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METHOD VALIDATION AND DEVELOPMENT OF MONTELUKAST SODIUM BY USING RP-HPLC METHOD

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I. INTRODUCTION

Pharmaceutical Analysis plays a very vital role in the quality assurance and quality control of bulk drugs and their formulations. Pharmaceutical analysis is a specialized branch of analytical chemistry which involves separating, identifying and determining the relative amounts of components in a sample of matter. It is concerned with the chemical characterization of matter both quantitative and qualitative.

In recent years, several analytical techniques have been evolved.

1.1 Spectrophotometric methods

Spectrophotometry is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colourless compounds in the near ultraviolet path of spectrum (200-380nm). The photometric methods of analysis are based on the Boger-Lambert-Beer's law, which establishes the absorbance of a solution is directly proportional to the concentration of the analyte. The fundamental principle of operation of spectrophotometer covering UV region consists in that light of definite interval of wavelength passes through a cell with solvent and falls on to the photoelectric cell that transforms the radiant energy into electrical energy measured by a galvanometer.

The important applications are

- Identification of many types of organic, inorganic molecules and ions.

- Quantitative determination of many biological, organic and inorganic species.
- Monitoring and identification of chromatographic of effluents.

1.2 Hplc method development

The term ‘Chromatography’ covers those processes aimed at the separation of the various species of a mixture on the basis of their distribution characteristics between a stationary and a mobile phase.

1.2.1 Modes of chromatography

Modes of chromatography are defined essentially according to the nature of the interactions between the solute and the stationary phase, which may arise from hydrogen bonding, Vander walls forces, electrostatic forces or hydrophobic forces or basing on the size of the particles (e.g. Size exclusion chromatography).

Different modes of chromatography are as follows

- Normal Phase Chromatography
- Reversed Phase Chromatograph
- Ion-Exchange Chromatography
- Size Exclusion Chromatography

Reversed phase chromatography

The objective was to make less polar or non polar so that polar solvents can be used to separate water-soluble polar compounds. Since the ionic nature of the chemically modified silica is now reversed i.e. it is non-polar or the nature of the phase is reversed. The chromatographic separation carried out with such silica is referred to as reversed- phase chromatography.

A large number of chemically bonded stationary phases based on silica are available commercially. Silica based stationary phases are still most popular in reversed phase chromatography however other adsorbents based on polymer (Styrene-divinyl benzene copolymer) are slowly gaining ground.

Simple compounds are better retained by the reversed phase surface, the less water- soluble (i.e. the more non-polar) they are. The retention decreases in the following order: Aliphatic > induced dipoles (i.e. CCl4) > permanent dipoles (e.g.CHCl3) > weak Lewis- bases (ethers, aldehydes, ketones) > strong Lewis bases (amines) > weak Lewis acids (alcohols, phenols) >

strong Lewis acids (carboxylic acids). Also the retention increases as the number of carbon atoms increases.

As a general rule the retention increases with increasing contact area between sample molecule and stationary phase i.e. with increasing number of water molecules, which are released during the adsorption of a compound. Branched chain compounds are eluted more rapidly than their corresponding normal isomers.

Chemically bonded octadecyl silane (ODS) an alkaline with 18 carbon atoms it is the most popular stationary phase used in pharmaceutical industry. Since most pharmaceutical compounds are polar and water soluble, the majority of HPLC methods used for quality assurance, decomposition studies, quantitative analysis of both bulk drugs and their formulations use ODS HPLC columns. The solvent strength in reversed phase chromatography is reversed from that of adsorption chromatography (silica gel) as stated earlier. Water interacts strongly with silanol groups, so that, adsorption of sample molecules become highly restricted and they are rapidly eluted as a result.

Exactly opposite applies in reversed phase system water cannot wet the non-polar (hydrophobic) alkyl groups such as C18 of ODS phase and therefore does not interact with the bonded moiety. Hence water is the weakest solvent of all and gives slowest elution rate. The elution time (Retention time) in reversed phase chromatography increases with increasing amount of water in the mobile phase.

In normal phase chromatography, the stationary phase is a polar adsorbent and the mobile phase is generally a mixture of non-aqueous solvents.

The silica structure is saturated with silanol groups at the end. These OH groups are statistically disturbed over the whole of the surface. The silanol groups represent the active sites (Very polar) in the stationary phase.

These situations arise when the molecule has one or several atoms with lone pair electron or a double bond. The adsorption strengths and hence k’ values (elution series) increase in the following order. Saturated hydrocarbon < olefins < aromatics < organic halogen compounds < sulphides < ethers< esters < aldehydes and ketones < amines < sulphones < amides < carboxylic acids. The strength of interactions depends not only on the functional groups in the sample molecule but also on steric factors. If a molecule has several functional groups, then the most polar one determines the reaction properties.

The aminopropyl and cyanopropyl phases provide opportunities for specific interactions between the analyte and the stationary phases and thus offer additional options for the optimizations of separations.

Resolution with water in weak mobile phase may be most conveniently achieved by drying the solvents and then adding a constant concentration of water or some very polar modifier such as acetic acid or triethylamine (TEA) to the mobile phase. The addition of such polar modifiers serves to deactivate the more polar shape as well as the reproducibility of the retention times.

Chromatographic methods can be classified most practically according to the stationary and mobile phases, as shown in the table 1:

Table 1: Classification of chromatographic methods.

Stationary phase	Mobile phase	Method
Solid	Liquid	Adsorption column, thin-layer, ion exchange, High performance liquid chromatography.
Liquid	Liquid	Partition, column, thin-layer, HPLC, paper chromatography.
	Gas	Gas–Liquid Chromatography

The various components of a HPLC system are herewith described. The modern form of column chromatography has been called high performance, high pressure, and high-resolution and high-speed liquid chromatography.

High-Performance Liquid Chromatography (HPLC)is a special branch of column chromatography in which the mobile phase is forced through the column at high speed. As a result the analysis time is reduced by 1-2 orders of magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possible increasing the column efficiency substantially.

The essential equipment consists of an eluent, reservoir, a high-pressure pump, and an injector for introducing the sample, a column containing the stationary phase, a detector and recorder. The development of highly efficient micro particulate bonded phases has increased the versatility of the technique and has greatly improved the analysis of multi component.

Solvent container — Pump — damping unit — Injection port --Column
|
Recorder — Detector --- Effluent

1.3 System components

Solvent delivery system: The mobile phase is pumped under pressure from one or several reservoirs and flows through the column at a constant rate. With micro particulate packing, there is a high-pressure drop across a chromatography column. Eluting power of the mobile phase is determined by its overall polarity, the polarity of the stationary phase and the nature of the sample components. For normal phase separations eluting power increases with increasing polarity of the solvent but for reversed phase separations, eluting power decreases with increasingsolvent polarity. Optimum separating conditions can be achieved by making use of mixture of two solvents. Some other properties of the solvents, which need to be considered for a successful separation, are boiling point, viscosity, detector compatibility, flammability and toxicity.

The most important component of HPLC in solvent delivery system is the pump, because its performance directly effects the retention time, reproducibility and detector sensitivity. Among the several solvent delivery systems (Direct gas pressure, pneumatic intensifier, reciprocating etc.) reciprocating pump with twin or triple pistons is widely used, as this system gives less baseline noise, good flow rate reproducibility etc.

Solvent degassing system

The constituents of the mobile phase should be degassed and filtered before use. Several methods are employed to remove the dissolved gases in the mobile phase. They include heating and stirring, vacuum degassing with an aspirator, filtration through 0.45 filters, vacuum degassing with an air-soluble membrane, helium purging ultrasonication or purging or combination of these methods. HPLC systems are also provided an online degassing system, which continuously removes the dissolved gases from the mobile phase.

Gradient elution devices

HPLC columns may be run isostatically, i.e., with constant eluent or they may be run in the gradient elution mode in which the mobile phase composition varies during run. Gradient elution is a means of overcoming the problem of dealing with a complex mixture of solutes.

Sample introduction systems

Two means for analyte introduction on the column are injection in to a flowing stream and a stop flow injection. These techniques can be used with a syringe or an injection valve. Automatic injector is a microprocessor-controlled version of the manual universal injector. Usually, up to 100 samples can be loaded in to the auto injector tray. The system parameters such as flow rates, gradient, run time, volume to be injected, etc. are chosen, stored in memory and sequentially executed on consecutive injections.

Liquid chromatographic detectors

The function of the detector in HPLC is to monitor the mobile phase as it emerges from the column. Generally, there are two types of HPLC detectors, bulk property detectors and solute property detectors.

1. Bulk property detectors

These detectors are based on differential measurement of a property, which is common to both the sample and the mobile phase. Examples of such detectors are refractive index, conductivity and dielectric constant detectors.

2. Solute property detectors

Solute property detectors respond to a physical property of the solute, which is not exhibited by the pure mobile phase. These detectors measure a property, which is specific to the sample, either with or without the removal of the mobile phase prior to the detection. Solute property detectors which do not require the removal of the mobile phase before detection include spectrophotometric (UV or UV-Vis) detector, fluorescence detectors, polarographic, electro-chemical and radio activity detectors, whilst the moving wire flame ionization detector and electron capture detector both require removal of the mobilephasebefore detection.

UV-Vis and fluorescent detectors are suitable for gradient elution, because many solvents used in HPLC do not absorb to any significant extent.

Column and Column-packing materials

The heart of the system is the column. In order to achieve high efficiency of separation, the column material (micro-particles, 5- 10 µm size) packed in such a way that highest numbers of theoretical plates are possible. Silica (SiO_2, H_2O) is the most widely used substance for the manufacture of packing materials. It consists of a network of siloxane linkages (Si-O-Si) in a rigid three dimensional structure containing inter connecting pores. Thus a wide range of commercial products is available with surface areas ranging from 100 to 800 m^2/g. and particle sizes from 3 to 50 □m.

The most popular material is octadecyl-silica (ODS-Silica), which contains C18 chains, but materials with C2, C6, C8 and C22 chains are also available. During manufacture, such materials may be reacted with a small mono functional silane (e.g. trimethyl chloro silaneto reduce further the number of silanol groups remaining on the surface (end-capping). The useful pH range for columns is 2 to 8, since siloxane linkages are cleaved below pH-2 while at pH values above eight silica may dissolve.

In HPLC, generally two types of columns are used, normal phase columns and reversed phase columns. Using normal phase chromatography, particularly of non-polar and moderately polar drugs can make excellent separation. It was originally believed that separation of compounds in mixture takes place slowly by differential adsorption on a stationary silica phase.

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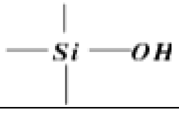
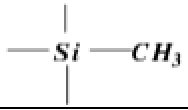
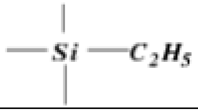
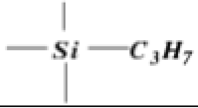
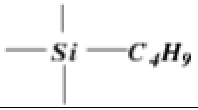
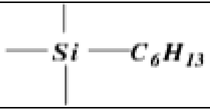
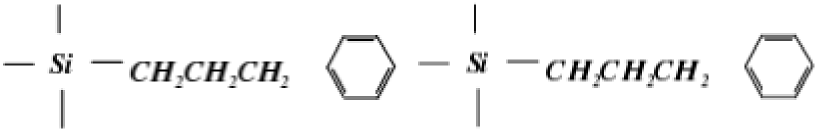
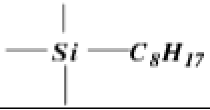
The silanol groups on the surface of silica give it a polar character, which is exploited in adsorption chromatography using non-polar organic eluents. Silica can be drastically altered by reaction with organ chloro silanes or organ alkoxy silanes giving Si-O-Si-R linkages with the surface. The attachment of hydrocarbon change to silica produces a non-polar surface suitable for reversed phase chromatography where mixtures of water and organic solvents are used as eluents. The most popular material is octadecyl-silica (ODS-Silica), which contains

C18 chains, but materials with C2, C6, C8 and C22 chains are also available. During manufacture, such materials may be reacted with a small mono functional silane (e.g. trimethyl chloro silane) to reduce further the number of silanol groups remaining on the surface (end-capping). There is a vast range of materials which have intermediate surface polarities arising from the bonding to silica of other organic compounds which contain groups such as phenyl, nitro, amino and hydroxyl. Strong ion exchangers are also available in which sulphonic acid groups or quaternary ammonium groups are bonded to silica. The useful pH range for columns is 2 to 8, since siloxane linkages are cleaved below pH-2 while at pH values above eight, silica may dissolve.

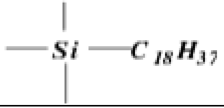
In HPLC, generally two types of columns are used, normal phase columns and reversed phase columns. Using normal phase chromatography, particularly of non-polar and moderately polar drugs can make excellent separation. It was originally believed that separation of compounds in mixture takes place slowly by differential adsorption on a stationary silica phase. However, it now seems that partition plays an important role, with the compounds interacting with the polar silanol groups on the silica or with bound water molecules.

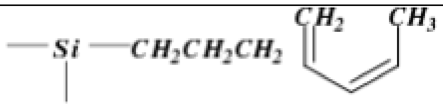
Phase	**Description**
Si	**Silica** Classic normal phase material. Suitable for separating polar non-ionic organic compounds.  $-\mathrm{Si}-\mathrm{OH}$
C1	**TMS, SAS**, Tri methyl silane Reversed phase material. Unique selectivity for polar and multifunctional compounds. Least retentive of all alkyl group bonded phases for non-polar solvents.  $-\mathrm{Si}-\mathrm{CH_3}$
C2	**RP-2, Dimethyl** Reversed phase material, less retentive than C4, C8, or C18. More retentive than C1.  $-\mathrm{Si}-\mathrm{C_2H_5}$
C3	**Propyl:** Reversed phase material, used in hydrophobic interaction chromatography (HIC) of proteins and peptides.  $-\mathrm{Si}-\mathrm{C_3H_7}$

C4	**Butyl:** Reversed phase material, useful for ion-pairing chromatography offers less retention than C8 and C18 phases for non-polar solutes. When bonded to 300 Å silica, it is an ideal phase for analysing large proteins and hydrophobic peptides.  $-Si-C_4H_9$
6	**Hexyl:** Reversed phase material, useful for ion-pairing chromatography. Less retentive than C8 and C18 phases.
	 $-Si-C_6H_{13}$
C8	MOS, RP-8, LC8, Octyl  Reversed phase material, similar selectivity to C18 but less retentive. Wide applicability (e.g. pharmaceuticals, nucleosides, steroids). When bonded to300 Å silica, it is an ideal phase for peptides, peptide mapping and small hydrophilic proteins.  $-Si-C_8H_{17}$

While normal phase seems the passage of a relatively non-polar mobile phase over a polar stationary phase, reversed phase chromatography is carried out using a polar mobile phase such as methanol, acetonitrile, water, buffers etc., over a non-polar stationary phase. Ranges of stationary phases (C18, C8, -NH2, -CN, -phenyl etc.) are available and very selective separations can be achieved. The pH of the mobile phase can be adjusted to suppress the ionization of the drug and thereby increase the retention on the column. For highly ionized drugs ion-pair chromatography is used.

Bonded phases for hplc and their abbreviations

C18	**ODS, RP-18, LC18, Octadecyl** Classic reversed phase material is most retentive for non-polar solutes and is excellent for ion-pairing chromatography. It is having wide applicability for the assay of nucleosides, nucleotides, steroids, pharmaceuticals, vitamins, fatty acids and environmental compounds when bonded to 300 Å silica, this phase is perfect for separating small hydrophilic peptides.  $-Si-C_{18}H_{37}$
C6H5	It is a reversed phase material and exhibits unique selectivity. It is useful for analyzing aromatic compounds. When bonded to 300 Å silica, this phase is useful for HIC.

	 Phenyl
CN	**CPS, PCN, Cyano,** $-Si-CH_2CH_2CH_2CN$ Cyanopropyl, Nitrile It can be employed as either a reversed phase or normal phase material. It is slightly polar and exhibits unique selectivity for polar compounds in both RP and NP modes. It equilibrates very rapidly and suitable for gradient separations. It has many pharmaceutical applications (e.g. tricyclic antidepressants).
NH2	APS, Amino, Amino propyl silyl $-Si-CH_2CH_2CH_2NH_2$ Can be employed as reversed phase, normal phase or weak anion exchange material. Reversed phase: useful for separating carbohydrates. Normal phase: alternative selectivity to silica, not deactivated by small amounts of water. Ion
	Exchange: weak anion exchanger when used with buffers separates anions and organic acids.
NO2	Nitro $-Si-NO_2$ Normal phase material. Separates aromatic compounds and compounds with double bonds.
OH	Diol, Glycerol $-Si-CH_2CH_2CH_2OCH_2CHOHCHOH$ Can be employed as either a reversed phase or normal phase material. Reversed phase: Used for Gel Filtration Chromatography (GFC) of proteins and peptides. Normal phase: Similar selectivity to silica not deactivated by small amounts of water.
SAX	SB,Quaternaryamine,Strong Base $-Si-CH_2CH_2CH_2N^{+}(CH_3)_3$ Ion exchange material. Strong anion exchangers (basic) are useful for separating nucleosides, nucleotides and organic acids.
SAX	SB,Quaternaryamine,Strong Base $-Si-CH_2CH_2CH_2N^{+}(CH_3)_3$ Ion exchange material. Strong anion exchangers (basic) are useful for separating nucleosides, nucleotides and organic acids.
SCX	SA, Sulfonic acid, Strong Acid $-Si-CH_2CH_2CH_2SO_2OH$ Ion exchange material. Strong cation exchangers (acidic) are useful for separating organic bases.
WAX	PEI, DEAE, Polyethyleneimine, Diethylaminoethyl, Weak Base $-Si-CH_2CH_2N(CH_2CH_3)_2$ Ion exchange material. Weak anion exchangers (basic) are most useful for analyzing acidic proteins and peptides.

