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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF FEXOFENADINE AND MONTELUKAST BY USING RP-UPLC

Christina Emette Jaldi [1] and Mangamma Kuna [*2]

School of Pharmaceutical Sciences, Jawaharlal Nehru Technological University, Kakinada-533003, East Godavari District, Andhra Pradesh, India.

Keywords:

Fexofenadine, RP-UPLC, Acetonitrile, Ortho Phosphoric acid

Correspondence to Author:
Mangamma Kuna

Assistant Professor, Department of Pharmaceutical Analysis, School of Pharmaceutical Sciences, Jawaharlal Nehru Technological University Kakinada, Kakinada-533003, East Godavari District, Andhra Pradesh, India.

E-mail: christinaemette@gmail.com

ABSTRACT: A simple, reliable, accurate, and precise method was developed for the simultaneous estimation of Fexofenadine and Montelukast in pharmaceutical dosage form. The chromatogram was run through Standard HSS C18 (2.1 × 100 mm, 1.8 µm). Mobile phase employed was 0.1% OPA Buffer and Acetonitrile taken in the ratio of 65:35 was pumped through column at a flow rate of 0.2 ml/min. The temperature was maintained at 30 °C. The optimized wavelength selected was 269 nm. The retention time of Montelukast and Fexofenadine was found to be 0.921 and 1.101 min. The Percent RSD of the Montelukast and Fexofenadine were and found to be 0.7% and 0.4% respectively. The Percent recovery was obtained as 99.22% and 99.67% for Montelukast and Fexofenadine respectively. The LOD and LOQ values obtained from regression equations of Montelukast and Fexofenadine were 0.06, 0.18 and 0.80, 2.44 respectively. Regression equation of Montelukast was y = 6333.8x + 755.96 and y = 4038.3x + 8964.8 of Fexofenadine. The decrease in the retention and run times in this method that has been developed was simple and economical that can be been easily adopted in regular Quality control test in industries.

INTRODUCTION:

Montelukast: Montelukast belong to a category of a potent and highly selective CysLT1 receptor antagonist, without demonstrated CysLT2 activity [1]. Montelukast, 2-[1-({(1R)-1-{3-[(E)-2-(7-chloro-quinolin-2yl) ethinyl] phenyl} 3-[2-(2-hydroxy-propan-2-yl) phenyl] propyl] sulfanyl} methyl) cyclopropyl] acetic acid is a leukotriene receptor antagonist (LTRA) used for the treatment of asthma and to alleviate symptoms of seasonal allergies [2].

QUICK RESPONSE CODE

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The molecular formula of Montelukast is $C_{35}H_{35}ClNO_3S.Na$ and the molecular weight is 608.17 g/mol. The usual mode of administration is rapidly absorbed orally with a bioavailability of 64%. The distribution volume ranges from 8-11L, with hepatic metabolism being occurred [3].

The mechanism of action involves blocking the action of leukotriene D4 on the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it. This reduces the bronchoconstriction otherwise caused by the leukotriene and leading to reduced inflammation. Due its method of operation, it is not useful for the treatment of acute asthma attacks but also does not interact with other allergy medications like theophylline. Montelukast has crystalline solid appearance and is soluble in ethanol (100 mM), DMSO (100 mM), methanol, water (>100 mg/ml at

25 °C) and DMF (~30 mg/ml) [4]. It is available in solid forms such as oral tablets, chewable tablets, and oral granules. Montelukast is used for the treatment of asthma [5]. Montelukast, like zafirlukast, is a leukotriene receptor antagonist used as an alternative for anti-inflammatory medications in the management and chronic treatment of asthma and exercise-induced bronchospasm (EIB). Montelukast does not affect by inhibiting enzymes like CYP2C9 or CYP3A4 which differentiates it from zafirlukast and therefore, does not affect the hepatic clearance of drugs metabolized by these enzymes. Side effects include headache, heartburn, tooth pain, sore throat, cough, stuffy nose, mild rash, fever, diarrhoea, stomach pain, nausea. The structure of Montelukast is represented in **Fig. 1**.

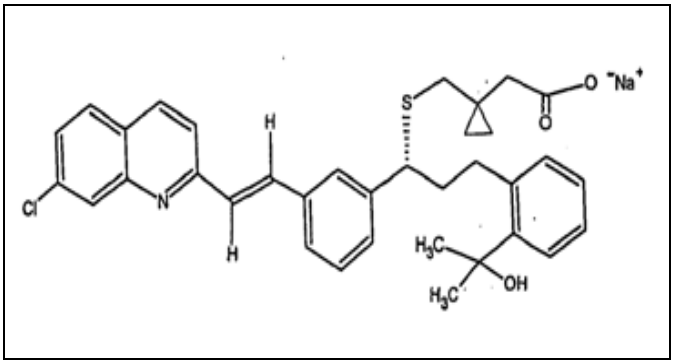


FIG. 1: STRUCTURE OF MONTELUKAST

Mechanism of Action: Montelukast acts by selectively antagonizing leukotriene D4 (LTD4) at the cysteinyl leukotriene receptor, CysLT1, in the human airway. The actions of leukotriene D4 is inhibited by Montelukast, which thereby prevents airway oedema, smooth muscle contraction, and enhanced secretion of thick, viscous mucus [6].

Fexofenadine: Fexofenadine belongs to a category of an antihistamine that inhibits Cox-1, Cox-2 and is a histamine H1 receptor agonist [7]. Fexofenadine, 2-(4-{1-hydroxy-4-[4-(hydroxyl diphenyl methyl) piperadin-1-yl] butyl} phenyl)-2-methyl propanoic acid 8 has a solid appearance and is freely soluble in DMSO (50 mM), and methanol. The molecular formula of Fexofenadine is $C_{32}H_{39}NO_4$ HCl and its respective molecular weight is 538.12 g/mol. Fexofenadine hydrochloride (Allegra) is an antihistamine drug commonly used in the treatment of hayfever and similar allergic symptoms. It was developed as a core successor and alternative to terfenadine. The causative reason for Fexofenadine for less drowsiness than first-generation histamine-receptor antagonists is it does not readily pass through the blood-brain barrier, which is similar to second and third-generation antihistamines [9]. Fexofenadine is a long-lasting, second-generation H1-receptor antagonist (antihistamine) which has a selective and peripheral H1-antagonist action. Histamine is a chemical that causes many signs that are part of allergic reactions, like swelling of tissues. Histamine is released from mast cells (known as histamine-storing cells) and attaches to other cells that have receptors for histamine. The attachment of the histamine to these receptors causes the cell to be "activated", thereby releasing other chemicals which produce the effects that we associate with allergy. Unlike other antihistamines, Fexofenadine does not enter the blood-brain barrier and, therefore, not causing drowsiness. Since fexofenadine does not block the potassium channel involved in repolarization of cardiac cells, it lacks the cardiotoxic potential of terfenadine [10]. The side effects include nausea, diarrhoea, menstrual cramps, dizziness, drowsiness, dry mouth, headache, muscle or back pain [11]. The structure of Fexofenadine is represented in **Fig. 2**.

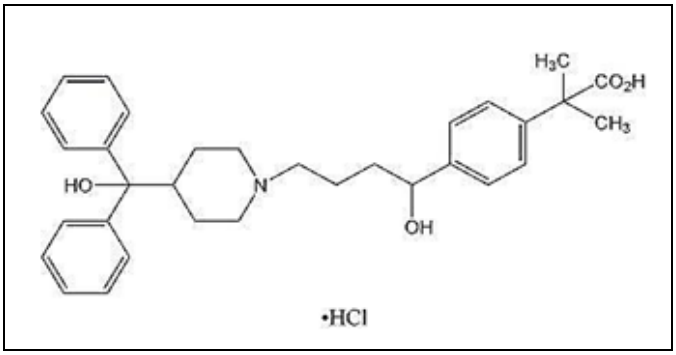


FIG. 2: STRUCTURE OF FEXOFENADINE

Mechanism of Action: Fexofenadine actively competes with free histamine for binding at H1-receptors in the GI tract, large blood vessels, and bronchial smooth muscles, and this action blocks the endogenous histamine, which in turn leads to temporary relief of the negative symptoms like nasal congestion, watery eyes brought on by histamine. Also, Fexofenadine does not have anti-dopaminergic, anti-cholinergic, alpha 1 or beta-adrenergic receptor blocking effects [12].

Review of Literature: Literature review 13-16 signifies the use of different mobile phases with wide a range of ratios and columns using RP-HPLC and UPLC methods.

Method	Author	Mobile Phase	Stationary Phase	Retention Time
RP-HPLC	M. Saeed Arayne	Methanol-phosphate buffer, 35:65(v/v)	Shim-pack CLC-ODS 150 × 4.6 mm	3.78 and 4.15min
UPLC	Mohammed Mustafa, S. Amutha Lakshmi	Acetonitrile: 20mM potassium dihydrogen phosphate 80:30 (v/v)	Waters c18 1.8 micron 4.6 × 50 mm	1.022 and 3.281 min
RP-HPLC	Hitesh Vekaria	50 mM Sodium acetate buffer: acetonitrile: methanol (25:35:40)	X-bridge C18 column (250 mm × 4.6 mm, 5 µm)	3.43 and 8.22 min
HPLC	R. M. Singh	Acetonitrile: 1 mM sodium acetate with acetic acid 90:10 v/v	Octadecylsilane column (C18)	3.4 min

Objective of Research:

- The scope of developing and validating an analytical method is to ensure the suitable strategy for a particular analyte which is more specific, accurate and precise.
- To develop a method that is rapid, sensitive, and cost-effective at the same time.
- To validate a developed RP-UPLC method for various parameters like Specificity, Linearity, Accuracy, Precision, Robustness, etc. as per the guidelines.

To ensure that the method can be used as quality control tool in the routine analysis of Fexofenadine and Montelukast in pure and pharmaceutical dosage form.

MATERIALS AND METHODOLOGY: The list of drugs, chemicals, and instruments used in this research are represented in below **Table 1**, **2** and **3** respectively.

TABLE 1: LIST OF DRUGS USED

S. no.	Name	Label Claim	Brand Used
1	Fexofenadine	120 mg	Allegra-M
2	Montelukast	10 mg	

TABLE 2: LIST OF CHEMICALS USED

S. no	Name	Grade	Make
1	Acetonitrile	HPLC	Rankem
2	Water	HPLC	Milli Q
3	Methanol	HPLC	Rankem
4	Ortho Phosphoric acid	HPLC	Rankem
5	Ammonium acetate	HPLC	Rankem
6	Potassium dihydrogen orthophosphate	HPLC	Rankem

TABLE 3: LIST OF INSTRUMENTS USED

S. No	Name of Instruments	Make
1	Analytical Balance	Sartorius
2	Ultra Sonicator	Enertech
3	pH Meter	Labindia-Pico^{+}
4	HPLC	Waters 2996
5	UV-Visible spectrophotometer	Shimadzu-1800
6	UPLC	Waters 2695

Method Development Parameters:

A) Selection of Suitable Solvent: According to solubility tests and literature review Fexofenadine and Montelukast were freely soluble in water, acetonitrile and methanol. As methanol is viscous in nature, water and acetonitrile mixture was chosen as diluent for present work.

B) Selection of Wavelength (for Detection): The UV-Spectrum of Fexofenadine and Montelukast was obtained separately by scanning the sample over the wavelength range 200 – 400 nm against blank as water.

Fexofenadine:

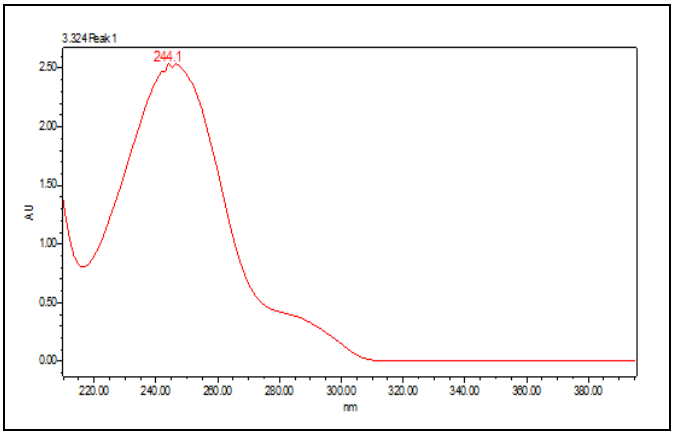


FIG. 3: U.V SPECTRA OF FEXOFENADINE

Montelukast:

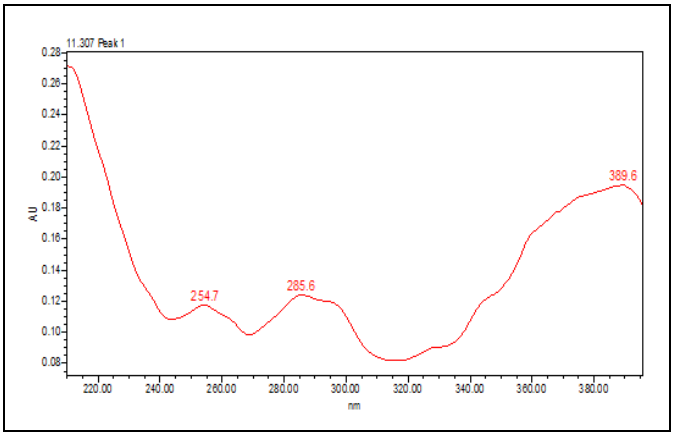


FIG. 4: U.V SPECTRA OF MONTELUKAST

After examination of the spectra, the wavelength 269 nm was selected for further analysis. Below are the **Fig. 3** and **4** that displays the U.V spectra of Fexofenadine and Montelukast respectively along with the U.V spectra for selection of wavelength in **Fig. 5**.

