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Trial- 4.**Chromatographic conditions**

Mobile phase : Phosphate Buffer : ACN
 Ratio : 55:45
 Column : Zodiac, C18 (250×4.6× 5μ)
 Wavelength : 275nm
 Flow rate : 1ml/min
 pH : 4.0

Preparation of mixed standard solution

Weigh accurately 80mg of Ambroxol and 40mg of Montelukast and 10mg of Levocetirizine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 80μg/ml of Ambroxol and 40μg/ml of Montelukast and 10μg/ml of Levocetirizine is prepared by (pipette out 2ml from Ambroxol and 1ml from Montelukast and 0.25 ml from Levocetirizine). diluting 3.25ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Chromatogram of Ambroxol, Montelukast and Levocetirizine

Assay**Preparation of samples for Assay****Preparation of mixed standard solution**

Weigh accurately 80mg of Ambroxol and 40mg of Montelukast and 10mg of Levocetirizine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 80μg/ml of Ambroxol and 40μg/ml of Montelukast and 10μg/ml of Levocetirizine is prepared by (pipette out 2ml from Ambroxol and 1ml from Montelukast and 0.25 ml from Levocetirizine). diluting 3.25ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution:

Tablets (each tablet contains (75mg+5mg+10mg) of Ambroxol, Montelukast and Levocetirizine) were

weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Ambroxol (80μg/ml) and Montelukast (40μg/ml) and Levocetirizine (10μg/ml) were prepared by dissolving weight equivalent to 75mg of Ambroxol and 10mg of Montelukast and 5mg of Levocetirizine and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 25ml with mobile phase. Further dilutions are prepared in 5 replicates of 80μg/ml of Ambroxol and 40μg/ml of Montelukast and 10μg/ml of Levocetirizine was made by adding 3.25ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of Ambroxol, Montelukast and Levocetirizine present in the formulation by using the formula given below, and results shown in above table:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation

AT: Peak area due to assay preparation

WS: Weight of Ambroxol, Montelukast and Levocetirizine in mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

VALIDATION**Specificity by Direct comparison method**

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of samples for Assay**Preparation of mixed standard solution**

Weigh accurately 80mg of Ambroxol and 40mg of Montelukast and 10mg of Levocetirizine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 80μg/ml of Ambroxol and 40μg/ml of Montelukast and 10μg/ml of Levocetirizine is prepared by (pipette out 2ml from Ambroxol and 1ml from Montelukast and 0.25 ml from Levocetirizine). diluting 3.25ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution

Tablets (each tablet contains (75mg+5mg+10mg) of Ambroxol, Montelukast and Levocetirizine) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Ambroxol (80μg/ml) and Montelukast (40μg/ml) and Levocetirizine (10μg/ml) were prepared by dissolving weight equivalent to 75mg of Ambroxol and 10mg of Montelukast and 5mg of Levocetirizine and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 25ml with mobile phase. Further dilutions

are prepared in 5 replicates of 80 µg/ml of Ambroxol and 40 µg/ml of Montelukast and 10 µg/ml of Levocetirizine was made by adding 3.25 ml of stock solution to 10 ml of mobile phase.

2. Linearity and range

Preparation of mixed standard solution

Weigh accurately 10 mg of Ambroxol and 10 mg of Montelukast and 10 mg of Levocetirizine in 25 ml of volumetric flask and dissolve in 25 ml of mobile phase and make up the volume with mobile phase. This stock solution contains 80 µg/ml of Ambroxol and 40 µg/ml of Montelukast and 10 µg/ml of Levocetirizine. This solution is used for recording chromatogram.

3. Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% & 150%.

4. Precision

Method precision

Prepared sample preparations of Ambroxol, Montelukast and Levocetirizine as per test method and injected 5 times in to the column.

Acceptance criteria

The % Relative standard deviation of Assay preparations of Ambroxol, Montelukast and Levocetirizine should be not more than 2.0%.

5. Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength. System suitability parameters were compared with that of method precision.

Acceptance criteria

The system suitability should pass as per the test method at variable conditions. Chromatogram of Ambroxol, Montelukast and Levocetirizine for Robustness (0.8 ml/min)

6. Limit of Detection

$$LOD = \frac{3.3\sigma}{S}$$

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.

Observation:

The LOD for this method was found to be 24.19 µg/ml of Ambroxol

The LOD for this method was found to be 18.85 µg/ml of Montelukast

The LOD for this method was found to be 7.23 µg/ml of Levocetirizine

7. Limit of Quantification

$$LOQ = \frac{10\sigma}{S}$$

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.

Observation: The LOQ for this method was found to be 73.31 µg/ml for Ambroxol

The LOQ for this method was found to be 57.12 µg/ml for Montelukast

RESULTS AND DISCUSSION

Determination of Working Wavelength (λ_{max})

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

RESULTS

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig. no. 8.1, 8.2 and 8.3 and the absorption curve shows characteristic absorption maxima at nm for ambroxol, nm for montelukast and nm for levocetirizine nm for the combination.

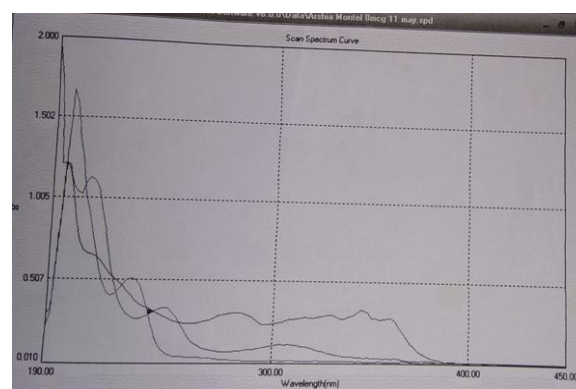


Fig. 1: UV-VIS spectrum of Ambroxol, Montelukast and Levocetirizine and the isobestic point was 275 nm. The Isobestic point was found to be 275 nm for Ambroxol, Montelukast and Levocetirizine combination.

METHOD DEVELOPMENT OF AMBROXOL, MONTELUKAST AND LEVOCETIRIZINE

Trial - 1

Chromatographic conditions

Mobile phase : Triethyl amine +ACN+Methanol

pH : 5.0
Ratio : 20:30:50
Column : BDS (250×4.6mm)5
Wavelength : 275nm
Flow rate : 1ml/min

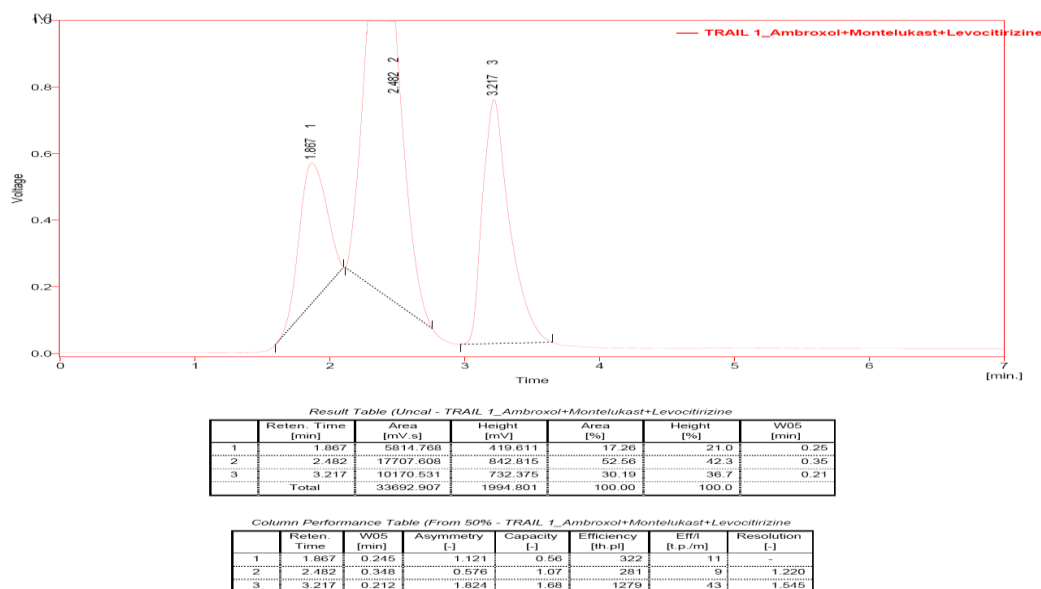


Fig 1.1: Chromatogram of Ambroxol, Montelukast and Levocetirizine.

Observation

- Efficiency was not good. Peak response was not satisfactory. Hence it is not optimized.

Trial- 2

Chromatographic conditions

Mobile phase : Water: ACN
Ratio : 50:50
Column : Zodiac C18Column (250×4.6 ×5μ)
Wavelength : 275nm
Flow rate : 1.2 ml/min

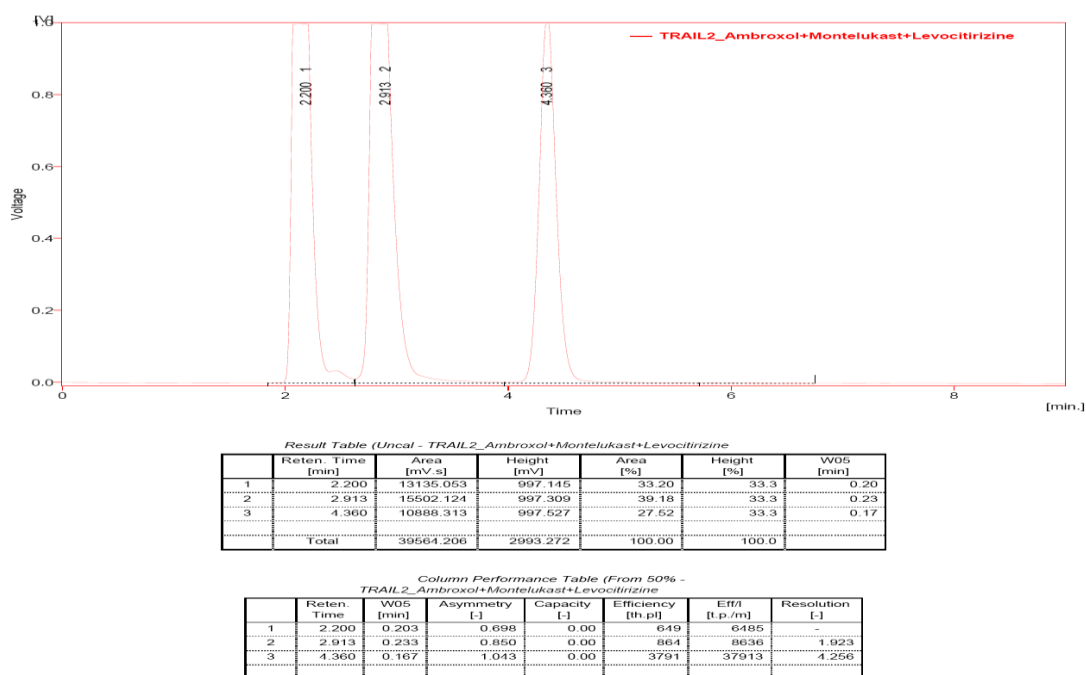


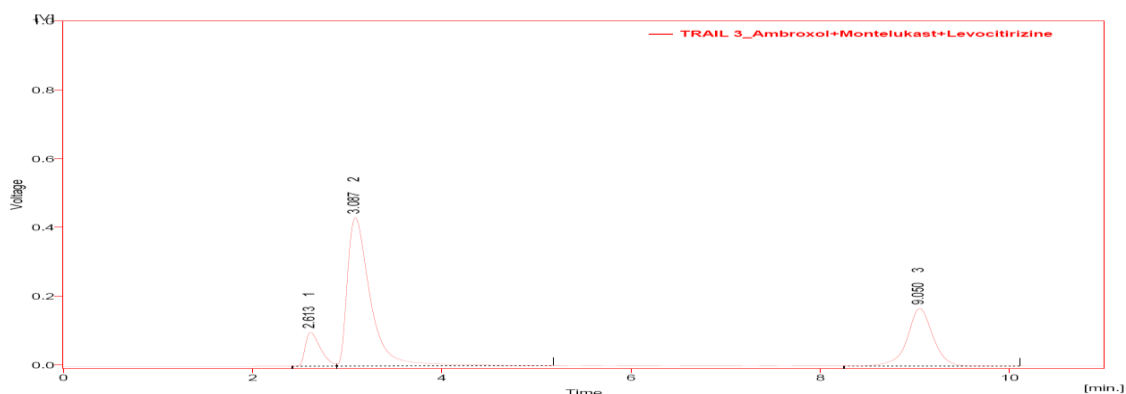
Fig 1.2: Chromatogram of Ambroxol, Montelukast and Levocetirizine.

Observation

Efficiency was not good. Resolution was not good.
Hence it is not optimized.

Trial- 3**Chromatographic conditions**

Mobile phase : Ammonium acetate: ACN
Ratio : 30:70
Column : Zodiac, C18 (250×4.6× 5μ)
Wavelength : 275nm
Flow rate : 1ml/min



Result Table (Uncal - TRAIL_3_Ambroxol+Montelukast+Levocetirizine)						
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.613	1095.597	97.970	9.48	14.1	0.18
2	3.087	7293.045	431.295	63.08	61.9	0.25
3	9.050	3172.697	167.761	27.44	24.1	0.28
Total		11561.339	697.016	100.00	100.0	

Column Performance Table (From 50% - TRAIL_3_Ambroxol+Montelukast+Levocetirizine)							Resolution [-]
	Reten. Time [min]	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/I [t.p./m]	
1	2.613	0.180	2.333	0.00	1168	11678	-
2	3.087	0.247	2.295	0.00	867	8675	1.306
3	9.050	0.277	1.035	0.00	5928	59278	13.410

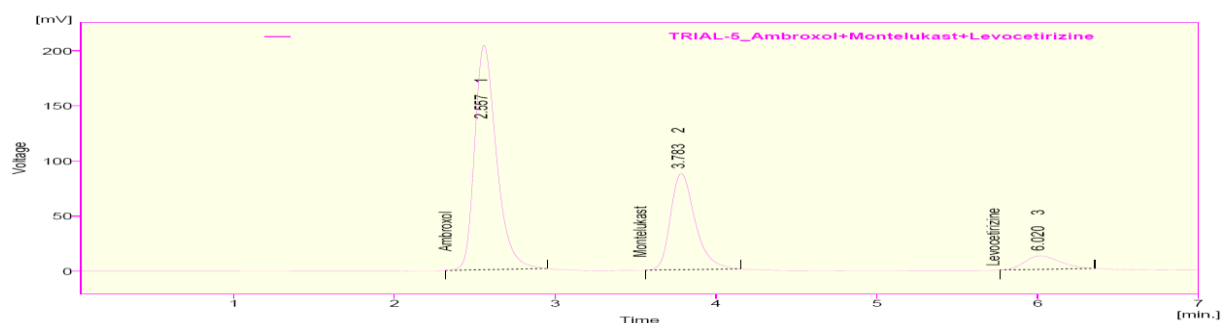
Fig 1.3: Chromatogram of Ambroxol, Montelukast and Levocetirizine.

