



ISSN: 2306-6091

International Journal of Pharmaceuticals and Health Care Research (IJPHR)

IJPHR | Vol.14 | Issue 4 | Oct - Dec 2026

www.ijphr.com

DOI : <https://doi.org/10.61096/ijphr.v14.iss4.2026.903-923>

RP-HPLC Method and Its Validation for Analysis of Fexofenadine and Montelukast HCL in Bulk and Pharmaceutical Dosage Form

Vanaparthi Sai Bhavani, DR. L. Harikiran

Department of Pharmaceutical Analysis, Princeton College of Pharmacy, Pocharam, Hyderabad, Telangana

*Corresponding Author: **Vanaparthi Sai Bhavani**

Email: guruveeru123@gmail.com



Published on:
14.10.2026
Published by:
Futuristic
Publications
2026| All rights
reserved.



Creative
Commons
Attribution 4.0
International
License.

Abstract: A Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and optimized for the simultaneous estimation of Fexofenadine and Montelukast in bulk and pharmaceutical dosage forms. The method was established by studying various chromatographic parameters to achieve optimal separation and accurate quantification of both drugs. The maximum absorbance for both drugs was found to be at 210 nm, with excellent peak purity. An injection volume of 10 μ L was chosen, which resulted in good peak area and resolution. The Altima C18 column was selected for its ability to provide clear, distinct peaks. The mobile phase, consisting of Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25), was optimized for optimal peak symmetry. The flow rate was maintained at 1.0 mL/min, ensuring satisfactory retention times and peak areas. The total run time was set to 7 minutes, with Fexofenadine and Montelukast eluting at 2.102 and 3.537 minutes, respectively. The method demonstrated excellent linearity over the concentration range of 5-25 mg/mL for Fexofenadine and 12.5-62.5 mg/mL for Montelukast. The method exhibited good recovery (98.0-102%) and precision, with relative standard deviations within acceptable limits. The method was validated for robustness and ruggedness, showing satisfactory results. This RP-HPLC method offers a reliable, efficient, and precise approach for the simultaneous determination of Fexofenadine and Montelukast in pharmaceutical formulations, ensuring its applicability for routine quality control and regulatory compliance.

Keywords: Fexofenadine and Montelukast, RP-HPLC, Validation, Accuracy.

INTRODUCTION

Introduction to HPLC^[1-28]

In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development and production. It is ideal for the analysis of many drugs in both dosage forms and biological fluids due to its simplicity, high specificity and good sensitivity.

High Performance Liquid Chromatography (HPLC) is a technique that has arisen from the application to liquid chromatography the use of an instrumentation that was originally developed for gas chromatography. High Pressure Liquid Chromatography was developed in the mid-1970 and was improved with the development of column packing material and the additional convenience of on-line detectors. The various components of HPLC are pumps (solvent delivery system), mixing unit, gradient controller and solvent degasser, injector (manual or automatic), guard column, analytical columns, detectors, recorders and/or integrators. Recent models are equipped with computers and software for data acquisition and processing. The mobile phase in HPLC refers to the solvent being continuously applied to the column or

stationary phase at a flow rate of 1-5 cm³/min. The mobile phase acts as a carrier for the sample solution. The chemical interactions of the mobile phase and sample with the column determine the degree of migration and separation of components contained in the sample. The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase.

1.1.1 Types of Chromatography^[1]

1. Normal-phase chromatography

Mechanism: Retention by interaction with the polar surface of the stationary phase with polar parts of the sample molecules.

Stationary phase: SiO₂, Al₂O₃, -NH₂, -CN, -Diol, -NO₂, etc.

Mobile phase: Heptane, hexane, cyclohexane, CHCl₃, CH₂Cl₂, dioxane, methanol, etc.

Application: Separation of non-ionic, non-polar to medium polar substances. Disadvantage: Lack of reproducibility of retention times as water or protic organic solvents change the hydration state of the silica or alumina chromatographic media.

2. Reversed-phase chromatography

Mechanism: Retention by interaction of the stationary phase's non-polar hydrocarbon chain with non-polar parts of the sample molecules.

Stationary phase: n-octadecyl (RP-18), n-octyl (RP-8), ethyl (RP-2), phenyl, (CH₂)_n-CN, (CH₂)_n-diol, etc.

Mobile phase: Methanol, acetonitrile, water, buffer (sometimes with additives of THF or Dioxane), etc.

Application: Separation of non-ionic and ion forming non-polar to medium polar substances (carboxylic acids, hydrocarbons). If ion forming substances (as carboxylic acids) are to be separated, a pH control by buffers is necessary.

3. Reversed-phase ion-pair chromatography

Mechanism: Ionic sample molecules are ionically bound to an ion-pair reagent. The ion- pair reagent contains an unpolar part suitable for interaction with the unpolar hydrocarbon chain of the stationary phase.

Stationary phase: Reversed phase materials (RP-18, RP-8, CN), etc.

Mobile phase: Methanol, acetonitrile, buffer with added ion-pair reagent in the concentration range of 0.001 to 0.01 M, etc.

Application: Ionic substances often show very poor retention in reversed phase chromatography. To overcome this difficulty an ion-pair reagent is added to the eluent.

4. Ion-exchange chromatography

Mechanism: Retention of reversible ionic bonds on charged groups of the stationary phase

Stationary phase:

	Strong	Weak
Cation exchanger	SO ₃ ⁻	COO ⁻
Anion exchanger	NR ₃ ⁺	NHR ₂ ⁺

Mobile phase: Aqueous buffer systems.

Application: Separation of substances which can form ions such as inorganic ions, organic acids, organic bases, proteins, nucleic acids.

1.1.2 Advantages of HPLC^[2]

- 1) It provides specific, sensitive and precise method for analysis of the different complicated sample.
- 2) There is ease of sample preparation and sample introduction.

- 3) There is speed of analysis.
- 4) The analysis by HPLC is specific, accurate and precise.
- 5) It offers advantage over gas chromatography in analysis of many polar, ionic substances, high molecular weight substances, metabolic products and thermolabile as well as nonvolatile substances.

1.1.3 Applications of HPLC^[2]

- a) Natural Products: HPLC is an ideal method for the estimation of various components in plant extracts which resemble in structure and thus demand a specific and very sensitive method e.g., analysis of digitalis, cinchona, liquorice, and ergot extracts.
- b) Stability studies: HPLC is now used for ascertaining the stability of various pharmaceuticals. With HPLC the analysis of the various degradation products can be done and thus stability indicating HPLC systems have been developed.
- c) Bioassays and its complementation: Complex molecules as antibiotics and peptide hormones are mainly analysed by bioassay which suffer from high cost, necessity replicates, poor precision and length of time required. Also bioassay gives an overall estimate of potency and gives no guidance about the composition. Thus HPLC can be used to complement bioassays and give an activity profile. It has been used for analysis of chloramphenicol, penicillins, clotrimoxazole, sulfas and peptides hormones.
- d) HPLC has also been used in the cosmetic industry for quality control of various cosmetics.

1.1.4 Instrumentation^[3-8]

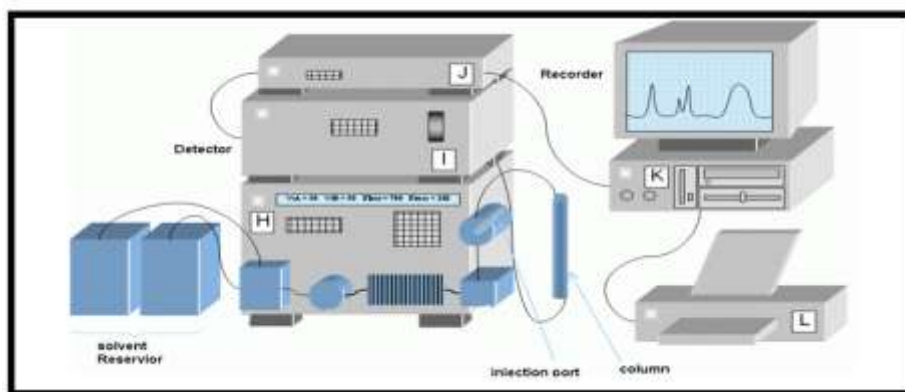


Fig 1: Diagram of HPLC Instrument^[3]

The basic components of HPLC are: ^[4-8]

1. Pumping System
2. Sample Introduction Device
3. Chromatographic Column
4. Detector
5. Data handling Device

1. Pumping System: The HPLC pump is very important component of the system. It delivers the constant flow of the mobile phase or phases so that the separation of the components of the mixture occur in a reasonable time. Its performance directly affects retention time, reproducibility and detector sensitivity. Three main types of pumps are used in HPLC to propel the liquid mobile phase through the system are as under;

a. Displacement pump: It produces a flow that tends to independent of viscosity and backpressure and also output is pulse free. But it possesses limited capacity (250 ml).

b. Reciprocating pump: It has small internal volume (35 to 400 μ l). It has high output pressure (up to 10,000 psi) and constant flow rates. But it produces a pulsed flow.

c. Pneumatic or constant pressure pump: They are pulse free, suffer from limited capacity as well as a dependence of flow rate on solvent viscosity and column back pressure. They are limited to pressure less than 2000 psi.