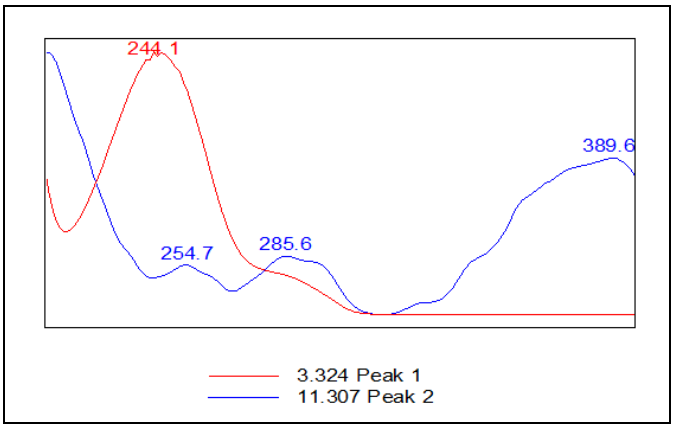


FIG. 5: U.V SPECTRA FOR SELECTION OF WAVELENGTH

C) Selection of chromatographic techniques: The choice of the method is based on the nature of the sample. The drugs are polarin nature. So, the reverse-phase chromatographic technique was selected for the present study. In the RP-UPLC technique, generally, non-polar columns are used as stationary phases. For The present study, SB C8 (3 × 100 mm, 1.8 µm) and HSS C18 (2.1 × 100 mm, 1.8 µm) columns were used as stationary phase.

D) Preparation of Solutions:

• **Preparation of Diluent:** Diluent was selected based on the solubility of the drugs. Acetonitrile and Water was taken in the ratio of 50:50 v/v.

• **Preparation of Standard Stock Solutions:** Accurately weighed 12 mg of Fexofenadine, 10 mg of Montelukast was transferred to 10 ml and 100 ml volumetric flasks separately, and 3/4th of diluent was added to each of these flasks and sonicated for 10 min. Flasks were made up with diluent and labelled as Standard stock solution 1 and Standard stock solution 2. (1200 µg/ml of Fexo and 100 µg/ml of Monte)

• **Preparation of Standard Working Solutions (100% solution):** 1ml from above each stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with diluent. (120 µg/ml of FEXO and 10 µg/ml of MONTE)

• **Preparation of Sample Stock Solutions:** Accurately weighed 5 tablets were taken, and the average weight of each of them was calculated. 1 tablet equivalent weight was transferred into a 100 ml volumetric flask, 50 ml of diluent was added to it and sonicated for 25 min; further the volume was made up with diluent and filtered by UPLC filters. (1200 µg/ml of FEXO and 100 µg/ml of MONTE)

• **Preparation of Sample Working Solutions (100% solution):** 1 ml of filtered sample stock solution was transferred to 10 ml volumetric flask each and made up with diluent. (120 µg/ml of FEXO and 10 µg/ml of MONTE)

• **Preparation of Buffer:** 0.01N KH_2PO_4 Buffer: 1.36 g of Potassium dihydrogen Orthophosphate was accurately weighed and transferred to 1000 ml volumetric flask. To this 900 ml of Milli-Q water added and sonicated for 20 min. Finally, make up the volume with water and then pH was adjusted to 5.4 with dilute Orthophosphoric acid solution.

0.1% OPA Buffer: 1 ml of orthophosphoric acid was diluted with HPLC grade water to 1000 ml.

Method Development Trails: The tabular representation of the method development trial data is represented in below **Table 4**.

TABLE 4: UPLC PARAMETERS FOR METHOD DEVELOPMENT TRIALS OF FEXOFENADINE AND MONTELUKAST

Parameters	Trial-1	Trial-2	Trial-3	Trial-4	Trial-5
Stationary Phase	SB C8 (3*100 mm, 1.8 µm)	HSS C18 (2.1*100 mm, 1.8 µm)	HSS C18 (2.1*100 mm, 1.8 µm)	HSS C18 (2.1*100 mm, 1.8 µm)	HSS C18 (2.1*100 mm, 1.8 µm)
Mobile Phase	Water: Methanol (50:50)	Water: Acetonitrile (50:50)	Potassium dihydrogen phosphate: Acetonitrile(50:50)	Ortho phosphoric acid: Acetonitrile (70:30)	Ortho phosphoric acid: Acetonitrile (50:50)
Diluent	Water: ACN (50:50)	Water: ACN (50:50)	Water: ACN (50:50)	Water: ACN (50:50)	Water: ACN (50:50)
Detection Wavelength	UV at 269 nm	UV at 269 nm	UV at 269 nm	UV at 269 nm	UV at 269 nm

Flow rate	0.1 ml/min	0.2 ml/min	0.2 ml/min	0.2 ml/min	0.2 ml/min
Injection Volume	10 µl	10 µl	10 µl	10 µl	10 µl
Column Temperature	30 ^{0}C	30 ^{0}C	30 ^{0}C	30 ^{0}C	30 ^{0}C
Run time	5 min	10 min	5 min	5 min	4 min
Observation	Only One peak was eluted	Only one peak was eluted	Both peaks were eluted but peak shapes were not good.	Both peaks were eluted but retention time was more.	Both peaks were eluted but with less resolution.

Optimized method:

Chromatographic Conditions:

Mobile phase : 65% OPA (0.1%): 35% Acetonitrile

Flow rate : 0.2 ml/min

Column : HSS C18 (2.1 × 100 mm, 1.8 µm)

Detector wave length : 269 nm

Column temperature : 30 °C

Injection volume : 10 µL

Run time : 2 min

Diluent : Water and Acetonitrile in the ratio 50:50

Results : Both peaks were eluted with good peak shape, Plate count and good tailing factor so, further. Process is carried out.

Method Validation:

System Suitability Parameters: The system suitability parameters were determined by preparing standard solutions of Fexofenadine (120 ppm) and Montelukast (10 ppm) and the solutions were injected six times and the parameters like Peak tailing, Resolution and USP plate count were determined. The % RSD for the area of six standard injections should not be more than 2.

Specificity: Specificity is simply checking of the interference in the optimized method. When no interfering peaks are found in blank and placebo at retention times of these drugs, this method was said to be specific.

Precision: Preparation of Standard stock solutions: Accurately weighed 12 mg of Fexofenadine, 10 mg of Montelukast and transferred to 10 ml and 100 ml volumetric flasks separately and 3/4th of diluent was added to both of these flasks and sonicated for 10 min. Flasks were made up with diluent and labelled as Standard stock solution 1 and Standard stock solution 2. (1200 µg/ml of Fexo and 100 µg/ml of Monte)

Preparation of Standard Working Solutions (100% Solution): 1 ml from each stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with diluent. (120 µg/ml of FEXO and 10 µg/ml of MONTE)

Preparation of Sample Stock Solutions: 5 tablets were accurately weighed, and the average weight of each tablet was calculated. 1 tablet equivalent weight was transferred into a 100 ml volumetric flask, 50 ml of diluent was added to it and sonicated for 25 min, further, the volume was made up with diluent and filtered by UPLC filters (1200 µg/ml of FEXO and 100 µg/ml of MONTE)

Preparation of Sample Working Solutions (100% Solution): 1 ml of above filtered sample stock solution was transferred to 10 ml volumetric flask and made up to mark with diluent. (120 µg/ml of FEXO and 10 µg/ml of MONTE)

Linearity:

25% Standard Solution: 0.25 ml each of standard stock solutions 1 and 2 was pipetted out and made up to 10 ml. (30 µg/ml of FEXO and 2.5 µg/ml of MONTE)

50% Standard Solution: 0.5 ml each of standard stock solutions 1 and 2 was pipetted out and made up to 10 ml. (60 µg/ml of FEXO and 5 µg/ml of MONTE)

75% Standard Solution: 0.75 ml each of standard stock solutions 1 and 2 was pipetted out and made up to 10 ml. (90 µg/ml of FEXO and 7.5 µg/ml of MONTE)

100% Standard Solution: 1.0 ml each of standard stock solutions 1 and 2 was pipetted out and made up to 10 ml. (120 µg/ml of FEXO and 10 µg/ml of MONTE)

125% Standard Solution: 1.25 ml each of standard stock solutions 1 and 2 was pipetted out and made up to 10 ml. (150 µg/ml of FEXO and 12.5 µg/ml of MONTE)

150% Standard Solution: 1.5 ml each of standard stock solutions 1 and 2 was pipetted out and made up to 10 ml (180 µg/ml of FEXO and 15 µg/ml of MONTE)

Accuracy:

Preparation of Placebo Solution: The weight equivalent to 1 tablet placebo was transferred into a 100 ml volumetric flask, 50 ml of diluents was added and sonicated for 25 min, further the volume was made up to the mark with diluent and filtered by UPLC filters.

Preparation of 50% Spiked Solution: 0.5 ml of standard stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each placebo stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0 ml of standard stock solution was taken into a 10 ml volumetric flask, to that 1.0ml from each sample stock solution was pipetted out and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5 ml of standard stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each placebo stock solution was pipetted out, and made up to the mark with diluent.

Robustness: Minute deliberate possible changes are made in method parameters like Flow rate, mobile phase ratio, and temperature but no significant change in the result was observed and is within range as per ICH Guidelines. Robustness conditions like Flow minus (0.1 ml/min), Flow plus (0.5 ml/min), mobile phase minus (25B:75A), mobile phase plus (45B:55A), temperature minus (25 ℃) and temperature plus (35 ℃) was maintained, and samples were injected in a duplicate manner.

Limit of detection:

LOD Sample Preparation: 0.25ml each of standard stock solutions 1 and 2 was pipetted out and transferred to two separate 10 ml volumetric flasks and made up with diluents. From the above solutions, 0.1 ml each of Fexofenadine, Montelukast solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents. (0.3 µg/ml of FEXO and 0.025 µg/ml of MONTE)

Limit of Quantification:

LOQ Sample Preparation: 0.25 ml of standard stock solutions from each was pipetted out and transferred to two separate 10 ml volumetric flask and made up to the mark with diluent.

From the above solutions, 0.3 ml each of Fexofenadine, Montelukast solutions respectively were transferred to 10 ml volumetric flasks and made up with the same diluent. (0.9 µg/ml of FEXO and 0.075 µg/ml of MONTE)

Assay:

Preparation of Standard Stock Solutions: Accurately weighed 12 mg of Fexofenadine, 10 mg of Montelukast were taken and transferred to 10 ml and 100 ml volumetric flasks separately. To that $3/4^{th}$ of diluent was added to both of these flasks and sonicated for 10 min. Flasks were made up with diluent and labelled as Standard stock solution 1 and Standard stock solution 2. (1200 µg/ml of Fexo and 100 µg/ml of Monte)

Preparation of Standard working solutions (100% solution): 1 ml from the above each stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with diluent. (120 µg/ml of FEXO and 10 µg/ml of MONTE)

Preparation of Sample Stock Solutions: 5 accurately weighed tablets were taken, and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50 ml of diluent was added and sonicated for 25 min; further the volume was made up with diluent and filtered by UPLC filters (1200 µg/ml of FEXO and 100 µg/ml of MONTE)

Preparation of Sample Working Solutions (100% Solution): 1 ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up to the mark with diluent. (120 µg/ml of FEXO and 10 µg/ml of MONTE).

Procedure: Separately inject both the standard (6 injections) and sample preparation (6 injections) into the chromatographic system and record the peak area responses.

Assay % = (sample area) / (standard area) × (standard weight) / (sample weight) × (potency) / 100 × 100

Formulation used: Allegra-M

Labelled Claim:
Fexofenadine: 120 mg

Montelukast: 10 mg

Potency:
Fexofenadine: 99.8%

Montelukast: 99.8%

Degradation Studies: Standards and degraded samples are injected, and the percentage of drug degraded in the solution by applying different conditions like acid, alkali, oxidative, photolytic, thermal, and neutral analysis is calculated.

Procedure:
Oxidation: To 1 ml stock solution of Fexofenadine and Montelukast, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately and the solutions were kept for 30 min at 60 ºC.

The resultant solution was diluted to obtain 120 µg/ml and 10 µg/ml of Fexofenadine and Montelukast solutions, and 10 µl of each were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Acid Degradation Studies: To 1 ml stock solution of Fexofenadine and Montelukast, 1 ml of 2N Hydrochloric acid was added and refluxed for 30 min at 60 ºC.

The resultant solution was diluted to obtain 120 µg/ml and 10 µg/ml solutions and 10 µl of each were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies: To 1 ml of stock solution Fexofenadine and Montelukast, 1 ml of 2N sodium hydroxide was added and refluxed for 30 min at 60 ºC. The resultant solution was diluted to obtain 120 µg/ml and 10 µg/ml of Fexofenadine and Montelukast solutions, and 10 µl of each were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Dry heat Degradation Studies: The standard drug solution was placed in an oven for 1 h at 105 ºC to study dry heat degradation. The resultant solution was diluted to obtain 120 µg/ml and 10 µg/ml of Fexofenadine and Montelukast solutions, and 10 µl of each were injected into the system and the chromatograms were recorded to assess the stability of the sample.