WCX	CM, Carboxymethyl, Weak Acid $-Si-CH_2COOH$ Ion exchange material. Weak cation exchangers (acidic) are most useful for analyzing basic proteins and peptides.

Derivatization

In HPLC derivatization is used to enhance the sensitivity and selectivity of detection when available detectors are not satisfactory for the underivatized compounds. Both ultra violet absorbing and fluorescence derivatives have been widely used. Ultra violet derivatization reagents include N-succinimidyl p-nitro phenyl acetate, phenyl hydrazine and 3, 5-dinitro benzyl chlorides, while fluorescent derivatives can be formed with reagents such as dansyl chloride, 4-bromo methyl-7-methoxy-coumarin and fluoresce amine. Derivative formation can be carried out before the sample is injected on to the column or by online chemical reactions between the column out let and the detector.

Gradient elution

Gradient elution or solvent programming is the change of solvent composition during a separation in which the solvent strength increases from the beginning to the end of the separation. It is well suited to the analysis of samples of unknown complexity since good resolution is automatically provided for a wide range of sample polarities. There are two types of gradient systems: Low-pressure gradient mixtures and high- pressure gradient mixtures. In the former the solvents are mixed at atmosphere pressure and then pumped to the column, where as in the later, solvents are pumped in to a mixing chamber at high pressure before going in to the column.

Performance calculations

Calculating the following values (Which can be include in a custom report) used to access overall system performance.

Relative retention, Theoretical plates, Capacity factor Resolution, Peak asymmetry, Plates per meter

The parameters used to calculate these system performance values for the separation of two chromatographic components.

Relative retention (Selectivity)

□ = (t2 - ta) / (t1 - ta)

Theoretical plates

$n = 16 (t / W)^{2}$

Capacity factor

$K' = (t2 / ta)^{-1}$

Résolution

R = 2 (t2 - t1) / (W2 + W1)

Peak asymmetry

T = W0.05 / 2f

Plates per meter

N = n / L

HETP

L/n

Where, □ = Relative retention.

t2 = Retention time of the second peak measured from point of injection. t1 = Retention time of the first peak measured from point of injection.

ta = Retention time of an inert peak not retained by the column, measured from point of injection.

n = Theoretical plates.

t = Retention time of the component.

W = Width of the base of the component peak using tangent method.

K' = Capacity factor.

R = Resolution between a peak of interest (p2) and the peak preceding it (p1) W2 = Width of the base of component peak 2.

W1 = Width of the base of component peak 1.

T = Peak asymmetry, or tailing factor.

W0.05 = Distance from the leading edge to the tailing edge of the peak, Measured at a point 5 % of the peak height from the baseline.

f = Distance from the peak maximum to the leading edge of the peak. L = length of column, in meters

N = Number of plates per meter

HPLC troubleshooting abnormal pressure

No pressure reading, no flow

Possible cause	**Solution**
Power off	Turn on power
Fuse blown	Replace fuse
Controller setting or failure	Verify proper settings, repair or replace controller
Broken piston	Replace piston
Air trapped in pump head	Degas mobile phase, bleed air from pump and prime pump
Insufficient mobile phase	Replenish reservoir, replace inlet frit if it is blocked
Faulty check valves	**Replace check valves**
Major leak	Tighten or replace fittings

Now pressure reading, flow is normal

Possible cause	**Solution**
Faulty meter	Replace meter
Faulty pressure transducer	Replace transducer

High back pressure

Possible cause	**Solution**
Flow rate set too high	Adjust settings
Blocked column frit	Backflush column if it is permitted, replace frit according to the manufacturer's instructions and warranty conditions or replace column
Improper mobile phase, precipitated buffer	Use correct mobile phase, wash column
Improper column	Use proper column
Injector blockage	Clear blockage or replace injector
Column temperature too low	Raise temperature
Controller malfunction	Repair or replace controller
Blocked guard column	Remove/replace guard column
Blocked in-line filter	Remove/replace in-line filter

You should find out, what caused high back pressure - column or system? We recommend following procedure

Remove column from the system and turn on pump. If high back pressure still appears, then the blockage is in the system:

- Blocked or crimped tubing
- Dirty pump frit
- Or clogged injection valve

If the pressure is normal, there is a problem with the column

- Clogged or damaged pre-column filter, guard column or frit
- Precipitation of sample or buffer in column

Low back pressure

Possible cause	**Solution**
Flow set too low	Adjust flow rate
Leak in the system	Locate and correct
Improper column	Use proper column

Column temperature too high	**Lower temperature**
Controller malfunction	Repair or replace controller

- **Fluctuating pressure**

See section *High back pressure*.

- **Pressure dropping to zero**

See sections *No pressure reading, no flow* and No pressure reading, flow is normal

- **Pressure dropping, but not to zero**

See section *Low pressure*

Pressure cycling

Possible case	**Solution**
Air in pump	Degas solvent and/or bleed air from the pump
Faulty check valve(s)	Replace check valve(s)
Pump seal failure	Replace pump seal
Insufficient degassing	Degas solvent and/or change degassing methods (e.g. use vacuum degasser)
Leak in system	Locate leak and correct it
Using gradient elution	Pressure cycling is normal due to viscosity change

- **Leaks**

Leaks are usually stopped by tightening or replacing a fitting. Be aware, however, that overtightened metal compression fittings can leak and plastic fingertight fittings can wear out. If a fitting leak does not stop when the fitting is tightened a little, take the fitting apart and inspect for damage (e.g. distored ferrule, or particles on the sealing surface). If the fitting or ferrule is damaged, replace it with new one.

- **Leaky fittings**

Possible cause	**Solution**
Loose fitting	Tighten the fitting
Stripped fitting	Replace the fitting
Overtightened fitting	Loosen and retighten the fitting. If the fitting is damaged, replace it.
Overtightened fitting	Loosen and retighten the fitting. If the fitting is damaged, replace it.
Dirty fitting	Disassemble fitting and clean it. If the fitting is damaged, replace it.
Mismatched parts	Use all parts from the same brand/type.

Leaks at the pump

Possible cause	**Solution**
Loose check valves	Tighten check valve (do not overtighten) or replace check valve
Loose fittings	Tighten fittings (do not overtighten)
Mixer seal failure	Repair or replace

Pump seal failure	**Repair or replace the seal**
Pulse damper failure	Replace pulse damper
Proportionin valve failure	Check diaphragms, replace if leaky and/or check for fitting damage, replace
Purge valve	Tighten valve or replace it if it is faulty

Injector leaks

Possible cause	**Solution**
Rotor seal failure	Rebuild or replace rotor
Blocked loop	Clean or replace loop
Loose injector port seal	Adjust
Improper syringe needle diameter	Use correct syringe

Waste line siphoning	Keep waste line above surface vaste, with proper slope
Waste line blockage	Replace waste line

Column leaks

Possible cause	**Solution**
Loose end fitting	Tighten end fitting
Column packing in ferrule	Disassemble, rinse ferrule, reassemble
Improper frit thickness	Use proper frit(*)

**Note: When the particle size of stationary phase is 3 to 4 µm, use frit 0.5 µm. When particle size of stationary phase is 5 to 20 µm, use frit 2 µm.*

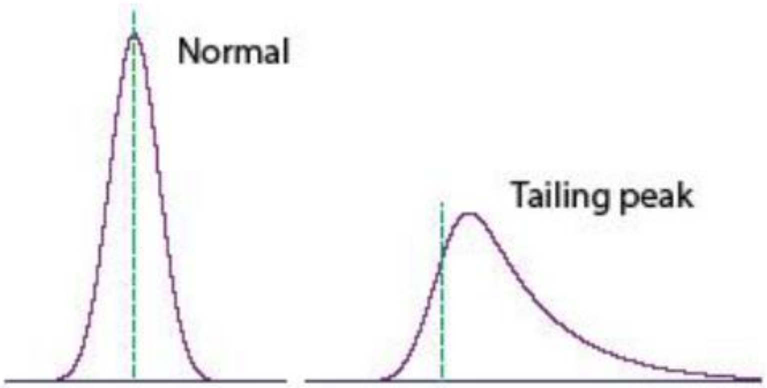
Detector leaks

Possible cause	**Solution**
Cell gasket failure	Prevent excessive backpressure or replace gasket
Cracked cell window(s)	Replace cell window(s)
Leaky fittings	Tighten or replace fittings
Blocked waste line	Replace waste line
Blocked flow cell	Rebuild or replace flow cell

Problems with the chromatogram

Many issues in the LC system appear as changes in the chromatogram. Some of these can be solved by changes in the instrument, however, other problems require modification of the assay procedure. Setting the proper column type, pre-column or guard column, tubing, detector cell and mobile phase are keys to *good chromatography*.

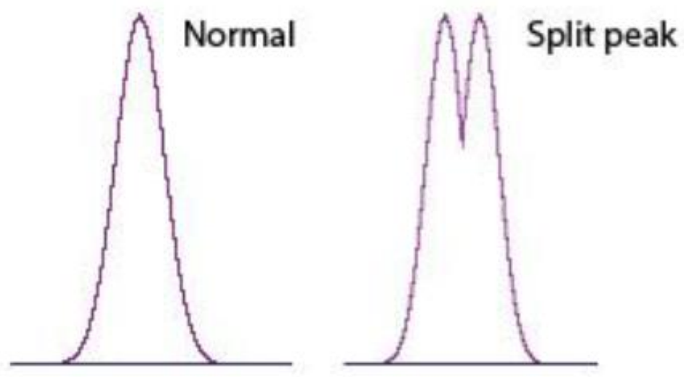
Peak tailing



Possible cause	**Solution**
Blocked frit	Reverse flush column (if it is allowed) or replace frit (if it is allowed) or replace column
Column void	Fill void
Interfering peak	Use longer column or change mobile phase and/or column selectivity
Wrong mobile phase pH	Adjust pH. For basic compounds, lower pH usually provides more symmetric peaks
Sample reacting with active sites	Add ion pair reagent or volatile basic modifier or change column

Peak fronting

Split peaks



Possible cause	**Solution**
Contamination on guard or analytical column inlet	Remove guard column and attempt analysis. Replace guard column if necessary. If analytical column is obstructed, reverse and flush (if it is allowed). If problem persists, column may be fouled with strongly retained contaminants. Use appropriate restoration procedure (see column care information). If problem persists, inlet is probably plugged. Change frit or replace column.
Sample solvent incompatible with mobile phase	Change solvent. whenever possible, inject samples in mobile phase.

Distortion of larger peaks

The peak distortion can be caused by sample overload. Reduce the sample size.

Distortion of early peaks

The distortion of early eluting peaks can be caused by wrong injection solvent. Reduce the injection volume, or use weaker injection solvent.

Tailing, early peaks more than later ones

Possible cause	Solution
Extra-column effects	Replumb the system (shorter, narrower tubing), use smaller volume detector cell

Increased tailing as k' increases

Possible cause	Solution
Secondary retention effects, reversed-phase mode	Add triethylamine (Basic samples) or add acetate (acidic samples) or add salt or buffer (Ionic samples) or try a different column.
Secondary retention effects, normal-phase mode	Add triethylamine (Basic compounds) or add acetic acid (acidic compounds) or add water (poly-functional compounds). Only for normal-phase methods which use water-miscible solvents. You can also try a different LC method.
Secondary retention effects, ion-pair chromatography	Add triethylamine (Basic samples)

Acidic or basic peaks tail

Possible cause	Solution
Inadequate buffering	Use 50 to 100 mM buffer concentration, use buffer with pKa equal to pH of mobile phase

Extra peaks

Possible cause	Solution
Ghost peaks	Impurities in the sample, reagents or material used. Change clean-up procedure and/or check possible source of contamination (Glassware, Vials, Used reagents, solvents, etc...)
Late eluting peak frompreviousinjection	Increase run time or gradient slope and/or increase flow rate

Method optimisation

Selection of stationary phase/column: Selection of the column is the first and the most important step in method development.

Some of the important parameters considered while selecting chromatographic columns are:

- Length and diameter of the column.
- Packing material.
- Shape of the particles.
- Size of the particles.

The column is selected depending on the nature of the solute and the information about the analyte. Reversed phase mode of chromatography facilitates a wide range of columns like dimethyl silane (C2), butylsilane (C4), octylsilane (C8), octadecylane (C18), base deactivated silane (C18) BDS phenyl, cyanopropyl (CN), nitro, amino etc. C18 was chosen for this study since it is most retentive one. The sample manipulation becomes easier with this type of column

Generally longer columns provide better separation due to higher theoretical plate numbers. Columns with 5-µm particle size give the best compromise of efficiency, reproducibility and reliability. In this case, the column selected had a particle size of 5 µm and a internal diameter of 4.6 mm.

A useful and practical measurement of peak shape is peak asymmetry factor and peak tailing factor. Peak asymmetry is measured at 10% of full peak height and peak tailing factor at 5%. Reproducibility of retention times and capacity factor is important for developing a rugged and repeatable method.

Selection of mobile phase: The primary objective in selection and optimization of mobile phase is to achieve optimum separation of all the individual impurities and degradants from each other and from analyte peak.

In liquid chromatography, the solute retention is governed by the solute distribution factor, which reflects the different interactions of the solute – stationary phase, solute – mobile phase and the mobile phase – stationary phase .For a given stationary phase, the retention of the given solute depends directly upon the mobile phase, the nature and the composition of which has to be judiciously selected in order to get appropriate and required solute retention.

The mobile has to be adapted in terms of elution strength (Solute retention) and solvent selectivity (solute separation) Solvent polarity is the key word in chromatographic separations since a polar mobile phase will give rise to low solute retention in normal phase and high solute retention in reverse phase LC. The selectivity will be particularly altered if the buffer pH is close to the pKa of the analytes; the solvent strength is a measure of its ability to pull analyte from the column. It is generally controlled by the concentration of the solvent with the highest strength.

The following are the parameters, which shall be taken into consideration while selecting and optimizing the mobile phase.

- Buffer,
- pH of the buffer
- Mobile phase composition.

Selection of detector

The detector was chosen depending upon some characteristic property of the analyte like UV absorbance, fluorescence, conductance, oxidation, reduction etc. characteristics that are to be fulfilled by a detector to be used in HPLC determination are,

- High sensitivity, facilitating trace analysis
- Negligible baseline noise. To facilitate lower detection
- Low dead volume
- Non destructive to sample
- Inexpensive to purchase and operate

For the greatest sensitivity λ_{max} should be used. Higher wavelengths give greater selectivity.

1.4 Method validation

Method validation can be defined as (ICH) “establishing documented evidence which provides a high degree of assurance that specific activity will consistency produce a desired result or product meeting its predetermined specifications and quality characteristics.

Method validation is an integral part of the method development; it is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, an and drug products. Simply, method validation is the process of proving that and potency of the drug substances analytical method is acceptable for its intended purpose.

For chromatographic methods used in analytical applications there is more consistency in validation practice with key analytical parameters

(a) Recovery (b) Response function (c) Sensitivity (d) Precision (e) Accuracy (f) limits of detection (g) Limit of quantitation (h) Ruggedness (i) Robustness (j) stability (k) system suitability

(a) Recovery: The absolute recovery of analytical method is measured as the response of a processed spiked matrix standard expressed as a percentage of the response of pure standard which has not been subjected to sample pre treatment and indicates whether the method provides a response for the entire amount of analyte that is present in the sample.

$$\text{Absolute recovery} = \frac{\text{Response of an analyte spike into matrix (processed)}}{\text{Response of analyte of pure standard (unprocessed)}} \times 100$$

b) Sensitivity: The method is said to be sensitive if small changes in concentration cause large changes in response function. The sensitivity of an analytical method is determined from the slope of the calibration line. The limits of quantification (LOQ) or working dynamic range of bio analytical method are defined as the highest and lowest concentrations, which can determined with acceptable accuracy. It is suggested that, this be set at □ 15% for both the upper and lower limit of quantitation respectively. Any sample concentration that falls outside the calibration range cannot be interpolated from the calibration line and extrapolation of the calibration curve is discouraged. If the concentration is over range, the sample should be diluted in drug-free matrix and re- assayed.

c) Precision: The purpose of carrying out a determination is to obtain a valid estimate of a ‘true’ value. When one considers the criteria according to which an analytical procedure is selected, precision and accuracy are usually the first time to come to mind. Precision and accuracy together determine the error of an individual determination. They are among the most important criteria for judging analytical procedures by their results.