There are two type of elution process, i.e. isocratic and gradient

Isocratic: In this system, the things are kept constant throughout the run. In the case of pumping of mobile phase, the mobile phase composition is kept constant throughout the run. The nominal flow rate accuracy required is $\pm 1\%$ of the set flow

Gradient: There is some change purposely incorporated during the particular sample run to achieve a better or/and faster separation. In case of pumping mobile phase, the composition of mobile phase is continuously varied during the particular run. The gradient accuracy of $\pm 1\%$ of the step gradient composition is typical.

2. Sample Introducing Device

It is not possible to use direct syringe injection on column like GC, as the inlet pressure in LC is too high. Insertion of the sample onto the pressurized column must be as a narrow plug so that the peak broadening attributable to this step is negligible. The injection system itself should have no dead (void) volume. There are three important ways of introducing the sample into injection port.

a. Loop injection: In which, a fixed amount of volume is introduced by making use of fixed volume loop injector.

b. Valve injection: In which, a variable volume is introduced by making use of an injection valve.

c. On column injection: In which, a variable volume is introduced by means of a syringe through a septum.

3. Chromatographic Column

Column is a heart of chromatography. The column is usually made up of heavy glass or stainless steel tubing to withstand high pressure. The columns are usually 10-30 cm long and 4-10 mm inside diameter containing stationary phase at particle diameter of 25 μm or less. Columns with an internal diameter of 5 mm give good results because of compromise between efficiency, sample capacity, and the amount of packing and solvent required.



Fig 2: Different Types of Columns^[3]

Column packing:

The packing used in modern HPLC consists of small, rigid particles having a narrow particle size distribution. There are three main types of column packing in HPLC.

a. Porous, polymeric beds: Porous, polymeric beds based on styrene divinyl benzene co-polymers used for ion exchange and size exclusion chromatography. For analytical purpose these have now been replaced by silica based, packing which are more efficient and more stable.

b. Porous layer beds: Consisting of a thin shell (1-3 μm) of silica or modified silica on an spherical inert core (e.g. Glass). After the development of totally porous micro particulate packings, these have not been used in HPLC.

c. Totally Porous silica particles (dia. < 10 μm): These packing have widely been used for analytical HPLC in recent years. Particles of diameter > 20 μm are usually dry packed. While particles of diameter <

20 µm are slurry packed in which particles are suspended on a suitable solvent and the slurry so obtained is driven into the column under pressure.

4. Detector

The function of the detector in HPLC is to monitor the mobile phase as it merges from the column. There are several detectors available in the market. However UV Visible detector, photo diode array detector, fluorescence detector, conductometric and coulometric detector are more commonly used. The new ELSD detector is proving to be important detector, while the MS detector is outstanding. Detectors are usually of two types:

a. Bulk property detectors: It compares overall changes in a physical property of the mobile phase with and without an eluting solute e.g. refractive index, dielectric constant or density.

b. Solute property detectors: It responds to a physical property of the solute, which is not exhibited by the pure mobile phase e.g. UV absorbance, fluorescence or diffusion current.

5. Data handling Device

Computer-based system that controls all components of HPLC instrument (eluent composition (mixing of different solvents); temperature, injection sequence, etc.) and acquires data from the detector and monitors system performance (continuous monitoring of the mobile phase composition, temperature, back pressure, etc.)

1.1.5 Methods for Quantitative Analysis^[1,9,10]

Normalized peak area

External standard calibration

Internal standard calculation method

Standard addition calculation method

1. Area normalization method

After integrating all significant peaks in a chromatogram, total peak area may be calculated. Area (%) of any individual peak is called normalized peak area. This technique is widely used particularly in preliminary method development.

$$\% A = \frac{\text{Area of peak A}}{\text{Total area of peaks (A + B + C + D)}} \times 100$$

2. External standard calibration

The external standard is the same substance as that being analysed in the sample. This method of calculation is most commonly used. The external standard must be pure or its composition known through prior analysis. Many times, the standard is costly or is in short supply so a secondary standard can be employed.

By injecting standard solution in different concentrations, peak response is plotted vs concentration. Unknown samples are analysed in similar manner and their concentrations determined from the calibration curve. The calibration curve must cover the range of unknown sample.

$$RF = \frac{\text{Standard peak area}}{\text{Concentration of the sample}}$$

$$\text{Sample concentration} = \frac{\text{Sample peak area}}{RF}$$

Accuracy and precision of the results can further be enhanced if the external standard also contains sample matrix.

3. Internal standard calculation method

It is the addition of known quantity of a foreign substance (internal standard-IS) to the analyzed sample, the response coefficient of which is known or arbitrarily fixed. Addition of internal standard is essential for the sample requiring significant pre-treatment such as derivatisation, extraction to reduce chances of error due to these steps as it is expected to mimic the behaviour of analyte in such re-treatment steps. A calibration curve is produced by analyzing different concentrations of the pure drug with constant amount of internal standard and from the chromatogram. Calculate the ratio (Rs) for each concentration of the analyte.

$$R_s = \frac{\text{Area of drug}}{\text{Area of the internal standard}}$$

Plot this ratio against concentration of the pure drug. The slope of this plot is the response factor.

The requirements for internal standards are that; it must completely resolve peak with no interferences. It should elute close to the compound of interest and behave equivalent to the compound of interest for analysis like pretreatments and derivative formations. It should also be stable, unreactive with sample components, column packing and the mobile phase and commercially available in high purity. This technique gives reliable, accurate, and precise results. If the internal standard is truly inert, the method is useful for determining the rate of analyte conversion in a chemical reaction.

4. Standard addition calculation method

The principle involved is that, the analytical signal is proportional to concentration, the initial analyte content is determined through measurements of this signal before and after the addition of known amount of the analyte to the analyzed sample. The method of standard addition, also denoted as “spiking,” is used when an analyte is to be quantified inside the matrix, the effect of which are likely to affect the chromatographic peak behavior.

In peak area method standard solution containing known quantities of all component of the mixture is chromatographed several times. The mean of peak area of each component is calculated and used to calibrate the detector response. Samples are also chromatographed several times; area for each sample is co-related with peak area of the standard.

1.1.6 Parameters Used in Chromatogram Characterization^[11,12]

1.1.6.1 Van Deemter Equation^[11]

Chromatography relates the variance per unit length of a separation column to the linear mobile phase velocity by considering physical, kinetic and thermodynamic properties of a separation. These properties include pathways within the column, diffusion (axial and longitudinal) and mass transfer kinetics between stationary and mobile phases. In liquid chromatography, the mobile phase velocity is taken as the exit velocity that is the ratio of the flow rate in ml/sec to the cross-sectional area of the ‘column-exit flow path.’ For a packed column, the cross-sectional area of the column exit flow path is usually taken as 0.6 times the cross-sectional area of the column. Alternatively, the linear velocity can be taken as the ratio of the column length to the dead time. The Van Deemter equation is:

$$H = A + \frac{B}{u} + Cu$$

$$H = A + \frac{B}{u} + \left(\frac{C_s}{C_m}\right)u$$

Where, H = Height equivalent of a theoretical plate (HETP)

u = Velocity (flow rate) of the mobile phase

A = Multipath term

B = Longitudinal diffusion term

C = Mass transfer term for mobile and stationary phases

The lower the resulting value of H, greater is the efficiency of the procedure. So, ideally a scientist will want to minimize all three terms in order to minimize H. The other three terms refer to factors that come into play while the chromatography is performed.

A - Multipath Term (Eddy Diffusion Factor)

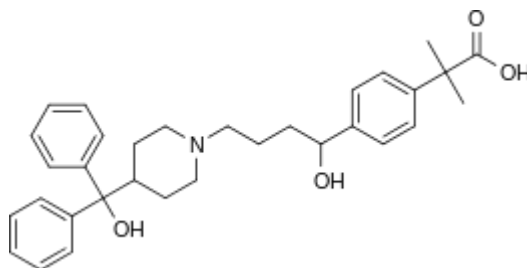
It is concerned with different paths travelled by molecules in a particular column. This leads to band broadening coefficient A.

The length of these pathways may differ significantly thus residence time in column for molecules of the same species is also variable.

The solute molecule then reached at the end of the column over a time interval which

MATERIALS AND METHODS

Drug : Fexofenadine
Synonym : Terfenadine carboxylate
Drug category : Anti-Allergic Agents
Structure:



Chemical name/ Nomenclature / IUPAC Name: (±)-4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-α, α-dimethyl benzeneacetic acid

Molecular Formula: C₃₂H₃₉NO₄

Molecular Weight: 501.68 gm/mole.