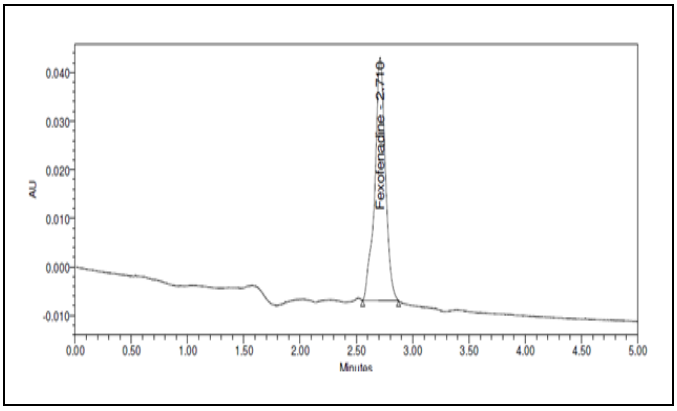
Photo-stability Studies: The photochemical stability of the drug was studied by exposing the 1200 µg/ml and 100 µg/ml of Fexofenadine and Montelukast drug solutions to UV Light by keeping the beaker in UV Chamber for 1 hour or 200 Watt-hour/m^2 in a photostability chamber. The resultant solution was diluted to obtain 120 µg/ml and 10 µg/ml of Fexofenadine and Montelukast solutions and 10 µl of each were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies: Stress testing was studied under neutral conditions by refluxing the drug in water at 60 ºC for 1 h. The resultant solution was diluted to obtain 120 µg/ml and 10 µg/ml of Fexofenadine and Montelukast solutions and 10 µl of each were injected into the system and the chromatograms were recorded to determine the stability of the sample.

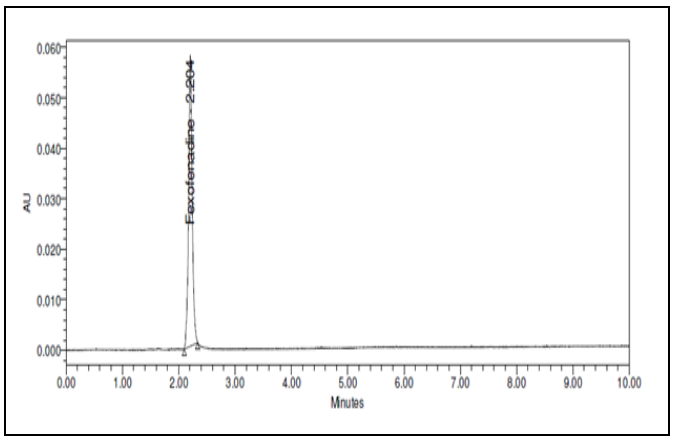
RESULTS AND DISCUSSION:

Method Development Trails: The trail chromatograms are observed in **Fig. 3** and **Fig. 4** displays the chromatogram of Optimized method.

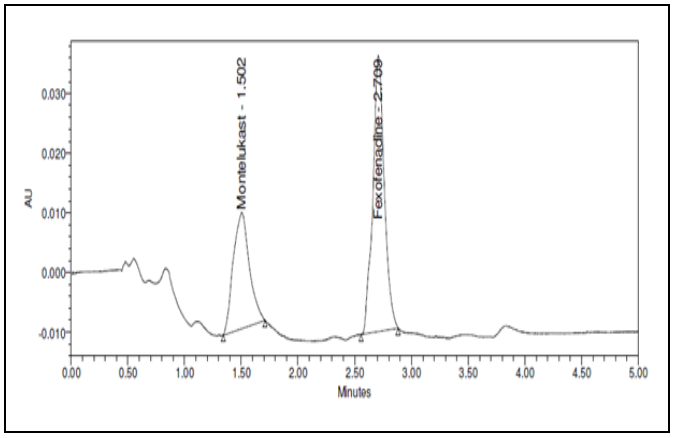
Trial Chromatogram-1:



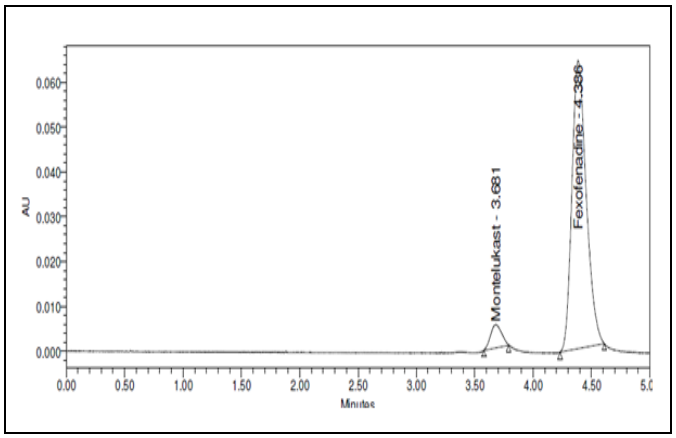
Trial Chromatogram 2:



Trial Chromatogram 3:



Trial Chromatogram 4:



Trial Chromatogram 5:

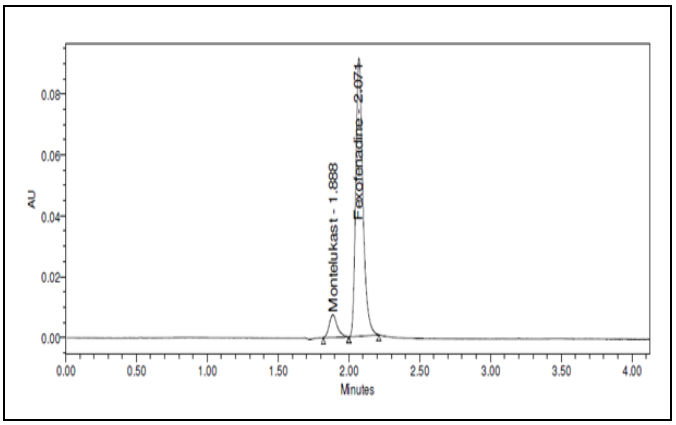


FIG. 3: TRIAL CHROMATOGRAMS OF FEXOFENADINE AND MONTELUKAST

Optimized Method:

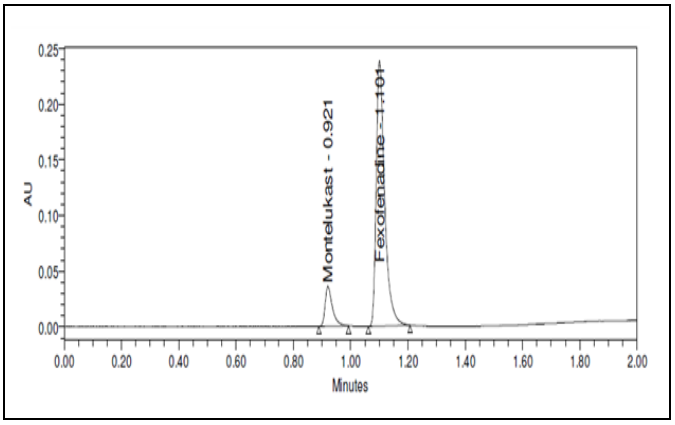


FIG. 4: OPTIMIZED CHROMATOGRAM

Observation: Montelukast and Fexofenadine were eluted at 0.921 min and 1.101 min, respectively with good resolution. Plate count and tailing factor was found to be satisfactory, so this method was to be optimized and validated.

Method Validation:

System Suitability: All the system suitability parameters were found to be within the range and satisfactory as represented in **Table 5**. The system suitability chromatograms of Montelukast and Fexofenadine are observed in **Fig. 5**.

TABLE 5: SYSTEM SUITABILITY PARAMETERS FOR MONTELUKAST AND FEXOFENADINE

S. no	Montelukast			Fexofenadine			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	0.920	6887	1.6	1.101	7265	1.5	3.6
2	0.920	7001	1.6	1.101	7321	1.5	3.6
3	0.921	6890	1.6	1.101	7228	1.6	3.6
4	0.921	7005	1.6	1.101	7367	1.6	3.6
5	0.921	6902	1.6	1.101	7271	1.6	3.6
6	0.921	6848	1.6	1.102	7237	1.5	3.6

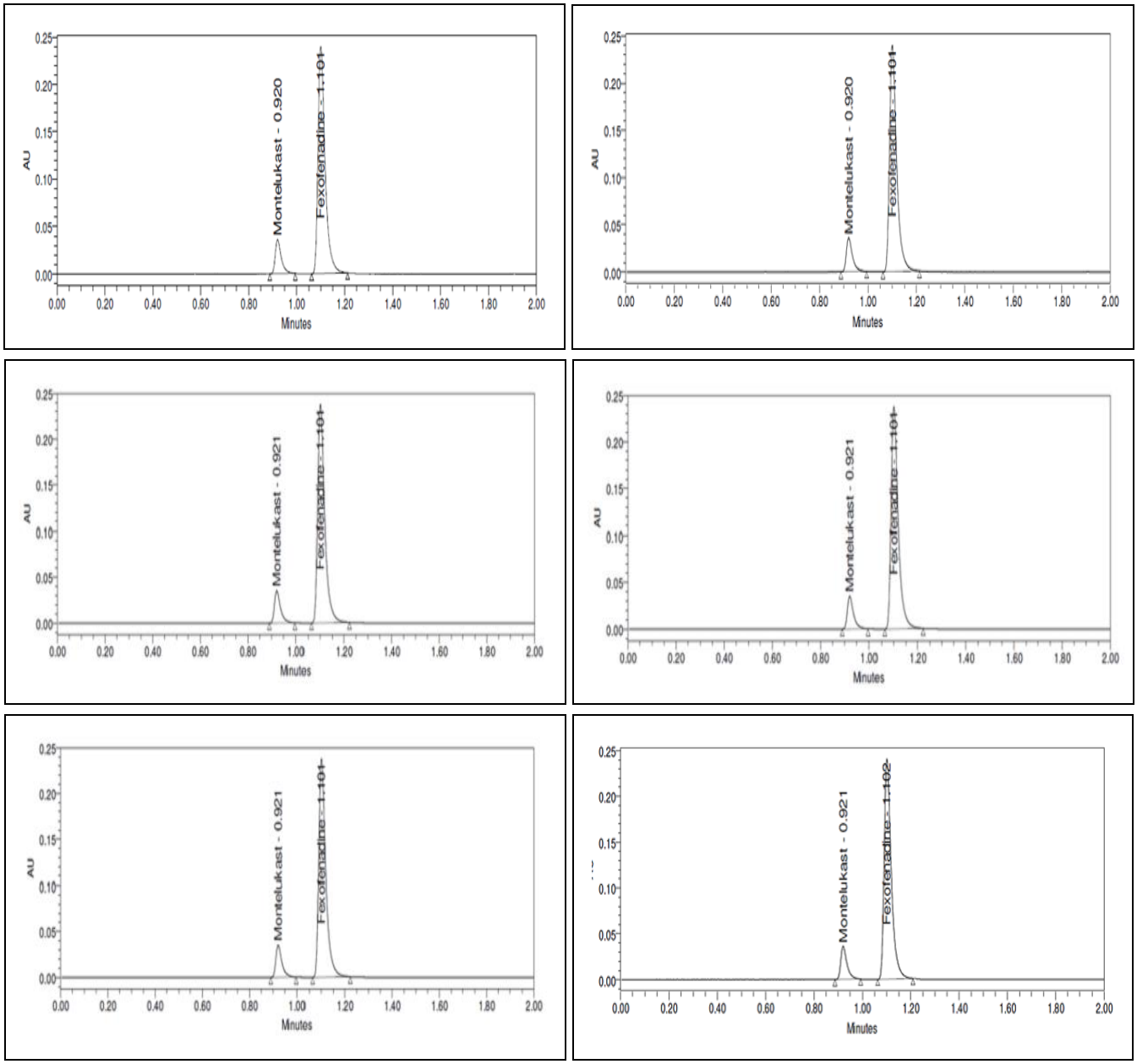


FIG. 5: SYSTEM SUITABILITY CHROMATOGRAMS OF MONTELUKAST AND FEXOFENADINE

Acceptance Criteria: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2, and resolution must be more than 2. All the suitable system parameters were passed and were found to be within limits.

Specificity: Retention times of Montelukast and Fexofenadine were 0.921 min and 1.101 min respectively.

We did not find any interfering peaks in blank and placebo at retention times of these drugs in this method.

So this method was concluded as specific. The below are **Fig. 6** and **7** that represent the chromate-gram of blank and placebo, respectively, and also the optimized chromatogram in **Fig. 8**.