Precision refers to the reproducibility of measurement within a set, that is, to the scatter of dispersion of a set about its central value. The term ‘set’ is defined as referring to a number (n) of independent replicate measurements of some property. One of the most common statistical terms employed is the standard deviation of a population of observation. Standard deviation is the square root of the sum of squares of deviations of individual results for the mean, divided by one less than the number of results in the set. The standard deviation S, is given by

$$S = \sqrt{\frac{1}{n-1}\sum_{i-1}^{n}(x_i - \overline{x})^2}$$

Standard deviation has the same units as the property being measured.

The square of standard deviation is called variance (S^2). Relative standard deviation is the standard deviation expressed as a fraction of the mean, i.e., S/x. It is sometimes multiplied by 100 and expressed as a percent relative standard deviation. It becomes a more reliable expression of precision.

$$\% \text{ Relative standard deviation} = S \times 100 / x$$

d) **Accuracy:** Accuracy normally refers to the difference between the mean x****, of the set of results and the true or correct value for the quantity measured. According to IUPAC accuracy relates to the difference between results (or mean) and the true value. For analytical methods, there are two possible ways of determining the accuracy, absolute method and comparative method.

Accuracy is best reported as percentage bias, which is calculated from the expression

$$\%\text{Bias} = \frac{(\text{measured value} - \text{true value})}{\text{true value}} \times 100$$

The accuracy of analytical method is then determined at each concentration by assessing the agreement between the measured and nominal concentrations of the analytes in the spiked drug – free matrix sampler.

e) **Limit of detection (LOD):** The limit of detection (LOD) of an analytical method may be defined as the concentration, which gives rise to an instrument signal that is significantly different from the blank. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sa), which may be related to LOD and the slope of the calibration curve, b, by

$$\text{LOD} = 3\ \text{Sa}/\ \text{b}$$

f) **Limit of quantitation (LOQ):** The LOQ is the concentration that can be quantitate reliably with a specified level of accuracy and precision. The LOQ represent the concentration of analyte that would yield a signal-to-noise ratio of 10.

$$LOQ = 10\ Sa/\ b$$

Where, Sa- the estimate is the standard deviation of the peak area ratio of analyte to IS (5 injections) of the drugs. b -is slope of the corresponding calibration curve.

g) **Ruggedness:** Method Ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, source of reagents, chemicals, solvents etc. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

h) **Robustness:** The concept of robustness of an analytical procedure has been defined by the ICH as “a measure of its capacity to remain unaffected by small but deliberate variations in method parameters”. The robustness of a method is the ability to remain unaffected by small changes in parameters such as pH of the mobile phase, temperature, organic solvent strength and buffer concentration etc. to determine the robustness of the method experimental conditions were purposely altered and chromatographic characters were evaluated.

i) **System suitability:** System suitability experiments can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation have been completed. (or) The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The criteria selected will be based on the actual performance of the method as determined during its validation. For example, if sample retention times form part of the system suitability criteria, their variation (SD) during validation can be determined system suitability might then require that retention times fall within a □3 SD range during routine performance of the method.

II. Drug profile

Montelukast

Summary

Montelukast is a leukotriene receptor antagonist used as part of an asthma therapy regimen, to prevent exercise include bronchoconstriction, and to treat seasonal allergic rhinitis.

Brand name

Singulair.

Drug bank accession number

DB00471.

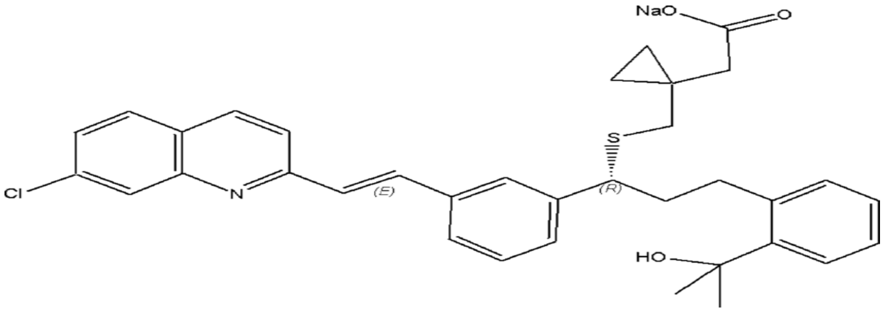
Background

Montelukast was first approved for clinical use by the US FDA in 1998 as Mercks brand name Singulair. The medication is the member of the leukotriene receptor antagonist (LTRA) category of drugs. Although capable of demonstrating effectiveness, the use of inhaled corticosteroids or other agents in asthma step therapy. Regardless, in 2008-2009, there were FDA-led investigations into the possibility of montelukast to elicit neuropsychiatric effects like agitation, hallucinations, suicidal behaviour, and others in individuals who used the medication. And included in the official prescribing information for montelukast, the drug still sees extensive use worldwide via millions of prescriptions annually and has since become available as a brand name product.

Type: Small molecule.

Groups: Approved.

Structure



Weight

Average: 586.183

Monoisotopic: 585.21044242

Chemical formula

C35H35ClNNaO3S

Synonyms

Montelukast, montelukastum

Pharmacology

Indication

Montelukast is indicated for

(a) The prophylaxis and chronic treatment of asthma in adults and pediatric patients who are 12 months of age and older, although other regional health authorities specifically note this indication for adults and adolescents who are 15 years and older and and also include indications for preventing day and night -time symptoms ,and the treatment of acetyl salicylic acid sensitive asthma.

(b) The prevention of exercise-induced bronchoconstriction (EIB) in patients who are 6 years of age and older, although other regional health authorities specifically note this indication for adults and adolescents who are 15 years and older, and

(c) The relief of symptoms of seasonal allergic rhinitis in patients 2 years of age and older and perennial allergic rhinitis in patients 6 months of age and older, although other regional health authorities specifically note the relief of seasonal allergic rhinitis symptoms for adults and adolescents who are 15 years and older. Furthermore, some formulations like chewable montelukast tablets may also be specifically indicated by particular regulatory bodies for the prophylaxis and chronic treatment of asthma, including the prevention of day and night-time symptoms, the treatment of acetylsalicylic acid based asthma, and the prevention of exercise-induced bronchoconstriction in adult and pediatric patients aged 2 and older, between the ages 2 and 5, or between the ages of 6 and 14 years.

Moreover, when employed for such indications montelukast is considered effective as monotherapy or when combined with other medications indicated for the maintenance treatment of chronic asthma. For instance, montelukast and inhaled corticosteroids can be used concomitantly to demonstrate additive effects to control asthma or to decrease the necessary inhaled corticosteroids dose while still maintaining clinical stability. additionally, in patients who continue to experience asthma symptoms, montelukast can also be combined with an ‘as required’ short-acting beta- agonist, an inhaled corticosteroids paired with a long-acting beta-agonist.

Pharmacodynamics

Montelukast is a leukotriene receptor antagonist that demonstrates a marked affinity and selectivity to the cysteinyl leukotriene receptor type-1 in preference to many other crucial airway receptors .As a consequence, the agent can elicit substantial blockage f LTD4 leukotriene-mediated bronchoconstriction with doses as 5mg. Moreover, a placebo-controlled, cross over study (n=12)demonstrated that montelukast is capable of inhibiting early and late phase bronchoconstriction caused by antigen challenge by 75% and 57% respectively.

In perticular, it has been documented that montelukast can cause bronchodilation as within 2 hours of oral administration. this action can also be additive to the bronchodilation caused by the concominent use of beta agonist. Nevertheless, clinical investigations performed with adults 15 years of age and older revealed that no additional clinical benefits is obtained when doses of montelukast greater than 10mg a day are used.

Mechanism of action

Cysteinyl leukotrienes(CysLT) like LTC4, LTD4, and LTE4, among others, are eicosanoids released by a variety of cells like mast cells and eosinophils. When such CysLT bind type-1 receptor located on respiratory airway smooth muscle cells, airway macrophages, and on various pro-inflammatory cells like eosinophills and some specific myeloid stem cells activities that facilitate the pathophysiology of asthma and allergic rhinitis are stimulated.

Subsequently, montelukast is a leukotriene receptor antagonist that binds with high affinity and selectivity to the CysLT type 1 receptor, which consequently assist in inhibiting any physiological actions of CysLTs like LTD4, LTC4, and LTE4 at the receptor may facilitate asthma or allergic rhinitis.

Route of elimination

It has been reported that montelukast and its metabolites are almost exclusively excreted in the bile and into the feces.

Half- life

Studies have demonstrated that the mean plasma half-life of montelukast varies from 2.7 to 5.5 hours when observed in healthy young adults.

Clearance

The plasma clearance documented for montelukast is an average of 45mL/min when observed in healthy adults.

Adverse effects

Improve decision Support & research Toxicity

The adverse effect associated with overdosage of montelukast included abnormal pain, somnolence, thirst, headache, vomiting, psychomotor hyperactivity, and less frequently, convulsion.

The oral LD50 value determined for mice and rats is >5000mg/kg.

Montelukast has not been studied in pregnant women. Consequently, it should be used during pregnancy only if clearly needed.

Additionally, as it is unknown whether montelukast is excreted into human breast milk, there is also caution regarding the use of the medication in nursing mothers.

The plasma half-life of montelukast is somewhat prolonged in elderlypatients altrhough no dosage adjustment is generally necessary.

III. Literature and Review

1. **FAIZ WARIS, ET ALL, 2024 . …** Ketorolac tromethamine (KT) is a nonsteroidal anti-inflammatory drug (NSAID) derived from heteroaryl acetic acid, functioning as a nonselective cyclooxygenase (COX) inhibitor in its racemate form. The primary objective of pharmaceutical treatment is to maintain therapeutically effective and non-toxic concentrations of medications in the blood or tissues. Controlled drug delivery has advanced significantly in the past quarter-century, leading to increased commercial success of some medications reformulated as sustained release delivery systems, resulting in extended patent protections. KT, as an NSAID, is highly effective and can be administered orally by dissolving it in water. Chemometry plays a crucial role in refining and validating HPLC methods, generating critical data from reported tests.
2. **Swati M. Andhale, ET ALL, 2022 …This** study aimed to develop and validate an RP-HPLC method for quantifying Bilastine and Montelukast, adhering to ICH validation guideline Q2R1. Conducted at Y. B. Chavan College of Pharmacy, Aurangabad, MS, India, between January 2020 and October 2021, the methodology involved optimizing

chromatographic parameters through trial runs. System suitability was confirmed by repeated injections of Bilastine (200µg/mL) and Montelukast (200µg/mL). The method's validation, per ICH Q2R1, encompassed accuracy, precision, robustness, limit of detection and quantitation, among others. Results demonstrated %RSD for retention time (RT) and mean peak area as 0.09, 0.35, and 0.35, 0.56 for Bilastine and Montelukast, respectively. The method fulfilled ICH guidelines, with correlation coefficients, slopes, and y-intercepts of 0.9971, 17595, 217883, and 0.998, 35458, 17147, respectively, for Bilastine and Montelukast. Precision exhibited %RSD of repeatability and intermediate precision < 2 across the range. Method accuracy ranged from 96.95-101.41 %w/w and 97.37-101.89 %w/w for Bilastine and Montelukast, respectively. Robustness results fell within acceptable limits. Recovery by spike method ranged from 96.37-98.88 %w/w for Bilastine and 96.11-100.06%w/w for Montelukast. In conclusion, the study achieved its goals, successfully developing and validating an RP-HPLC method for Bilastine and Montelukast quantification in accordance with ICH Q2R1 guidelines.

3. **Anusha Kunala, ET ALL, 2022...** This study aimed to develop and validate a new RP-HPLC method for estimating Bilastine and Montelukast in bulk and in their combined tablets. Utilizing an Ascent is C18 column (150 x 4.6mm, 2.6µ) with a mobile phase of 0.1% OPA: acetonitrile (50:50 v/v) at a flow rate of 1ml/min and a detection wavelength of 230nm, good separation of both analytes was achieved. The stability of the analytes was tested under various stress conditions, including hydrolysis with acid and base, peroxide oxidation, and thermal degradation. Bilastine and Montelukast eluted at 2.25min and 2.78 min, respectively, with isocratic elution. The method exhibited linear response ranges of 2.5 to 15µg/ml for Bilastine and 5 to 30 µg/ml for Montelukast. The LOD and LOQ for Bilastine were 0.1 and 0.31µg/ml, respectively, and for Montelukast, they were 0.3 and 0.91µg/ml. Degradant peaks from Bilastine and Montelukast were distinguishable. The method demonstrated sensitivity, accuracy, cost-effectiveness, and stability.

4. **Shweta P Ghode, ET ALL, 2021**1 Department of Pharmaceutical Quality Assurance, JSPM’s Rajarshi Shahu College of Pharmacy and Research, Pune, Maharashtra, India 2 Rasiklal Makinchand Dhariwal Institute of Pharmaceutical Education & Research, Chinchwad, Pune, M Combination therapy of Montelukast (MNK) and Ebastine (EBA) provide anti-asthmatic effector the maintain treatment of asthma and relieve symptoms of

allergies. The objective of this study is development of a new simple, accurate, sensitive, and reproducible RP-HPLC method for simultaneous estimation of MNK and EBA in pharmaceutical formulation (tablet) using Ofloxacin (OFL) as an internal standard and validate the same as per ICH guidelines. The chromatogram separation was achieved on Qualisil-5 BDS C8 column (250 mm × 4.6 mm, 5μm) column with mobile phase acetonitrile: water (pH 2.8 with TFA) in the composition of 84:16 v/v at a flow rate of 1 mL/min using PDA detector at 254 nm at ambient column temperature, keeping the injection volume 20 μL. The retention time of OFL, MNK, and EBA was observed to be 2.107 min, 2.517 min, and 3.819 min, respectively. All the criteria for the validation (linearity, accuracy, precision, and robustness) were observed to be within the acceptation range. The calibration plots were obtained between 5-60 μg/mL for MNK and 5- 60 μg/mL for EBA with r2 values of 0.999 in each case. The recovery of MNK and EBA was found to be 98.99% and 99.40%, respectively with a % RSD of <2. This RP-HPLC method was found to be rapid, specific, precise, and accurate and can be used for the routine analysis of MNK and EBA in bulk as well as in tablet dosage form. The separation was complete with a shorter analysis time along with well good resolved peak.

5. **N. Anjaneyulu*, ET ALL, 2015….**A simple, sensitive, accurate, precise and rapid reverse phase high performance liquid chromatographic method has been developed and validated for the simultaneous determination of Tolperisone and Paracetamol from synthetic mixture. The chromatographic separation was performed on Imo Sil 5 C18 column (250 mm × 4.6 mm i.d, 5 μm particle size). Mobile phase consisted of a Acetonitrile and methanol in the ratio of 25:75, v/v at a flow rate of 1.0 ml/min. The detection wavelength was set at 261nm. The proposed method was validated for linearity, accuracy, precision, LOD and LOQ. The calibration was linear over the concentration range of 10-30 μg/ml for Tolperisone and 5-15 μg/ml for Paracetamol. The retention times were found to be 5.3 ± 0.14min for Paracetamol and 2.4 ± 0.13min for Tolperisone. The mean recoveries were 100.5 ± 0.34 and 98.2 ±0.80 for Tolperisone and Paracetamol, respectively. The method can be easily adopted for quality control analysis.

6. **Yakkala Lakshmi Manasa*, ET ALL, 2015** …..A simple, accurate, economical and precise reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the simultaneous determination of Fexofenadine hydrochloride and Montelukast Sodium. The separation was achieved on an X-Terra C18 column (100 x 4.6

mm, 5 μm) as stationary phase with a mobile phase comprising of 0.1% Triethylamine: Acetonitrile (30:70) in an isocratic mode, at a flow rate of 0.8 ml/min. The detection was monitored at 220 nm. The retention time of Fexofenadine hydrochloride and Montelukast Sodium were 1.425min and 3.364 min respectively. The linearity was found to be in the range of 35-105 μg/ml and 2.9-8.7 μg/ml for Fexofenadine hydrochloride and Montelukast Sodium respectively with correlation coefficient of 0.999. The proposed method was validated according to ICH guidelines for parameters like linearity, accuracy, precision and specificity. All validation parameters were within the acceptable range. The developed method was successfully applied for the estimation of Fexofenadine hydrochloride and Montelukast Sodium in pure and pharmaceutical dosage form.

7. **R. Naga Kishore, ET ALL, 2015….:**A simple, sensitive, accurate, precise and rapid reverse phase high performance liquid chromatographic method has been developed and validated for the simultaneous determination of Tolperisone and Paracetamol from synthetic mixture. The chromatographic separation was performed on Imo Sil 5 C18 column (250 mm × 4.6 mm i.d, 5 μm particle size). Mobile phase consisted of a Acetonitrile and methanol in the ratio of 25:75, v/v at a flow rate of 1.0 ml/min. The detection wavelength was set at 261nm. The proposed method was validated for linearity, accuracy, precision, LOD and LOQ. The calibration was linear over the concentration range of 10-30 μg/ml for Tolperisone and 5-15 μg/ml for Paracetamol. The retention times were found to be 5.3 ± 0.14min for Paracetamol and 2.4 ± 0.13min for Tolperisone. The mean recoveries were 100.5 ± 0.34 and 98.2 ±0.80 for Tolperisone and Paracetamol, respectively. The method can be easily adopted for quality control analysis.