Observation

- Efficiency of the peaks was not good an tailing was observed. Hence it is not optimized.

Trial- 4**Chromatographic conditions**

Mobile phase : Phosphate Buffer : ACN
Ratio : 55:45
Column : Zodiac, C18 (250×4.6× 5μ)
Wavelength : 275nm
Flow rate : 1ml/min
pH : 4.0



Result Table (Uncal - TRIAL-5_Ambroxol+Montelukast+Levocetirizine)				
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.557	1925.712	203.825	63.29
2	3.783	930.203	87.194	30.57
3	6.020	185.933	12.312	5.14
Total		3042.846	303.332	100.00

Column Performance Table (From 50% - TRIAL-5_Ambroxol+Montelukast+Levocetirizine)						
	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.557	0.143	1.576	1763	17626	-
2	3.783	0.163	1.568	2972	29724	4.707
3	6.020	0.243	1.411	3391	33908	6.473

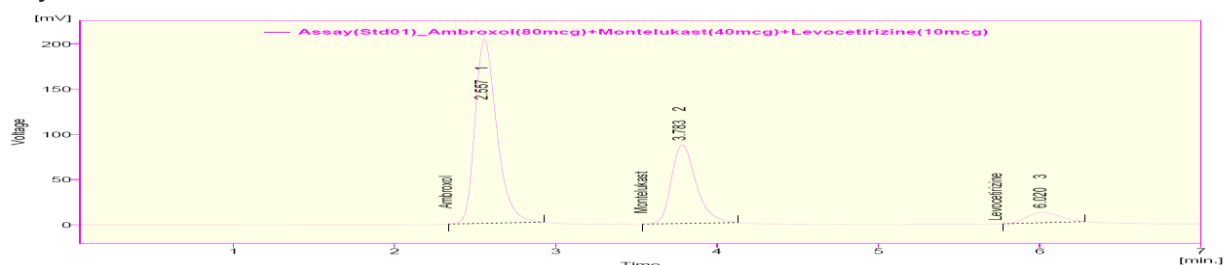
Fig 1.4: Chromatogram of Ambroxol, Montelukast and Levocetirizine.

Observation

- All the system suitability requirements were met.
- The peak Asymmetry factor was less than 2 for AMBROXOL, MONTELUKAST and LEVOCETIRIZINE.
- The efficiency was more than 2000 AMBROXOL, MONTELUKAST and LEVOCETIRIZINE
- Resolution between two peaks >1.5. Hence it is optimized

Table 2: Optimized chromatographic conditions.

Mobile phase	Phosphate buffer : Acetonitrile 55:45
Column	INERTSIL column,C18(150x4.6 ID) 5µm
Flow rate	1.2 ml/min
Column temperature	Room temperature(20-25°C)
Sample temperature	Room temperature(20-25°C)
Wavelength	275nm
Injection volume	20 µl
Run time	7min
Retention time	About 2.557 min for Ambroxol, 3.783 min for Montelukast and 6.020 min for Levocetirizine.

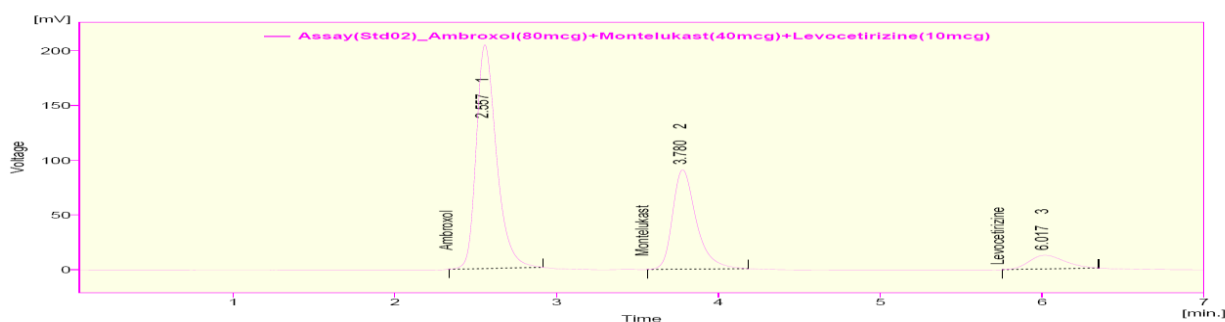
Assay

Result Table (Uncal - Assay(Std01)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.557	1912.194	203.507	63.73
2	3.783	923.623	87.059	30.78
3	6.020	161.673	11.685	5.49
Total		3000.390	302.252	100.00

Column Performance Table (From 50% - Assay(Std01)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.557	0.143	1.545	1763	17626	-
2	3.783	0.163	1.568	2972	29724	4.707
3	6.020	0.233	1.200	3688	36876	6.636

Fig 1.5: Chromatogram of Assay standard preparation-1.

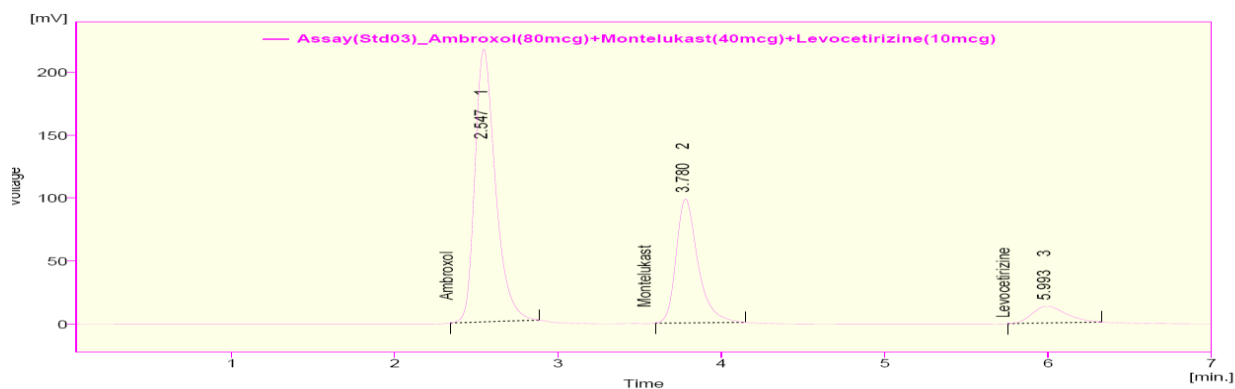
Result Table (Uncal - Assay(Std02)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.557	1785.598	204.414	61.60
2	3.780	922.694	90.823	31.83
3	6.017	190.394	12.574	6.57
Total		2898.685	307.811	100.00

Column Performance Table (From 50% - Assay(Std02)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.557	0.133	1.548	2037	20370	-
2	3.780	0.153	1.583	3367	33668	5.022
3	6.017	0.243	1.436	3387	33870	6.636

Fig 1.6: Chromatogram of Assay standard preparation-2.



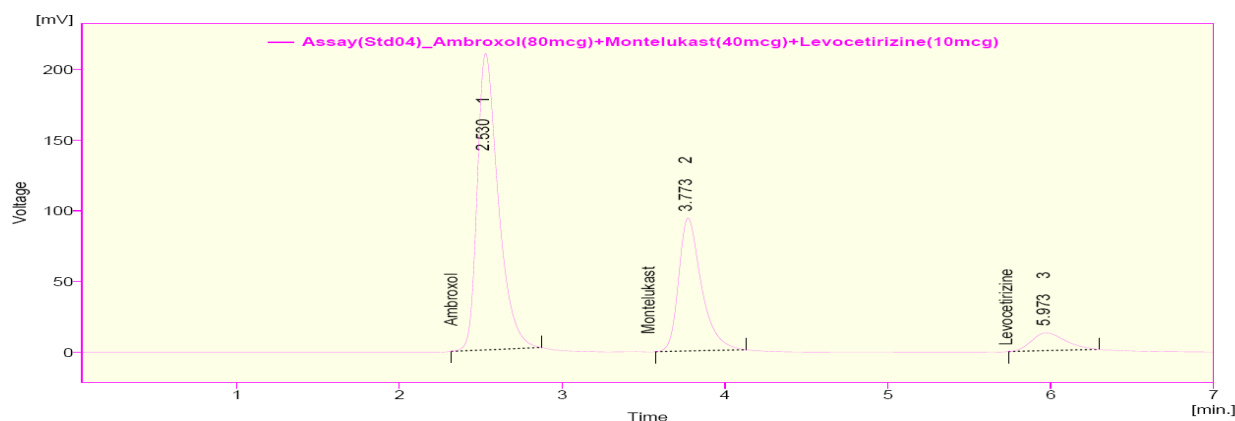
Result Table (Uncal - Assay(Std03)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.547	1894.838	216.405	63.17
2	3.780	913.510	98.518	30.45
3	5.993	191.406	13.343	6.38
Total		2999.755	328.266	100.00

Column Performance Table (From 50% - Assay(Std03)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	W05 [-]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.547	0.133	1.548	2021	20210	-
2	3.780	0.140	1.576	4039	40387	5.310
3	5.993	0.227	1.510	3873	38732	7.104

Fig. 1.7: Chromatogram of Assay standard preparation-3.



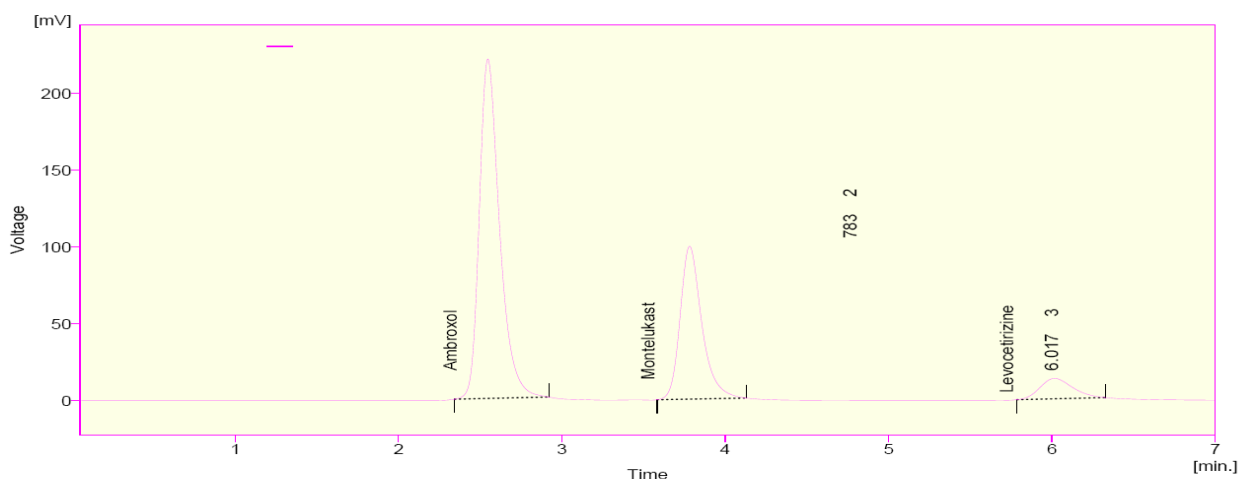
Result Table (Uncal - Assay(Std04)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.530	1888.580	209.559	63.28
2	3.773	914.480	93.991	30.64
3	5.973	181.491	12.595	6.08
Total		2984.550	316.146	100.00

Column Performance Table (From 50% - Assay(Std04)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	W05 [-]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.530	0.137	1.828	1899	18986	-
2	3.773	0.147	1.727	3667	36669	5.164
3	5.973	0.230	1.510	3737	37367	6.874

Fig. 1.8: Chromatogram of Assay standard preparation-4.



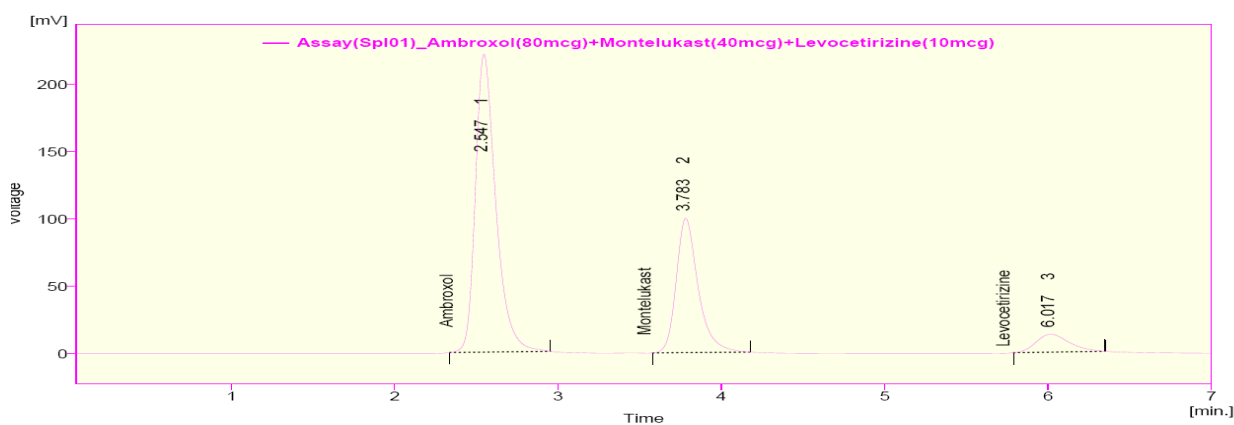
Result Table (Uncal - Assay(Std05)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.547	1910.621	221.214	63.33
2	3.783	921.942	99.659	30.56
3	6.017	184.485	13.312	6.11
	Total	3017.048	334.185	100.00

Column Performance Table (From 50% - Assay(Std05)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.547	0.130	1.600	2126	21260	-
2	3.783	0.137	1.500	4246	42455	5.458
3	6.017	0.220	1.385	4144	41436	7.369

Fig 1.9: Chromatogram of Assay standard preparation-5.