Official Pharmacopoeia: EP

PHYSICOCHEMICAL PROPERTIES:

Description (Physical State): Solid

Solubility: water solubility Slightly soluble

Storage Conditions: should be stored at controlled room temperature between 20-25 deg C

Dosage: Tablet

Melting point: 142.5 °C

pKa (strongest acidic): 4.04

Log P: 5.6

PHARMACOKINETIC PROPERTIES:

Bioavailability : 30-41%

Half-life : 14.4 hours

Absorption : 33%

Protein binding : 60-70%

Metabolism : Approximately 5% of the total dose is metabolized, by cytochrome P450 3A4 and by intestinal microflora.

Adverse effects/Side effects: nausea, diarrhea, upset stomach, menstrual cramps.

PHARMACODYNAMICS: Fexofenadine is a second-generation, long lasting H1-receptor antagonist (antihistamine) which has a selective and peripheral H1-antagonist action. Histamine is a chemical that causes many of the signs that are part of allergic reactions, such as the swelling of tissues. Histamine is released from histamine-storing cells (mast cells) and attaches to other cells that have receptors for histamine. The attachment of the histamine to the receptors causes the cell to be "activated," releasing other chemicals which produce the effects that we associate with allergy. Fexofenadine blocks one type of receptor for histamine (the H1 receptor) and thus prevents activation of cells by histamine. Unlike most other antihistamines, Fexofenadine does not enter the brain from the blood and, therefore, does not cause drowsiness. Fexofenadine lacks the cardiotoxic potential of terfenadine, since it does not block the potassium channel involved in repolarization of cardiac cells.

Mechanism of action: Like other H1-blockers, Fexofenadine competes with free histamine for binding at H1-receptors in the GI tract, large blood vessels, and bronchial smooth muscle. This blocks the action of endogenous histamine, which subsequently leads to temporary relief of the negative symptoms (eg. nasal congestion, watery eyes) brought on by histamine. Fexofenadine exhibits no anticholinergic, antidopaminergic, alpha1-adrenergic or beta-adrenergic-receptor blocking effects.

Therapeutic efficacy/ Indications: For management of Seasonal allergic rhinitis

Contraindications: Allegra tablets, Allegra Odt and Allegra oral suspension are contraindicated in patients with known hypersensitivity to fexofenadine and any of the ingredients of Allegra. Rare cases of hypersensitivity reactions with manifestations such as angioedema, chest tightness, dyspnea, flushing and systemic anaphylaxis have been reported.

INTERACTIONS:

Drug interactions: 2,5-Dimethoxy-4-ethylamphetamine 2,5-Dimethoxy-4-ethylamphetamine may decrease the sedative activities of Fexofenadine.

3,4-Methylenedioxyamphetamine 3,4-Methylenedioxyamphetamine may decrease the sedative activities of Fexofenadine.

4-Bromo-2,5-dimethoxyamphetamine 4-Bromo-2,5-dimethoxyamphetamine may decrease the sedative activities of Fexofenadine.

7-Nitroindazole The risk or severity of adverse effects can be increased when 7-Nitroindazole is combined with Fexofenadine.

Acepromazine The risk or severity of adverse effects can be increased when Acepromazine is combined with Fexofenadine.

Food interactions: Grapefruit and grapefruit juice should be avoided throughout treatment as grapefruit can significantly decrease serum levels of this product.

Take without regard to meals.

DRUG FORMULATION:

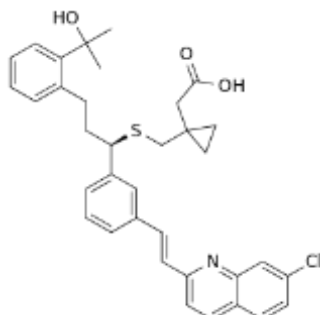
S.No	Drug name	Label Claim	Brand name	Company
1	Fexofenadine	120mg	Allegra	Mylan

Drug : Montelukast

Synonym : Montelukast

Drug category : Anti-Asthmatic Agents

Structure:



Chemical name/ Nomenclature / IUPAC Name : (R,E)-2-((1-(3-(2-(7-Chloroquinolin-2-yl)vinyl)phenyl)-3-(2-(2-hydroxypropan-2-yl)phenyl)propylthio)methyl)cyclopropyl)acetic acid

Molecular Formula : C₃₅H₃₆ClNO₃S

Molecular Weight : 586.184 gm/mole.

Official Pharmacopoeia : USP, EP

PHYSICOCHEMICAL PROPERTIES:

Description (Physical State): Solid

Solubility: Water Solubility 8.2e-06 mg/mL

Storage Conditions: Store montelukast sodium tablets at 20 o to 25 oC (68 o to 77 oF), excursions permitted to 15 o to 30 oC (59 o to 86 oF)

Dosage: Tablet

pKa (strongest acidic): 4.4

Log P: 7.25

PHARMACOKINETIC PROPERTIES:

Bioavailability : 63–73%

Half-life : 2.7–5.5 hours

Absorption : Rapidly absorbed following oral administration (bioavailability is 64%)

Volume of Distribution : 8 to 11 L

Protein binding : 99%

Excretion : Coupled with estimates of montelukast oral bioavailability, this indicates that montelukast and its metabolites are excreted almost exclusively via the bile.

Adverse effects/Side effects : Feeling confused, Not able to focus, Bad dreams ,Not able to sleep ,Sleepwalking.

PHARMACODYNAMICS: Montelukast, like zafirlukast, is a leukotriene receptor antagonist used as an alternative to anti-inflammatory medications in the management and chronic treatment of asthma and exercise-induced bronchospasm (EIB). Unlike zafirlukast, montelukast does not inhibit CYP2C9 or CYP3A4 and is, therefore, not expected to affect the hepatic clearance of drugs metabolized by these enzymes.

Mechanism of action: Montelukast selectively antagonizes leukotriene D₄ (LTD₄) at the cysteinyl leukotriene receptor, CysLT₁, in the human airway. Montelukast inhibits the actions of LTD₄ at the CysLT₁ receptor, preventing airway edema, smooth muscle contraction, and enhanced secretion of thick, viscous mucus.

Therapeutic efficacy/ Indications: For the treatment of asthma

Adverse reactions: Churg-Strauss syndrome / Delayed / Incidence not known

vasculitis / Delayed / Incidence not known

erythema multiforme / Delayed / Incidence not known

Contraindications: Hypersensitivity to any component of this product.

INTERACTIONS:

Drug interactions: Abiraterone The metabolism of Montelukast can be decreased when combined with Abiraterone.

Amiodarone The metabolism of Montelukast can be decreased when combined with Amiodarone.

Amodiaquine The serum concentration of Amodiaquine can be increased when it is combined with Montelukast.

Aprepitant The serum concentration of Montelukast can be increased when it is combined with Aprepitant.

Atazanavir The metabolism of Montelukast can be decreased when combined with Atazanavir.

Food interactions: Take without regard to meals.

DRUG FORMULATION:

S.No	Drug name	Label Claim	Brand name	Company
1	Montelukast	4,5,10MG	Singulair	STADA

RESULTS AND DISCUSSION

Trails

Trail 1:

Mobile phase : ACN: Water (70:30%v/v)

Column : Symmetry C18 (4.6 ×150mm, 5µm particle size) Make; waters

Flow rate : 1.1ml/min

Wavelength : 210 nm

Column temp : 40°C

Injection Volume : 10 µl

Run time : 4 minutes

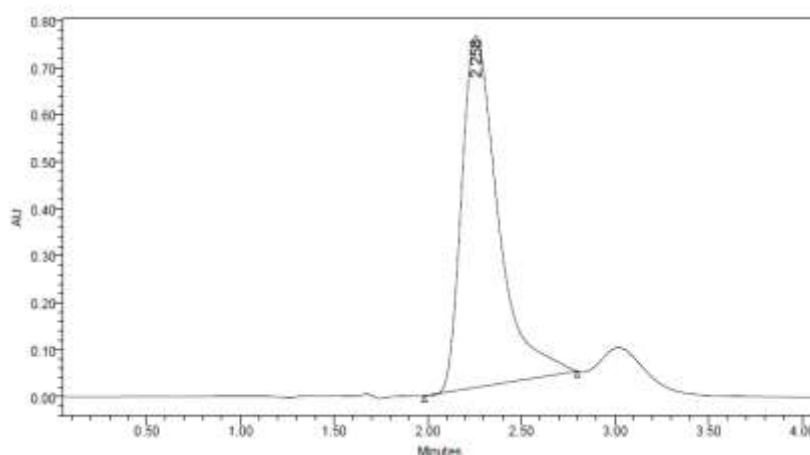


Fig: chromatogram for trail 1

Table: peak results for trail 1

S.No	Peak Name	R _t	Area	Height	USP Resolution	USP Tailing	USP Plate count
1	Fexofenadine	2.258	10177443	750855		0.81	642

Observation: In a separation of Fexofenadine and Montelukast peak was obtained only for one compound because there may be less solubility. So, we go for further trails.