8. **Sonal More*, ET ALL, 2015**:….The present work deals with development of HPLC method for simultaneous estimation of L-dopa and Diosgenin in marketed aphrodisiac formulation containing Mucuna pruriens seed and Tribulus terristris fruit powder as the ingredients. Chromatographic separation of the drugs was carried out on a Hemochrom Instil [(C18 (250 X 4.6 mm, 5 μm)] using the mobile phase ACN: 0.1%orthophosphoric acid in water (20:80 v/v), pumped at the flow rate of 0.6ml/min with UV-detection at 216nm. The two markers were satisfactorily resolved with retention time 3.781 and 5.255 for L- dopa and Diosgenin, respectively. The method was validated and shown to be linear for L-dopa and Diosgenin. The correlation coefficients for L-dopa and Diosgenin are 0.9954 and 0.9961 respectively. The developed method was found to be rapid,

simple, specific and reliable for the determination of L-dopa and Diosgenin from these marketed aphrodisiac formulation.

9. **S.A.Patel, ET ALL, 2013…**:This research paper describes simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method for the simultaneous determination of Montelukast sodium and Bambuterol hydrochloride in combined dosage form. The sample was analyzed by reverse phase C18 column (Phenomenex C18, 5µ, 250mm xIntroductionMontelukast sodium (MTKT), 1-[({(R)-m-[(E)-2-(7chloro-2-quinolyl)vinyl]-a-[o-(1- hydroxyl 1methylethyl)phenethyl]benzyl} thio)methyl]cyclopropaneacetate sodium is a leukotriene receptorantagonist, used in the treatment of asthma.[2],[3] It isnot official in IP, BP and USP. Various analytical methods, such as liquid chromatography withfluorescence detection.[4]151.[6] stereoselective HPLC forMTKT and its S-enantiomer simultaneous HPLC and derivative spectroscopic method with loratadine.[8] stability indicating HPLC method for Montelukast sodium in tablets and human plasma have beenreported. Bambuterol hydrochloride (BAM), (RS)- 5(2-tert-butylamino-1- hydroxyethyl) - m -phenylene bis (dimethylcarbamate) hydrochloride is a direct acting sympathomimetic with predominantly -adrenergicactivity (82 - agonist)).[10] It is an ester prodrug of b$_2$adrenergic agonist terbutaline[2] Bambuterolhydrochloride is official in BP, Different HPLC4.6mm) as stationary phase; methanol: acetonitrile : 1% trichloroacetic acid in the ratio of 80:10:10 v/v/v as a mobile phase at a flow rate of 1.0ml/min. Quantification was achieved with ultraviolet detection at 220 nm. The retention time for Montelukast sodium and Bambuterol hydrochloride was found to be 3.17 and 2.35 min, respectively. The linearity for both the drugs was in the range of 0.5-10 µg/ml with mean accuracies 100.06 ± 0.49 and 99.95 ± 0.63 for Montelukast sodium and Bambuterol hydrochloride, respectively. The method was successively applied to pharmaceutical formulation no chromatographic interferences from the tablet excipients were found. The method retained its accuracy and precision when the standard addition technique was applied.

10. **J. S. Shah, ET ALL, 2013…:**A simple, specific, accurate, and stability- indicating reversed-phase high-performance liquid chromatographic method was developed for the simultaneous determination of montelukast and fexofenadine hydrochloride, using a Lichrospher® 100, RP-18e column and a mobile phase composed of methanol:0.1% *o*-phosphoric acid (90:10 v/v), pH 6.8. The retention times of montelukast and fexofenadine

hydrochloride were found to be 10.16 and 12.03 min, respectively. Linearity was established for montelukast and fexofenadine hydrochloride in the range of 2-10 µg/ml and 24-120 µg/ml, respectively. The percentage recoveries of montelukast and fexofenadine hydrochloride were found to be in the range of 99.09 and 99.81%, respectively. Both the drugs were subjected to acid and base hydrolysis, oxidation, photolytic, and thermal degradation conditions. The degradation products of montelukast and fexofenadine hydrochloride were well resolved from the pure drug with significant differences in their retention time values. This method can be successfully employed for simultaneous quantitative analysis of montelukast and fexofenadine hydrochloride in bulk drugs and formulations.

11. **Jitendra Patel, ET ALL, 2012…**A reversed-phase-liquid chromatographic (RP-HPLC) method was developed for the determination of Montelukast Sodium (MTKT) and Desloratadine (DSL) in their marketed formulation. A reversed-phase C-18 column (250 mm × 4.8 mm i.d., particle size 5 µm) column with mobile phase consisting of methanol: water: Acetic acid (90:10:0.05 v/v/v) was used. The flow rate was 1.0 ml/ min and effluents were monitored at 280 nm. The retention times of Montelukast Sodium and Desloratadine were found to be 7.61±0.2 min and 2.23±0.3 min,respectively. The method was validated in terms of specificity,linearity, range, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ). The method showed good linearity in the range of 40-140 µg/ml for Montelukast Sodium and 20-70 µg/ml for Desloratadine. The % recoveries of Montelukast Sodium and Desloratadine were found to be between 99.54-101.25 and 99.54-101.40. The percentage RSD for the method precision was found to be less than 2%. The proposed method was successfully applied to the estimation of Montelukast Sodium and Desloratadine in combined capsule dosage forms.

12. **Sachin Gholve, ET ALL, 2015….**A reversed-phase high-performance liquid chromatography method is developed and validated for the determination of Montelukast Sodium (Montelukast) in bulk drug and pharmaceutical dosage form. The chromatographic determination was performed on Agilent 1220 Infinity LC with EZ chrome elite software with variable wavelength detector. The separation was conducted by using Zobrax Eclipse XDB-C18 column (4.6×150×5µm) with mobile phase consisting Methanol:Acetonitrile:Water (60:30:10). The mobile phase was delivered at the flow rate of 1.0 ml/min. The eluent was monitored at wavelength 344 nm and found a sharp and

symmetrical peak with retention time 3.582. The method was validated for linearity, accuracy, precision, system suitability, and stability. The method was found to be linear over the concentration range 5-30µg/ml with coefficient R2 - 0.999. The developed HPLC technique is precise, specific, accurate and stable. Statistical analysis proves that the method is reproducible, selective and suitable to be applied for analysis of montelukast sodium in commercial pharmaceutical dosage form for routine quality control application.

13. **Hitesh Vekaria, ET ALL, 2013** simple, fast and precise reverse phase high performance liquid chromatographic method has been developed for the simultaneous determination of Montelukast Sodium (MONT) and Fexofenadine hydrochloride (FEXO). The chromatographic separation was achieved on X- bridge C18 column (250 mm × 4.6 mm, 5 µm) as stationary phase with a mobile phase comprising of 50 mM Sodium acetate buffer:acetonitrile:methanol (25:35:40) adjust pH 8.2 with 5% *o*-phosphoric acid at a flow rate of 1.0 mL/min, column temperature of 40 ± 2 °C and UV detection at 210 nm. The retention time of Montelukast Sodium and Fexofenadine hydrochloride were 3.43 min, and 8.22 min respectively. The linearity were found to be in the range of 12.5–37.5 µg/mL and 150 450 µg/mL for Montelukast Sodium and Fexofenadine hydrochloride with correlation coefficient greater than 0.999. The proposed methods were validated as per ICH guidelines and successfully applied for the determination of investigated drugs in tablets.

14. **B. Mohammed Ishaq, ET ALL 2015….:**A simple, selective and sensitive reverse phase high performance liquid chromatography (Rp-HPLC) method has been proposed for the simultaneous quantitative determination of levocetirizine dihydrochloride (LEV) and Montelukast sodium (MON) in pure form as well as in its pharmaceutical formulation. The chromatography was carried out on Waters C18 analytical column (15cm × 4.6 mm, 5µ) using a mobile phase of methanol: water (75:25 v/v). The flow rate was 1.0 ml/min with detection at 235 nm. The retention time of LEV and MON were found to 2.88 and 3.83 min respectively. The linearity for LEV and MON were in the range of 50-150 µg/mL and 100- 300 µg/mL respectively. The recoveries of LEV and MON were found to be 100.00% and 99.00%, respectively. The proposed method was validated and successfully applied to the estimation of LEV and MON in combined tablet dosage forms.

15. **R. R. Jain, ET ALL, 2013…** A novel, simple, sensitive, and rapid spectrophotometric method was developed for the simultaneous estimation of montelukast sodium (MONTE) and desloratadine (DES). The method involved solving simultaneous equations based on measurement of absorbance at two wavelengths: 285.6 nm and 245 nm, corresponding to the λmax values of MONTE and DES, respectively. Beer's law was obeyed in the concentration range of 4-24 µg/mL for MONTE and 2–12 µg/mL for DES. The method was validated for accuracy, precision, and recovery studies. It demonstrated precision, reproducibility, selectivity, specificity, and accuracy for quantitative estimation of MONTE and DES in combination. With its wide linearity range, sensitivity, accuracy, and simple procedure, the method is deemed appropriate for routine analysis and quality control assays of their tablets. Validation was performed according to the ICH guidelines.

16. **Sathis Kumar Dinakaran, ET ALL, 2014…** A simple, economic, and accurate absorption ratio method was developed for the simultaneous estimation of Montelukast Sodium (MTK) and Simvastatin (SMV) in bulk and tablet dosage form. 0.1M NaOH was utilized as a diluent to dissolve MTK and SMV. Absorptions were observed at 244 nm (Isosbestic point) and 295 nm (λmax of MTK), selected based on overlapping spectra of SMV and MTK. The linearity range was determined to be 2-10 µg/ml at 244 nm (r2 = 0.998±0.001) and 295 nm (r2 = 0.998±0.0008). The method was deemed simple, precise, accurate, and rapid for the simultaneous determination of SMV and MTK in bulk and tablet dosage form using the absorption ratio method. Recovery study was conducted to confirm method accuracy, and validation was performed according to ICH guidelines.

17. **Nikesh S. Rana, ET ALL, 2014…** A simple spectrophotometric method was developed for the simultaneous estimation of Montelukast Sodium and Ebastine from tablet dosage form. The method utilized absorbance correction, measuring absorbance at two wavelengths: 345 nm (where Ebastine has no absorbance) and 253 nm (where both drugs exhibit considerable absorbance). Linearity was established in the range of 5-25g/ml for Montelukast Sodium and Ebastine. Accuracy and precision were evaluated, meeting ICH guidelines. The method demonstrated good reproducibility and recovery with % RSD within the desired range. Rapid, specific, precise, and accurate, this method can be effectively applied for routine analysis of Montelukast Sodium and Ebastine in their combined tablet dosage form.

18. **Ghada M Hadad, ET ALL, 2023….** A novel and eco-friendly reversed-phase HPLC approach with diode array and fluorescence detection modes was designed for simultaneous estimation of Bilastine and Montelukast, two co- formulated antihistaminic drugs. The Quality by Design (QbD) approach was employed to accelerate method development and test method robustness. A full factorial design was used to evaluate the effect of variable factors on chromatographic response. Chromatographic separation was achieved using isocratic elution on a C18 column with a mobile phase consisting of 92% methanol, 6% acetonitrile, and 2% phosphate buffer with 0.1 (v/v) triethylamine adjusted to pH 3, pumped at a flow rate of 0.8 mL/min with an injection volume of 20 μL. The developed stability-indicating HPLC approach was used to assess the stability of Montelukast (MNT) under various stress conditions, including hydrolytic (acid–base), oxidative, thermal, and photolytic stress conditions. All conditions resulted in relevant degradation pathways, and MNT degradation followed pseudo-first-order kinetics. Kinetic parameters (rate constant and t1/2) were calculated, and a proposed degradation pathway was postulated under the described experimental conditions.

IV. AIM AND OBJECTIVES

Aim

Simultaneous estimation of montelukast by using uv spectroscopy

Objectives

1. Identify the mechanisam of action of montelukast
2. Describe the possible adverse effects of montelukast
3. Summarize the indications for montelukast
4. Employ interorofessional tea stratergies for enhancing care coordination and communication to advance the safe use of montelukast for asthma and imoorove outcomes.

V. Experimental work

5. Methodology validation (Materials and Methods)

Instruments-Instruments

HPLC –Waters Model

NO.2690/5 series Compact System Consisting of

□	Inertsil-C18	ODS
column.□	Electronic	balance
(Sartorious)□	Sonicator (Clean)	FAST

Substances containing chemicals

- Methanol HPLC Grade.
- Buffer (KH_2PO_4) HPLC Grade.

Raw Equipment (Unprocessed materials)

MONTELUKAST is working standard.

Stock solution preparation: Take 100mg MONTELUKAST working standard in 100ml V.F add methanol sonicate it 30minets, (That is 1000ppm solution).

Further Dilution (or) Trails Solution: Take 10ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minutes (That 100ppm solution)

5.1 Development of an hplc method

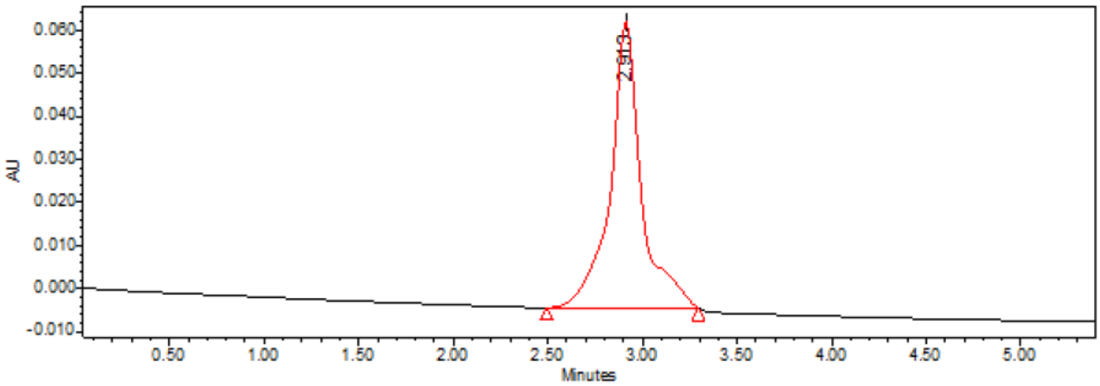
The goal of this study was to improve the assay technique for simultaneous quantification of MONTELUKAST on literature surveys. As a result, the trials detailed below show how the optimization was accomplished.

Trail: 1

Step mobile: Degassed Acetonitrile:Water 90:10 .

Chromatographic conditions

Flow rate : 1.0ml / min
Column : Inertsil-C18, plate ODS
Wave longitude detector : 225nm
Tempo in the column : Ambient
Size of injection : 20µl
Time to run : 6min
Retention time : 2.913



Inference: The two summits are entirely Blended and Cannot be distinguished.

Fig. 1: Trial 1 chromatogram.

S. NO.	Name of the peak	Retention time(min)
1.	Montelukast	2.913

Trail 2

Mobile phase: Degassed Acetonitrile and methanol in the ratio of 45:55 V/V.

Chromatographic conditions

Flow rate : 1.0ml / min

Column : Inertsil-C18, plate ODS

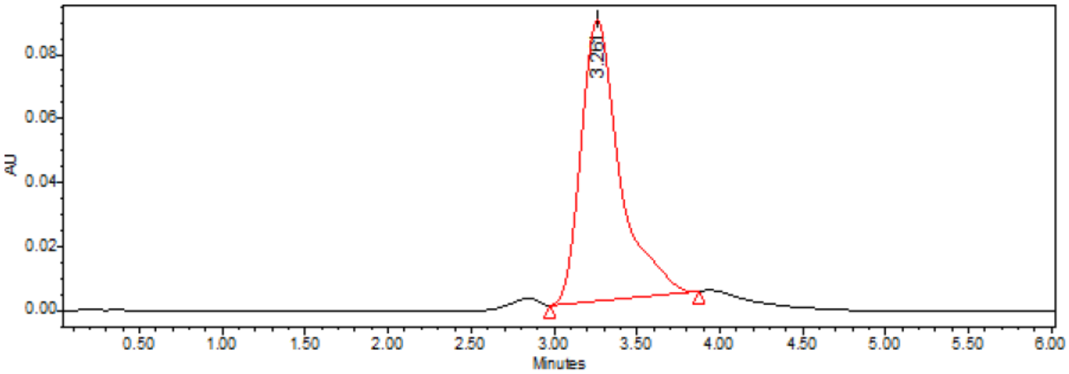
Wave longitude detector : 225nm

Tempo in the column : Ambien

Size of injection : 20µl

Time to run : 6min

Retention time : 3.261



Inference: The forms of the peaks are not appealing.

Fig. 2: Trial 2 chromatogram.