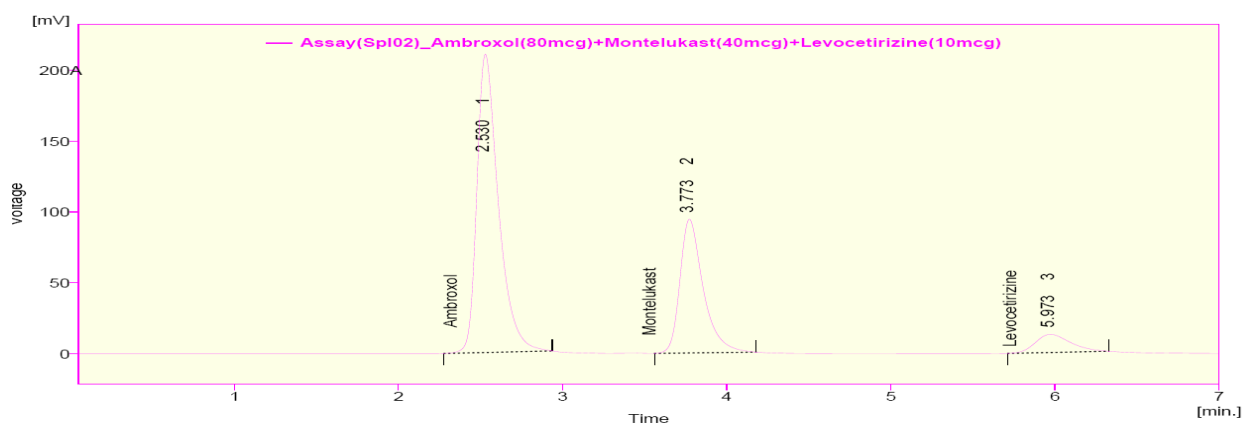
Result Table (Uncal - Assay(Spl01)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.547	1924.730	221.519	63.18
2	3.783	933.238	99.902	30.63
3	6.017	188.631	13.410	6.19
	Total	3046.599	334.831	100.00

Column Performance Table (From 50% - Assay(Spl01)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.547	0.130	1.633	2126	21260	-
2	3.783	0.137	1.529	4246	42455	5.458
3	6.017	0.220	1.442	4144	41436	7.369

Fig. 2: Chromatogram of Assay sample preparation-1.



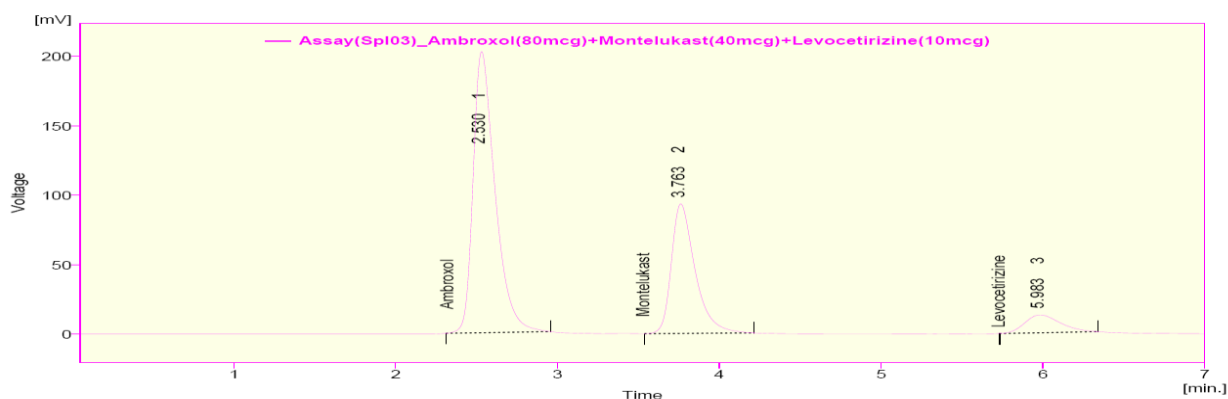
Result Table (Uncal - Assay(Spl02)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.530	1922.400	210.333	63.20
2	3.773	928.623	94.306	30.53
3	5.973	190.581	12.837	6.27
Total		3041.604	317.476	100.00

Column Performance Table (From 50% - Assay(Spl02)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.530	0.137	1.862	1899	18986	-
2	3.773	0.147	1.758	3667	36669	5.164
3	5.973	0.237	1.528	3529	35291	6.754

Fig 2.1: Chromatogram of Assay sample preparation-2.



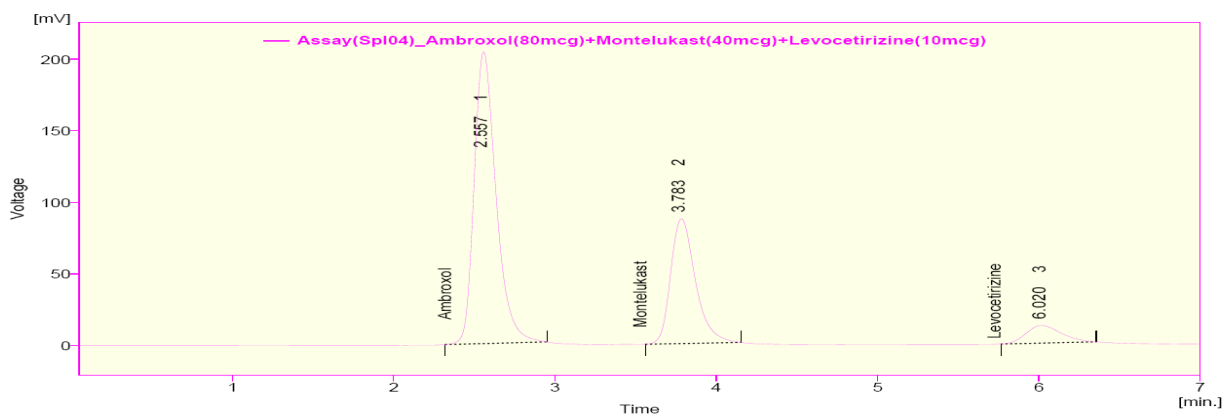
Result Table (Uncal - Assay(Spl03)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.530	1915.267	202.602	62.59
2	3.763	948.698	93.408	31.00
3	5.983	196.092	12.896	6.41
Total		3060.058	308.907	100.00

Column Performance Table (From 50% - Assay(Spl03)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.530	0.143	1.931	1726	17261	-
2	3.763	0.160	1.735	3487	34872	4.948
3	5.983	0.243	1.500	3350	33496	6.642

Fig 2.2: Chromatogram of Assay sample preparation-3.



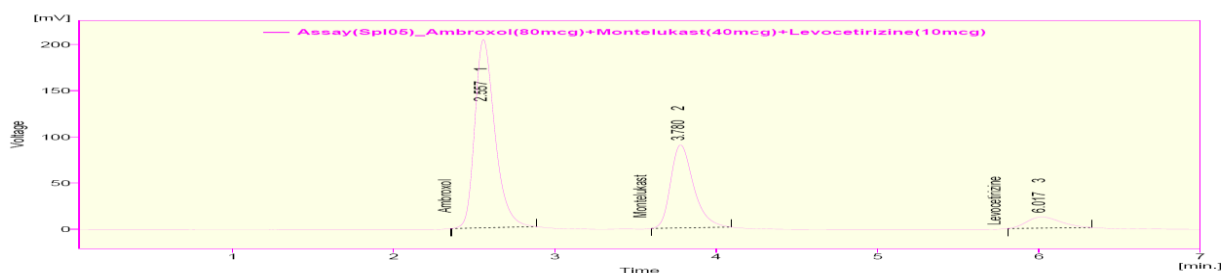
Result Table (Uncal - Assay(Spl04)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.557	1925.712	203.825	63.29
2	3.783	930.203	87.194	30.57
3	6.020	186.932	12.312	6.14
Total		3042.846	303.332	100.00

Column Performance Table (From 50% - Assay(Spl04)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.557	0.143	1.576	1763	17626	-
2	3.783	0.163	1.568	2972	29724	4.707
3	6.020	0.243	1.411	3391	33908	6.473

Fig 2.3: Chromatogram of Assay sample preparation-4.



Result Table (Uncal - Assay(Spl05)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.557	1767.839	203.938	62.47
2	3.780	886.391	89.861	31.32
3	6.017	176.636	12.059	6.20
Total		2629.765	305.858	100.00

Column Performance Table (From 50% - Assay(Spl05)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.557	0.133	1.516	2037	20370	-
2	3.780	0.153	1.543	3367	33668	5.022
3	6.017	0.237	1.471	3561	35605	6.749

Fig 2.4: Chromatogram of Assay sample preparation-5.

Table 3 Assay Results

	Ambroxol		Montelukast		Levocetirizine	
	Standard Area	Sample Area	Standard Area	Sample Area	Standard Area	Sample Area
Injection-1	1912.194	1924.730	923.523	933.238	164.673	188.631
Injection-2	1785.598	1922.4	922.694	928.623	190.394	190.581
Injection-3	1894.838	1915.267	913.51	948.698	191.406	196.092
Injection-4	1888.58	1925.712	914.48	930.203	181.491	186.932
Injection-5	1910.621	1767.839	921.942	886.391	184.485	175.535
Average Area	1878.366	1891.190	919.2298	925.4306	182.4898	187.5542
Assay(%purity)	100.682689		100.674565		102.7751688	

Observation: The amount of Ambroxol, Montelukast and Levocetirizine present in the taken dosage form was found to be 100.68%, 100.67% and 102.77% respectively.

VALIDATION

1. Specificity

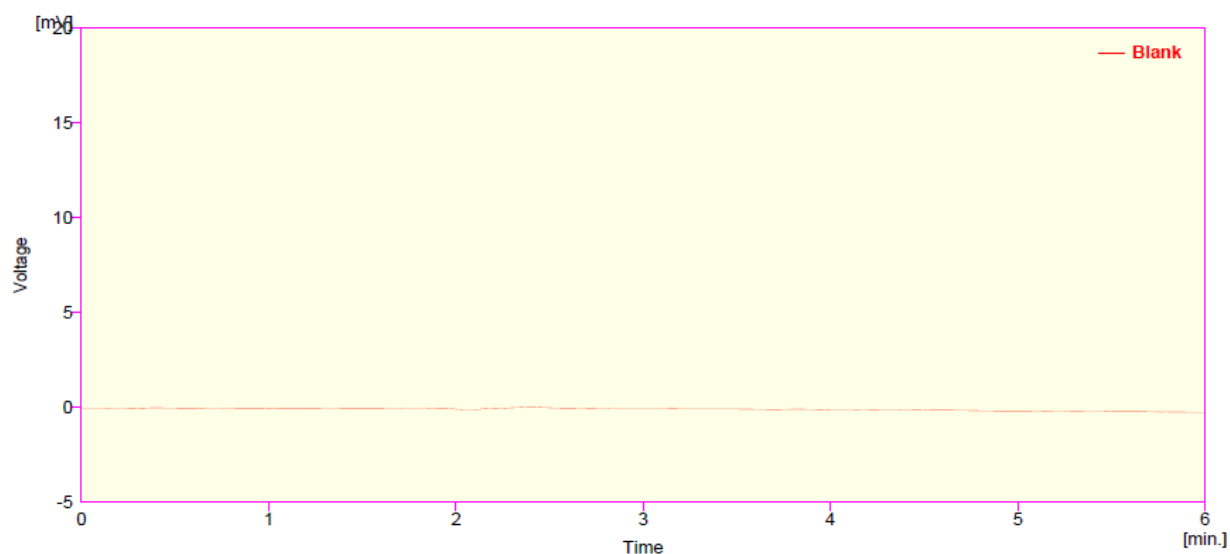


Fig 3.1: Blank chromatogram for specificity.

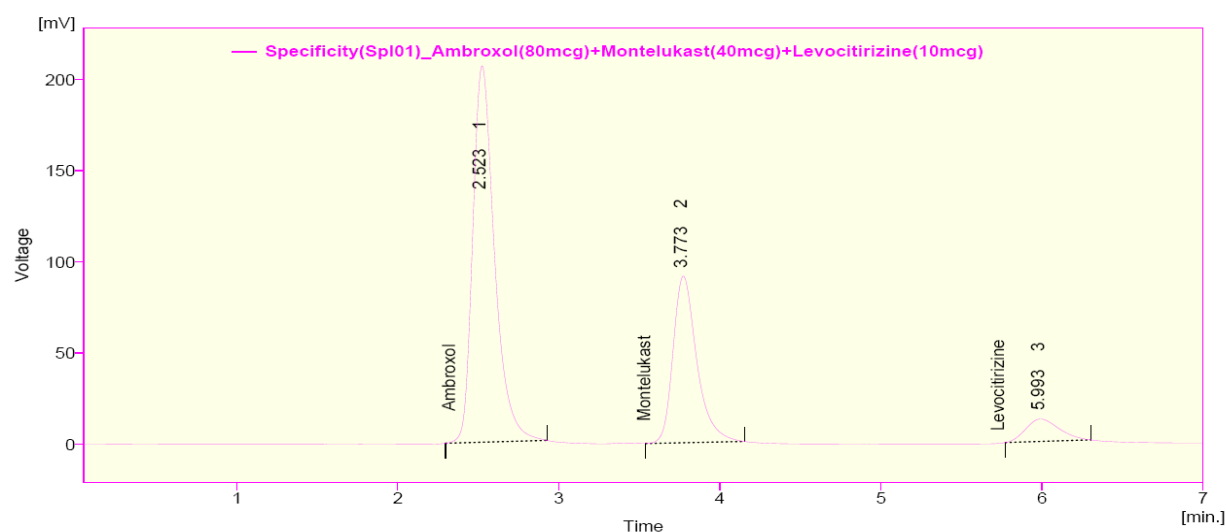


Fig 3.2: Chromatogram for specificity of Ambroxol, Montelukast and Levocetirizine sample.