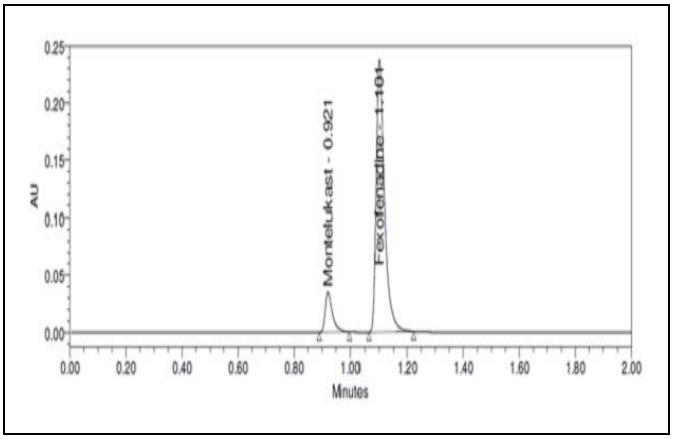


FIG. 6: CHROMATOGRAM OF BLANK

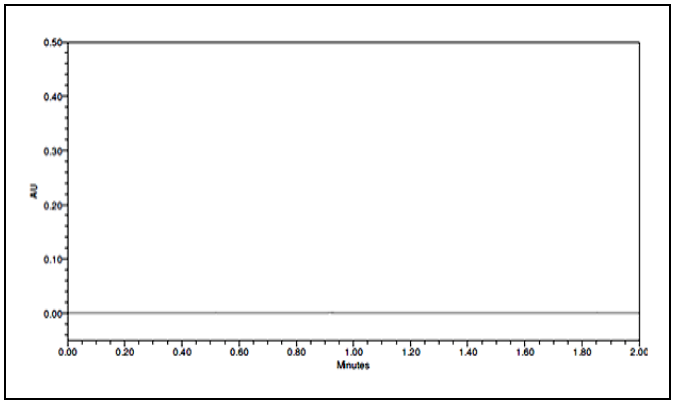


FIG. 7: CHROMATOGRAM OF PLACEBO

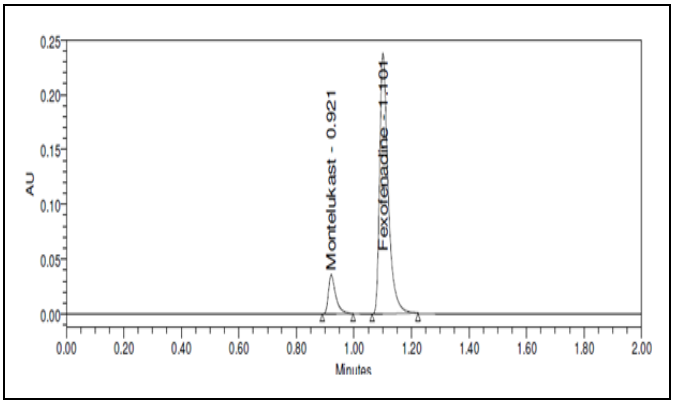


FIG. 8 OPTIMIZED CHROMATOGRAM

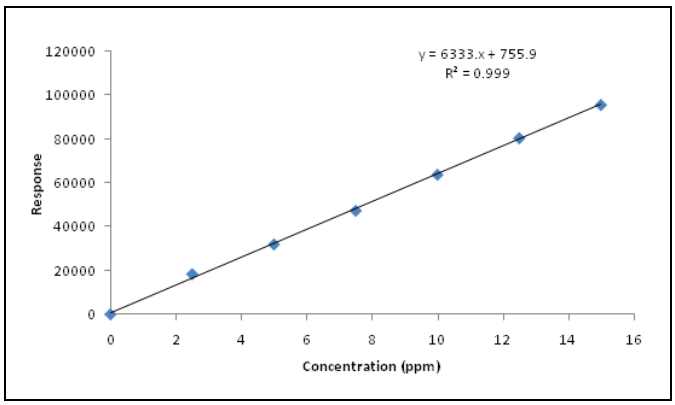
Acceptance Criteria: There should be no presence of any interfering compounds or matrices.

TABLE 6: LINEARITY TABLE FOR MONTELUKAST AND FEXOFENADINE

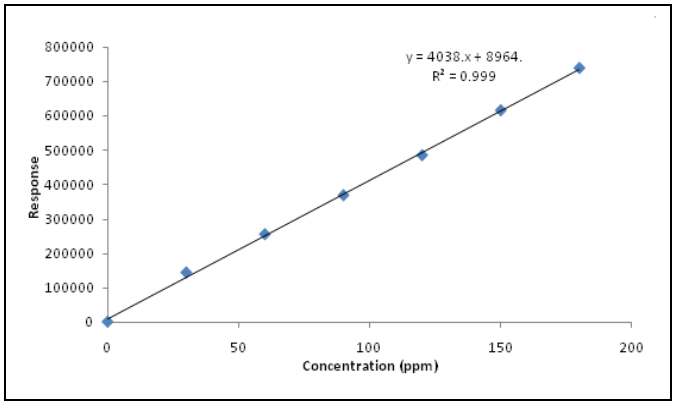
Montelukast		Fexofenadine	
Concentration (µg/mL)	**Peak area**	**Concentration (µg/mL)**	**Peak area**
0	0	0	0
2.5	18394	30	143493
5	31956	60	254913
7.5	47363	90	368462
10	63833	120	485044
12.5	80535	150	615862
15	95736	180	739095

Average areas were mentioned above and linearity equations obtained for Montelukast was y = 6333.8x + 755.96 and of Fexofenadine was y = 4038.3x + 8964.8 as observed in **Graph 1** and **Graph 2,** respectively. The correlation coefficient obtained was 0.999 and 0.999 for Montelukast and Fexofenadine, respectively. Hence, linearity was passed in this method.

Linearity: Six linear concentrations of Montelukast (2.5-15 µg/ml) and Fexofenadine (30-180 µg/ml) were injected in a duplicate manner, and the chromatograms are observed in **Fig. 9**. The linearity data is represented in **Table 6**.

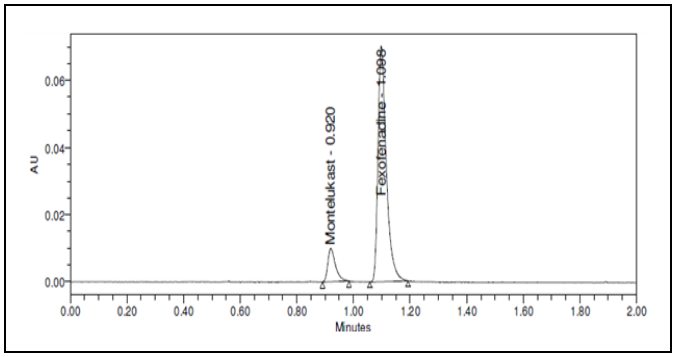


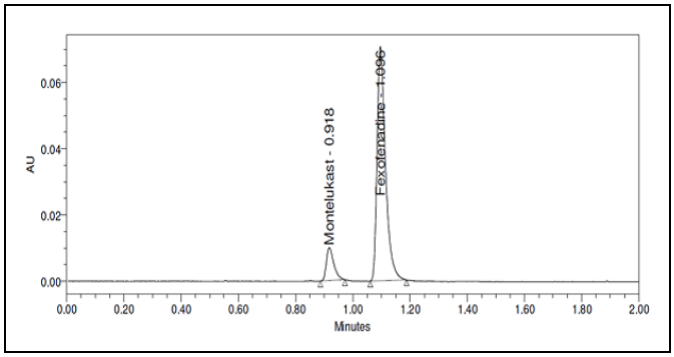
GRAPH 1: CALIBRATION CURVE OF MONTELUKAST



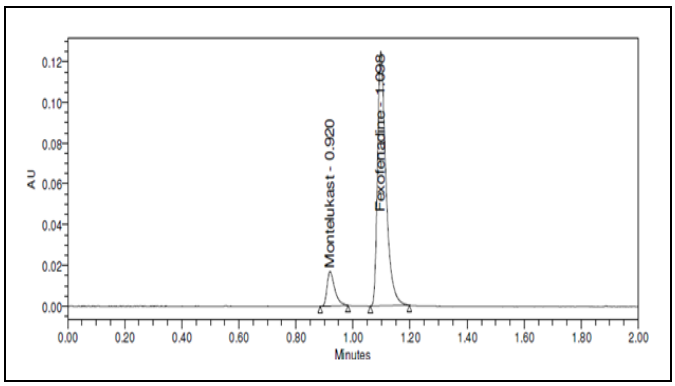
GRAPH 2: CALIBRATION CURVE OF FEXOFENADINE

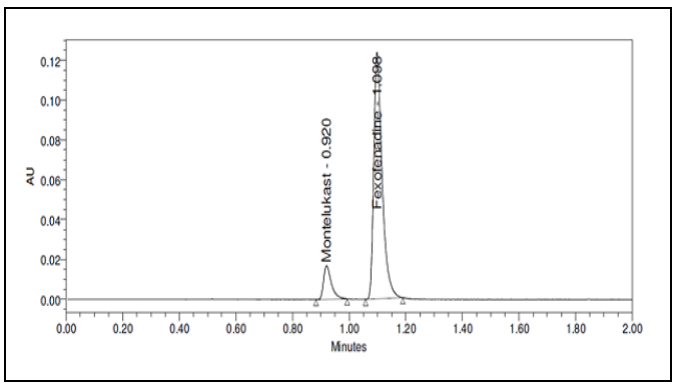
Linearity at 25%:



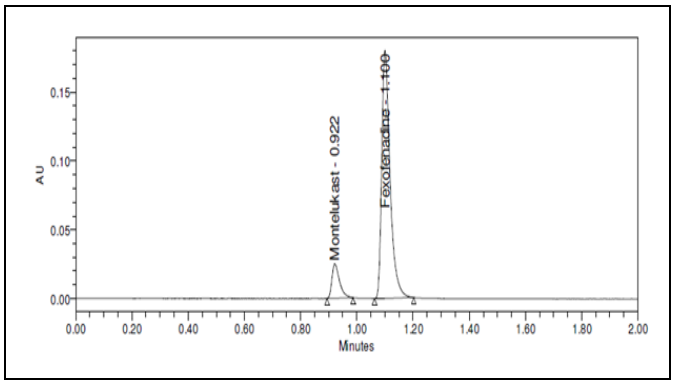


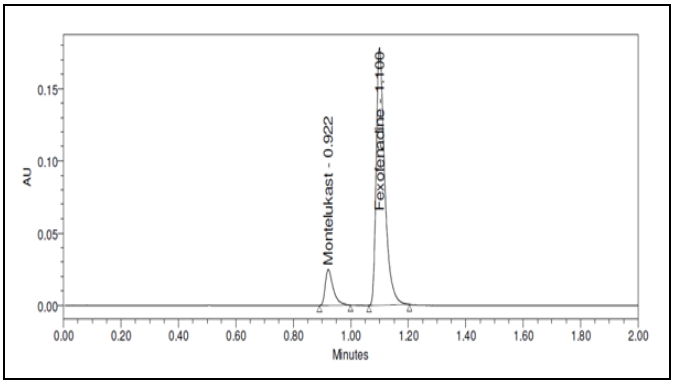
Linearity at 50%:



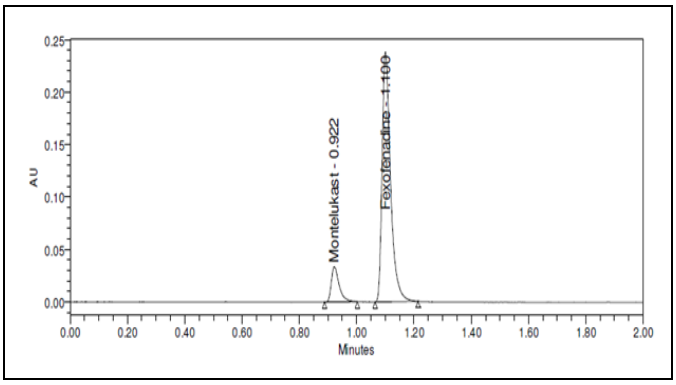


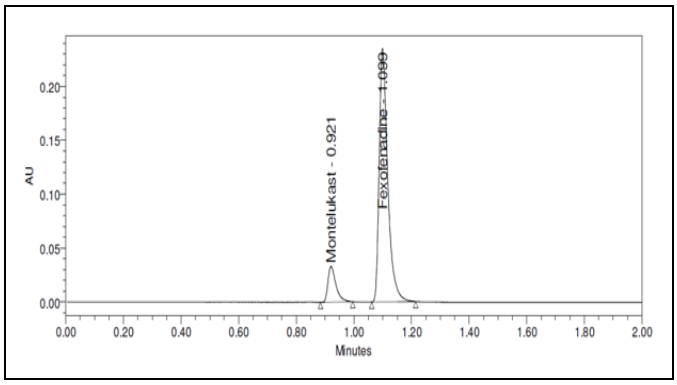
Linearity at 75%:



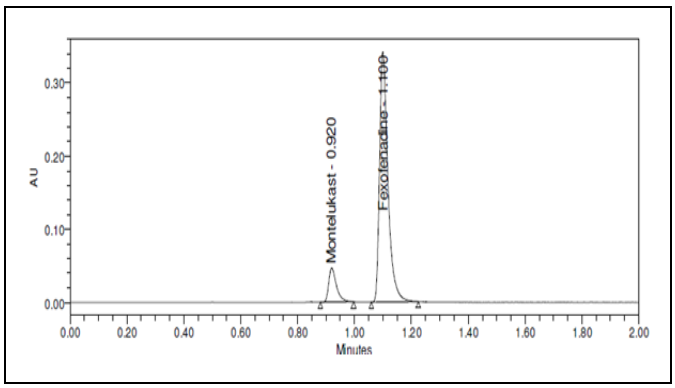


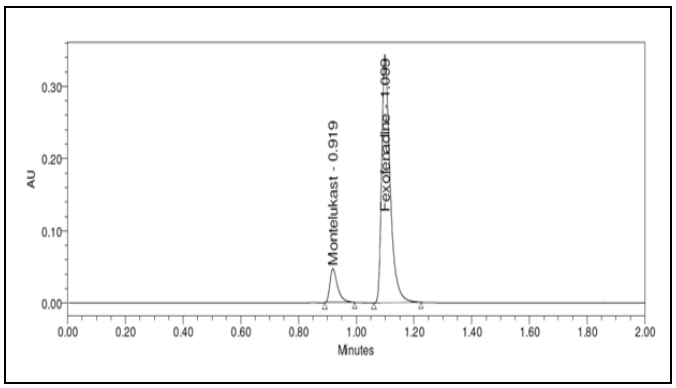
Linearity at 100%:





Linearity at 125%:





Linearity at 150%:

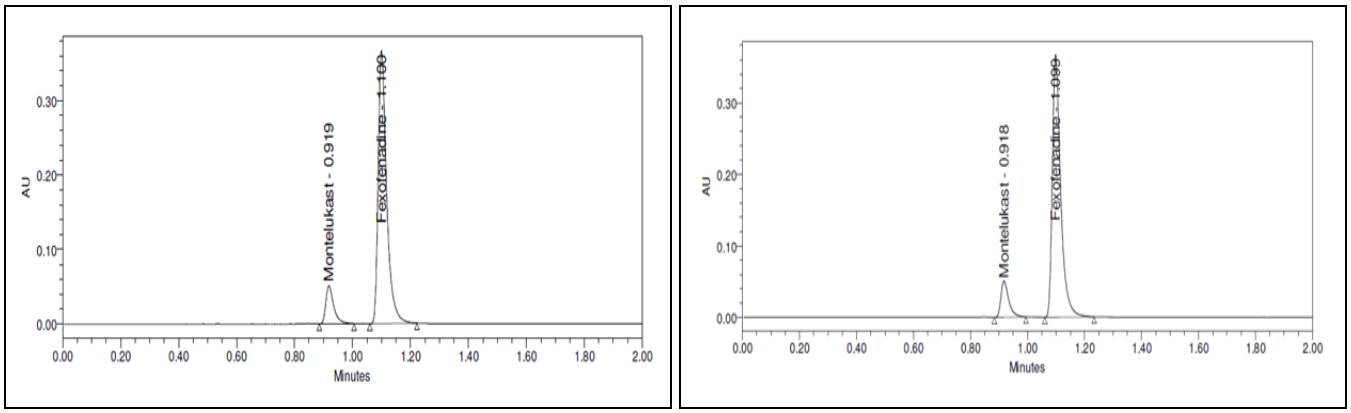


FIG. 9: LINEARITY CHROMATOGRAMS OF MONTELUKAST AND FEXOFENADINE

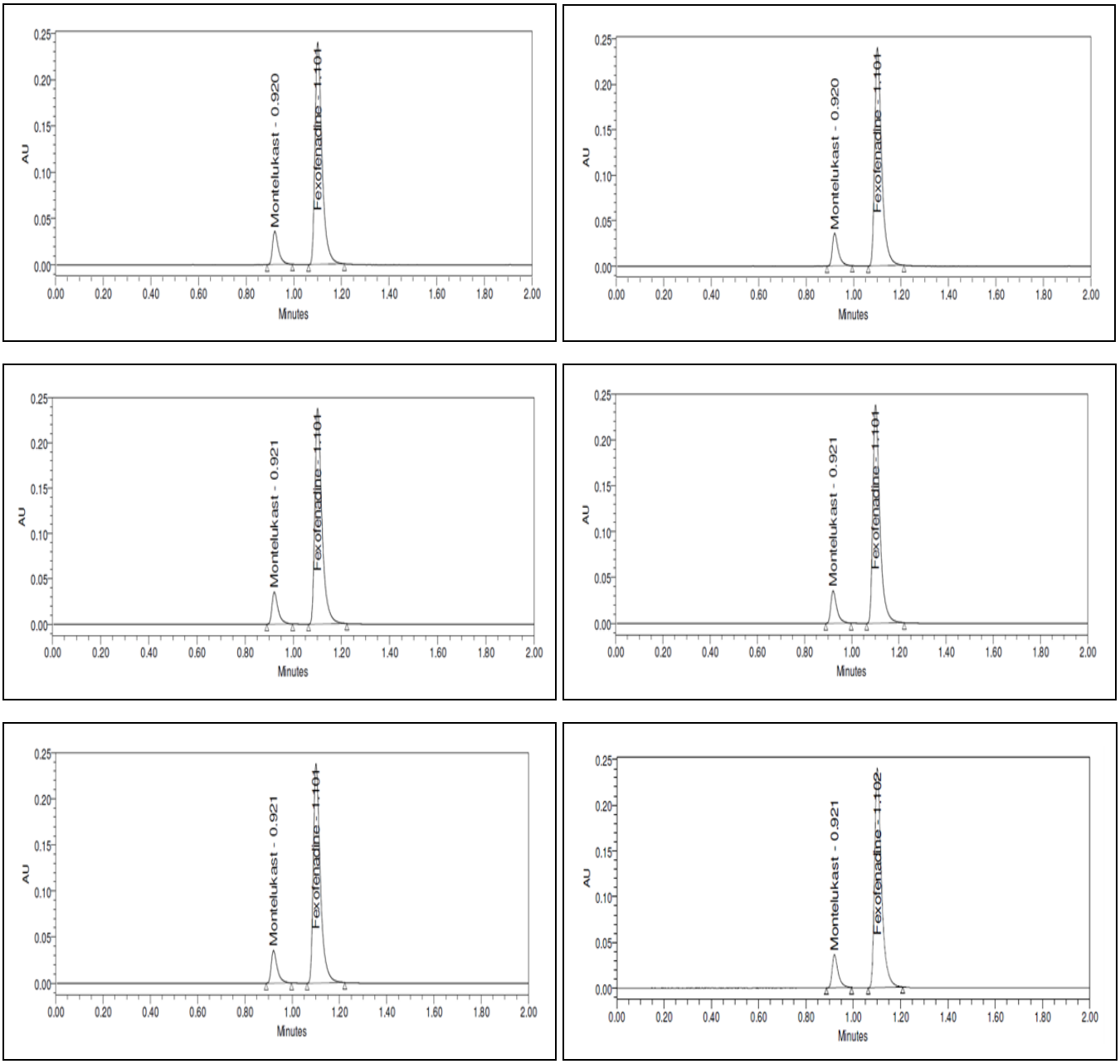


FIG 10: SYSTEM PRECISION CHROMATOGRAM (6 DETERMINANTS)

Acceptance Criteria: Correlation coefficient [R2] ≥ 0.999

Precision:

A. System Precision: Six injections were given from a single volumetric flask of working standard solution and the obtained areas, standard deviation and %RSD were mentioned below in **Table 7**. The %RSD obtained as 0.9% and 0.3%, respectively, for Montelukast and Fexofenadine. The resultant chromatograms are observed in **Fig. 10**.

TABLE 7: SYSTEM PRECISION TABLE OF MONTELUKAST AND FEXOFENADINE

S. no.	Area of Montelukast	Area of Fexofenadine
1	64134	488378
2	63209	488109
3	63405	488088
4	62801	484745
5	63006	486898
6	62394	489540
Mean	63158	487626
S.D	591.7	1643.5
% RSD	0.9	0.3

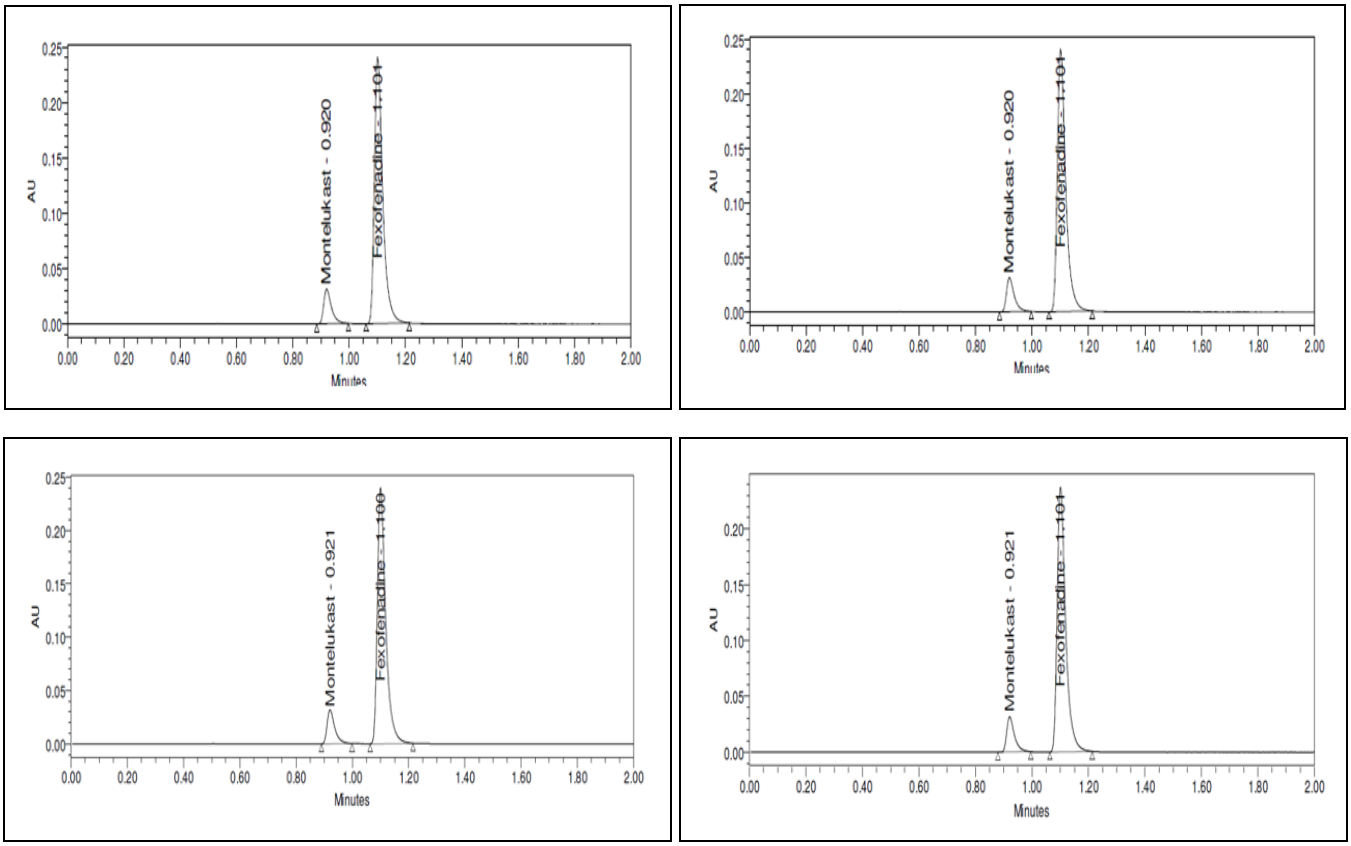
Acceptance Criteria: %RSD should be NMT 2. The limit of Precision being less than "2", the system precision was passed in this method.

B. Repeatability: Multiple samplings from a sample stock solution were done and six working sample solutions of the same concentrations were prepared, in which each injection from each working sample solution was given, and the areas obtained were mentioned in **Table 8**. The resultant chromatograms are observed in **Fig. 11**.

Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.7% and 0.4%, respectively, for Montelukast and Fexofenadine.

TABLE 8: REPEATABILITY TABLE OF MONTELUKAST AND FEXOFENADINE

S. no	Area of Montelukast	Area of Fexofenadine
1	63026	492006
2	62875	491072
3	63727	495089
4	62539	492730
5	63471	493991
6	62919	489784
Mean	63093	492445
S.D	432.2	1929.8
%RSD	0.7	0.4



