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A Review on Bioanalytical Chromatographic Method Development for Quantification & Validation of Cysteinyl Leukotriene Receptor-Antagonists in Plasma Matrices

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ABSTRACT

Both Qualitative and Quantitative analysis plays a significant role in promising the safety and therapeutic efficacy of drugs in variety dosage forms. A bioanalytical method is a set of procedures involved in the collection, processing, storage, and analysis of a biological matrix for a chemical compound. Bioanalytical studies are employed to obtain a quantitative measure of the drug or its metabolites for the study of pharmacokinetics, toxicokinetic, bioequivalence and exposure-response like pharmacokinetic/ pharmacodynamic studies. Leukotriene receptor antagonists are widely used for the treatment and management of bronchial asthma and allergic rhinitis in different dosage forms. Drugs of this class are Zafirlukast, Montelukast and Pranlukast which are being potent drugs and are more than 99% bound to plasma proteins presenting special challenges in the development and validation of analytical methods from a variety of matrices. The main objective of this review is discussion on various analytical methods used, different solvents used as mobile phase and their retention times to understand final optimized chromatographic method which could be useful for the assessment of Pharmacokinetic parameters. Among different analytical methods, HPLC, LC-MS, UV-Visible spectroscopy and spectroflourimetric techniques are the most widely preferred techniques applied by the researchers worldwide. This review article gives information about various types of extraction procedures of the drug in plasma matrices to create an optimized method for method development and to offer practical approaches for determining validation parameters like specificity, selectivity, recovery, lower limit of quantitation (LOQ), limit of detection (LOD), linearity, range, accuracy, precision, stability, ruggedness and robustness of High performance liquid chromatographic methods (HPLC) to support pharmacokinetic studies. Accurate and sensitive analytical methods for quantitation of drugs and their metabolites are very important for the successful conduct of preclinical and clinical pharmacology studies..

Keywords: Bioanalytical, Leukotriene receptor antagonists, Pranlukast, Montelukast, Zafirlukast, validation

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INTRODUCTION

Asthma is a respiratory illness marked by recurrent episodes of airway obstruction, an exaggerated bronchoconstriction response to environmental stimuli, and varying degrees of airway inflammation. Asthma is common, affecting at least 5% of the adult population and often arising in childhood [4]. The therapy of asthma consists of various combinations of inhaled and oral medications, with parenteral agents used during severe attacks. The major classes of agents used for asthma include beta adrenergic agonists, xanthine derivatives, corticosteroids, antileukotrienes, monoclonal antibodies, and miscellaneous agents. Hepatotoxicity is rare with most antiasthma medications but can occur, particularly with the antileukotrienes. Only oral and parenteral antiasthma agents have been linked to drug induced liver disease. [5]

OVER REVIEW OF LEUKOTRIENE RECEPTOR ANTAGONISTS

Antileukotrienes or Leukotriene receptor antagonists are the drugs which works as leukotriene-related enzyme inhibitor (arachidonate5-lipoxygenase) or leukotriene receptor antagonist (cysteinyl leukotriene receptors) and consequently opposes the function of the inflammatory mediators like leukotrienes which are produced by the immune system and serve to promote bronchoconstriction action, inflammation and mucus secretion in asthma and COPD conditions. [1]

The Cysteinyl leukotrienes (C4, D4 and E4) are products of arachidonic acid metabolism and are released from various cells, including mast cells and Eosinophils. These Eicosanoids bind to CysLT receptors. The CysLT Type-1 receptor is found in the human airway smooth muscle cells and airway macrophages and on other proinflammatory cells. In asthmatic patients, leukotriene mediated effects include airway edema, smooth muscle contraction, and altered cellular activity which associated with the inflammatory process. In allergic rhinitis, CysLTs are released from the nasal mucosa after allergen exposure and precipitate the symptoms of allergic rhinitis. [2]

MECHANISM OF ACTION

There are two main approaches to block the actions of leukotrienes-

Inhibition of the 5-lipoxygenase pathway

5-lipoxygenase is the initial enzyme of Leukotriene pathway and catalyzes the insertion of molecular oxygen in to arachidonic acid. Drugs that inhibit the 5-lipoxygenase enzyme will inhibit [3] the synthetic pathway of leukotriene metabolism

Examples of 5-LOX inhibitors include drugs, such as Meclofenamate sodium and Zileuton.

Some chemicals found in trace amounts in food, and some dietary supplements, also have been shown in inhibit 5-LOX, such as Baicalein, Caffeic acid, Curcumin, Hyperforin and St John's wort.

Antagonism of cysteinyl-leukotriene type 1 receptors

Agents such as Montelukast and Zafirlukast block the actions of cysteinyl leukotrienes at the CysLT1 receptor on target cells such as bronchial smooth muscle via receptor antagonism. [4] These modifiers have been shown to improve asthma symptoms, reduce asthma exacerbations and limit markers of inflammation such as eosinophil counts in the peripheral blood and bronchoalveolar lavage fluid. This demonstrates that they have anti-inflammatory proper

Plasma Drug Concentration

Measurement of drug concentrations in blood, plasma, or serum after drug administration is the most direct and objective way to determine systemic drug bioavailability. By appropriate blood sampling, an accurate description of the plasma drug concentration–time profile of the therapeutically active drug substance(s) can be obtained using a validated drug assay method.

Leukotriene receptor antagonists are rapidly absorbed following oral administration and mean peak plasma concentration (Cmax) is achieved in 3 to 4 hours (Tmax). They are more than 99% bound to plasma proteins. Hence a suitable Bio-analytical method is useful in the estimation of the pharmacokinetic profile of these drugs.