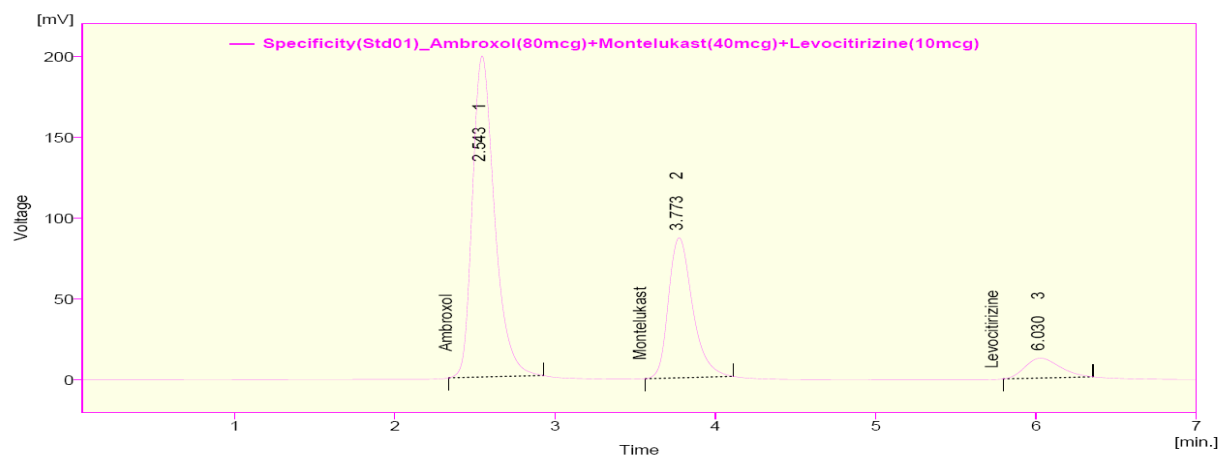
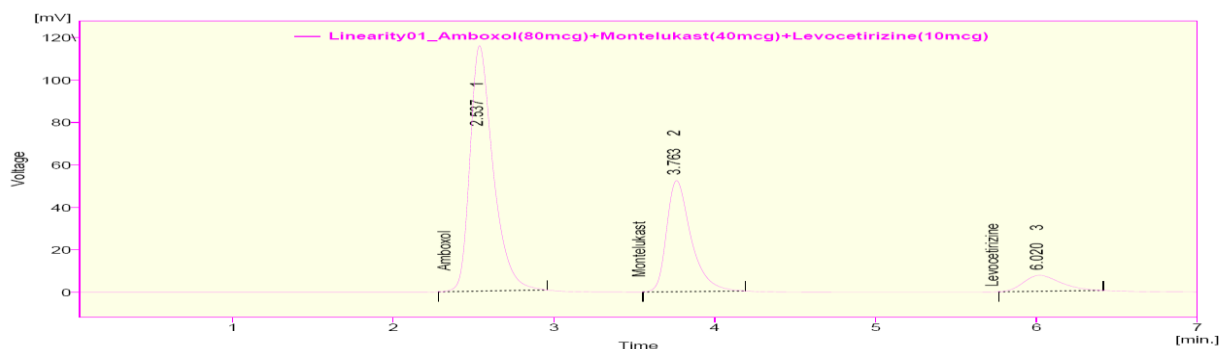


Fig 3.3: Chromatogram for Specificity of Ambroxol, Montelukast and Levocetirizine standard.

Observation

It is observed from the above data, diluents or excipient

peaks are not interfering with the Ambroxol, Montelukast and Levocetirizine peaks.

2. Linearity and range

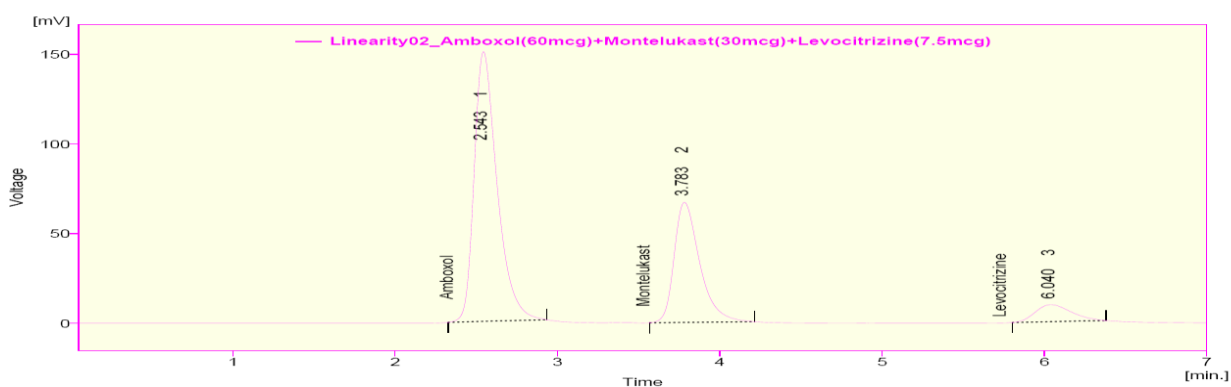
Result Table (Uncal - Linearity01_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.537	1161.176	115.667	63.32
2	3.763	551.668	52.335	30.08
3	6.020	120.949	7.503	6.60
Total		1833.793	175.505	100.00

Column Performance Table (From 50% - Linearity01_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/1 [t.p./m]	Resolution [-]
1	2.537	0.153	1.706	1516	15162	-
2	3.763	0.160	1.771	3065	30649	4.607
3	6.020	0.257	1.571	3048	30476	6.374

Fig 3.4: Chromatogram of Ambroxol, Montelukast and Levocetirizine preparation-1.



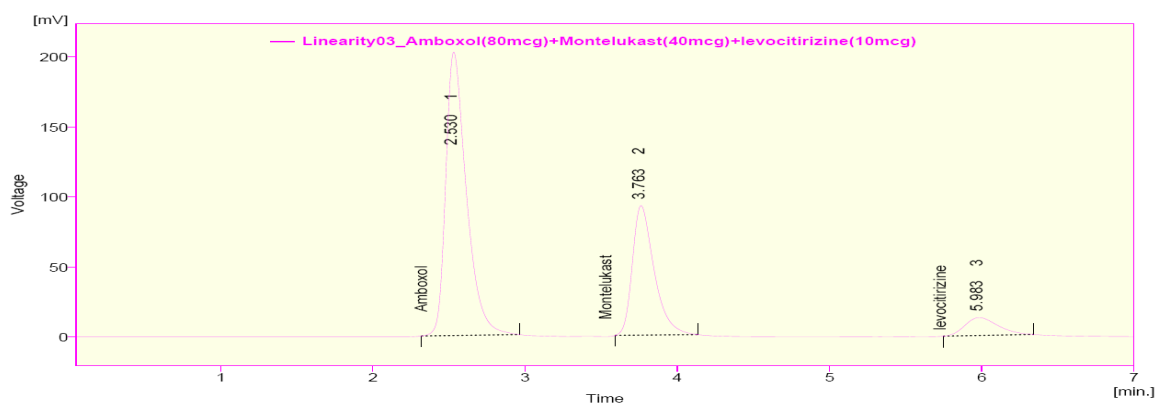
Result Table (Uncal - Linearity02_Ambroxol(60mcg)+Montelukast(30mcg)+Levocetirizine(7.5mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.543	1479.914	150.505	62.76
2	3.783	750.005	96.400	31.02
3	6.040	159.637	9.525	6.22
Total		2293.546	227.035	100.00

Column Performance Table (From 50% - Linearity02_Ambroxol(60mcg)+Montelukast(30mcg)+Levocetirizine(7.5mcg)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/1 [t.p./m]	Resolution [-]
1	2.543	0.150	1.781	1593	15927	-
2	3.783	0.163	1.750	2972	29724	4.657
3	6.040	0.250	1.500	3234	32337	6.425

Fig 3.5: Chromatogram of Ambroxol, Montelukast and Levocetirizine preparation-2.



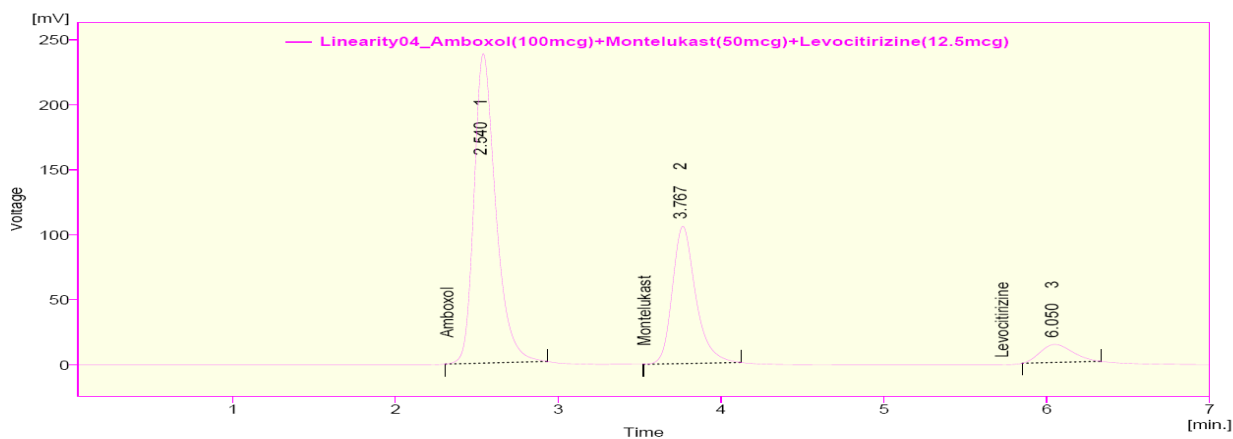
Result Table (Uncal - Linearity03_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.530	1915.883	202.585	63.21
2	3.763	931.185	92.636	30.39
3	5.983	194.041	12.828	6.40
Total		3031.109	308.049	100.00

Column Performance Table (From 50% - Linearity03_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.530	0.143	1.931	1726	17261	-
2	3.763	0.150	1.758	3487	34872	4.948
3	5.983	0.243	1.528	3350	33496	6.642

Fig 3.6: Chromatogram of Ambroxol, Montelukast and Levocetirizine preparation-3.



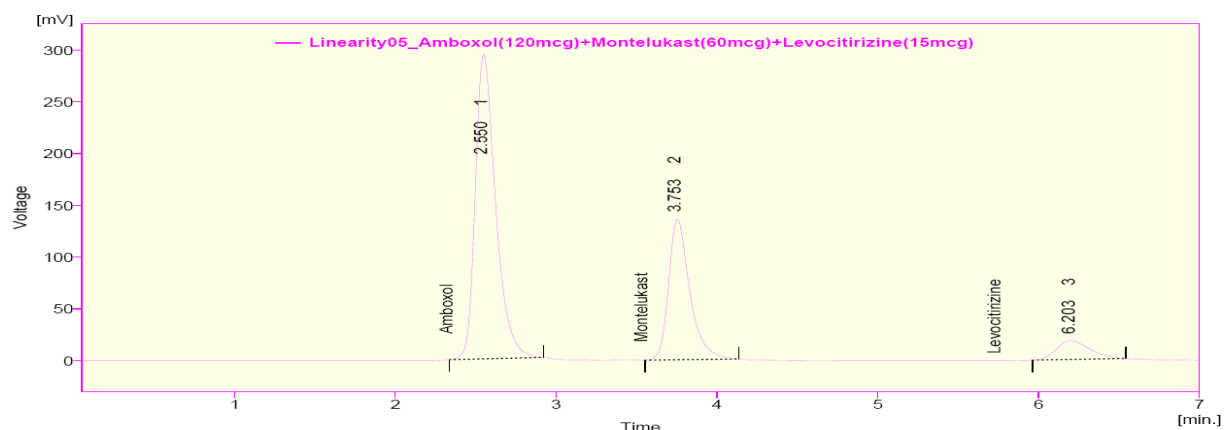
Result Table (Uncal - Linearity04_Ambroxol(100mcg)+Montelukast(50mcg)+Levocetirizine(12.5mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.540	2215.446	238.081	64.05
2	3.767	1071.398	105.604	30.40
3	6.050	232.171	13.912	5.56
Total		3459.016	357.597	100.00

Column Performance Table (From 50% - Linearity04_Ambroxol(100mcg)+Montelukast(50mcg)+Levocetirizine(12.5mcg)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.540	0.143	1.545	1740	17397	-
2	3.767	0.150	1.421	3493	34933	4.921
3	6.050	0.227	1.380	3947	39468	7.134

Fig 3.7: Chromatogram of Ambroxol, Montelukast and Levocetirizine preparation-4.



Result Table (Uncal - Linearity05_Ambroxol(120mcg)+Montelukast(60mcg)+Levocetirizine(15mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.550	2592.470	294.226	63.23
2	3.753	1246.308	135.866	30.40
3	6.203	261.464	18.250	6.38
Total		4100.243	448.341	100.00

Column Performance Table (From 50% - Linearity05_Ambroxol(120mcg)+Montelukast(60mcg)+Levocetirizine(15mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.550	0.137	1.667	1929	19287	-
2	3.753	0.137	1.656	4178	41785	5.181
3	6.203	0.227	1.481	4149	41494	7.936

Fig 3.8: Chromatogram of Ambroxol, Montelukast and Levocetirizine preparation-5.

Table 4: Linearity Preparations.

Preparations	Volume from standard stock transferred in ml			Volume made up in ml (with mobile phase)	Concentration of solution (µg /ml)		
					Ambroxol	Montelukast	Levocetirizine
Preparation 1	1	0.5	0.12	10	40	20	5
Preparation 2	1.5	0.75	0.18	10	60	30	7.5
Preparation 3	2	1	0.25	10	80	40	10
Preparation 4	2.5	1.25	0.31	10	100	50	12.5
Preparation 5	3	1.5	0.375	10	120	60	15

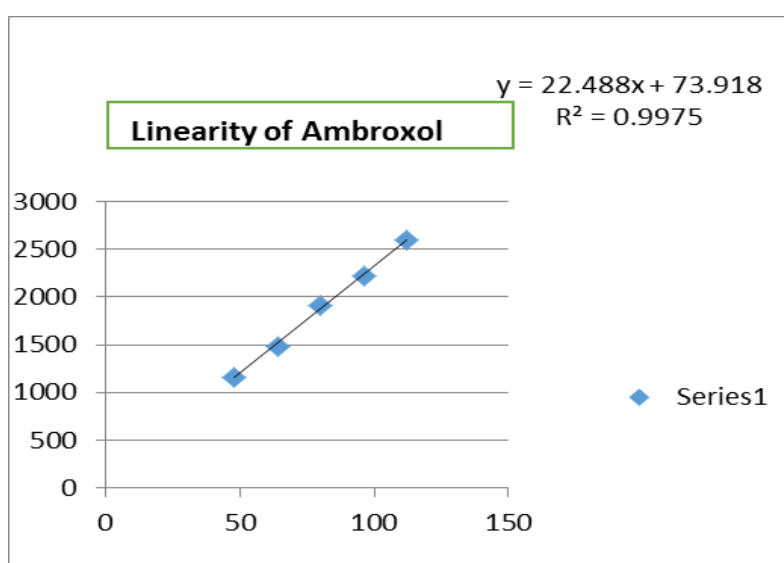


Fig 4.1: Linearity graph of Ambroxol.