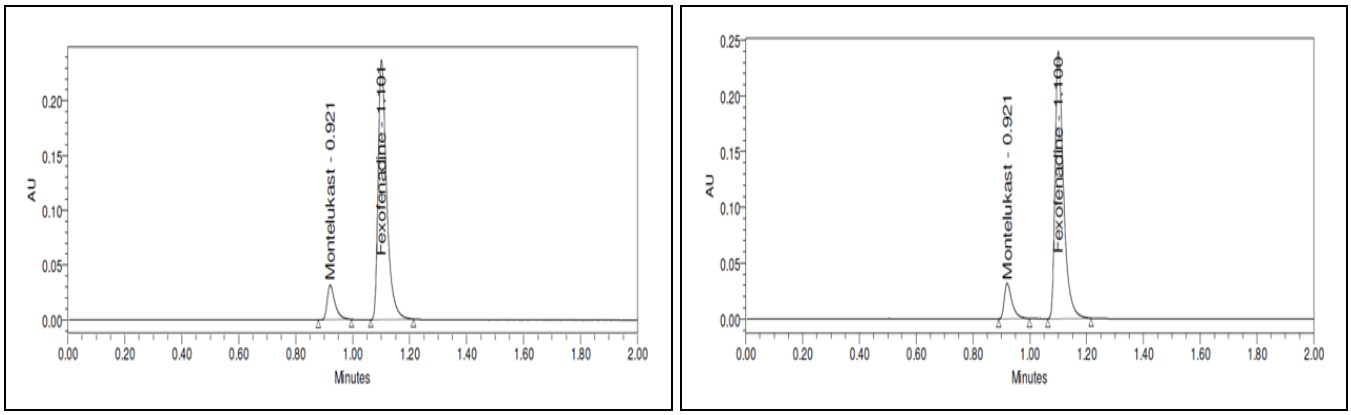


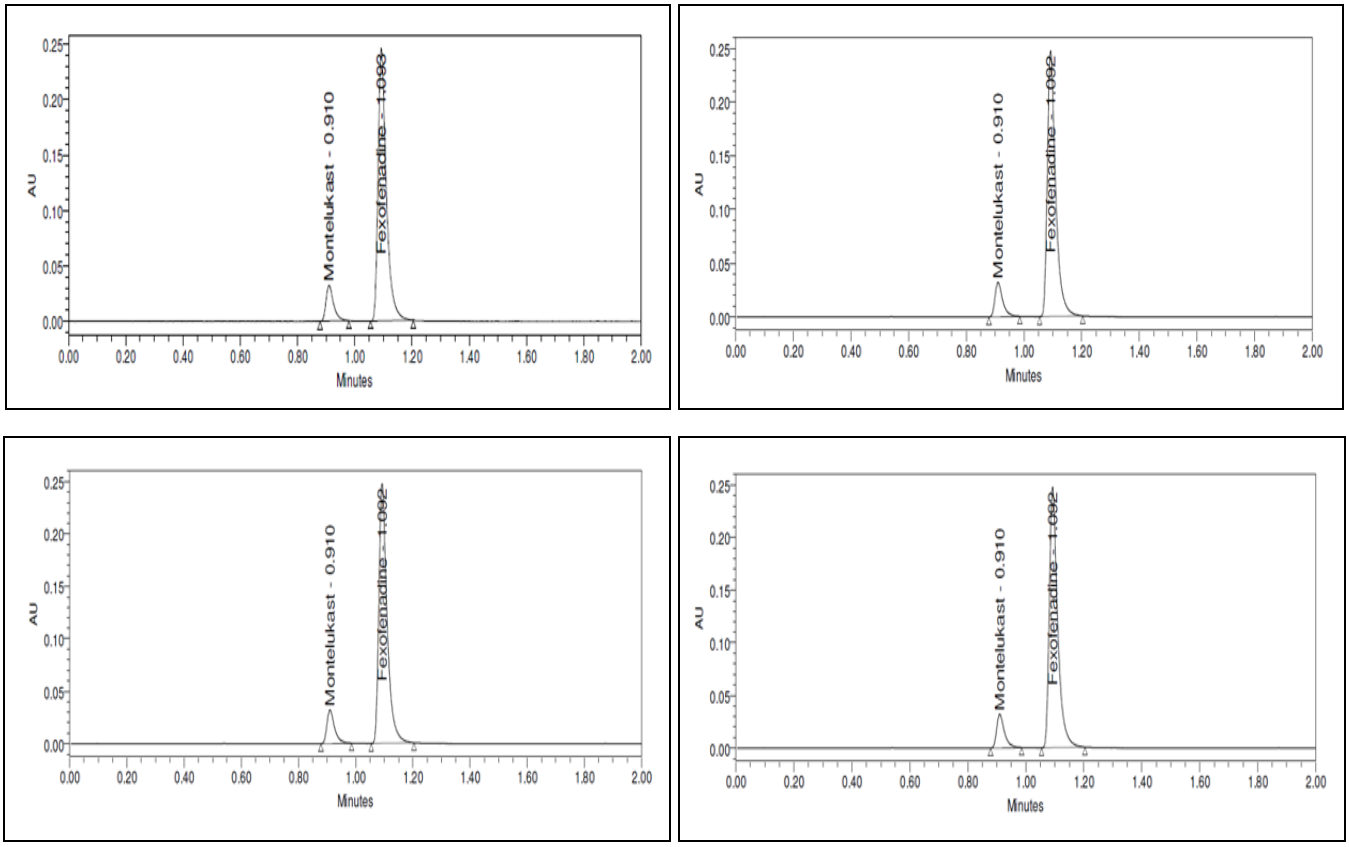
FIG. 11: REPEATABILITY CHROMATOGRAMS (6 DETERMINANTS)

Acceptance Criteria: %RSD should be NMT 2. The limit of Precision being less than "2", the system precision was passed in this method

C. Intermediate Precision (Day-Day Precision): Multiple sampling from a sample stock solution was done, and six working sample solutions of the same concentrations were prepared, in which each injection from each working sample solution was given on the next day of the sample preparation, and the areas obtained were mentioned in the below **Table 9**. The resultant chromatograms were observed in **Fig. 12**. Average area, standard deviation, and % RSD were calculated for two drugs and obtained as 1.1% and 0.3%, respectively, for Montelukast and Fexofenadine.

TABLE 9: INTERMEDIATE PRECISION TABLE OF MONTELUKAST AND FEXOFENADINE

S. no	Area of Montelukast	Area of Fexofenadine
1	59884	491409
2	58821	491484
3	59009	494900
4	59700	491684
5	58390	493112
6	59863	491534
Mean	59278	492354
S.D	625.7	1402.0
%RSD	1.1	0.3



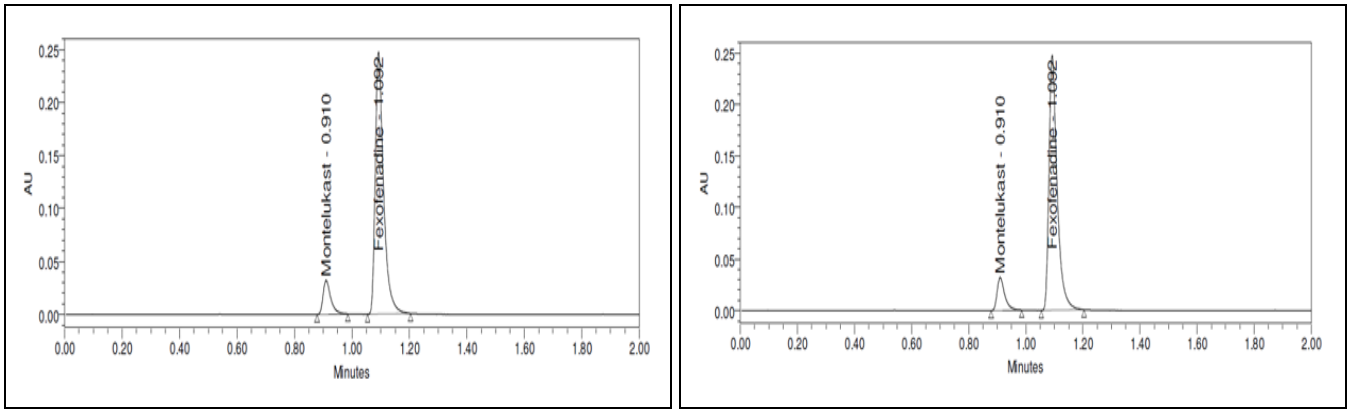


FIG. 12: INTER DAY PRECISION CHROMATOGRAMS

Acceptance Criteria: %RSD should be NMT 2. The limit of Precision being less than "2", the system precision was passed in this method.

Accuracy: Three levels of Accuracy samples, *i.e.*, 50%, 100%, 150% were prepared by the standard addition method.

Triplicate injections were given for each level of accuracy, and mean %Recovery was obtained as 99.22% and 99.67% for Montelukast and Fexofenadine, respectively, as represented in the below **Table 10** for Montelukast and **Table 11** for Fexofenadine. The resultant chromatograms are observed in **Fig. 13**.

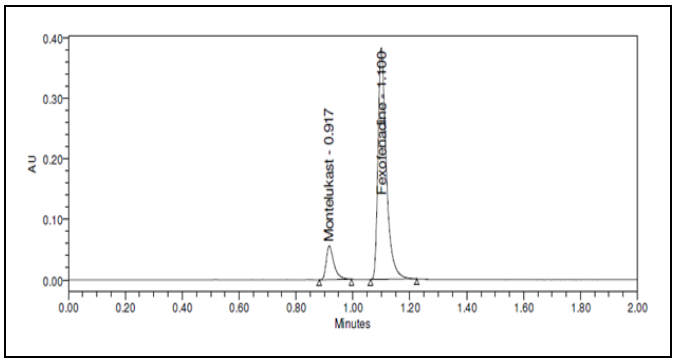
TABLE 10: ACCURACY TABLE OF MONTELUKAST

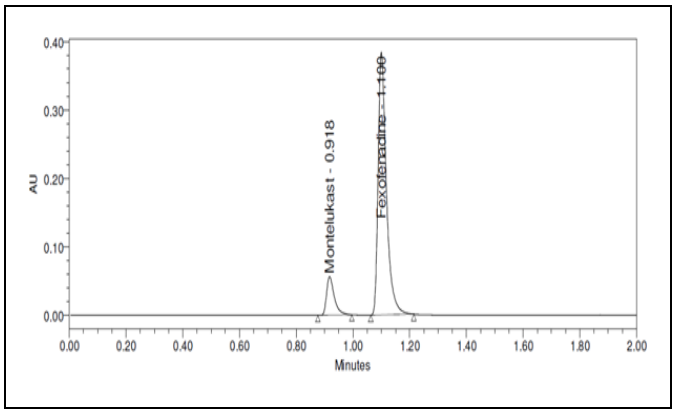
% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5	4.91	98.14	99.22%
	5	4.95	99.06	
	5	5.05	100.97	
100%	10	9.92	99.21	
	10	9.95	99.55	
	10	9.84	98.36	
150%	15	15.07	100.44	
	15	14.75	98.33	
	15	14.84	98.92	

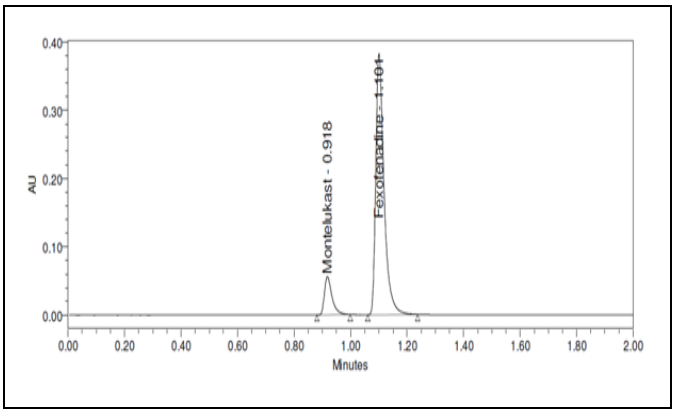
TABLE 11: ACCURACY TABLE OF FEXOFENADINE

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	60	59.94	99.91	99.67%
	60	59.96	99.94	
	60	60.10	100.17	
100%	120	119.80	99.83	
	120	119.06	99.22	
	120	119.50	99.58	
150%	180	177.54	98.64	
	180	180.75	100.41	
	180	178.84	99.35	

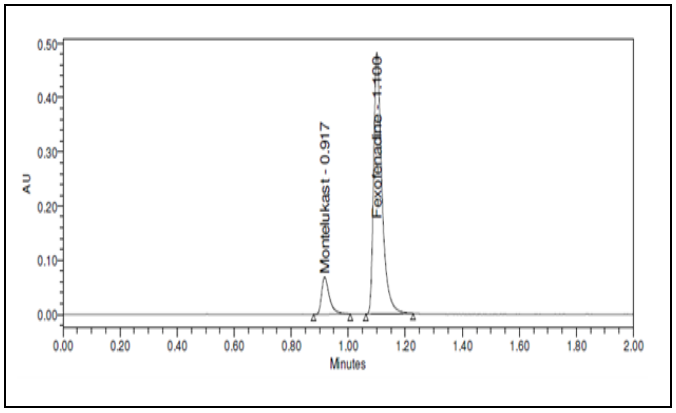
Accuracy at 50%:

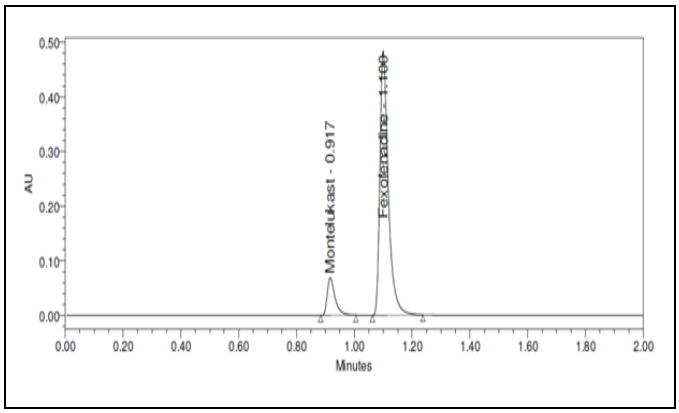


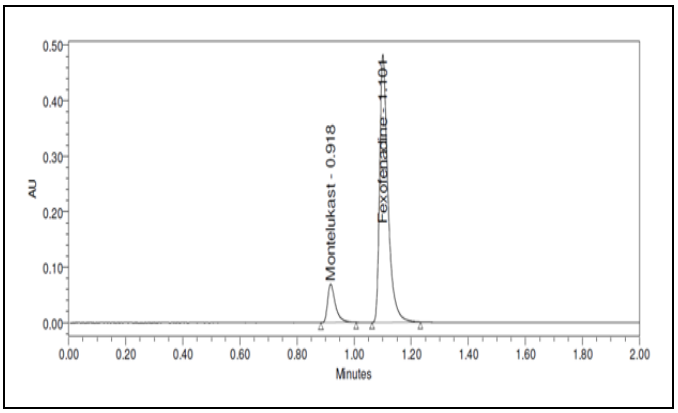




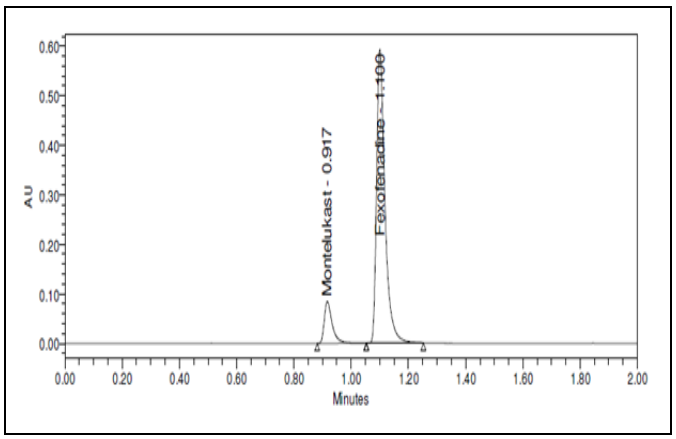
Accuracy at 100%:







Accuracy at 150%:



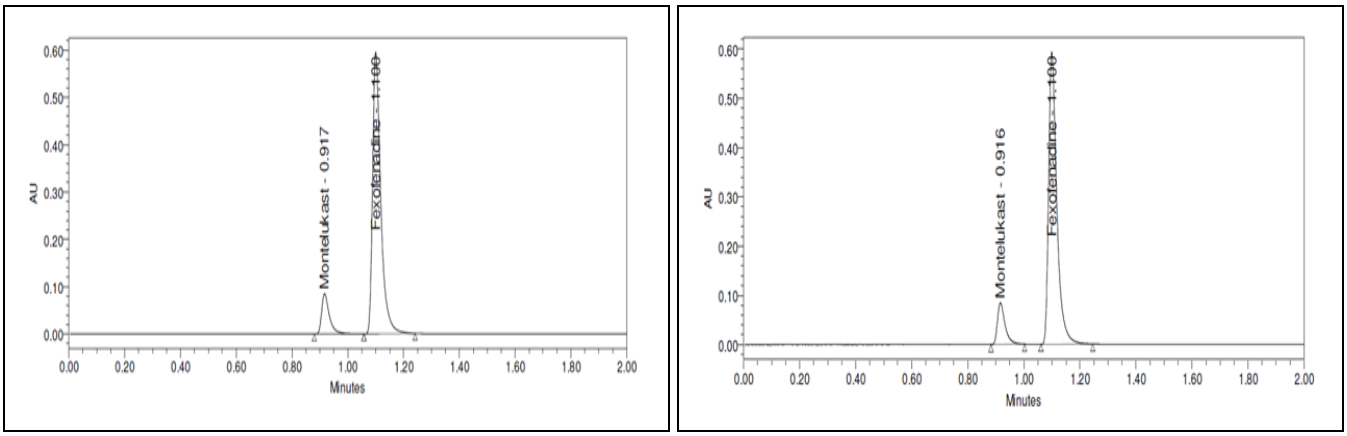


FIG 13: ACCURACY CHROMATOGRAMS OF MONTELUKAST AND FEXOFENADINE AT 3 CONCENTRATIONS

Acceptance Criteria: %Recovery should be within 98-102%.

Sensitivity: The LOD and LOQ sample preparations of Montelukast and Fexofenadine were injected and determined by using a signal-to-noise approach, and the data was tabulated in **Table 12**.

The LOD and LOQ chromatograms of Montelukast and Fexofenadine were observed in **Fig. 14** and **15,** respectively.

TABLE 12: SENSITIVITY TABLE OF MONTELUKAST AND FEXOFENADINE

Drug	LOD	LOQ
Montelukast	0.06	0.18
Fexofenadine	0.80	2.44

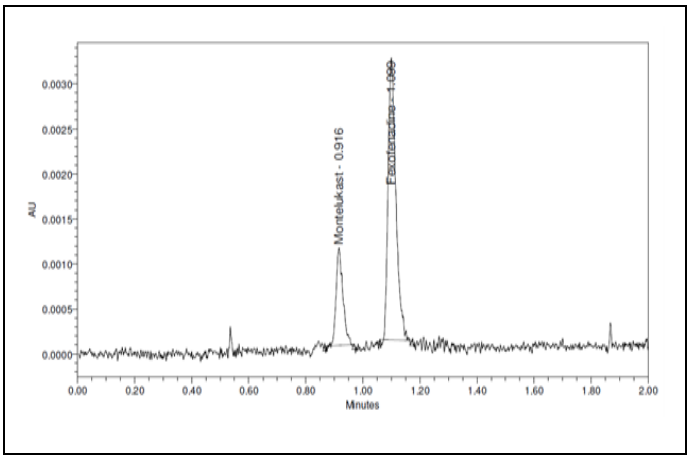


FIG. 14: LOD CHROMATOGRAM OF MONTELUKAST AND FEXOFENADINE

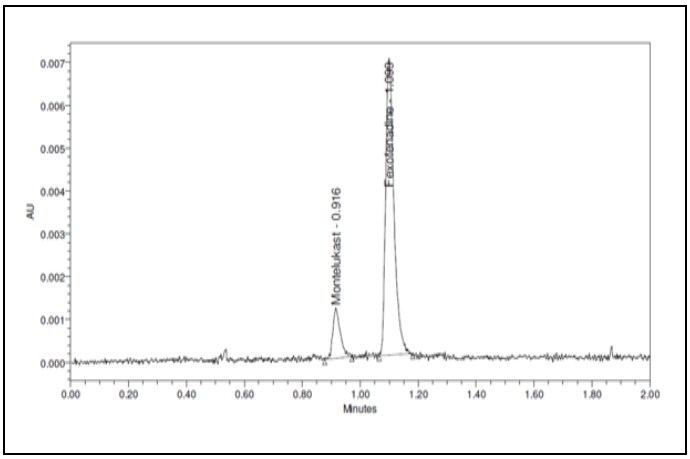


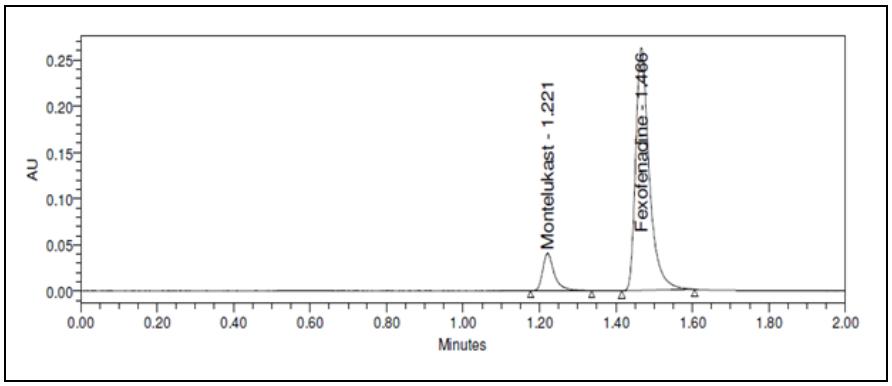
FIG. 15: LOQ CHROMATOGRAM OF MONTELUKAST AND FEXOFENADINE

Robustness: Robustness conditions like Flow minus (0.1 ml/min), Flow plus (0.5 ml/min), mobile phase minus (25B:75A), mobile phase plus (45B:55A), temperature minus (25 °C) and temperature plus (35 °C) were maintained and samples were injected in a duplicate manner, and their resultant chromatograms were observed in **Fig. 16** along with the below-tabulated data in **Table 13**.

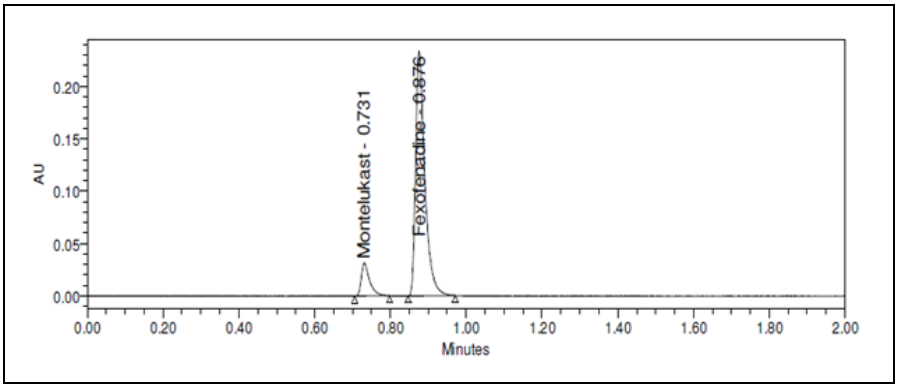
TABLE 13: ROBUSTNESS DATA FOR MONTELUKAST AND FEXOFENADINE

S. no	Condition	%RSD of Montelukast	%RSD of Fexofenadine
1	Flow rate (-) 0.1 ml/min	1.4	0.6
2	Flow rate (+) 0.5 ml/min	0.6	0.5
3	Mobile phase (-) 25B:75A	1.7	0.4
4	Mobile phase (+) 45B:55A	1.1	0.3
5	Temperature (-) 25 °C	0.5	0.7
6	Temperature (+) 35 °C	1.1	0.3

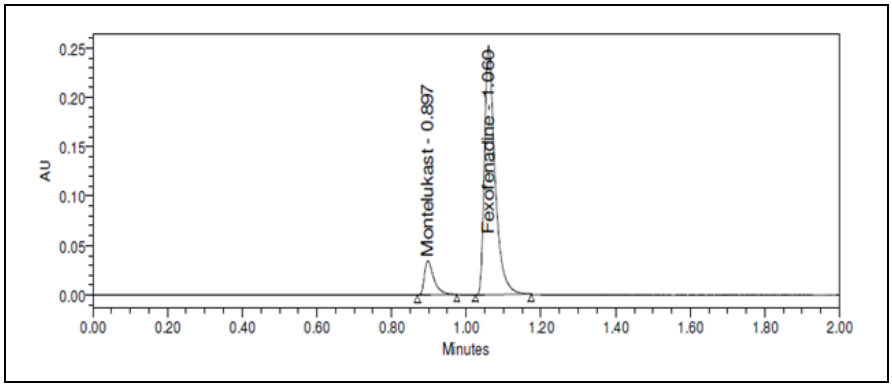
Flow Minus Chromatogram of Montelukast and Fexofenadine:



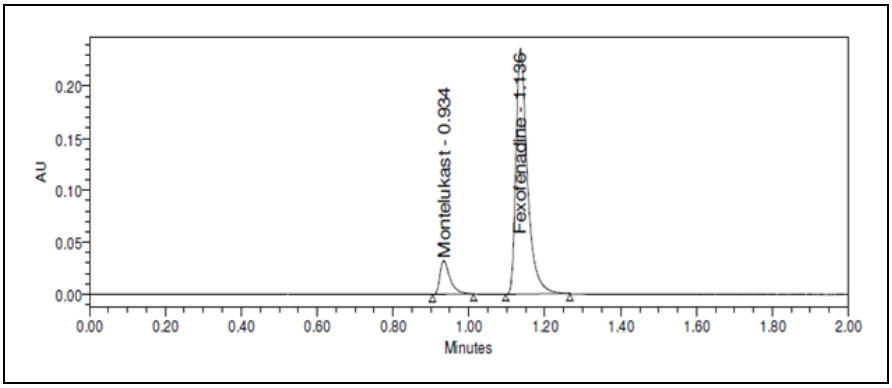
Flow Plus Chromatogram of Montelukast and Fexofenadine:



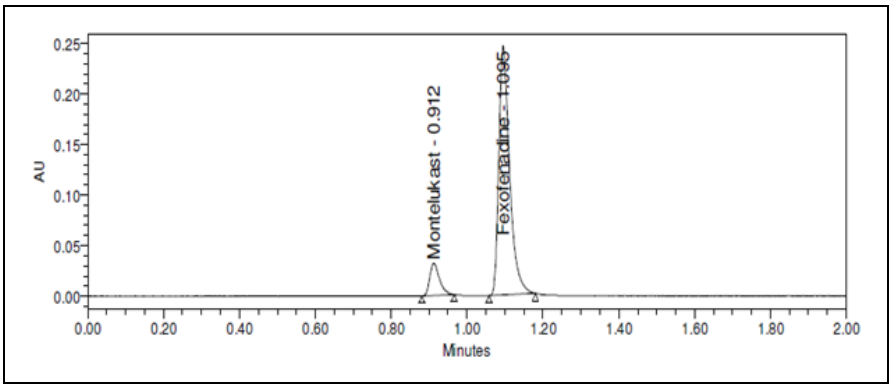
Mobile Phase Minus Chromatogram of Montelukast and Fexofenadine:



Mobile Phase Plus Chromatogram of Montelukast and Fexofenadine:



Temperature Minus Chromatogram of Montelukast and Fexofenadine



Temperature Plus Chromatogram of Montelukast and Fexofenadine:

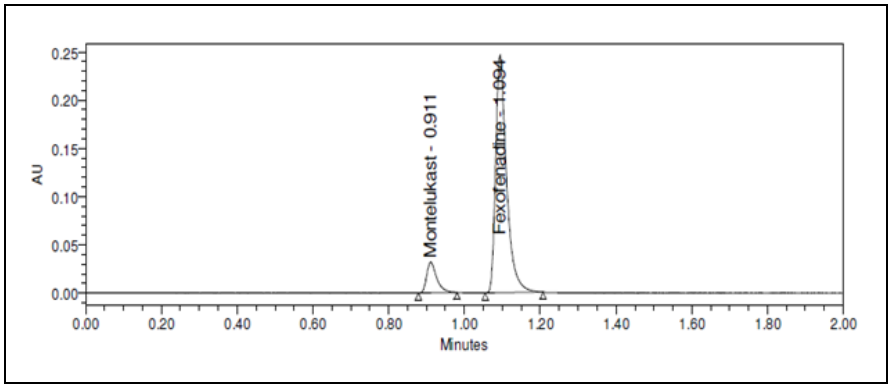


FIG. 16: ROBUSTNESS CHROMATOGRAMS OF MONTELUKAST AND FEXOFENADINE

Acceptance Criteria: %RSD should be NMT 2. System suitability parameters of Montelukast and Fexofenadine were found to be not much affected, and all the method parameters were passed, and %RSD was found to be within the limit.