Trail 2:

Mobile phase : Water: Methanol (15:85v/v)

Column : Zodiac C18 (4.6×250mm) 5µ

Flow rate : 1.2 ml/min

Wavelength : 210 nm

Column temp : 40°C

Injection Volume : 9 μ l

Run time : 2 minutes

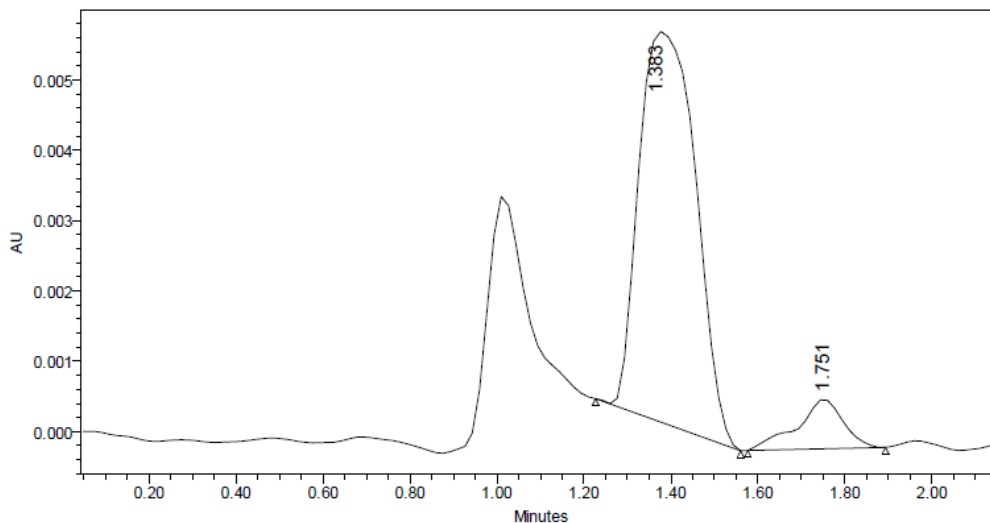


Fig: chromatogram for trail 2

Table: peak results for trail 2

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Fexofenadine	1.383	50680	5598	1.9	1.23	595
2	Montelukast	1.751	5203	697		0.83	846

Observation: The separations of two compounds were obtained but improper separation is obtained and resolution is not good. So, we go for further trails.

Trail 3:

Mobile phase : Methanol: TEA Buffer pH 4.5: ACN (55:35:10% v/v)

Column : Zodiac C18 (4.6×250mm 5 μ m)

Flow rate : 0.8 ml/min

Wavelength : 210 nm

Column temp : 40°C

Injection Volume : 20 μ l

Run time : 10 minutes

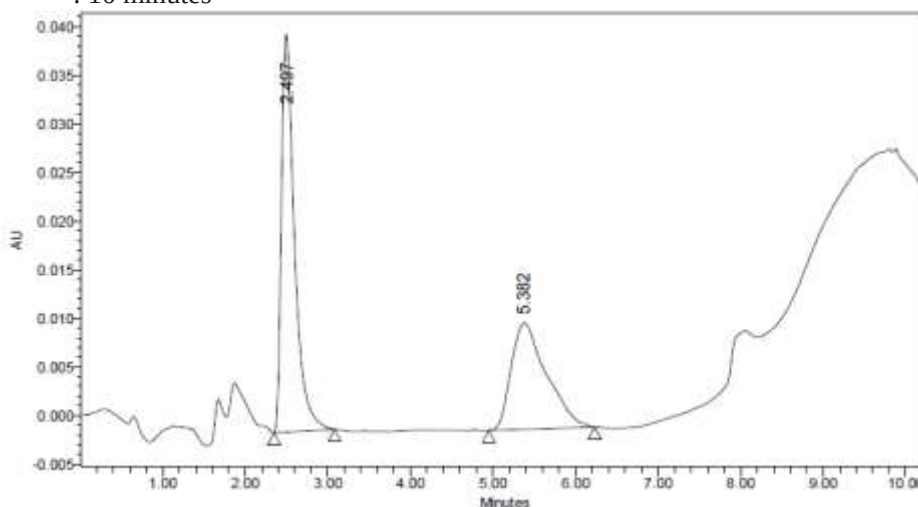


Fig: chromatogram for trail 3

Table: peak results for trail 3

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Fexofenadine	2.497	450040	40970		2.5	1292
2	Montelukast	5.382	323454	10940	1.39	3.5	677

Observation: The separations of two compounds were obtained but base line noise was very high, peak symmetry was not available. We go for further trail as the void peaks are obtained.

Trail 4:

Mobile phase : Methanol: TEA Buffer pH 4.5: Acetonitrile (55:30:15)

Column : Symmetry C18 5 μ m (4.6 $\times$ 250mm) Make; waters

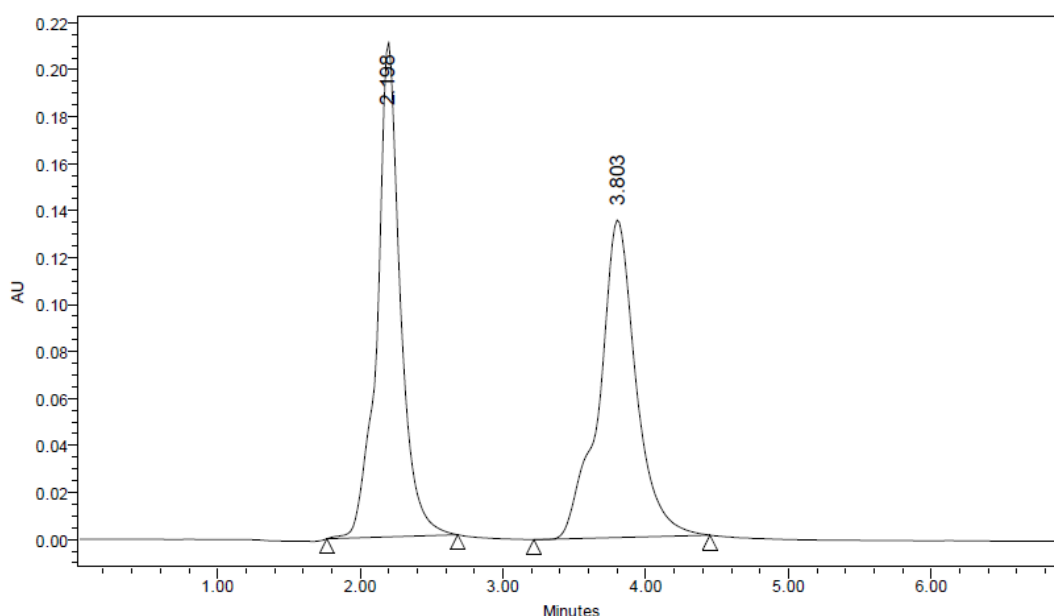
Flow rate : 1 ml/min

Wavelength : 210 nm

Column temp : 40°C

Injection Volume : 10 μ l

Run time : 7 minutes



S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Fexofenadine	2.198	2384517	210516		1.0	1140
2	Montelukast	3.803	2439720	135275	3.02	1.0	1345

Observation: After repeating the same conditions got proper peaks, base line is good and we can go for repeatability. Therefore separations of two peaks, base line, peak symmetry, resolution are proper.

Optimised Chromatogram (Standard)

Mobile phase : Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25)

Column : Altima C18 (4.6 $\times$ 150mm, 5.0 μ m)

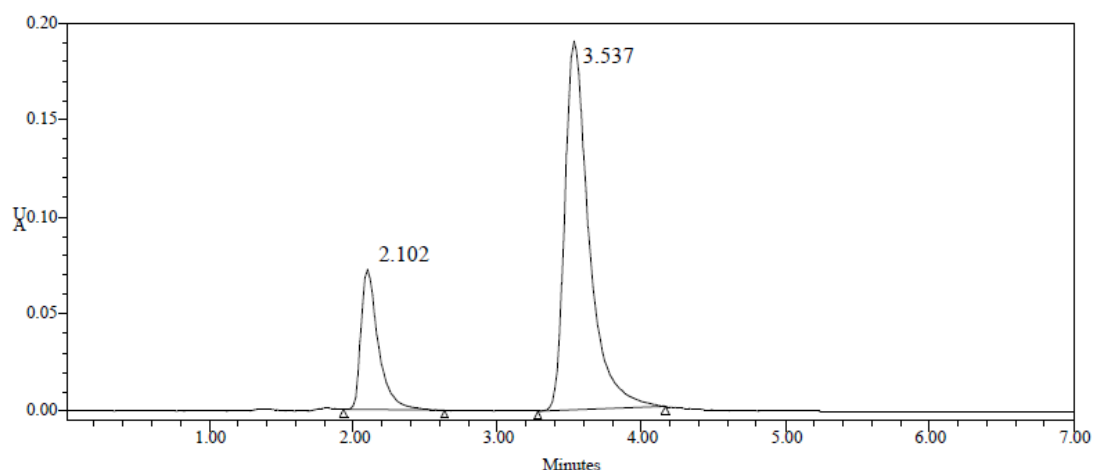
Flow rate : 1 ml/min

Wavelength : 210 nm

Column temp : 40°C

Injection Volume : 10 μ l

Run time : 7 minutes



Optimized Chromatogram

Table: peak results for optimised

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Fexofenadine	2.102	607323	72100		0.96	5586.0
2	Montelukast	3.537	2231111	190007	2.97	1.22	5371.0

Observation: From the above chromatogram it was observed that the Fexofenadine and Montelukast peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimised trial.