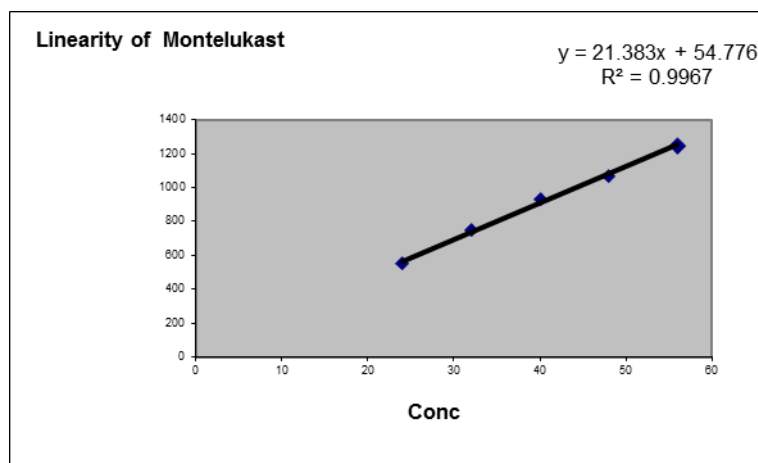


Fig. 4.2: Linearity graph of Montelukast.

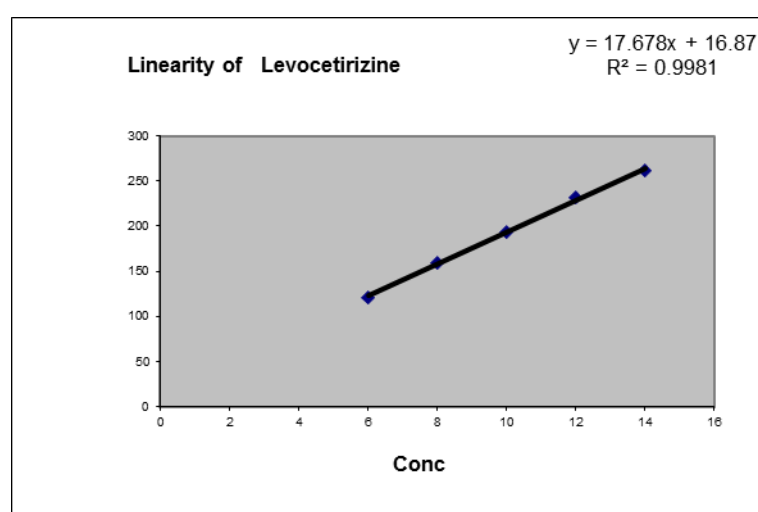


Fig. 4.3: Linearity graph of Levocetirizine.

Acceptance criteria

The relationship between the concentration of Ambroxol, Montelukast and Levocetirizine and area of Ambroxol, Montelukast and Levocetirizine should be linear in the specified range and the correlation should not be less than 0.99.

Observation

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Ambroxol, Montelukast and Levocetirizine is 0.997, 0.996 and 0.998. The relationship between the concentration of Ambroxol, Montelukast and Levocetirizine and area of Ambroxol, Montelukast and Levocetirizine is linear in the range examined since all

points lie in a straight line and the correlation coefficient is well within limits.

3. Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% & 150%.

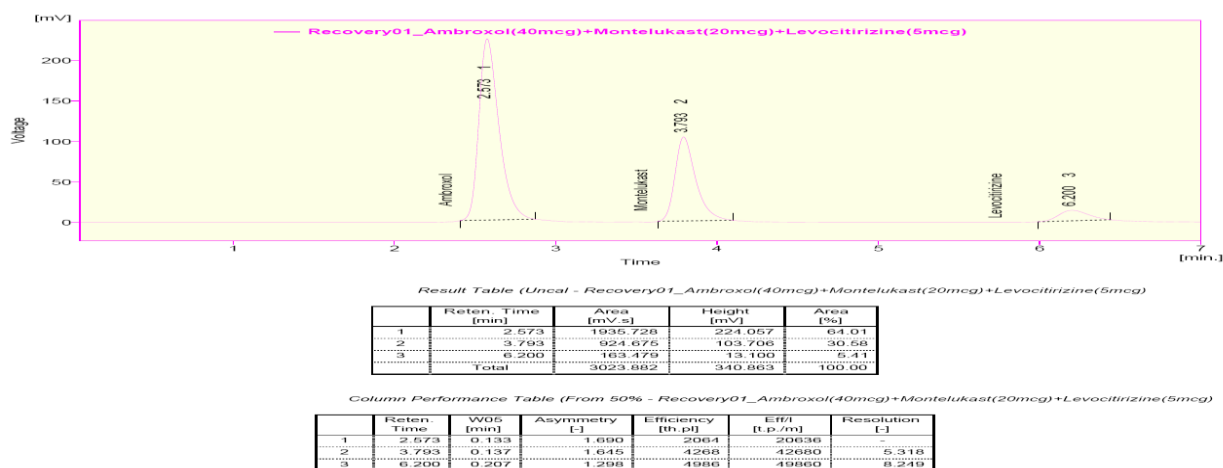


Fig 5.1: Chromatogram of 50% recovery (injection 1).

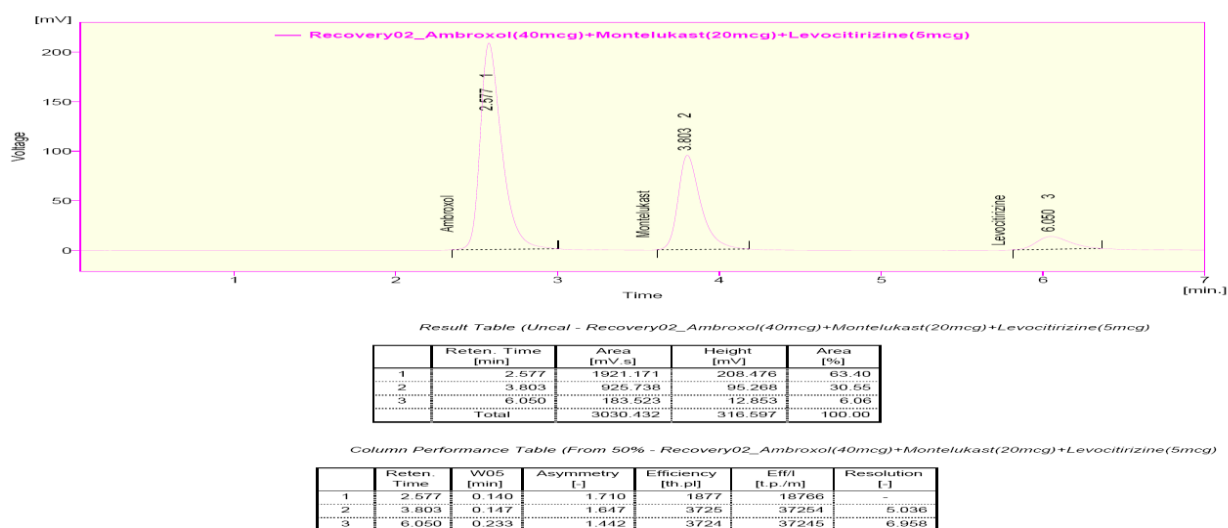


Fig 5.2: Chromatogram of 50% recovery (injection 2).

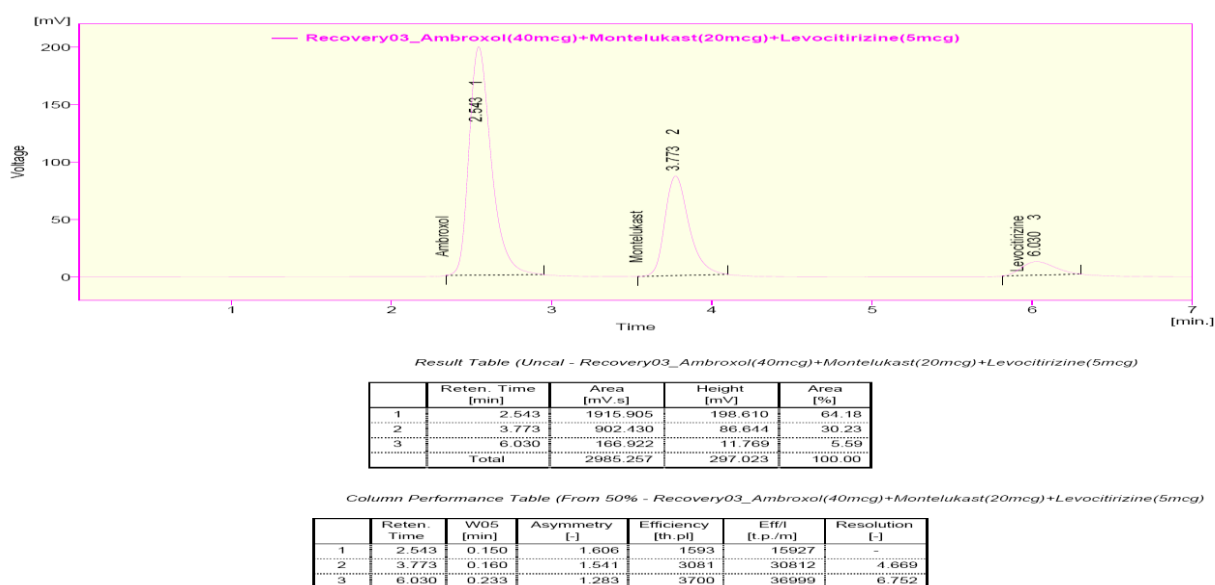
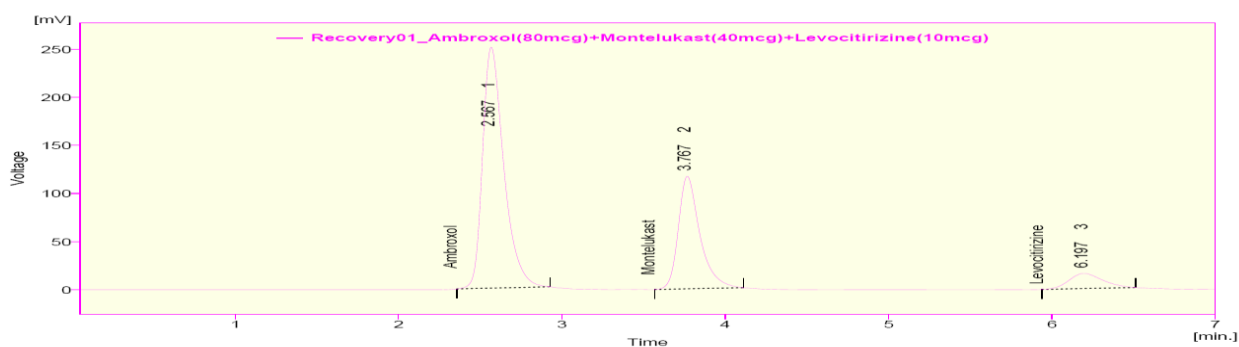


Fig 5.3: Chromatogram of 50% recovery (injection3).



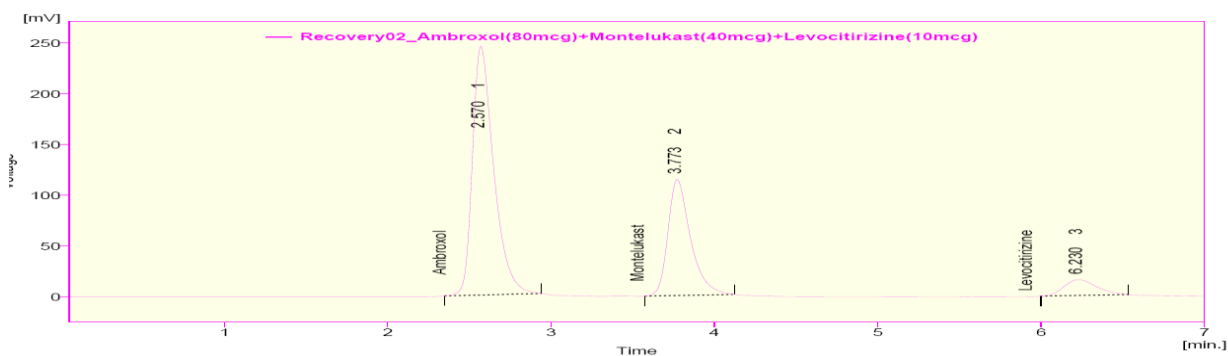
Result Table (Uncal - Recovery01_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.567	2261.821	250.403	63.59
2	3.767	1071.062	116.976	30.11
3	6.197	223.917	15.690	6.30
Total		3556.800	383.069	100.00

Column Performance Table (From 50% - Recovery01_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.567	0.137	1.733	1954	19540	-
2	3.767	0.137	1.688	4208	42082	5.167
3	6.197	0.230	1.415	4021	40213	7.799

Fig 5.4: Chromatogram of 100% recovery (injection 1).