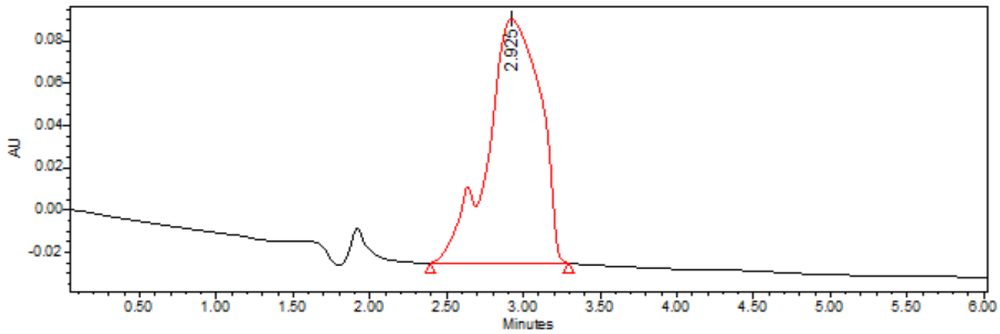
S. NO.	Name of the peak	Retention time(min)
1	Montelukast	3.261

Trail: 3

Mobile phase: Degassed Acetonitrile and Methanol in the ratio of 50:50 V/V.

Chromatographic Conditions

Flow rate	: 1.0ml / min
Column	: Inertsil-C18, plate ODS
Wave longitude detector	: 225nm
Tempo in the column	: Ambien
Size of injection	: 20µl
Time to run	: 6min
Retention time	: 2.925



Inference: The summits are not fully separated.

Fig. 3: Trial 3 chromatogram.

S. NO.	Name of the peak	Retention time(min)
1	Montelukast	2.925

VI. RESULTS AND DISCUSSION

6. Discussion of the results (Results and Discussions)

6.1 Advanced method (Optimized method)

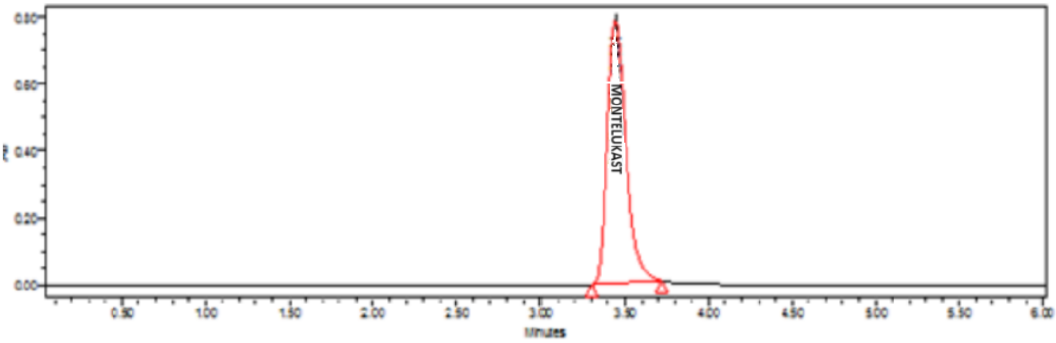
Mobile phase: Methanol: Water (75:25)V/V. Sonicate it 30minets, Filter this mobile phase through 0.45micron filter paper.

Optimized method stock solution preparation: Take 100mg MONTELUKAST working standard in 100ml V.F add methanol sonicate it 30minets, (That is 1000ppm solution).

Further Dilution (or) Optimized method solutions preparation: Take 4ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minutes (That 40ppm solution).

3. Chromatographic conditions

Parameters	Method
Stationary phase (column)	Inertsil -ODS C18(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol: Water (75:25)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	6 min
Column temperature (°C)	Ambient
Volume of injection loop (□l)	20
Detection wavelength (nm)	225nm
Drug RT (min)	3.444min



Inference: Got chromatogram at a Rt of 3.444 for standard.

Fig. 4: Standard chromatogram.

S. NO.	Name of the peak	Retention time(min)
1	Montelukast	3.444

6.3 Information of high value (Validation data)

6.3.1 Products for the system(System suitability)

Validation stock solution preparation: Take 100mg MONTELUKAST working standard in 100ml V.F add methanol sonicate it 30minets, (That is 1000ppm solution).

Validation parameters solutions preparation: Take 2ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minutes (That 20ppm solution).

Take 3ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minutes (That 30ppm solution).

Take 4ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minutes (That 40ppm solution).

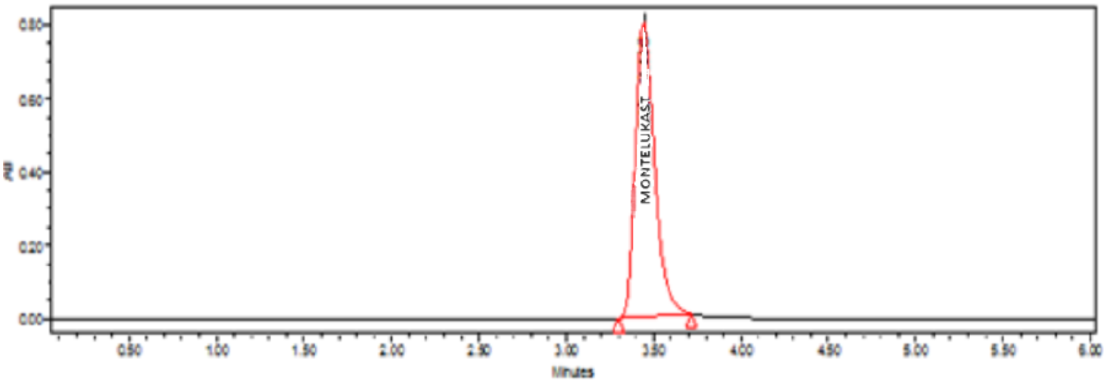
Take 5ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minutes (That 50ppm solution).

Take 6ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minutes (That 60ppm solution).

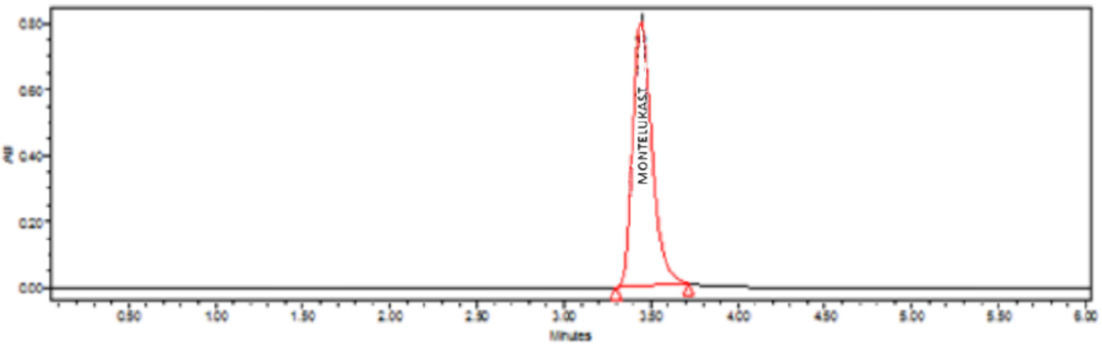
Take 7ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minutes (That 70ppm solution).

Table 3: Data of system suitability.

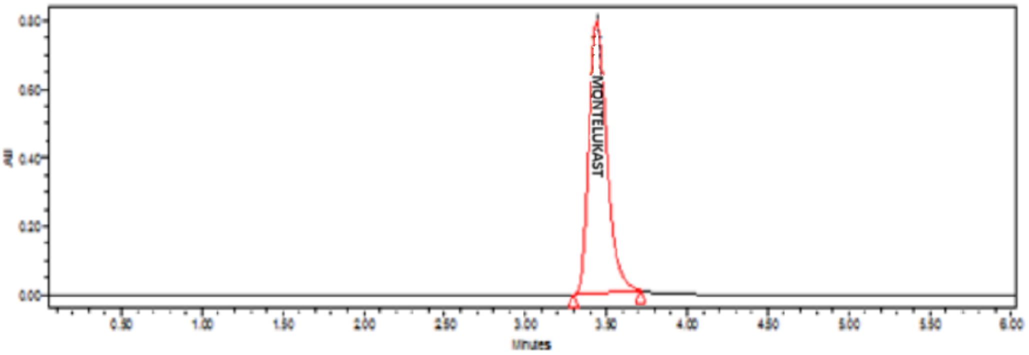
Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.446	2102936.24	11003	1.126
2	3.442	2102846.85	11040	1.126
3	3.441	2103011.54	11020	1.130
4	3.442	2102942.85	11038	1.127
5	3.440	2103038.45	11060	1.128
Mean	3.4422	2102955.18	11032	1.1274
SD	0.00228	74.7604	-------	-------
% RSD	0.066247	0.00355	-------	-------



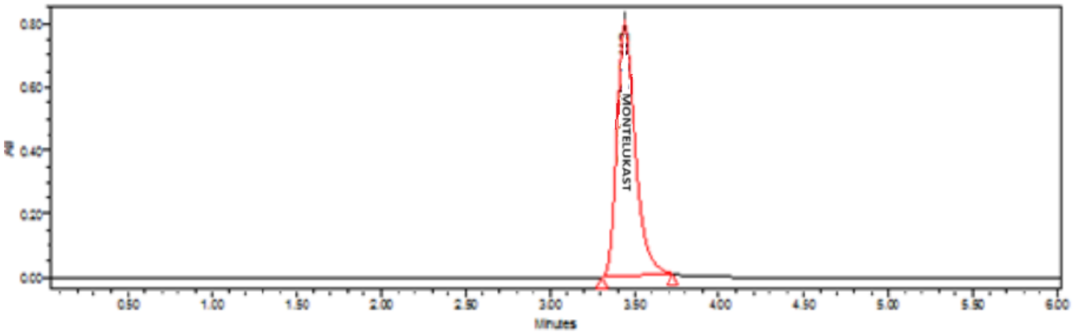
Inference: Standard Chromatogram-1 System Suitability.



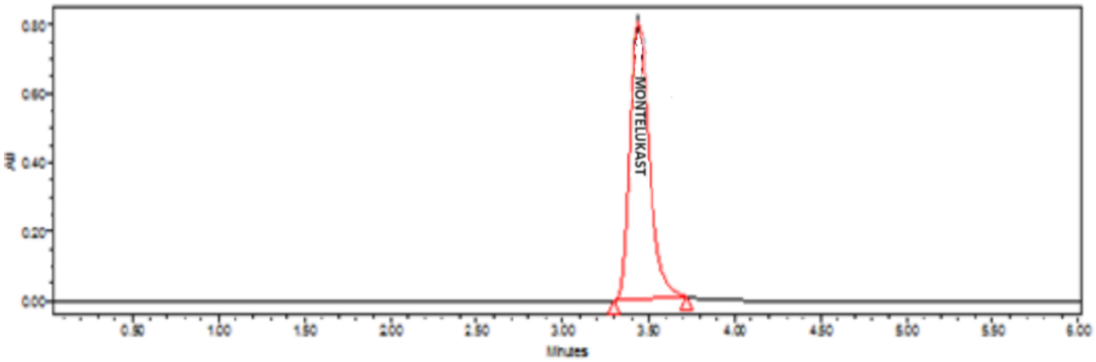
Inference: Norm Chromatogram-2 device appropriateness



Inference: For standard Chromatogram-3, a suitable system is required.



Inference: Applicability of the device for routine Chromatogram-4.

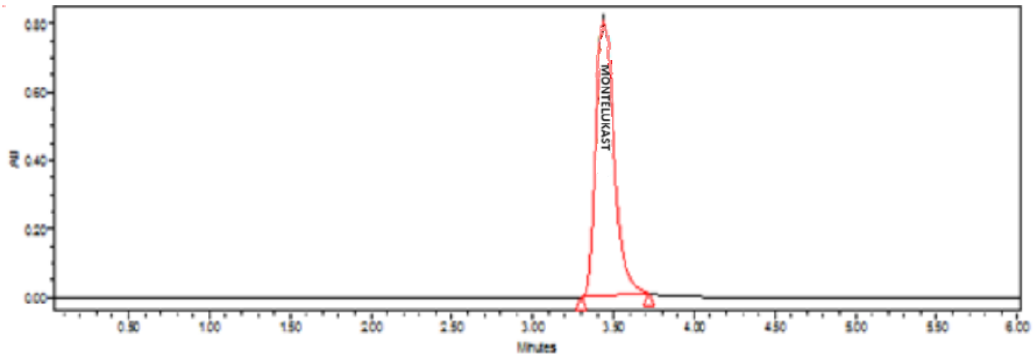


Inference: Norm Chromatogram-5 device appropriateness.

Fig. 5-9: System suitability chromatograms (standards 1-5).

6.3.2 Description (Specificity)

Fig 10: Blank chromatograph.



Inference: Got a peak for std at an Rt of 3.440min

Fig. 11: Chromatogram standard.

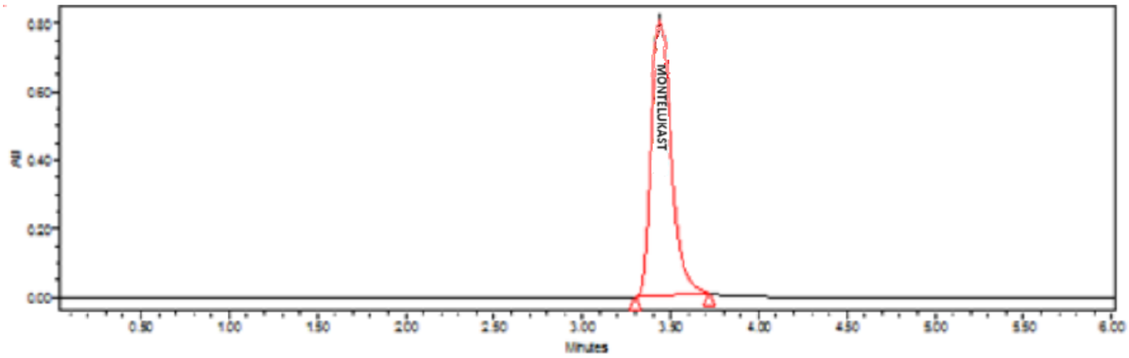
6.3.2 Prequire (Precision)

6.3.2.1 Repetition (Repeatability)

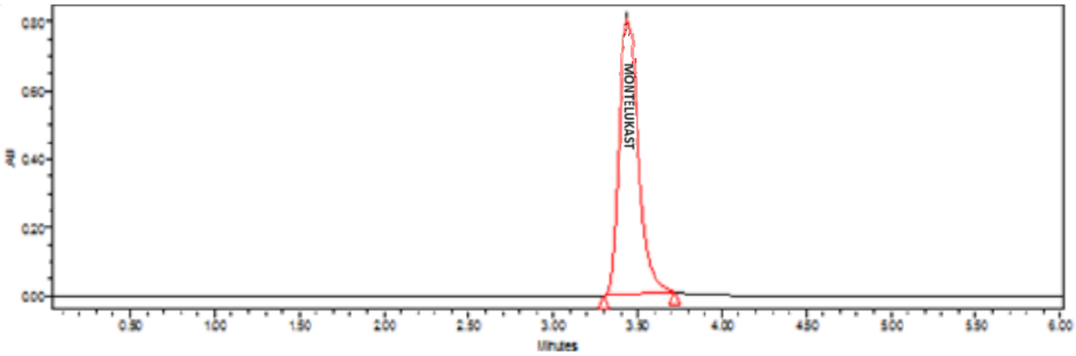
(a) Precise system (System precision)

Table 4: Data of Repeatability (System precision).

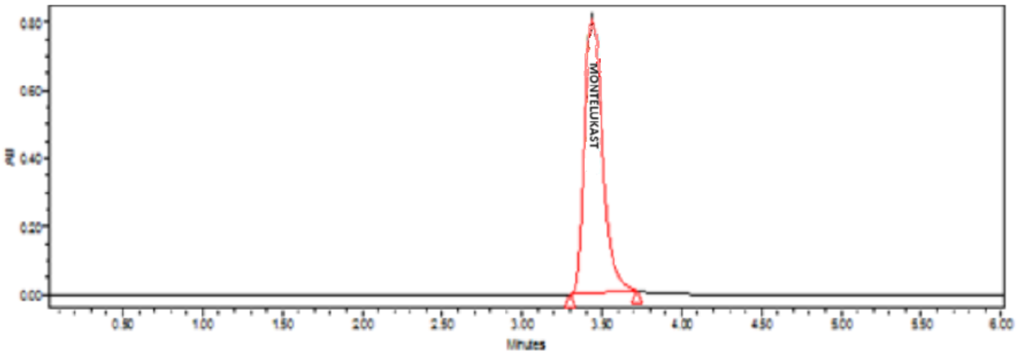
	Injection	**Peak Areas of montelukast**	**%Assay**
Concentration 40ppm	1	2102965.54	100.22
	2	2102912.84	100.22
	3	2102886.52	100.22
	4	2103045.69	100.23
	5	2102946.46	100.22
Statistical **Analysis**	**Mean**	2102951.41	100.22
	SD	60.8501	0.00290
	% RSD	0.00289	0.00290



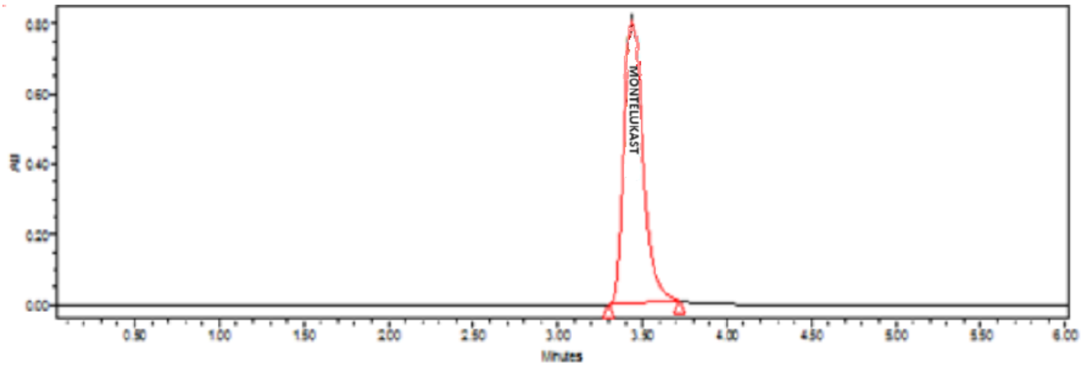
Inference: Precision chromatograph devices (standard-1).