ASSAY: Allegra, bearing the label, claim Fexofenadine 120 mg, Montelukast 10 mg. The assay was performed based on the above formulation. The peak area responses of standard and sample (six injections) preparations were recorded for Montelukast and Fexofenadine separately and tabulated below in **Table 14** and **15** respectively. The resultant chromatograms were observed in **Fig. 17**.

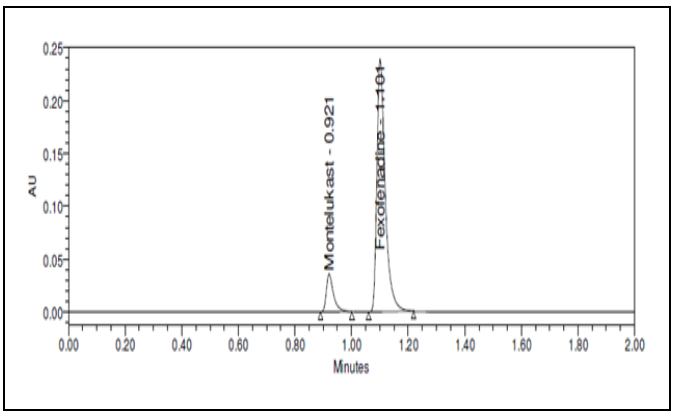
TABLE 14: ASSAY DATA OF MONTELUKAST

S. no.	Standard Area	Sample area	% Assay
1	64134	63026	99.59
2	63209	62875	99.35
3	63405	63727	100.70
4	62801	62539	98.82
5	63006	63471	100.29
6	62394	62919	99.42
Mean	63158	63093	99.70
S.D	591.7	432.2	0.68
%RSD	0.9	0.7	0.69

TABLE 15: ASSAY DATA OF FEXOFENADINE

S. no.	Standard Area	Sample area	% Assay
1	488378	492006	100.70
2	488109	491072	100.51
3	488088	495089	101.33
4	484745	492730	100.84
5	486898	493991	101.10
6	489540	489784	100.24
Mean	487626	492445	100.79
S.D	1643.5	1929.8	0.3950
%RSD	0.3	0.4	0.4

CHROMATOGRAM OF WORKING STANDARD SOLUTION:



Chromatogram of Working Sample Solution:

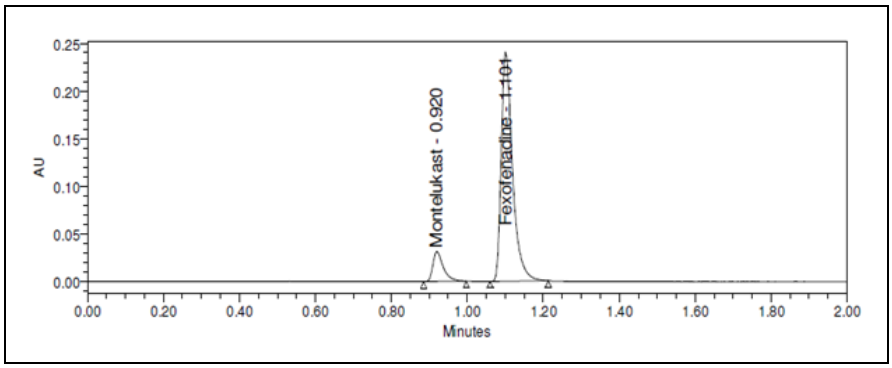


FIG 17: ASSAY CHROMATOGRAMS OF MONTELUKAST AND FEXOFENADINE

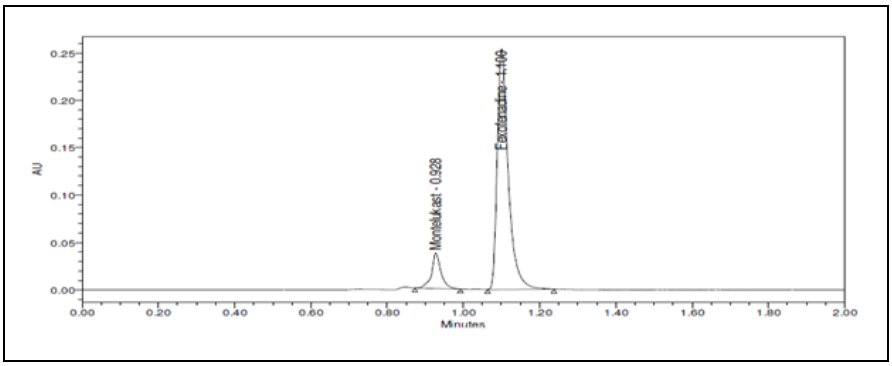
Acceptance Criteria: % Assay should be within 98-102.

Degradation Studies: Samples were injected, and the percentage of drug degraded in the solution by applying different conditions like acid, alkali, oxidative, photolytic, thermal, and neutral analysis was calculated and tabulated in the below **Table 16**. The resultant chromatograms were observed in **Fig. 18**.

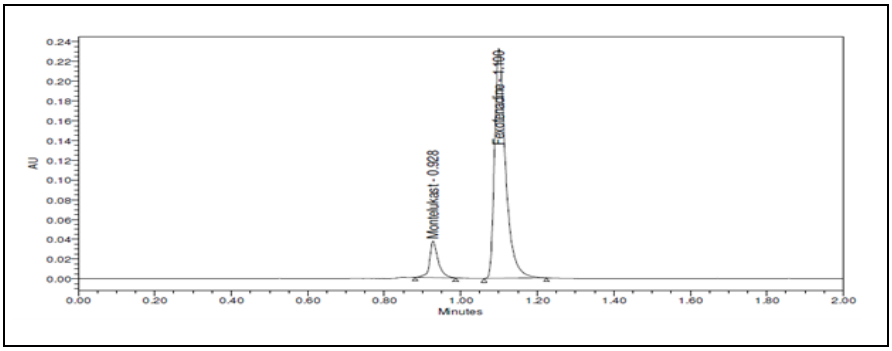
TABLE 16: DEGRADATION DATA

Type of Degradation	Montelukast			Fexofenadine		
	Area	% Recovered	% Degraded	Area	% Recovered	% Degraded
Acid	60376	95.40	4.60	464248	95.02	4.98
Base	61443	97.09	2.91	474314	97.08	2.92
Peroxide	62049	98.05	1.95	479726	98.18	1.82
Thermal	62657	99.01	0.99	485381	99.34	0.66
UV	62858	99.33	0.67	484950	99.25	0.75
Water	62658	99.01	0.99	485114	99.29	0.71

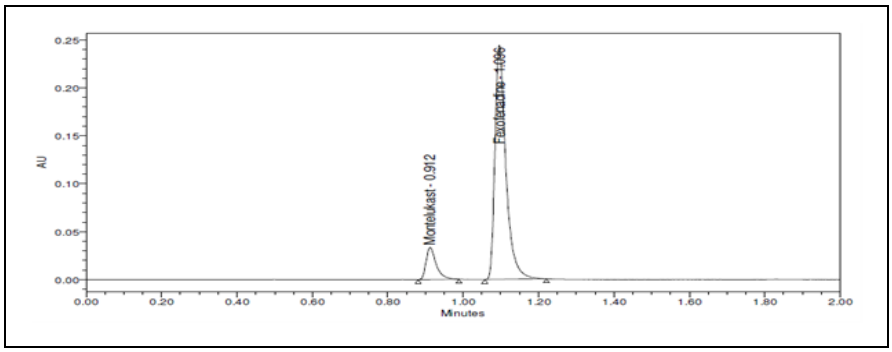
Acid Degradation Chromatogram:



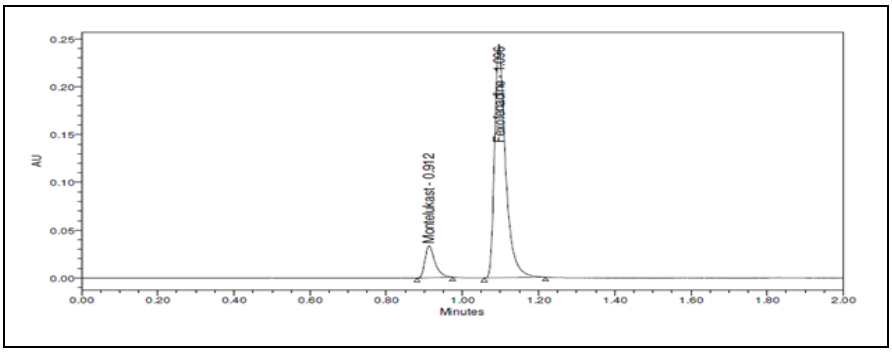
Base Degradation Chromatogram:



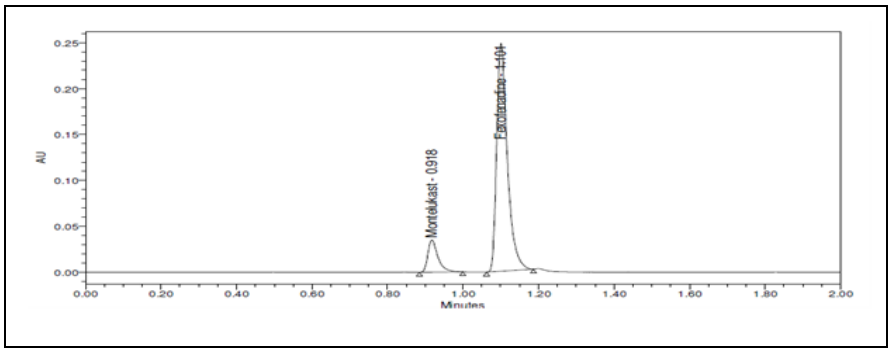
Peroxide Degradation Chromatogram:



Thermal Degradation Chromatogram:



UV Degradation Chromatogram:



Water Degradation Chromatogram:

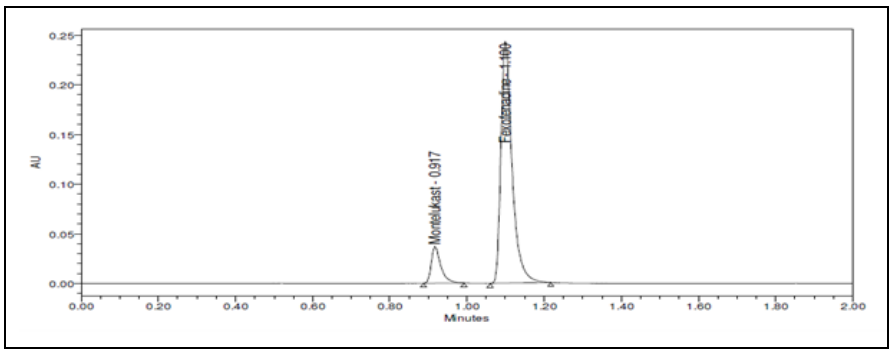


FIG. 18: DEGRADATION CHROMATOGRAMS

CONCLUSION: A novel approach was used to develop and validate a simple, accurate, precise, sensitive, rapid, specific, efficient and reproducible RP-UPLC method for the simultaneous estimation

of the Fexofenadine and Montelukast. The values of the theoretical plate number, tailing factor, retention times, and peak area were found to be within limits. Hence, it is concluded that the system is suitable for the assay of Fexofenadine and Montelukast in pharmaceutical formulation. The proposed method was found to be highly specific, accurate, and precise because it did not show any interference from sample and blank. The Robustness of the method was checked in terms of varying Flow rate, Column temperature and Mobile phase composition. The standard was able to give system suitability parameters within the limit, which indicates that the developed method is Robust. Retention time of Montelukast and Fexofenadine were found to be 0.921 min and 1.101 min. The developed method was found to be simple and have a short run time, which makes the analysis rapid. The forced degradation studies were used to describe the stable nature of both the drugs in a combined drug formulation. They were performed by using HCl (Acid), NaOH (Base), H_2O, H_2O_2 (Peroxide), Thermal, UV radiation. So the method has been successfully used to perform long-term and accelerated stability studies of Fexofenadine and Montelukast formulation.

Retention times were decreased, so that run time was also decreased. Hence, it can be concluded that the proposed method was a good approach for obtaining reliable results and that the developed method can be adopted in regular quality control tests in industries, approved testing laboratories, and clinical pharmacokinetic studies.

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CONFLICTS OF INTEREST: We declare that we have no conflicts of interest.

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