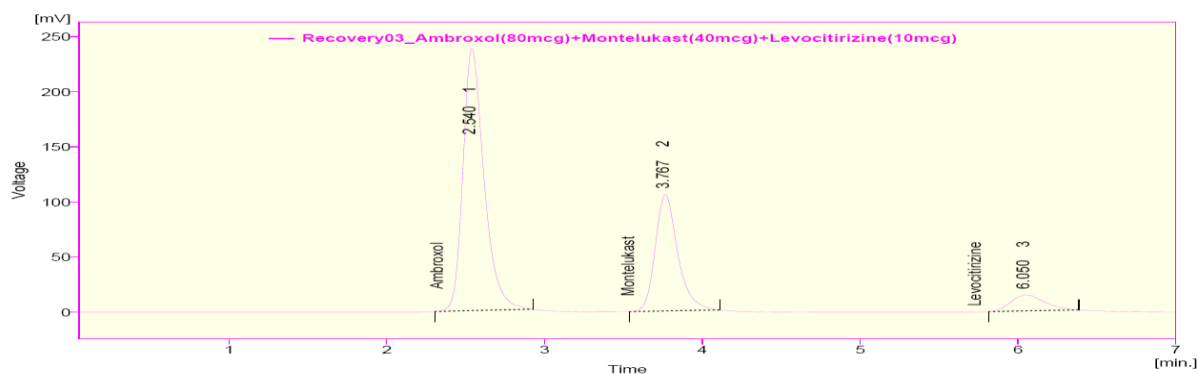
Result Table (Uncal - Recovery02_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.570	2116.645	245.399	62.86
2	3.773	1101.370	114.559	31.09
3	6.230	180.373	15.573	6.05
Total		3542.389	375.532	100.00

Column Performance Table (From 50% - Recovery02_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.570	0.137	1.828	1959	19591	-
2	3.773	0.143	1.697	3839	38394	5.058
3	6.230	0.220	1.420	4443	44426	7.957

Fig 5.5: Chromatogram of 100% recovery (injection 2).



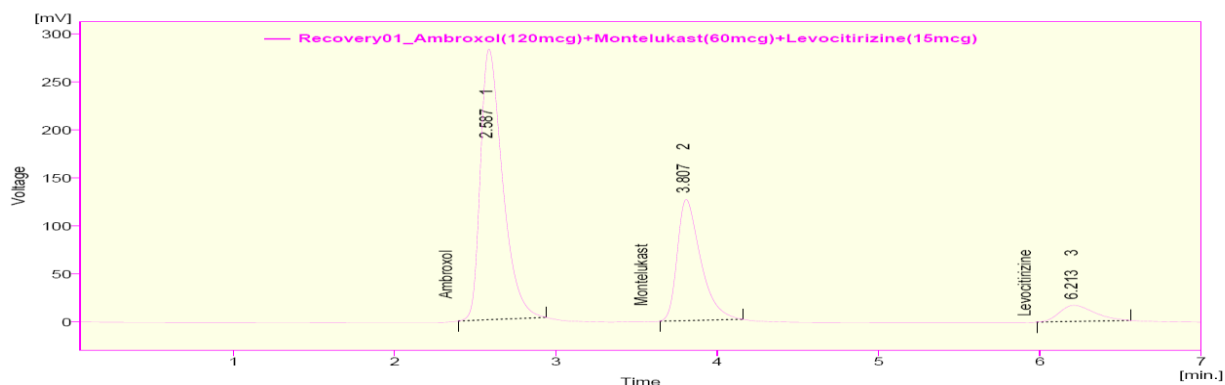
Result Table (Uncal - Recovery03_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.540	2212.172	238.016	63.66
2	3.767	1047.547	105.511	30.14
3	6.050	190.394	14.628	6.20
Total		3470.114	358.154	100.00

Column Performance Table (From 50% - Recovery03_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.540	0.143	1.545	1740	17397	-
2	3.767	0.150	1.432	3493	34933	4.921
3	6.050	0.237	1.453	3620	36203	6.950

Fig 5.6: Chromatogram of 100% recovery (injection 3).



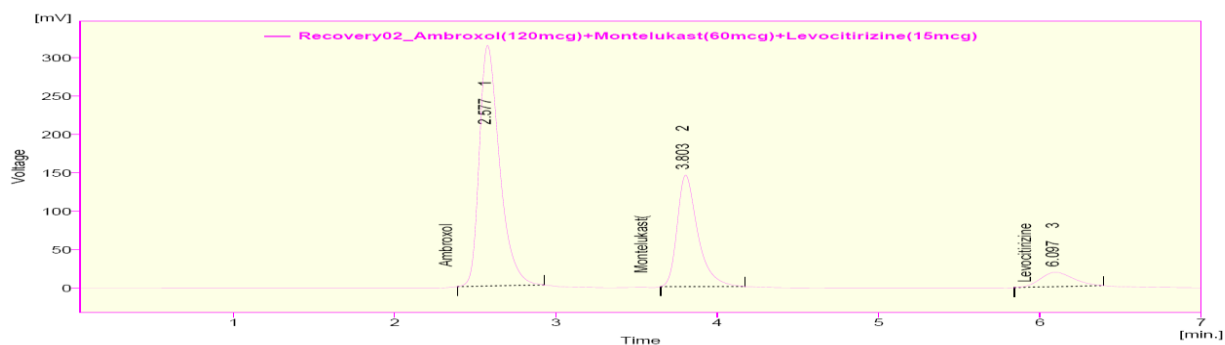
Result Table (Uncal - Recovery01_Ambroxol(120mcg)+Montelukast(60mcg)+Levocetirizine(15mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.587	2695.715	281.943	63.89
2	3.807	1272.049	126.183	30.15
3	6.213	90.577	16.822	5.96
Total		4219.340	424.947	100.00

Column Performance Table (From 50% - Recovery01_Ambroxol(120mcg)+Montelukast(60mcg)+Levocetirizine(15mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.587	0.147	1.867	1723	17232	-
2	3.807	0.153	1.968	3414	34145	4.786
3	6.213	0.240	1.608	3713	37131	7.201

Fig 5.7: Chromatogram of 150% recovery (injection 1).



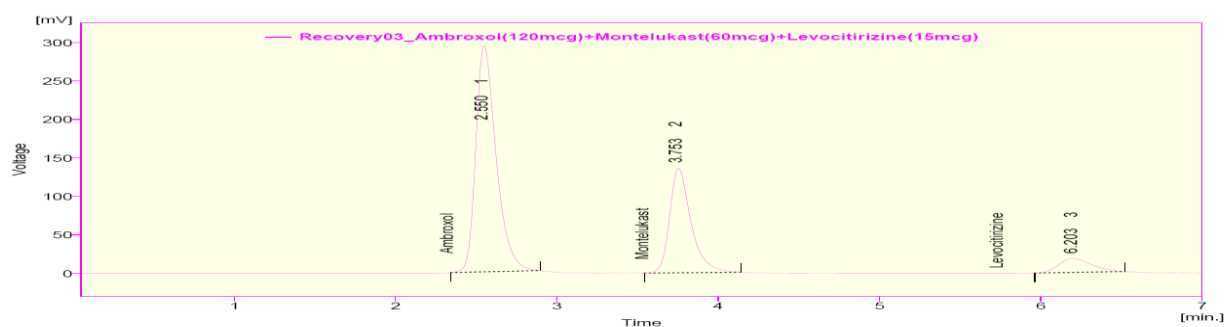
Result Table (Uncal - Recovery02_Ambroxol(120mcg)+Montelukast(60mcg)+Levocetirizine(15mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.577	2744.932	314.016	63.82
2	3.803	1250.244	145.407	30.00
3	6.097	92.681	18.791	6.18
Total		4300.856	478.214	100.00

Column Performance Table (From 50% - Recovery02_Ambroxol(120mcg)+Montelukast(60mcg)+Levocetirizine(15mcg))

	Reten. Time [min]	W05 [-]	Asymmetry [-]	Efficiency [th.pl]	Eff/1 [t.p./m]	Resolution [-]
1	2.577	0.133	1.724	2069	20689	-
2	3.803	0.133	1.733	4508	45078	5.414
3	6.097	0.230	1.340	3893	38926	7.428

Fig 5.8: Chromatogram of 150% recovery (injection 2).



Result Table (Uncal - Recovery03_Ambroxol(120mcg)+Montelukast(60mcg)+Levocetirizine(15mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.550	280.932	293.900	63.10
2	3.753	1248.559	135.918	30.58
3	6.203	94.863	18.113	6.27
Total		4083.353	447.932	100.00

Column Performance Table (From 50% - Recovery03_Ambroxol(120mcg)+Montelukast(60mcg)+Levocetirizine(15mcg))

	Reten. Time [min]	W05 [-]	Asymmetry [-]	Efficiency [th.pl]	Eff/1 [t.p./m]	Resolution [-]
1	2.550	0.137	1.667	1929	19287	-
2	3.753	0.137	1.656	4178	41785	5.181
3	6.203	0.227	1.423	4149	41494	7.936

Fig 5.9: Chromatogram of 150% recovery (injection 3).

Table 5.1: Acceptance criteria: The % recovery of Ambroxol, Montelukast and Levocetirizine should lie between 98% and 102%. Recovery results for Ambroxol.

Recovery level	Accuracy Ambroxol					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	% of Recovery	% of Mean Recovery	
50%	40	1935.728	1924.127	100.4066539	99.81222113	100.38
	40	1921.171		99.65157903		
	40	1915.905		99.37843041		
100%	80	2261.821	2233.55	105.5890552	104.2690875	
	80	2226.645		103.9469267		
	80	2212.172		103.2712807		
150%	120	2695.715	2673.19	97.87914738	97.06113831	
	120	2744.932				
	120	2578.911				

Table 5.2: Recovery results for Montelukast.

Table 5.2. Recovery Results for Montelukast.						
Recovery level	Accuracy Montelukast					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	%of Recovery	% of mean Recovery	
50%	20	924.675	917.614	100.7148548	102.83	100.43
	20	925.738		100.8306359		
	20	902.43		98.29194737		
100%	40	1101.37	1083.43	119.9603316	99.36	
	40	1101.37		119.9603316		
	40	1047.547		114.0979739		
150%	60	1272.049	1270.28	96.9853854	99.10	
	60	1290.244				
	60	1248.559				

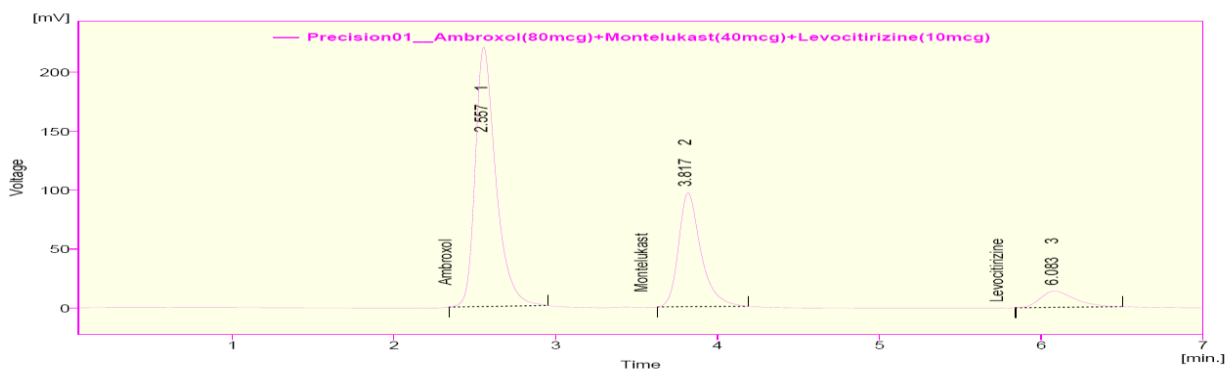
Table 5.3.

Recovery level	Accuracy Levocetirizine					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	%Recovery	% of mean Recovery	
50%	5	163.479	171.308	89.34824788	93.62713038	102.04
	5	183.523		100.303149		
	5	166.922		91.22999426		
100%	10	193.917	192.895	102.9839134	102.4251646	
	10	194.373		100.2331369		
	10	190.394		101.0584436		
150%	15	100.577	110.09	109.9392427	110.09	
	15	100.681				
	15	100.893				

Observation

The percentage mean recovery of Ambroxol,

Montelukast and Levocetirizine is 100.38%, 100.43% and 102.04% respectively.

4. Precision

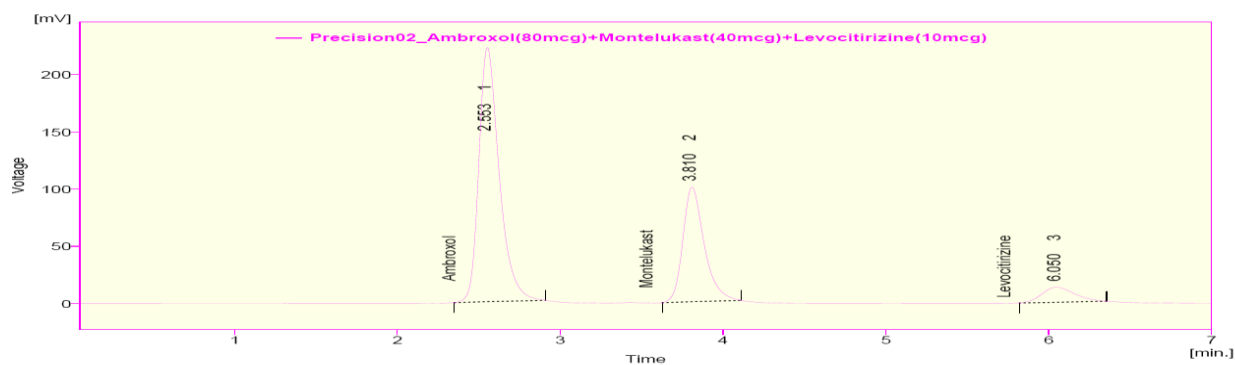
Result Table (Uncal - Precision01__Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.557	1961.318	220.058	63.42
2	3.817	926.701	96.643	29.97
3	6.083	204.535	13.773	6.61
Total		3092.554	330.474	100.00

Column Performance Table (From 50% - Precision01__Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/1 [t.p./m]	Resolution [-]
1	2.557	0.133	1.645	2037	20370	-
2	3.817	0.143	1.618	3928	39281	5.360
3	6.083	0.230	1.740	3876	38756	7.145

Fig 6.1: Chromatogram of precision injection 1.