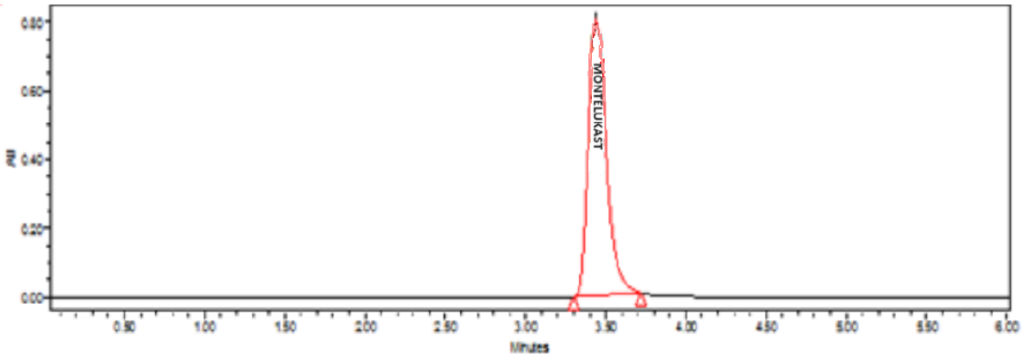
Inference: Precision chromatograph devices (standard-2).



Inference: Precision chromatograph devices (standard-3).



Inference: Precision chromatograph devices (standard-4).



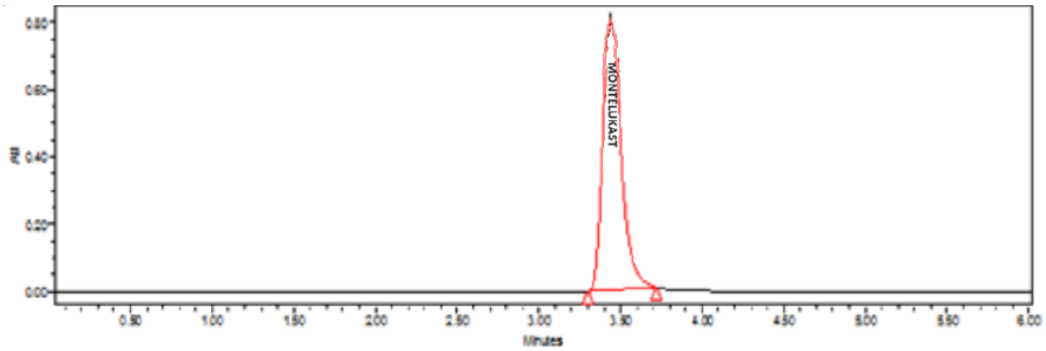
Inference: Precision chromatograph devices (standard-5).

Fig. 12-16: Detailed chromatograms of systems.

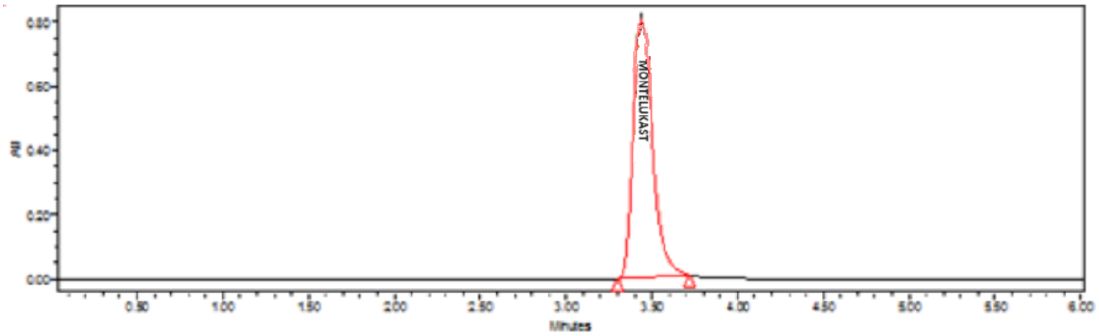
(b) Method precision

Table 5: Data of Repeatability (Method precision).

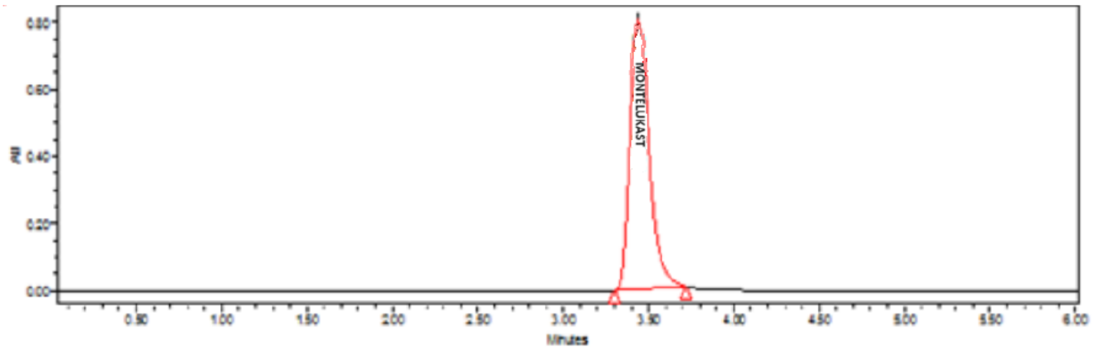
	Injection	Peak Areas of Montelukast	%Assay
Concentration 40ppm	1	2102877.32	100.22
	2	2102956.23	100.22
	3	2102997.12	100.23
	4	2103022.22	100.23
	5	2103075.84	100.23
	6	2103124.45	100.23
Statistical **Analysis**	**Mean**	2103008.86	100.23
	SD	87.4487	0.00418
	% RSD	0.00415	0.00417



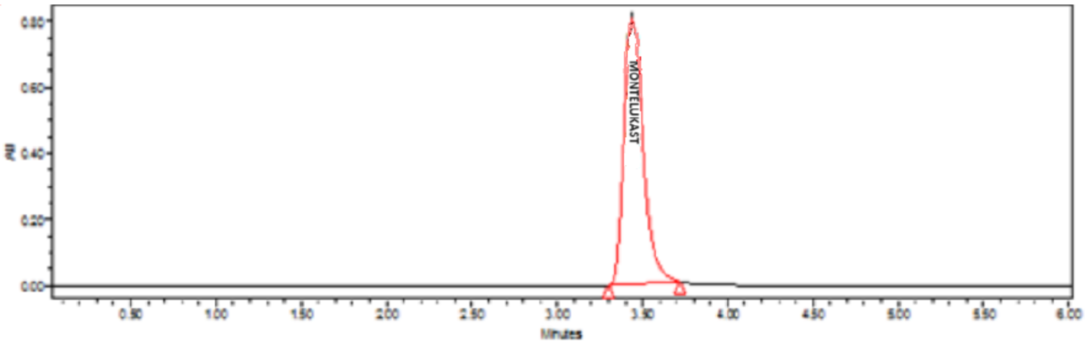
Inference: Chromatograph with high repeatability (Standard-1).



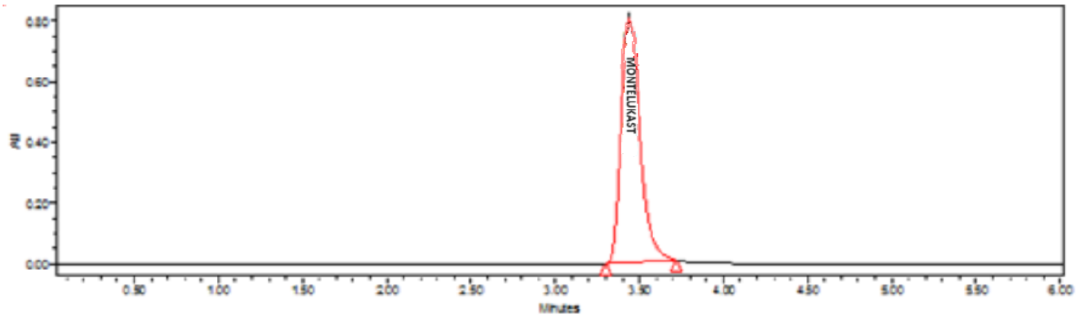
Inference: Chromatograph with high repeatability (Standard-2).



Inference: Chromatograph with high repeatability (Standard-3).



Inference: Chromatograph with high repeatability (Standard-4).



Inference: Chromatograph with high repeatability (Standard-5).

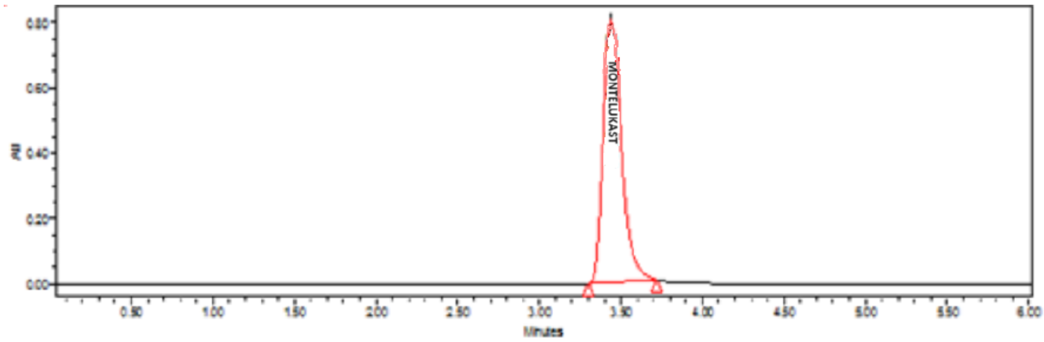


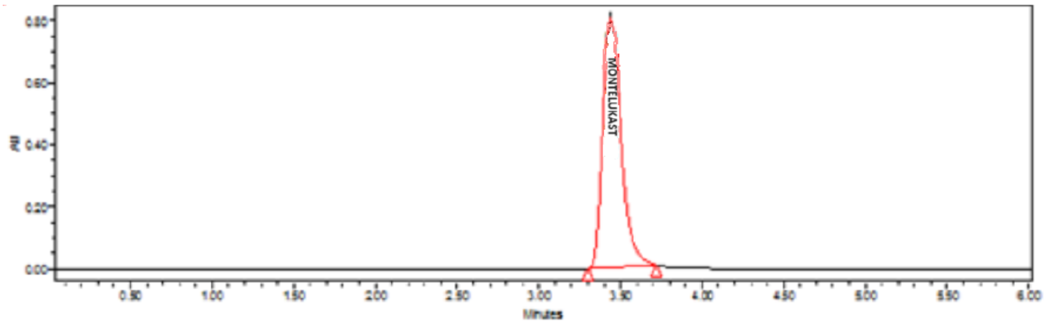
Fig. 17-22: Repeatability chromosomes (Repeatable chromatograms).

6.3.2.2 Intermediate precision

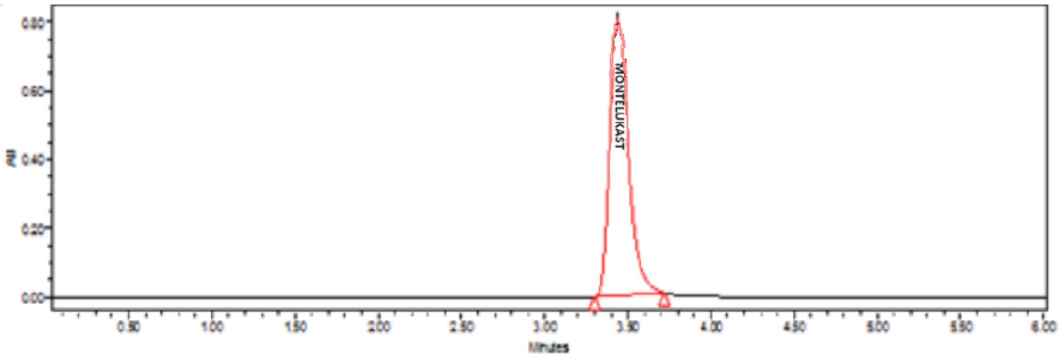
For **Analyst 1** ref: Table3.

Table 6: Data of Intermediate precision (Analyst 2).

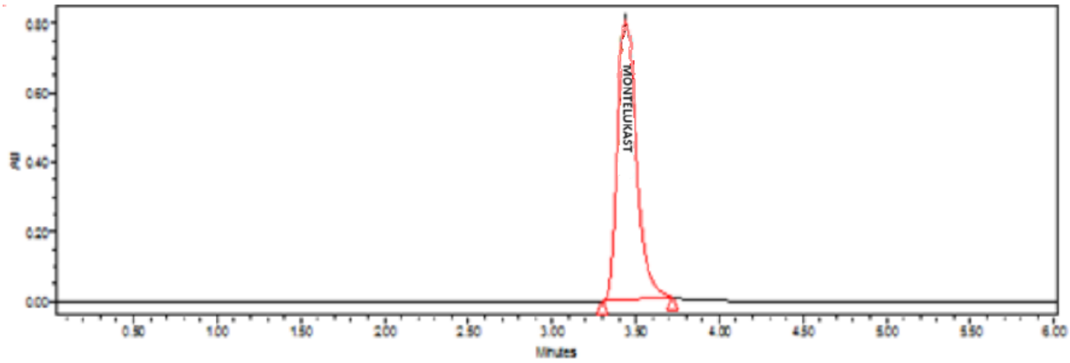
	Injection	**Peak Areas of Montelukast**	**%Assay**
Concentration 40ppm	1	2102937.51	100.22
	2	2102989.98	100.23
	3	2103018.18	100.23
	4	2102922.85	100.22
	5	2103145.48	100.23
	6	2102846.13	100.22
Statistical **Analysis**	**Mean**	2102976.68	100.22
	SD	101.8507	0.00486
	% RSD	0.00484	0.00485



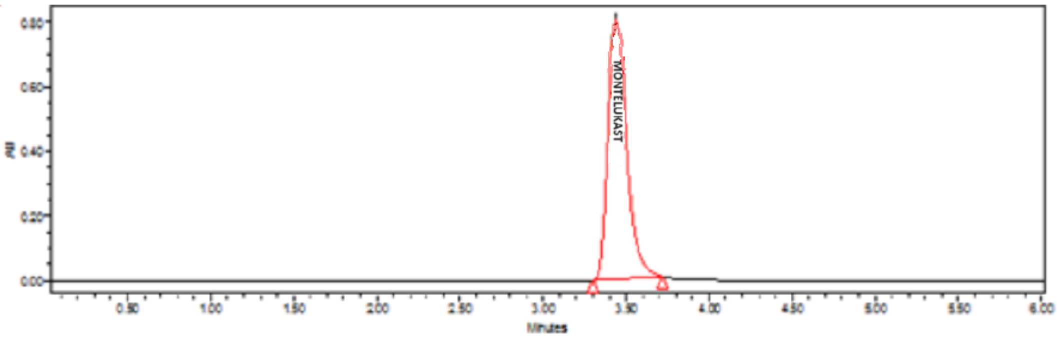
Inference: Chromatograph with a medium precision.1



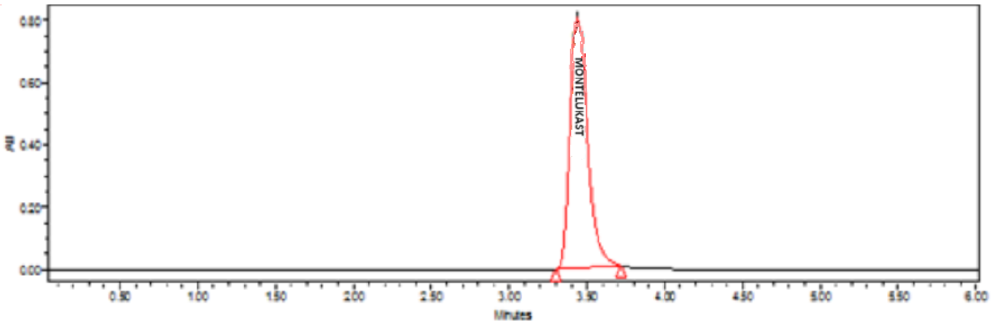
Inference: Chromatograph with a medium precision.2



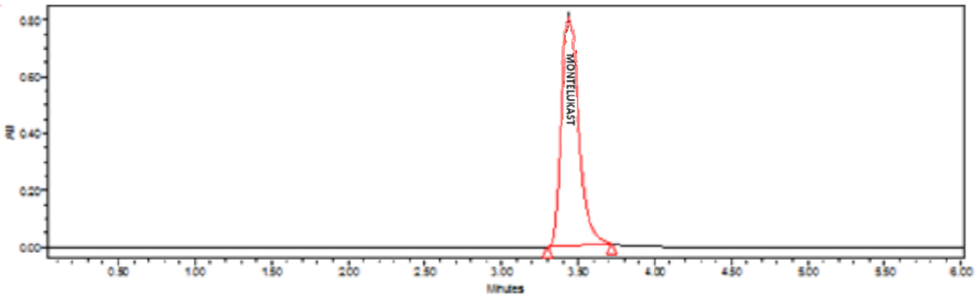
Chromatograph with a medium precision.3



Chromatograph with a medium precision.4



Chromatograph with a medium precision.5



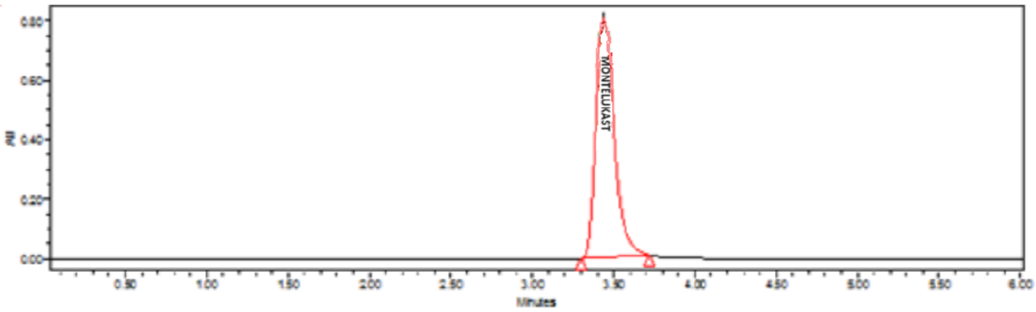
Chromatograph with a medium precision.6

Fig. 23-28: Chromatograms of intermediate precision.