Optimised Chromatogram (Sample)

Mobile phase : Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25)

Column : Altima C18 (4.6×150mm, 5.0 µm)

Flow rate : 1 ml/min

Wavelength : 210 nm

Column temp : 40°C

Injection Volume : 10 µl

Run time : 7 minutes

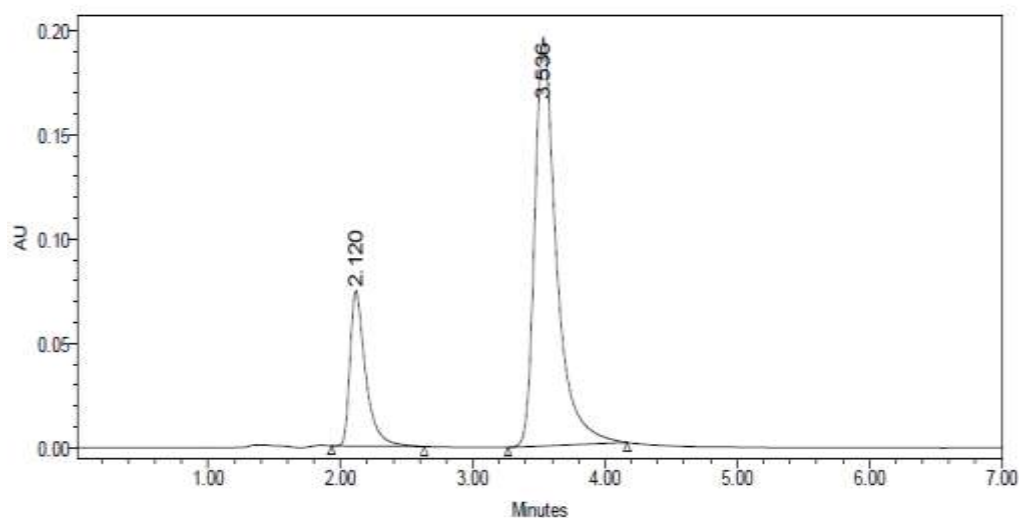


Fig: Optimised Chromatogram (Sample)

Table: Optimised Chromatogram (Sample)

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Fexofenadine	2.120	775610	130275		0.98	6253
2	Montelukast	3.536	555592	93740	5.06	1.23	7836

Acceptance criteria:

- Resolution between two drugs must be not less than 2
- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION

Blank:

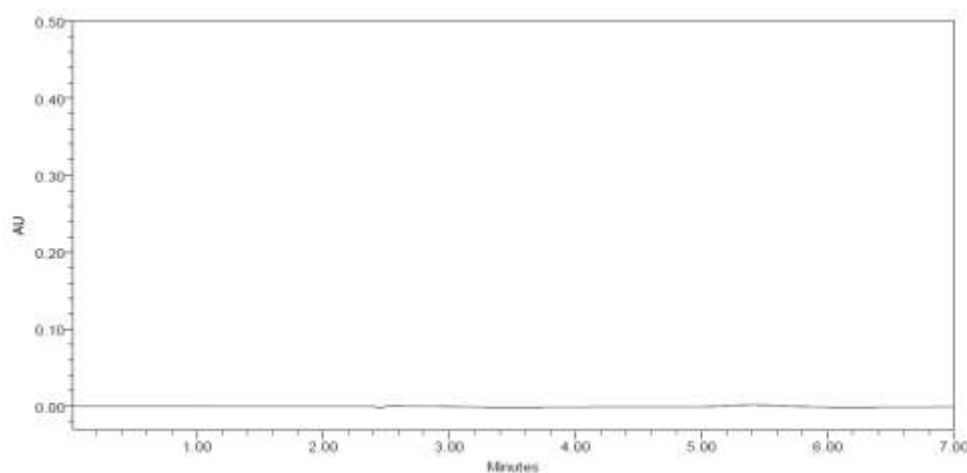


Fig: Chromatogram showing blank (mobile phase preparation)

System suitability:

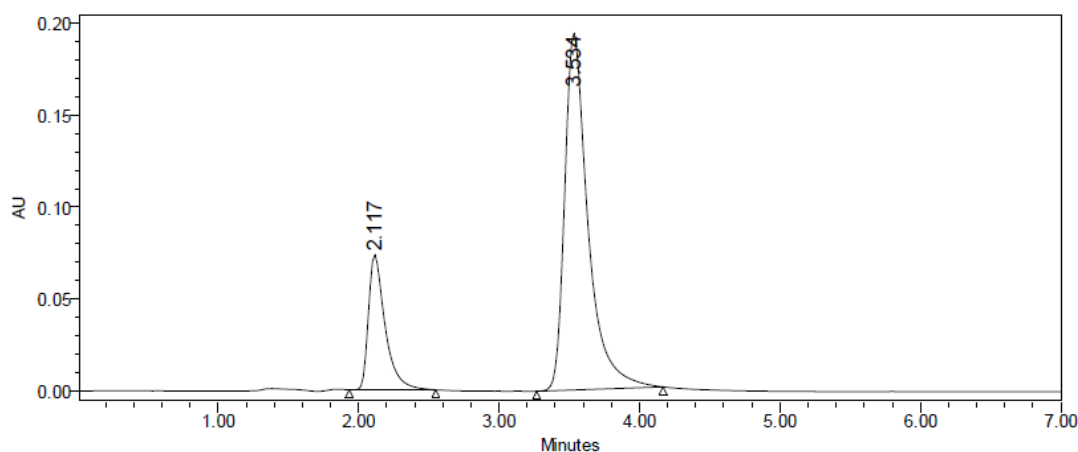


Fig: Chromatogram showing injection -1

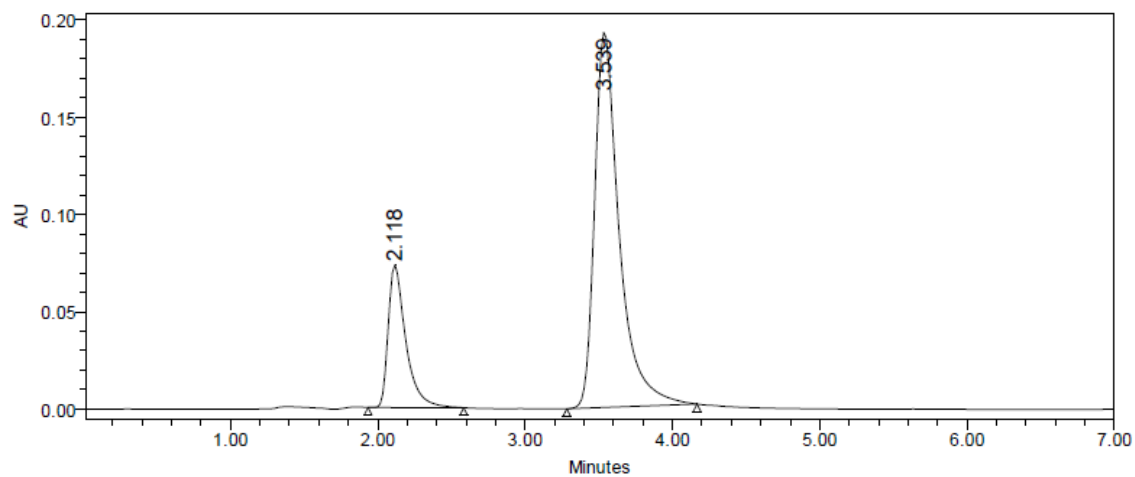


Fig: Chromatogram showing injection -2

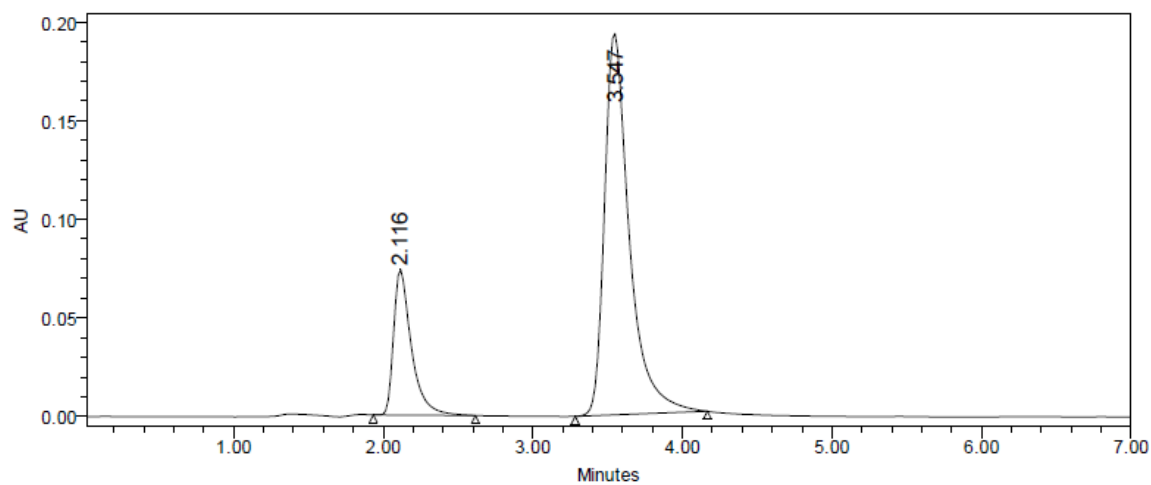


Fig: Chromatogram showing injection -3

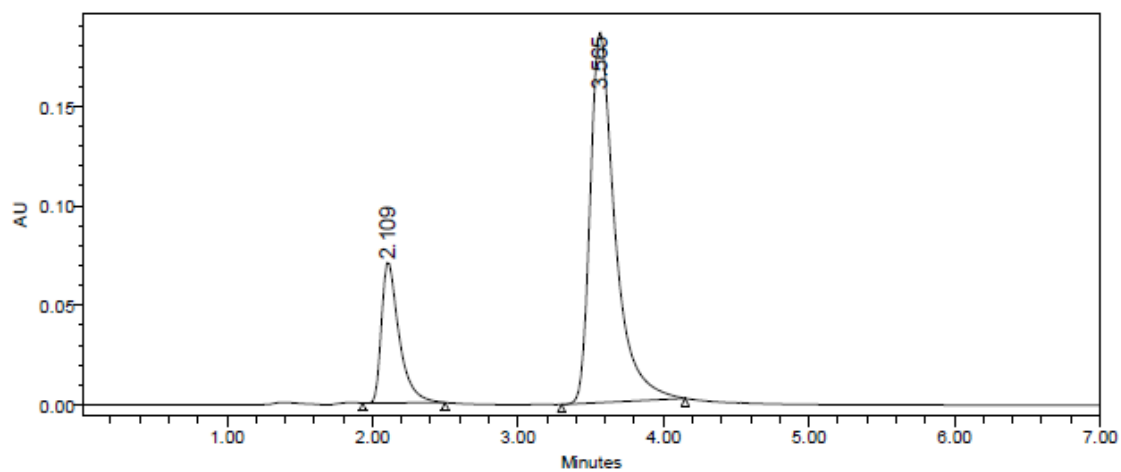


Fig: Chromatogram showing injection -4

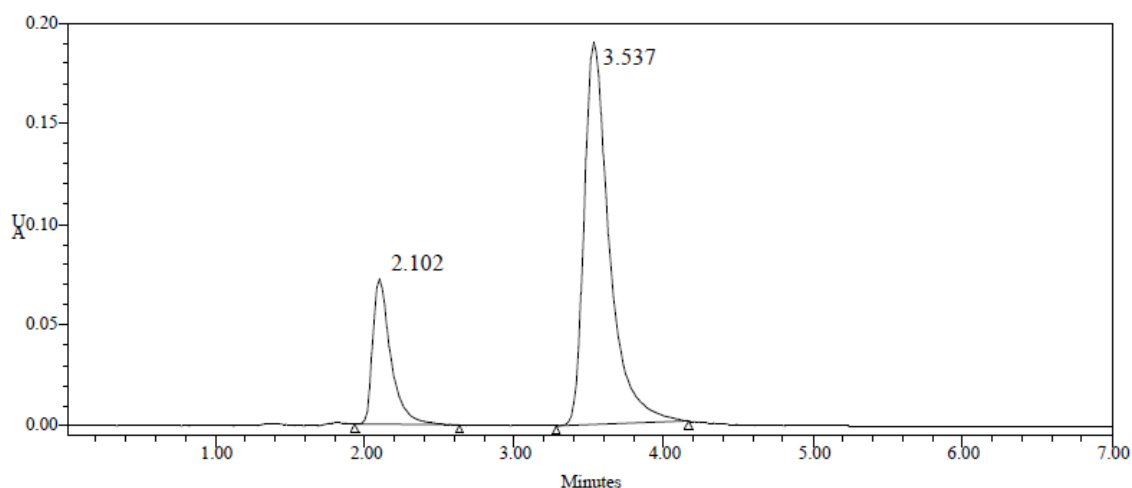


Fig: Chromatogram showing injection -5

Table: Results of system suitability for Fexofenadine

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Fexofenadine	2.117	608452	71498	5643	1.9
2	Fexofenadine	2.118	606820	126412	5432	1.6
3	Fexofenadine	2.116	608452	126471	5123	1.6
4	Fexofenadine	2.109	595267	129859	5207	1.7
5	Fexofenadine	2.102	596608	124691	5481	1.6
Mean			603119.8			
Std. Dev			6607.31			
% RSD			1.09			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table: Results of system suitability for Montelukast

S.no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Montelukast	3.547	2234724	188631	5043	1.2	2.07
2	Montelukast	3.539	2240080	2614821	5432	1.4	2.05
3	Montelukast	3.547	2234724	2321451	5987	1.5	2.0
4	Montelukast	3.565	2204466	2324710	5845	1.6	2.01
5	Montelukast	3.537	2209574	2531247	5371	1.6	2.01
Mean			2224714				
Std. Dev			16399.05				
% RSD			0.73				

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitates Fexofenadine and Montelukast in drug product.

Assay (Standard):