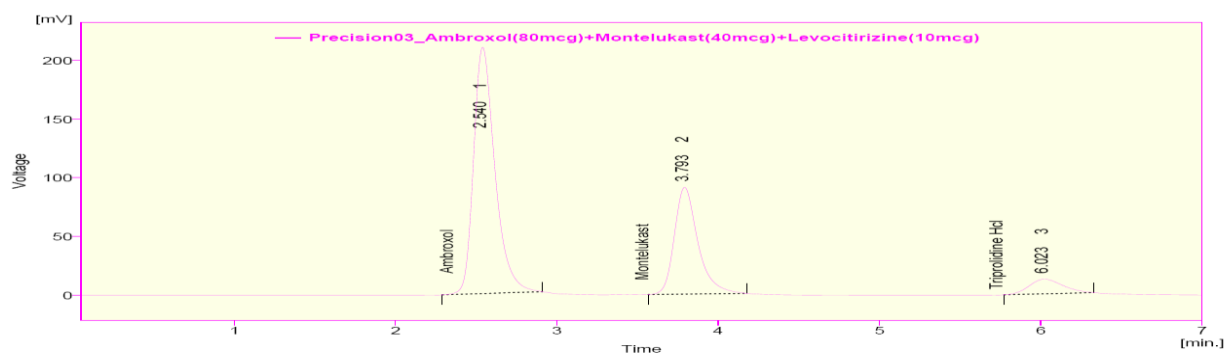
Result Table (Uncal - Precision02_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.553	1930.566	222.169	63.78
2	3.810	913.004	100.105	30.16
3	6.050	183.133	13.169	6.05
Total		3026.703	335.443	100.00

Column Performance Table (From 50% - Precision02_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.553	0.133	1.633	2032	20316	-
2	3.810	0.140	1.545	4103	41030	5.411
3	6.050	0.223	1.385	4065	40655	7.256

Fig 6.2: Chromatogram of precision injection 2.



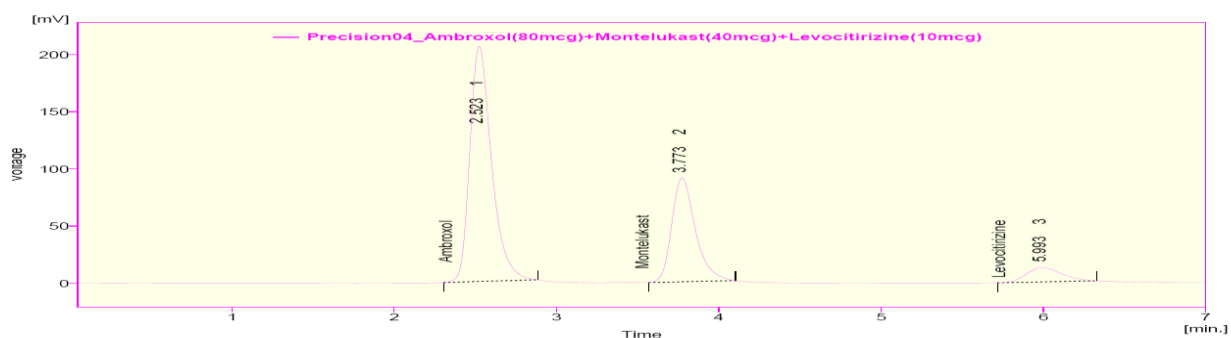
Result Table (Uncal - Precision03_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.540	1934.385	209.949	63.68
2	3.793	923.268	91.048	30.39
3	6.023	180.068	12.471	5.93
Total		3037.721	313.468	100.00

Column Performance Table (From 50% - Precision03_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.540	0.140	1.594	1824	18236	-
2	3.793	0.150	1.541	3543	35430	5.086
3	6.023	0.233	1.327	3692	36917	6.846

Fig 6.3: Chromatogram of precision injection 3.



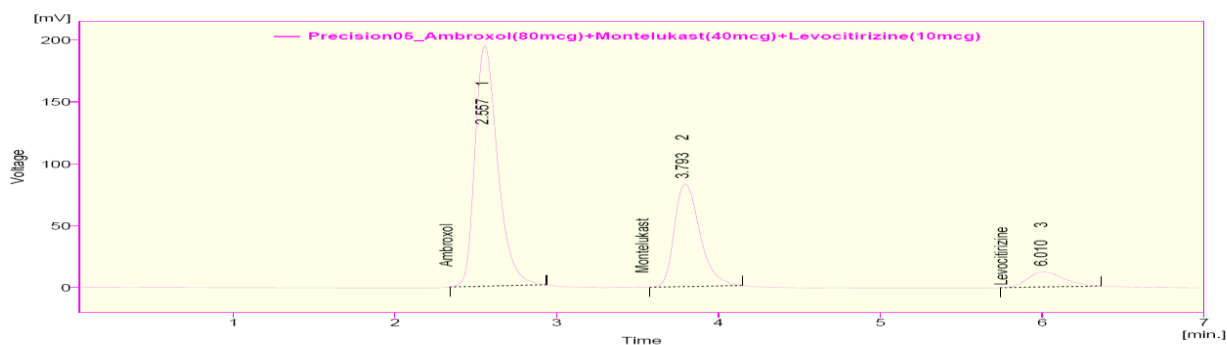
Result Table (Uncal - Precision04_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.523	1919.778	205.958	63.42
2	3.773	917.788	90.904	30.32
3	5.993	189.338	12.668	6.26
Total		3026.904	309.530	100.00

Column Performance Table (From 50% - Precision04_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.523	0.143	1.625	1717	17170	-
2	3.773	0.153	1.556	3355	33550	4.959
3	5.993	0.237	1.393	3553	35528	6.699

Fig 6.4: Chromatogram of precision injection 4.



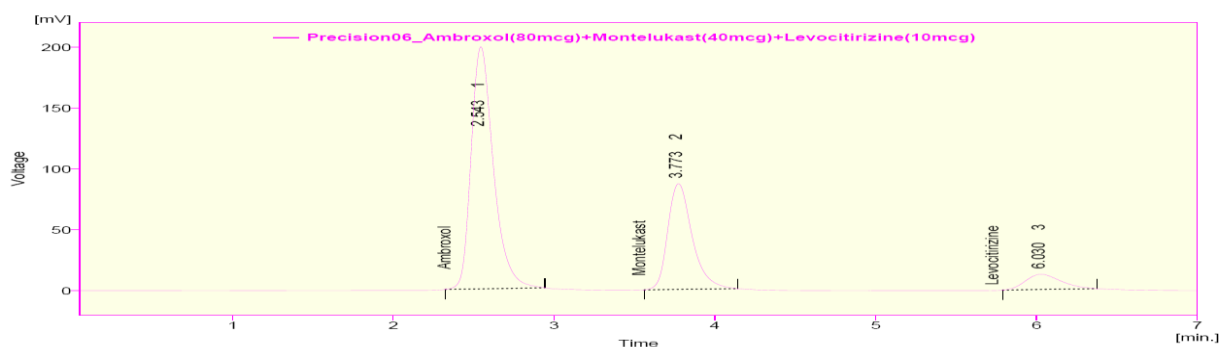
Result Table (Uncal - Precision05_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.557	1900.881	194.739	63.28
2	3.793	912.760	82.797	30.39
3	6.010	190.221	12.262	6.33
Total		3003.862	289.798	100.00

Column Performance Table (From 50% - Precision05_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.557	0.153	1.529	1540	15402	-
2	3.793	0.170	1.622	2758	27584	4.501
3	6.010	0.247	1.473	3289	32888	6.261

Fig 6.5: Chromatogram of precision injection 5.



Result Table (Uncal - Precision06_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.543	1920.401	198.814	63.46
2	3.773	915.150	86.915	30.24
3	6.030	190.515	12.447	6.30
Total		3026.066	298.177	100.00

Column Performance Table (From 50% - Precision06_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg))

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.543	0.150	1.606	1593	15927	-
2	3.773	0.160	1.568	3081	30812	4.669
3	6.030	0.243	1.455	3402	34021	6.585

Fig 6.6: Chromatogram of precision injection 6.

Table 6: Results for Method precision of Ambroxol, Montelukast and Levocetirizine

Ambroxol			Montelukast		
S.No.	Rt	Area	S.No.	Rt	Area
1	2.557	1961.318	1	3.817	926.701
2	2.553	1930.566	2	3.810	913.004
3	2.540	1934.385	3	3.793	923.268
4	2.523	1919.778	4	3.773	917.788
5	2.557	1900.881	5	3.793	912.76
6	2.543	1920.401	6	3.773	915.150
avg	2.5455	1927.888	avg	3.793	918.112
stdev	0.0131	20.091	stdev	0.018	5.727
%RSD	0.52	1.04	%RSD	0.48	0.62

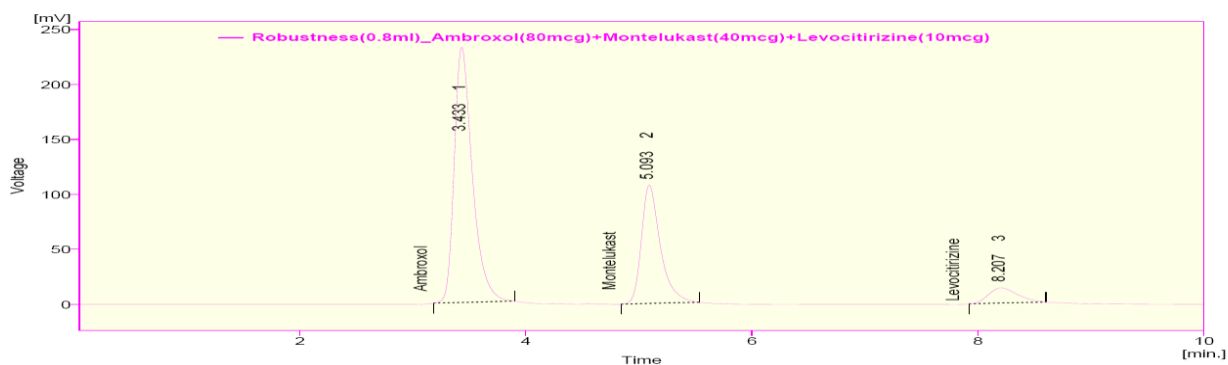
Levocetirizine		
S.No.	Rt	Area
1	6.083	204.535
2	6.05	183.133
3	6.023	180.068
4	5.993	189.338
5	6.01	190.221
6	6.03	190.515
Avg	6.0315	182.9683
Stdev	0.028906	3.292234
%RSD	0.479258	1.799346

Observation

Test results for Ambroxol, Montelukast and

Levocetirizine are showing that the %RSD of Assay results are within limits.

5. Robustness



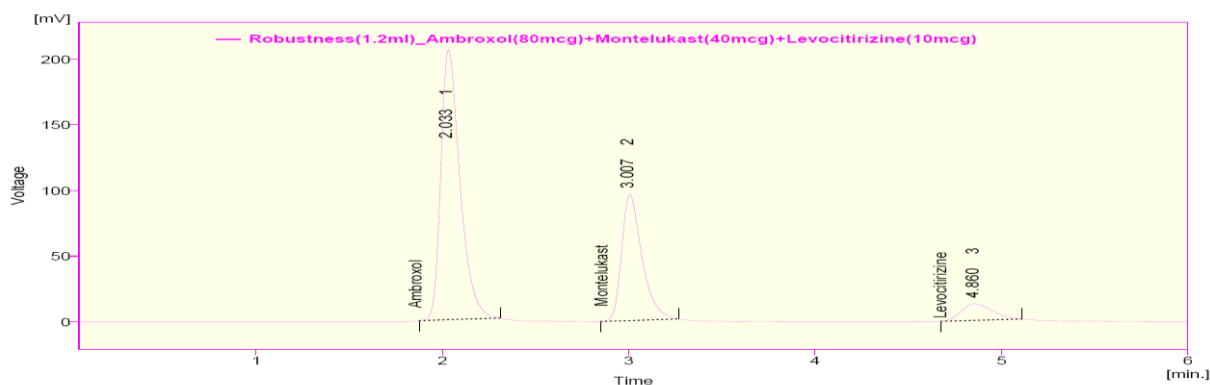
Result Table (Uncal - Robustness(0.8ml)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	3.433	2589.112	232.116	63.33
2	5.093	1253.362	107.663	30.66
3	8.207	245.552	13.787	6.01
	Total	4088.026	353.565	100.00

Column Performance Table (From 50% - Robustness(0.8ml)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	3.433	0.170	1.684	2260	22597	-
2	5.093	0.173	1.659	4784	47835	5.690
3	8.207	0.287	1.492	4540	45403	7.965

Fig 7.1: Chromatogram of Ambroxol, Montelukast and Levocetirizine for Robustness (0.8 ml/min).



Result Table (Uncal - Robustness(1.2ml)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.033	1468.878	205.691	62.66
2	3.007	729.939	95.999	31.14
3	4.860	145.480	12.581	6.21
	Total	2344.297	314.271	100.00

Column Performance Table (From 50% - Robustness(1.2ml)_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.033	0.110	1.708	1893	18930	-
2	3.007	0.117	1.731	3679	36795	5.054
3	4.860	0.190	1.429	3625	36247	7.112

Fig 7.2: Chromatogram of Ambroxol, Montelukast and Levocetirizine for Robustness (1.2 ml/min).

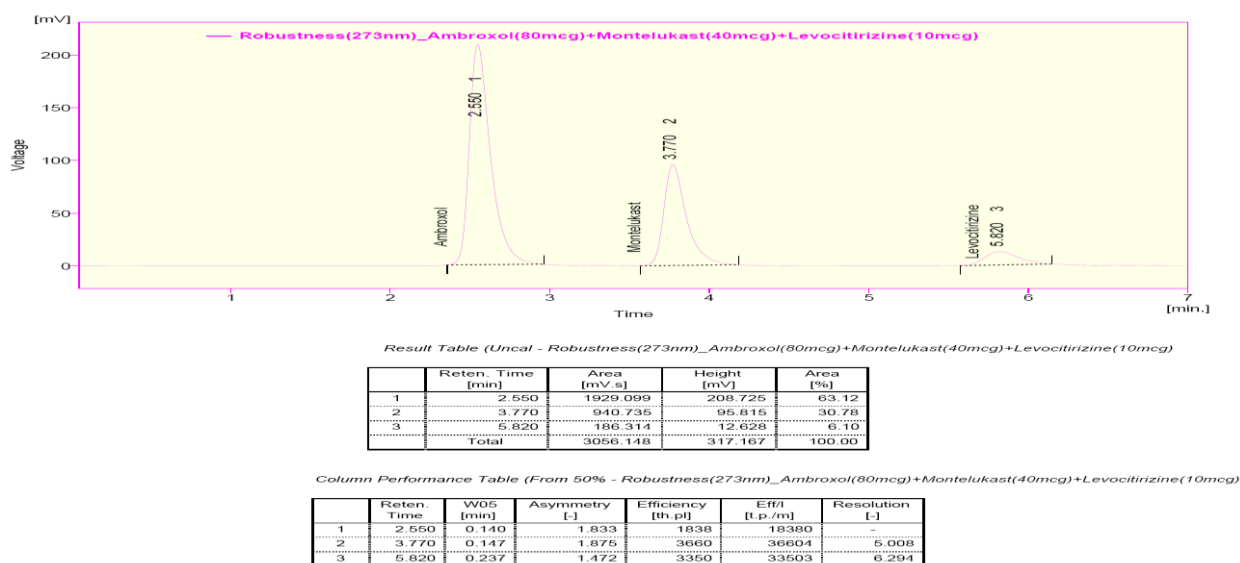


Fig 7.3: Chromatogram of Ambroxol, Montelukast and Levocetirizine for Robustness (273nm).