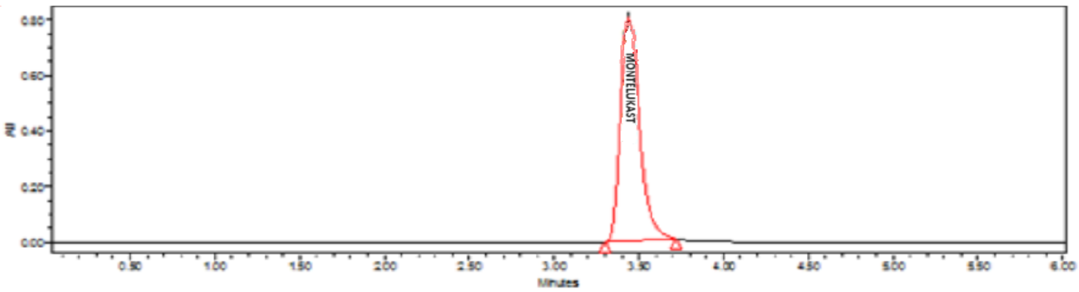
6.3.4 Accuracy

Table 7: Data of accuracy.

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	20	19.98	99.92	MEAN	100.12
50% Sample 2	20	19.98	99.92		
50% Sample 3	20	20.10	100.52	%RSD	0.3493
100 % Sample 1	40	40.09	100.22	MEAN	100.33
100 % Sample 2	40	40.09	100.23		
100 % Sample 3	40	40.21	100.53	%RSD	0.17665
150% Sample 1	60	60.19	100.33	MEAN	100.39
150% Sample 2	60	60.19	100.32	%RSD	0.1163
150% Sample 3	60	60.31	100.53		

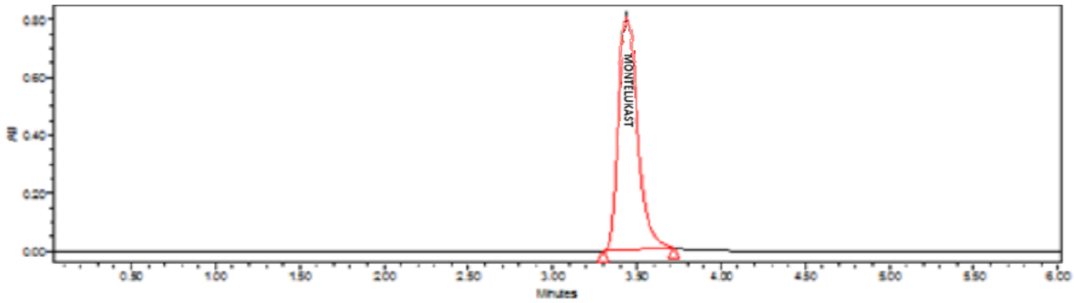


Inference: Standard 1 chromatogram.

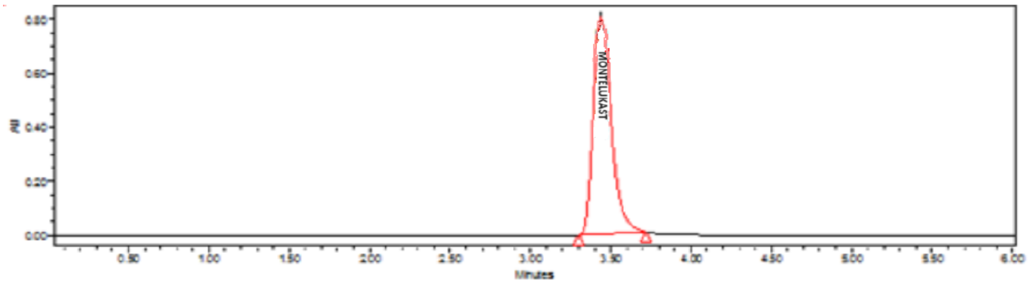


Inference: Standard 2 chromatogram.

Fig. 29 -30: Chromatographic precision (50 percent).

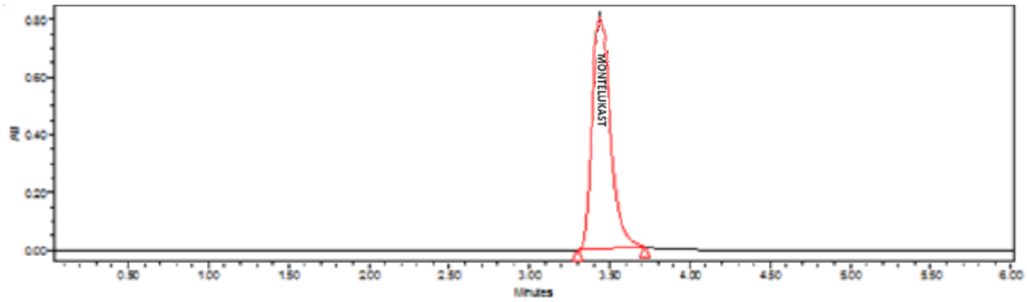


Inference: Standard 1 chromatogram.

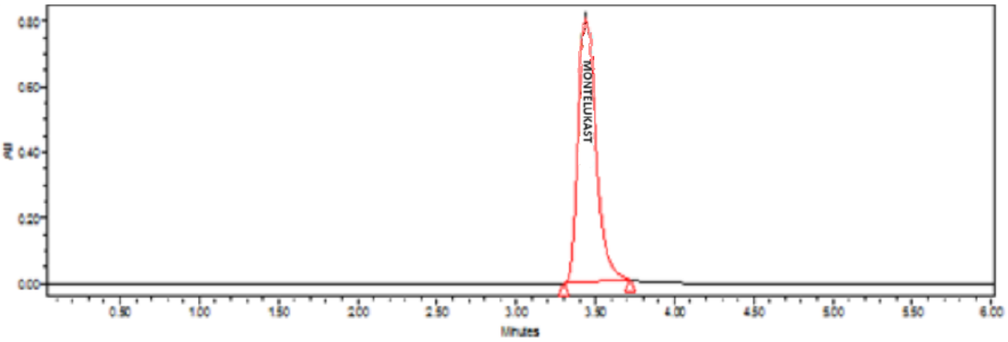


Inference: Standard 2 chromatogram.

Fig. 31-32: Chromatograms with extreme accuracy (100 per cent).



Inference: Standard 1 chromatogram.



Inference: Standard 2 chromatogram.

Fig. 33-34: Chromatograms are used to ensure precision (150 per cent).

6.3.5 Linearity

Table 8: Data of linearity.

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	52296
20	1051491.45	y-Intercept	6339
30	1577236.65	Correlation Coefficient	0.999
40	2102982.87		
50	2628727.42		
60	3154473.86		
70	3649285.27		

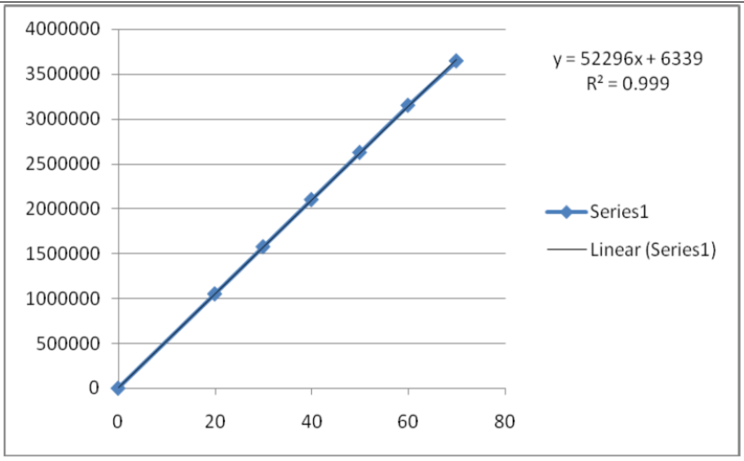
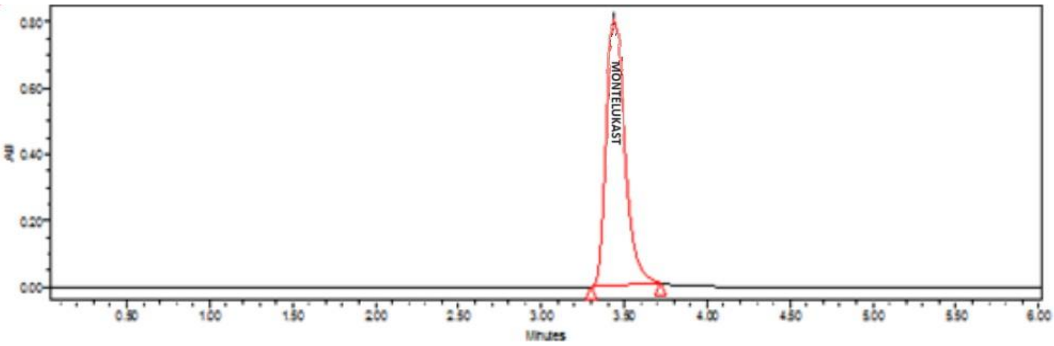
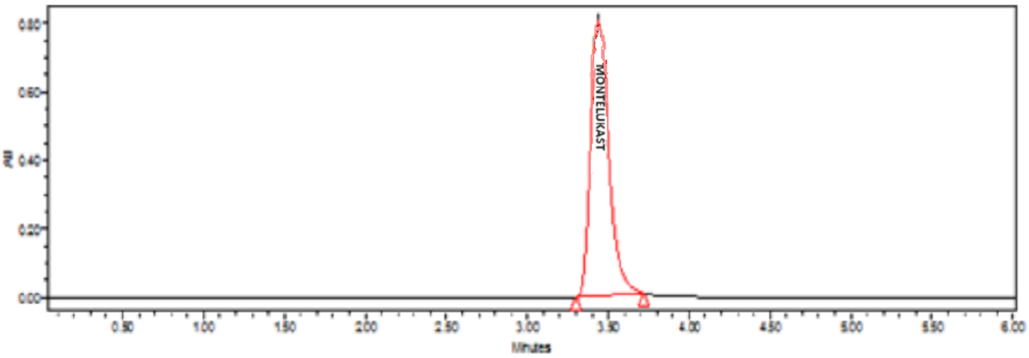


Fig. 41: linearity plot (Concentration Vs Response).

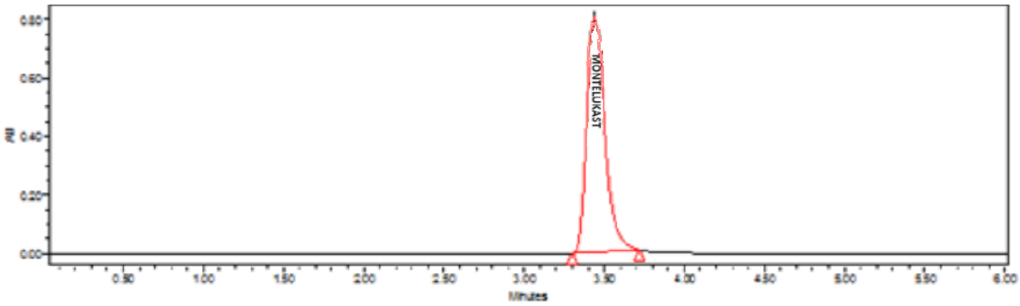


Inference: The standard chromatogram of 20 ppm.

Fig. 35: The chromatograms at 20 ppm are as follows.

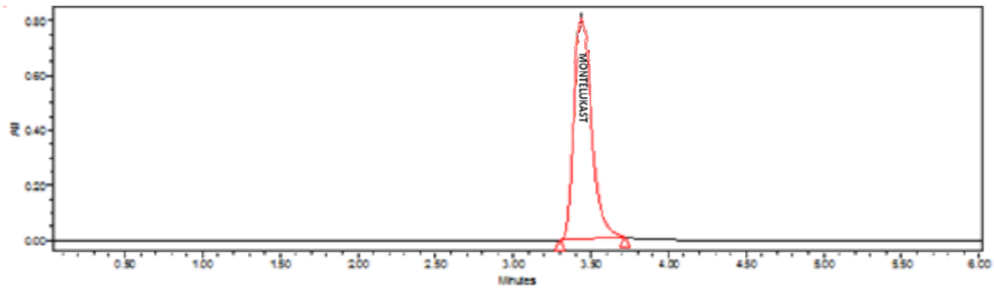


Inference: The standard chromatogram of 30 ppm.

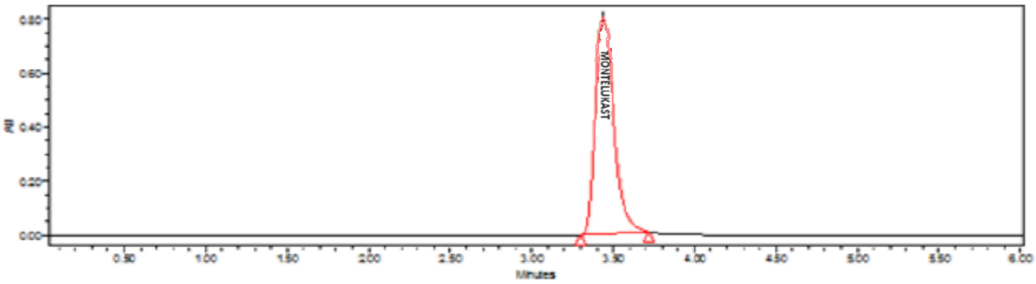


Inference: The standard chromatogram of 40 ppm.

Fig. 36-37: Forchromatograms at 30ppm and 40ppm.

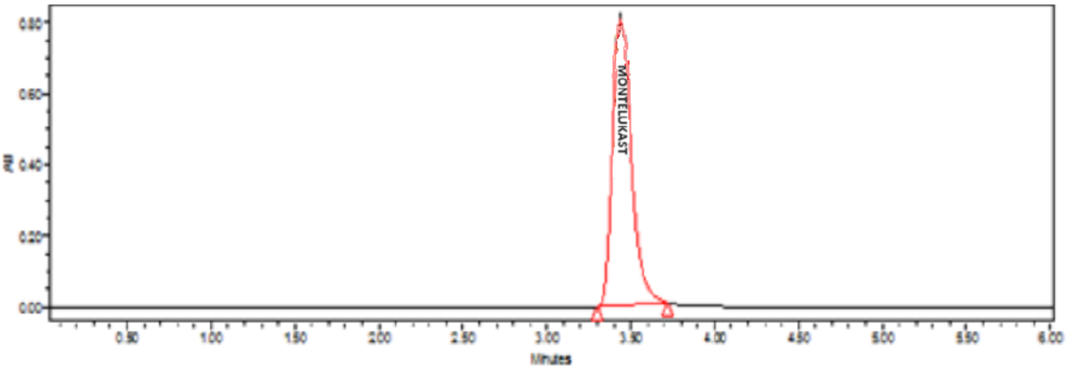


Inference: The standard chromatogram of 50 ppm.



Inference: The standard chromatogram of 60 ppm.