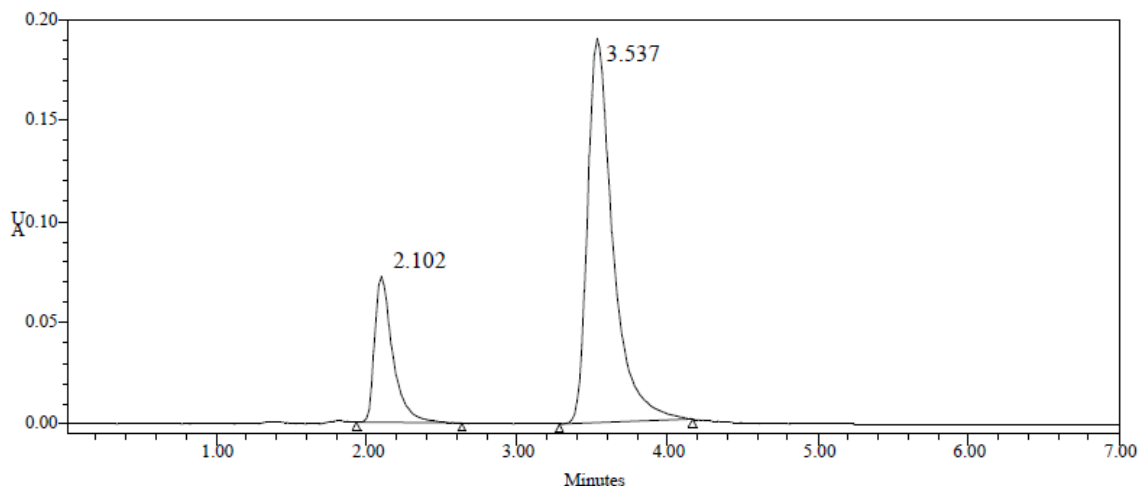


Fig: Chromatogram showing assay of standard injection -1

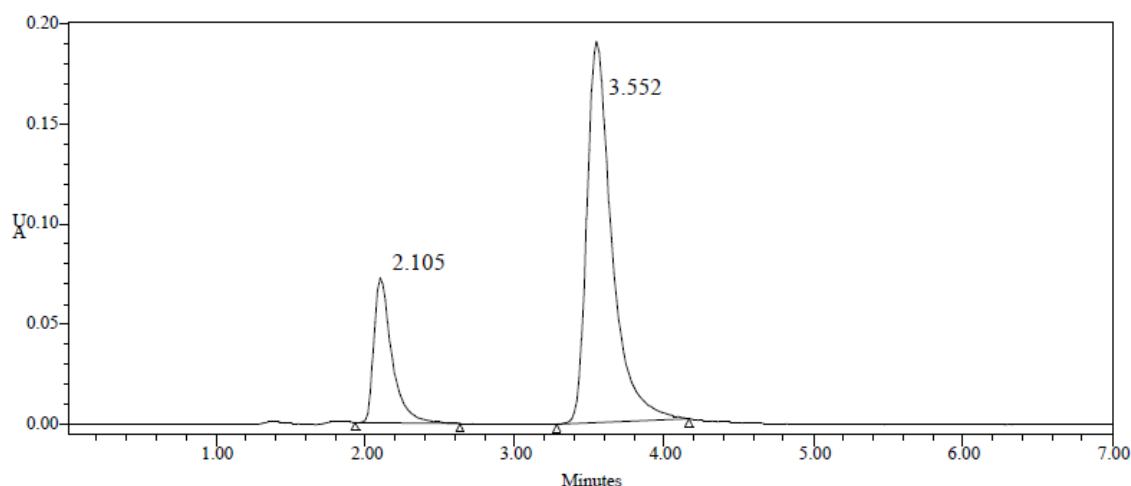


Fig: Chromatogram showing assay of standard injection -2

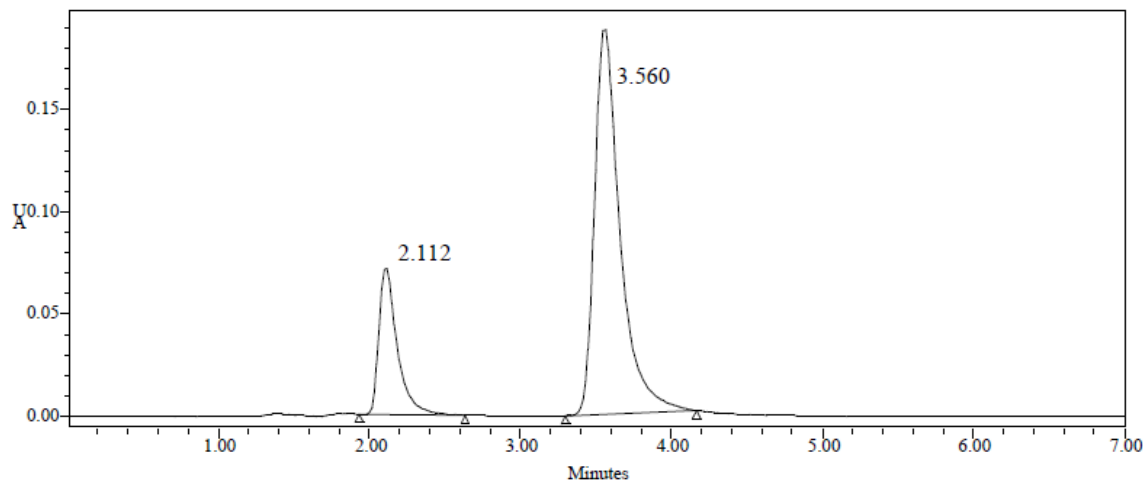


Fig: Chromatogram showing assay of standard injection -3

Table: Peak results for assay standard

S.no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Fexofenadine	2.102	607323	128898		1.7	2586	1
2	Montelukast	3.537	558777	2231111	2.04	1.6	2371	1
3	Fexofenadine	2.105	606379	127950		1.7	2636	2
4	Montelukast	3.552	578377	2220237	2.00	1.6	2414	2
5	Fexofenadine	2.112	606885	129769		1.7	2561	3
6	Montelukast	3.560	556966	2217353	2.04	1.6	2384	3

Assay (Sample):

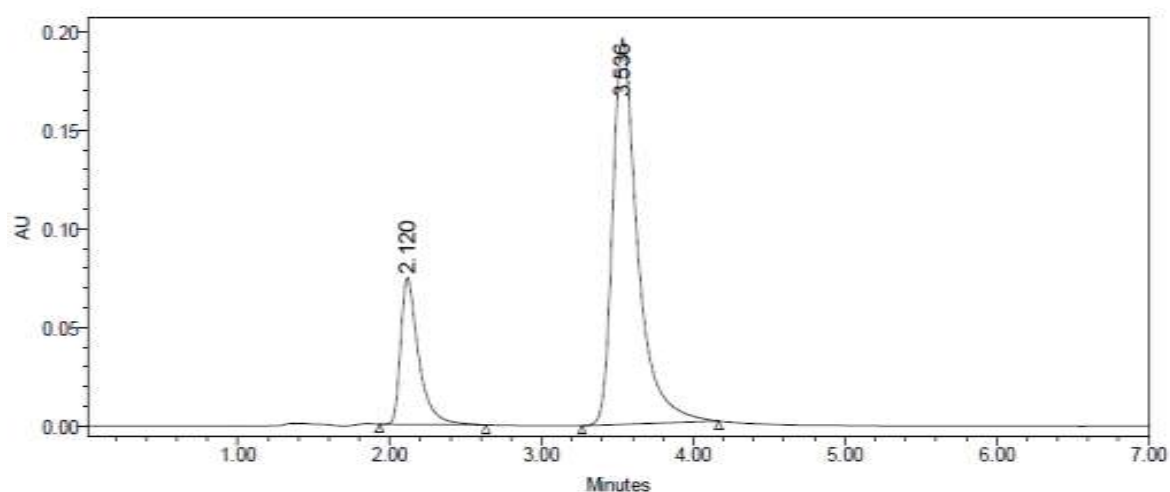


Fig: Chromatogram showing assay of sample injection-1

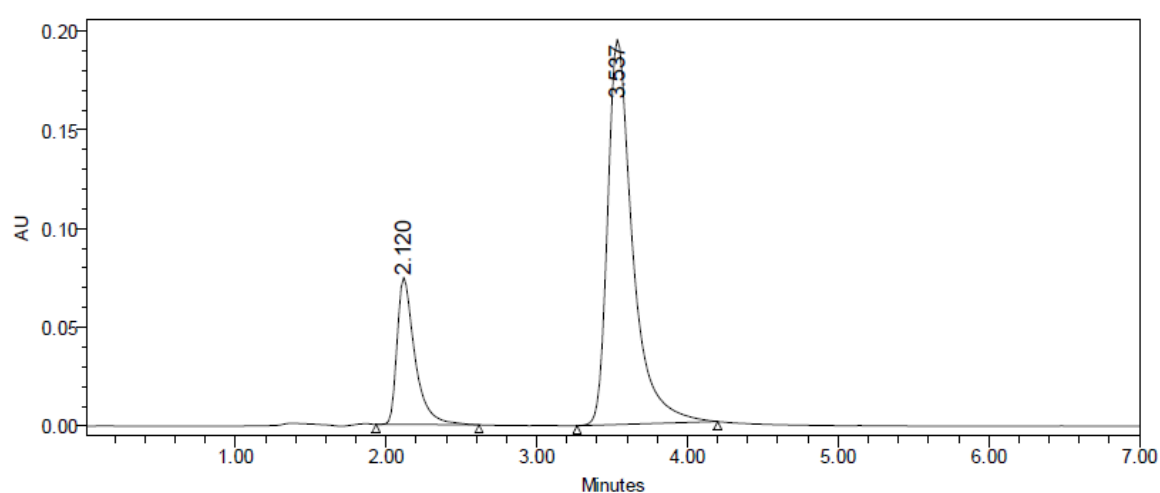


Fig: Chromatogram showing assay of sample injection-2

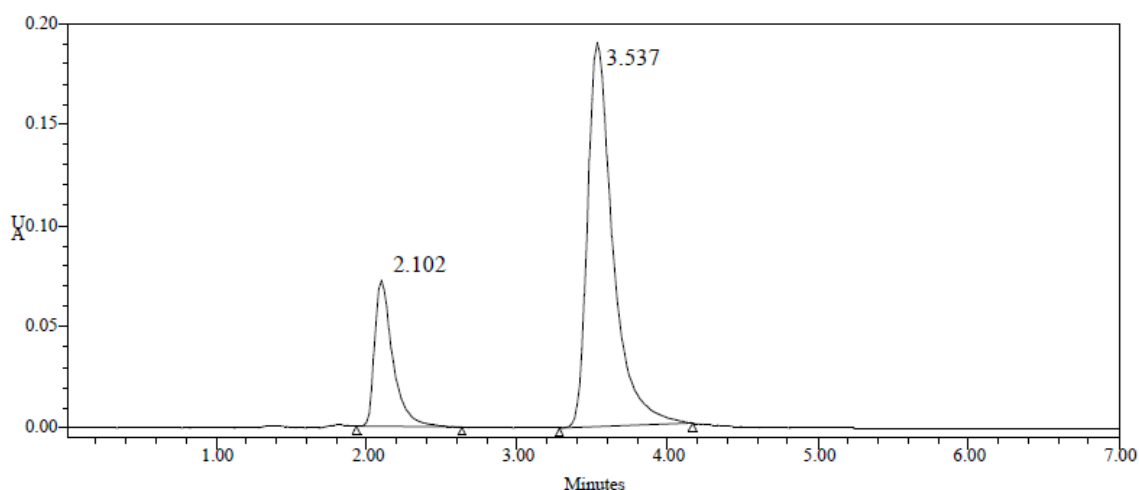


Fig: Chromatogram showing assay of sample injection-3

Table: Peak results for Assay sample

S.no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Fexofenadine	2.120	775610	130275		0.98	7253	1
2	Montelukast	3.536	555592	93740	2.06	1.23	8836	1
3	Fexofenadine	2.120	689956	73869		1.05	6530	2
4	Montelukast	3.537	575685	129125	2.04	0.99	7270	2
5	Fexofenadine	2.102	607323	128898		1.7	7586	3
6	Montelukast	3.537	558777	2231111	2.04	1.6	8371	3

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Fexofenadine and Montelukast in pharmaceutical dosage form was found to be 99.6%.