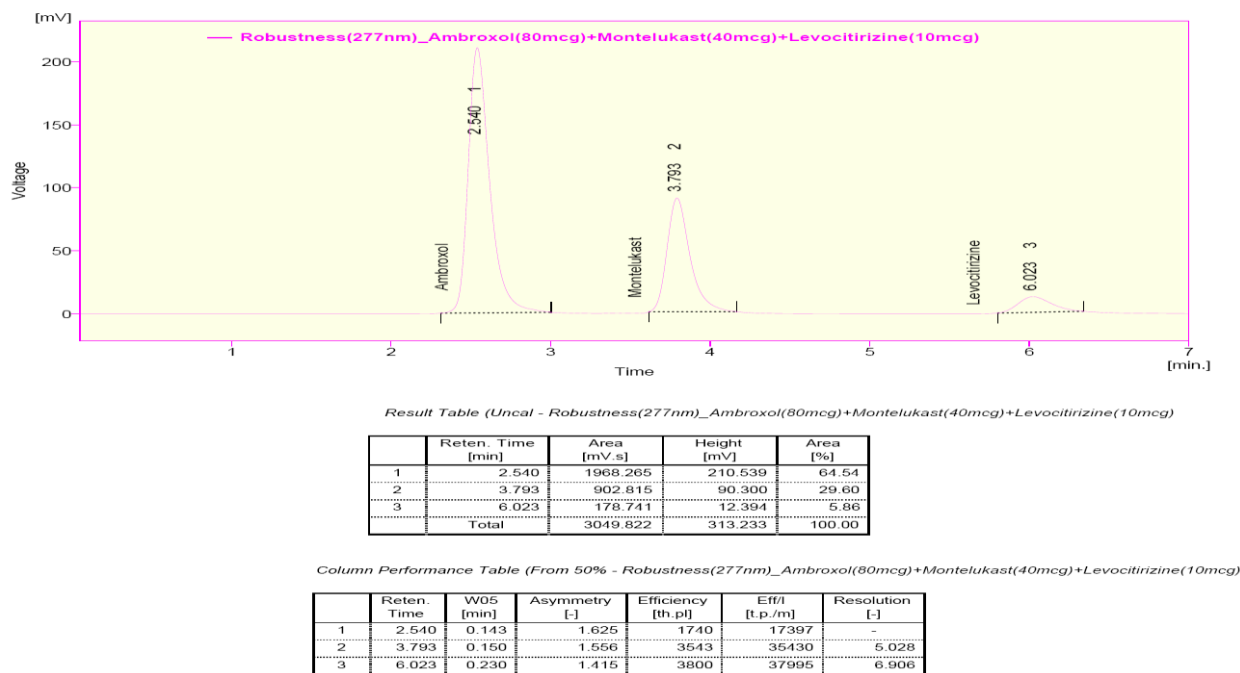


Fig 7.4: Chromatogram of Ambroxol, Montelukast and Levocetirizine for Robustness (277nm).

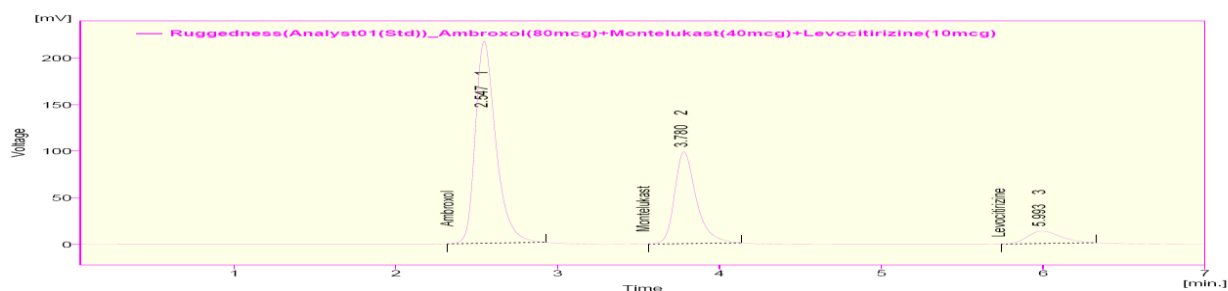
Table 7: Result of Robustness study.

Parameter	Ambroxol		Montelukast		Levocetirizine	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow						
0.8ml/min	3.433	1.684	5.093	1.659	8.207	1.492
1.2ml/min	2.033	1.708	3.007	1.731	4.860	1.429
Wavelength						
273nm	2.550	1.833	3.770	1.875	5.820	1.472
277nm	2.540	1.625	3.793	1.556	6.023	1.415

Observation

From the observation it was found that the system

suitability parameters were within limit at all variable conditions.

6. Ruggedness

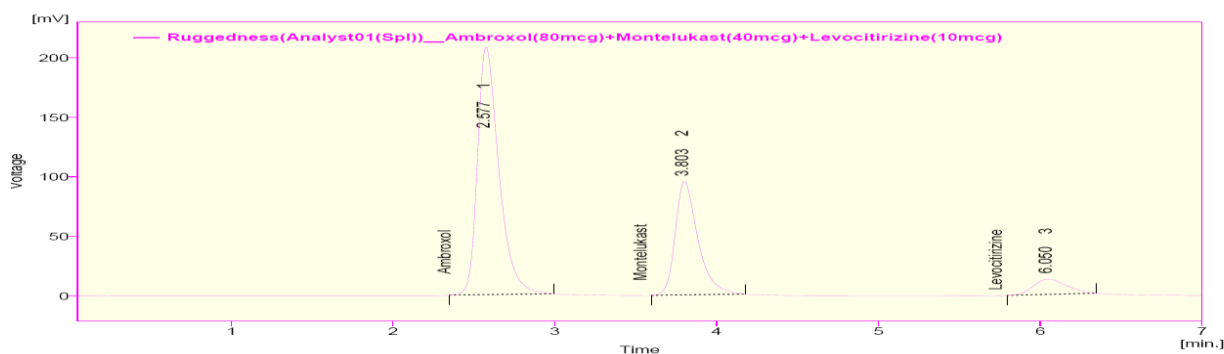
Result Table (Uncal - Ruggedness(Analyst01(Std))_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.547	1917.776	216.981	63.38
2	3.780	916.553	98.683	30.29
3	5.993	131.743	13.361	6.34
Total		3026.072	329.025	100.00

Column Performance Table (From 50% - Ruggedness(Analyst01(Std))_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	W05 [-]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.547	0.133	1.581	2021	20210	-
2	3.780	0.140	1.576	4039	40387	5.310
3	5.993	0.227	1.481	3873	38732	7.104

Fig 8.1: Chromatogram of Analyst 01 standard preparation.



Result Table (Uncal - Ruggedness(Analyst01(Spl))_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.577	1917.807	208.416	63.37
2	3.803	926.010	95.387	30.66
3	6.050	189.686	12.786	5.97
Total		3026.505	316.592	100.00

Column Performance Table (From 50% - Ruggedness(Analyst01(Spl))_Ambroxol(80mcg)+Montelukast(40mcg)+Levocetirizine(10mcg)

	Reten. Time [min]	W05 [-]	Asymmetry [-]	Efficiency [th.pl]	Eff/I [t.p./m]	Resolution [-]
1	2.577	0.140	1.677	1877	18766	-
2	3.803	0.147	1.647	3725	37254	5.036
3	6.050	0.227	1.385	3947	39468	7.082

Fig 8.2: Chromatogram of Analyst 01 sample preparation.

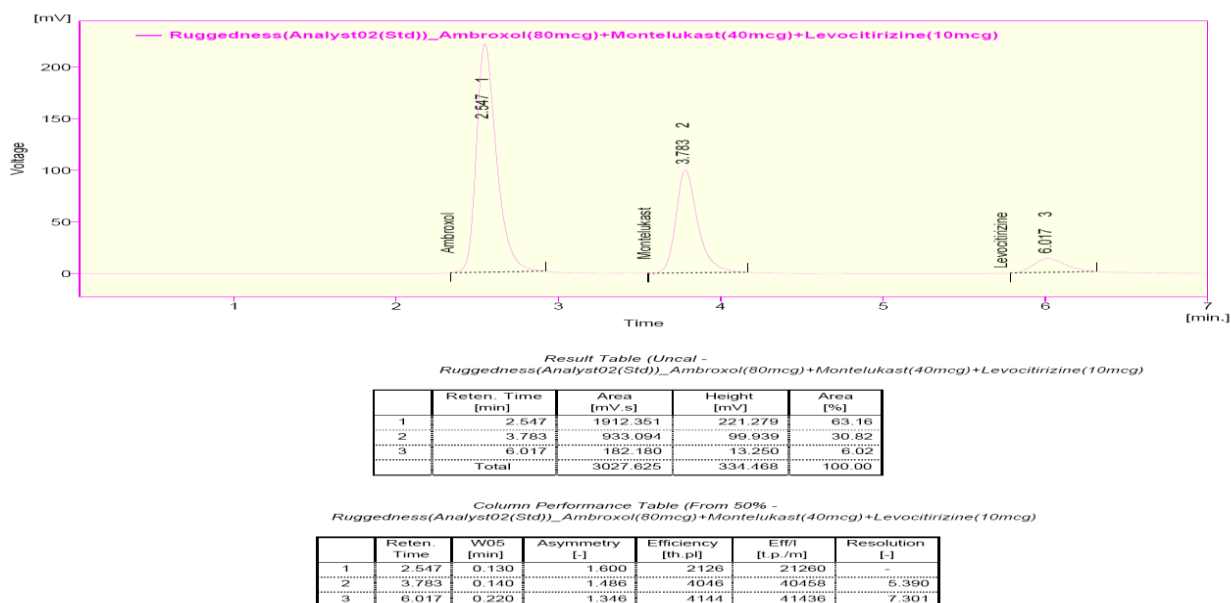


Fig 8.3: Chromatogram of Analyst 02 standard preparation.

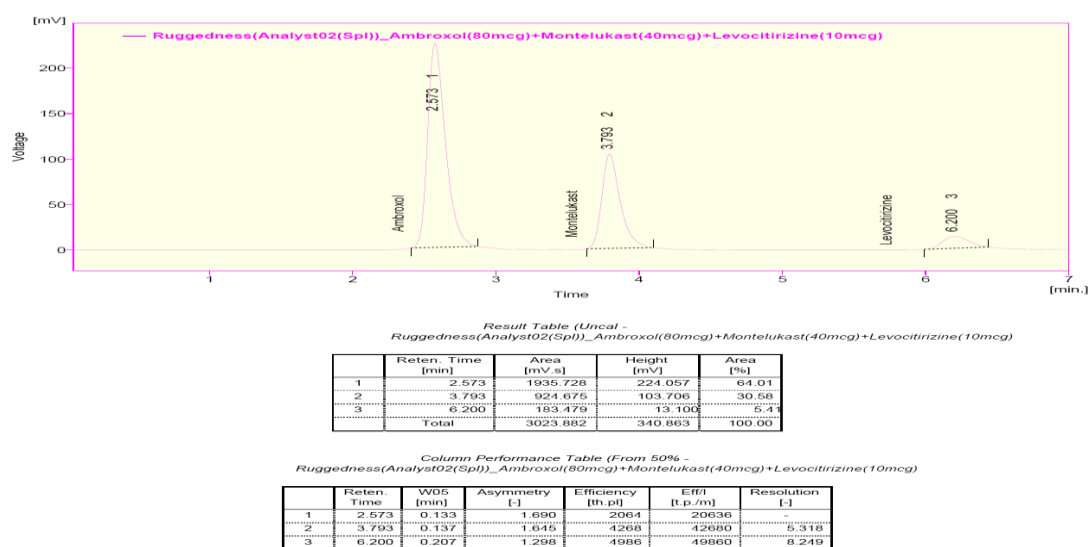


Fig 8.4: Chromatogram of Analyst 02 sample preparation.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts.

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

Table 8: Results for Ruggedness.

Ambroxol	%Assay	Montelukast	%Assay	Levocetirizine	%Assay
Analyst 01	100.0016	Analyst 01	101.2500095	Analyst 01	99.4497843
Analyst 02	101.2224	Analyst 02	99.0977329	Analyst 02	100.9166301
%RSD	0.857989	%RSD	0.759623932	%RSD	0.70354313

Observation

From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

7. Limit of Detection

$$LOD = \frac{3.3\sigma}{S}$$

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.

Observation:

The LOD for this method was found to be 24.19 µg/ml of Ambroxol

The LOD for this method was found to be 18.85 µg/ml of Montelukast

The LOD for this method was found to be 7.23 µg/ml of Levocetirizine

8. Limit of Quantification

$$LOQ = \frac{10\sigma}{S}$$

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.

Observation

The LOQ for this method was found to be 73.31 µg/ml for Ambroxol

The LOQ for this method was found to be 57.12 µg/ml for Montelukast

CONCLUSION

A simple and selective LC method was described for the determination of Melatonin and Zolpidem tartrate in tablet dosage forms. Chromatographic separation was achieved on a C₁₈ column using mobile phase consisting of a mixture of 50 volumes of Triethylamine and 50 volumes of Acetonitrile with detection of 280nm. Linearity was observed in the range 10-30 µg/ml for Melatonin (r² = 0.992) and 5-15 µg/ml for Zolpidem tartrate (r² = 0.995) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Ambroxol, Montelukast and Levocetirizine 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 industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic

studies in near future.

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