Fig. 38:39: 50 ppm chromatograms, 60 ppm chromatograms.



Inference: The standard chromatogram of 70 ppm.

Fig. 40: There are chromatograms available. 70 parts per million.

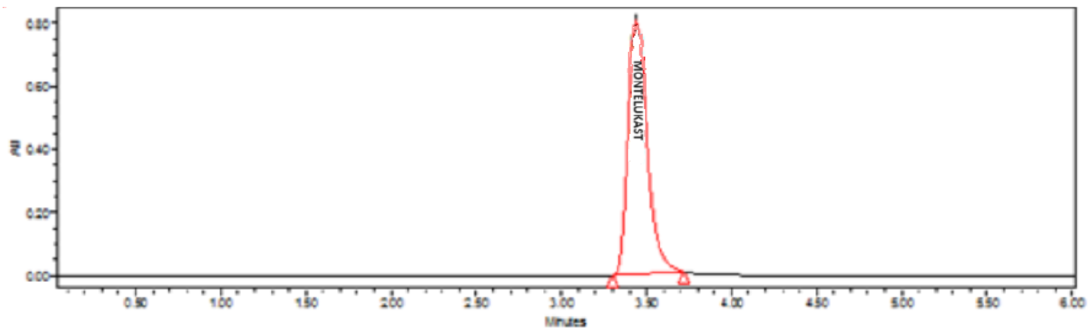
6.3.6 Ruggedness

System to system variability

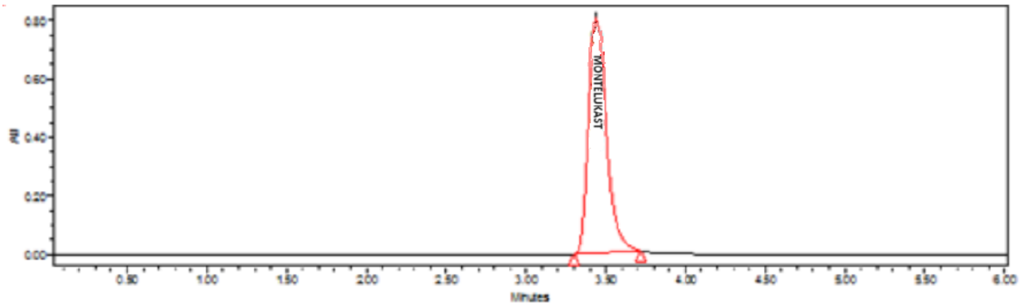
Refer to Table3 for System 1

Table 9: Data on System Variability System-2.

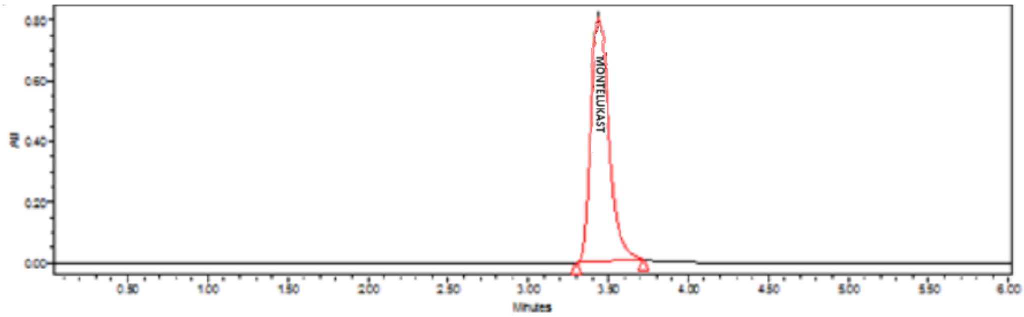
S. No.	Peak area	Assay % of Montelukast
1	2102837.37	100.22
2	2102956.99	100.22
3	2102890.92	100.23
4	2103072.69	100.23
5	2103127.56	100.23
6	2102954.32	100.22
Mean	2102973.30	100.22
%RSD	0.00519	0.00520



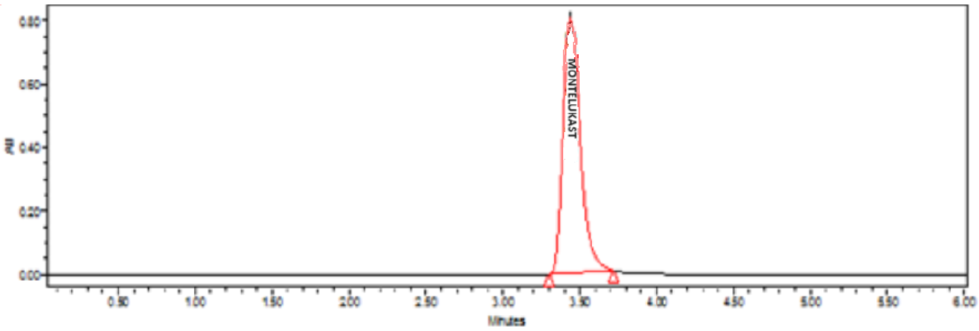
Inference: std- 1: Chromatogram showing system-to-system variability.



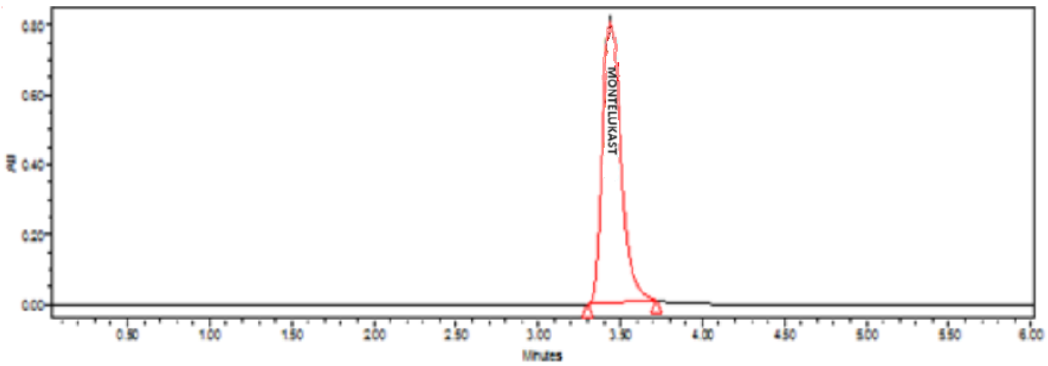
Inference: std- 2: Chromatogram showing system-to-system variability.



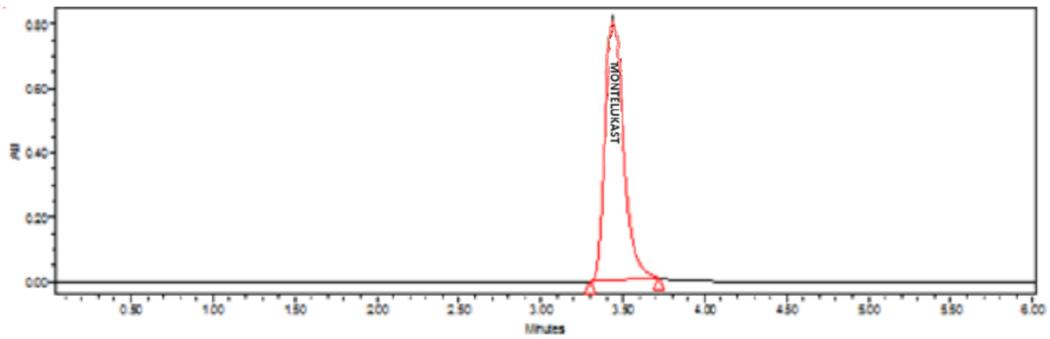
Inference: std- 3: Chromatogram showing system-to-system variability.



Inference: std- 4: Chromatogram showing system-to-system variability.



Inference: std- 5: Chromatogram showing system-to-system variability.



Inference: std- 6: Chromatogram showing system-to-system variability.

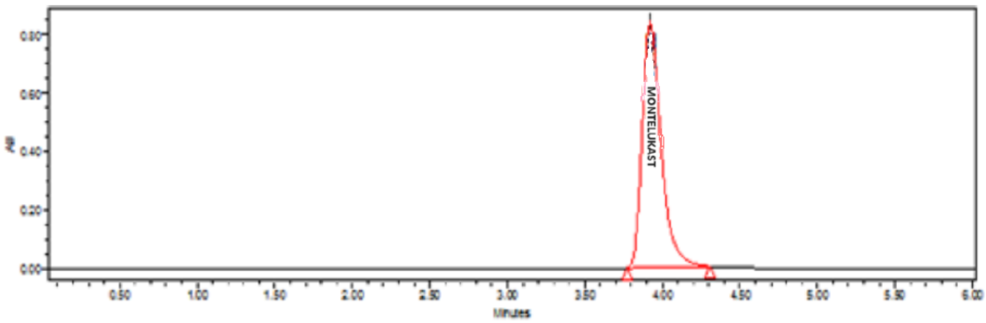
Fig. 41-46: System to system variability chromatograms.

6.3.7 Robustness

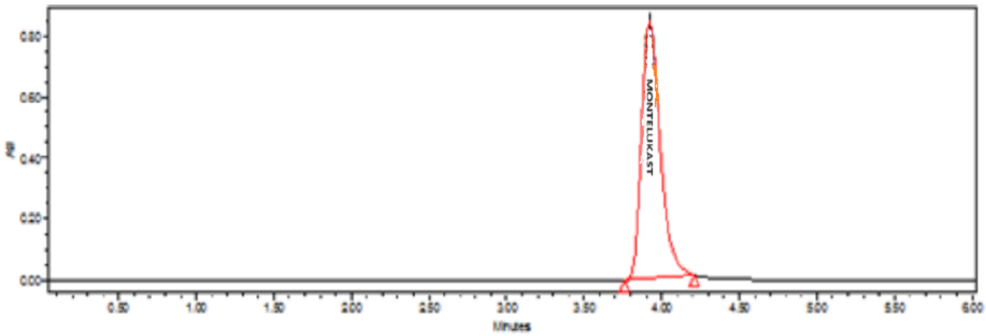
Table 10: There's proof that flux rate variability has an impact.

	Std Area	**Tailin g factor**		**Std Area**	**Tailin g factor**		**Std Area**	**Tailing factor**
Flow 0.8 ml	1432628.65	1.108	**Flow 1.0 ml**	2102946.64	1.112	**Flow 1.2 ml**	2843631.12	1.125
	1432568.45	1.112		2102928.28	1.114		2843589.98	1.123
	1432670.79	1.114		2103048.82	1.112		2843652.32	1.124
	1432521.56	1.120		2103010.01	1.113		2843628.62	1.124
	1432689.98	1.119		2102989.56	1.114		2843672.56	1.123
Avg	1432615.88	1.114	Avg	2102984.66	1.113	Avg	2843634.92	1.123
SD	70.3826	0.0049	SD	48.4957	0.001	SD	30.7947	0.0008
%RS D	0.00491	0.4467	%RS D	0.00230	0.0898	%RS D	0.0010	0.0744

a) Variation in flow rate (for 0.8 ml/min flow) has an effect

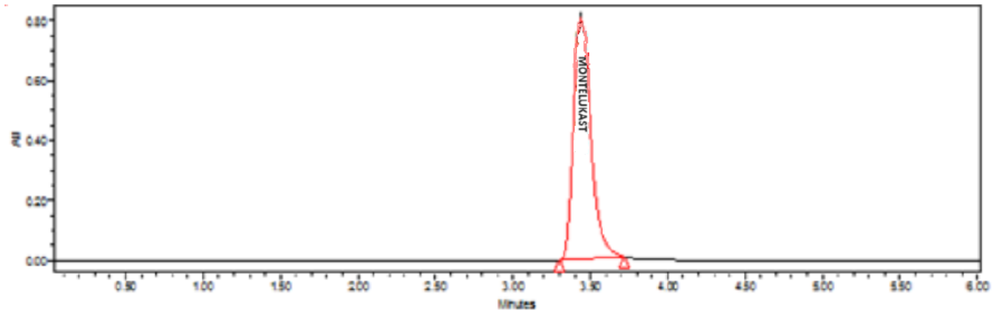


Inference: Standard for robustness chromatogram – 1.

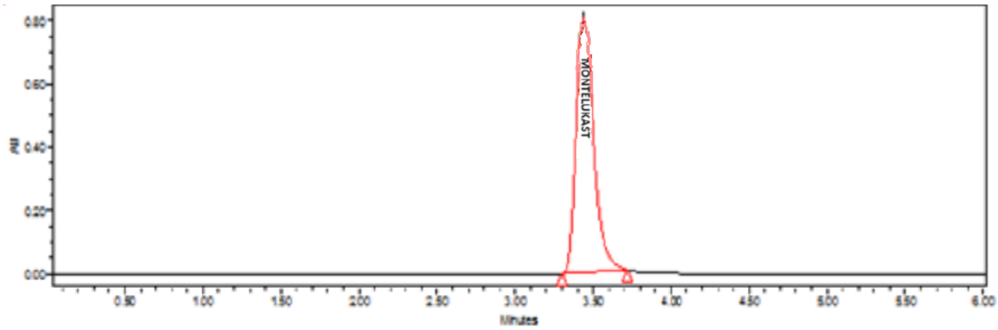


Inference: Standard for robustness chromatogram – 2.

Fig. 47-48: Robustness chromatograms.

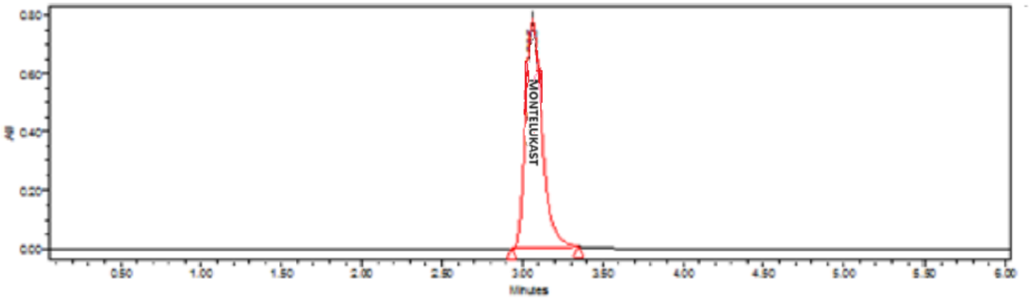


Inference: Standard for robustness chromatogram – 1.

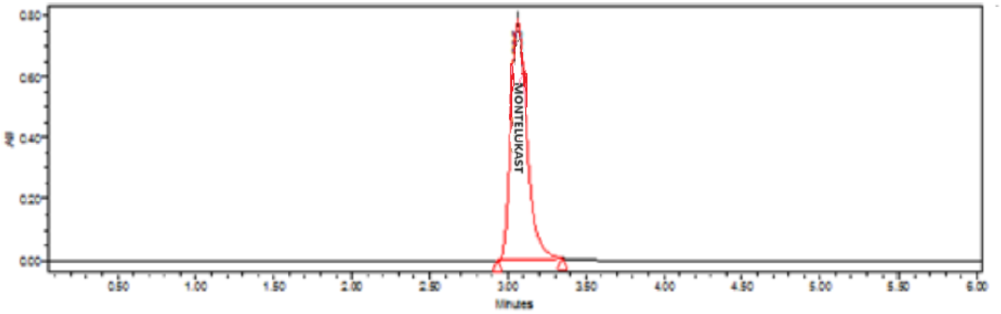


Inference: Standard for robustness chromatogram – 2.

Fig. 49-50: Chromatograms for a 1ml/min flow rate.



Inference: Standard for robustness chromatogram – 1.



Inference: Standard for robustness chromatogram – 2.

Fig. 51-52: 1.2ml/min chromatogram.

6.3.8 LOD AND LOQ (Limit of Detection and Limit of quantitation)

From the linearity plot the LOD and LOQ are calculated:

$$\text{LOD}=\frac{3.3\,\sigma}{S}=\frac{3.3\times 74.7604}{52296}=0.0047$$

$$\text{LOQ}=\frac{10\,\sigma}{S}=\frac{10\times 74.7604}{52296}=0.01429$$

VII. CONCLUSION

SUMMARY AND CONCLUSION

Different parameters were studied to create the analytical approach. For starters, the maximum absorbance of MONTELUKAST was discovered to be 225nm. The injection volume was set at 20 l, which resulted in a nice peak area. The Inertsil C18 column was employed in this work, and ODS picked a nice peak shape. The temperature of the ambient environment was determined to be adequate for the type of the medication solution. Because of the good peak area, adequate retention duration, and good resolution, the flow rate was set at 1.0ml/min. Different mobile phase ratios were investigated, however the mobile phase with a Methanol: Water (75:25) ratio was chosen because to its symmetrical peaks and high resolution. As a result, the planned research made use of this mobile phase.

The accuracy of both the system and the procedure was determined to be precise and well within range. The correlation coefficient and curve fitting were discovered during the linearity investigation. For both medicines, the analytical approach was shown to be linear throughout a range of 20-70ppm of the target concentration. Both robustness and ruggedness tests were passed by the analytical. The relative standard deviation in both circumstances was excellent.

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