LINEARITY

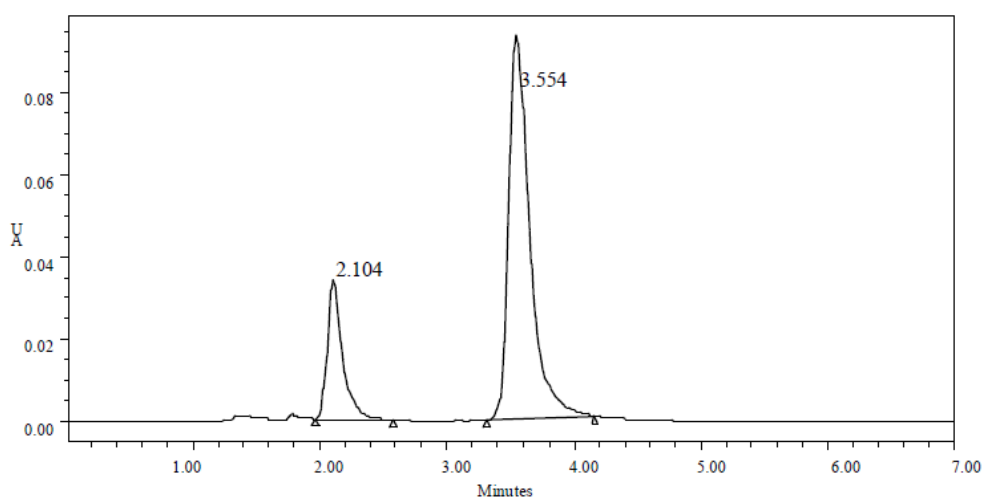


Fig: Chromatogram for linearity concentration-10µg/ml of Fexofenadine & 20 µg/ml of Montelukast

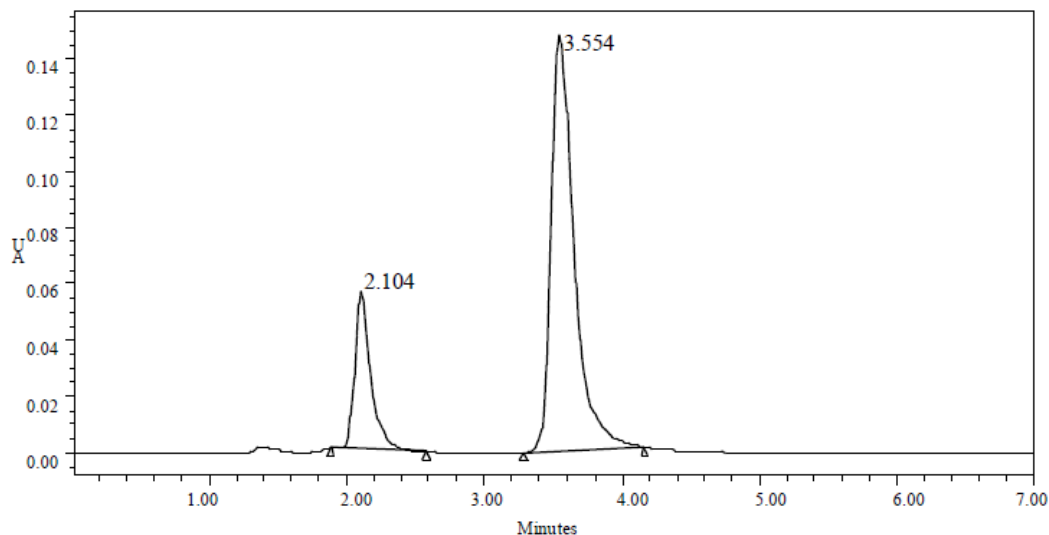


Fig: chromatogram for linearity concentration-20 µg/ml of Fexofenadine & 40µg/ml of Montelukast

CONCLUSION

Summary:

The present study developed and validated a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Fexofenadine and Montelukast in both bulk and pharmaceutical dosage forms. The method was optimized by adjusting several chromatographic parameters. The maximum absorbance for both drugs was found at 210 nm, ensuring excellent peak purity. The analysis was conducted using an Altima C18 column, with a mobile phase composed of Methanol: TEA Buffer pH 4.5: Acetonitrile in a 50:25:25 ratio. The flow rate was set to 1.0 mL/min, and a total run time of 7 minutes was chosen, with Fexofenadine and Montelukast eluting at 2.102 minutes and 3.537 minutes, respectively. The method showed linearity in the concentration ranges of 5-25 mg/mL for Fexofenadine and 12.5-62.5 mg/mL for Montelukast. Recovery values ranged from 98.0-102%, and the method demonstrated high precision with acceptable relative standard deviations. Validation tests for robustness and ruggedness were also performed, and the method passed with satisfactory results.

Conclusion:

The developed RP-HPLC method for the simultaneous estimation of Fexofenadine and Montelukast proved to be reliable, accurate, and precise. The method was validated and demonstrated excellent linearity, recovery, and method precision, making it suitable for routine pharmaceutical quality control and regulatory compliance. The optimized parameters, including the selection of the Altima C18 column, mobile phase composition, and flow rate, ensure that the method is efficient for the analysis of these drugs in both bulk and tablet formulations. The results indicate that this RP-HPLC method can be confidently used for the routine analysis and quality control of Fexofenadine and Montelukast in pharmaceutical products.

BIBLIOGRAPHY

1. Shethi PD. HPLC- Quantitative analysis of pharmaceutical formulations. 1st Ed. New Delhi: CBS Publishers & Distributors; 2001: 8-10, 101-103.
2. Kasture AV, Mahadik KR, Wadodkar SG, More HN. Pharmaceutical Analysis: Vol-II. 8th Ed. Pune: Nirali Prakashan; 2002: 48-57.
3. Prajapati GA. Method development and validation for simultaneous estimation of Hypertensive drugs by RP-HPLC. M.Pharm Thesis, Maliba Pharmacy College, Gujarat Technological University, Gujarat, India, 2011: 7-28.
4. Gabor S. HPLC in pharmaceutical Analysis: Vol. I. 1st Ed. London: CRC Press; 1990:101-173.
5. Jeffery GH, Bassett J. Vogel's textbook of Quantitative Chemical Analysis. 5th Ed. NewYork : John Wiley & Sons Inc; 1991: 217-235.

6. Hobart HW, Merritt LL, John AD. Instrumental Methods of Analysis. 7th Ed. New Delhi: CBS Publishers; 1988: 580-610.
7. Sharma BK. Instrumental Method of Chemical Analysis. 20th Ed. Meerut: Goel Publishing House; 2001: 54-83.
8. Ashutoshkar. Pharmaceutical Drug Analysis. 2nd Ed. New Delhi: New Age International Publisher; 2005: 455-466.
9. Ahuja S, Michael WD. Hand book of Pharmaceutical Analysis by HPLC. 1st Ed. London: Elsevier Academic Press; 2005: 44-54.
10. Snyder LR, Kirkland JL, Glajch JL. Practical HPLC Method Development. 3rd Ed. New York: Wiley; 1988: 227.
11. Skoog DA, West DM. Principles of Instrumental Analysis. 2nd Ed. Saunders Golden Sunburst Series. Philadelphia; 1980: 674-675, 690-696.
12. Snyder LR, Kirkland JL, Glajch JL. Practical HPLC Method Development. 2nd Ed. New York: Wiley; 1997: 1-19.
13. Valko K, Snyder LR, Glajch J. Retention in Reversed-Phase Liquid Chromatography as a function of mobile phase composition. J. Chromatogr. A. 1993; 656(2): 501-520.
14. Neue UD. HPLC Columns: Theory, Technology and Practice. 2nd Ed. New York: John Wiley & Sons; 1997: 174-186.
15. Kazakevich Y, Lobrutto R. HPLC for Pharmaceutical Scientists. 1st Ed. New Jersey: John Wiley & Sons Inc; 2007: 987-1051.
16. Peter's son P. RPLC column classification and the development of a column selection tool. ACD/Labs European Users' Meeting; 2003; Obernai, France.
17. Huber JFK, Vander LR, Ecker E, *et al*. Column switching in High Pressure Liquid Chromatography. J. Chromatogr. A. 1973; 83(2): 267-271.
18. Snyder LR, Schunk TC. Retention mechanism and the role of the mobile phase in normal-phase separation on amino-bonded-phase columns. J. Anal. Chem. 1982; 54(11): 1764-1772.
19. Yun KS, Zhu C, Parcher JF. Theoretical relationships between the void volume, mobile phase volume, retention volume, adsorption and Gibbs free energy in chromatographic processes. J. Anal. Chem. 1995; 67(4)