Pharmacokinetic Evaluation of the Data

For single-dose studies, including a fasting study or a food intervention study, the pharmacokinetic analyses include calculation for each subject of the area under the curve to the last quantifiable concentration (AUC0–t) and to infinity (AUC0–∞), T max, and C max. Additionally, the elimination rate constant, k, the elimination half-life, t 1/2, and other parameters may be estimated.

Statistical Evaluation of the Data

Bioequivalence is generally determined using a comparison of population averages of a bioequivalence metric, such as AUC and Cmax. The 90% confidence limits for the mean pharmacokinetic parameters [7] of the Test product were within 0.80–1.25 (80–125%) of the reference product means based on log transformation of the data. To establish bioequivalence, the calculated confidence interval should fall within a prescribed bioequivalence limit, usually, 80–125% for the ratio of the product averages.

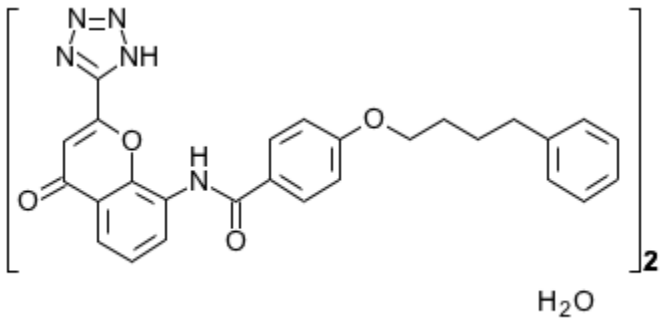
Application of a Validated Bioanalytical Method to Routine Drug Analysis:

- It should be reminded that the effort of a method validation is undertaken to guarantee during the routine analysis a quality of the measurement data as needed for the application for bioavailability, bioequivalence or pharmacokinetic studies such as

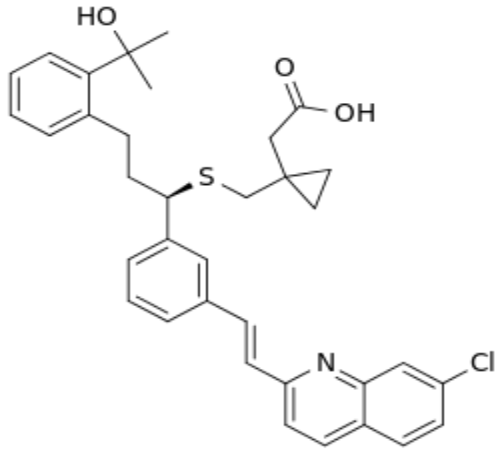
preclinical pharmacokinetic studies, preclinical toxicokinetic study, clinical toxicokinetic study regulatory toxicokinetic study etc.

- The different pharmacokinetic and bioequivalence studies require such validated bioanalytical methods, which meet the international rules and the selective and specific determination of the compound, the internal standard and metabolites.
- Pharmacokinetics describes the absorption, distribution, metabolism and elimination of drugs. Pharmacokinetic studies are important in the generic drug development, when the mentioned safety studies are not necessary to execute.
- Substitutability of the generic and original formulations is proved bioequivalence studies. The biological equivalence is investigated by studying the statistical accordance of pharmacokinetic parameters. (Refer Table no. 1 for overall Physical, chemical & biological properties of cysteinyl leukotriene receptor-antagonists).

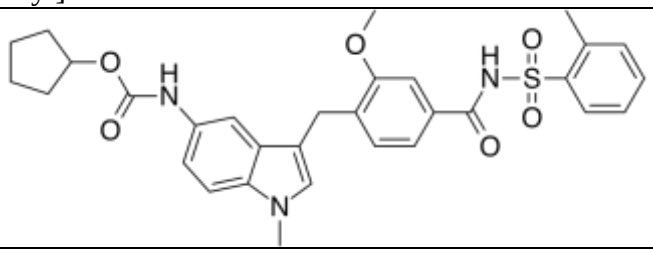
Table 1: Physical, chemical & biological properties of cysteinyl leukotriene receptor-antagonists

Drug name	**PRANLUKAST**
IUPAC name	N-(4-oxo-2-(2H-tetrazol-5-yl)-4H-chromen-8-yl)-4-(4-phenylbutoxy)benzamide hydrate
Chemical structure	 D02732
Molecular formula	$C_{27}H_{25}N_5O_5$
Molecular weight	499.51
Solubility	Insoluble in water (0.0032 mg/mL), soluble in organic solvents like DMSO (Dimethyl sulfoxide-10 mg/mL) & DMF (Dimethyl formamide-)
Mechanism of action	Pranlukast selectively antagonizes leukotriene D_4 (LTD_4) at the cysteinyl leukotriene receptor, $CysLT_1$, in the human airway. Pranlukast inhibits the actions of LTD_4 at the $CysLT_1$ receptor, preventing airway edema, smooth muscle contraction, and enhanced secretion of thick, viscous mucus.
Adverse effects	Headache, increased incidence of resp tract infection, GI disturbances, induced generalised pain, fever, myalgia, arthralgia.
Indication & Dosage	*Oral* Allergic rhinitis, Asthma *Adult:* 225 mg bid.

	Child: 3.5 mg/kg bid. Max dose: 10 mg/kg/day (not exceeding 450 mg daily).
Physical state	Crystalline solid
Long Term Storage	-20 Degrees Celsius
Melting point	236-238°C
Pharmacokinetics data	Metabolism: Hepatic (mainly CYP3A4) Bioavailability: 10 mg oral dose, the bioavailability of in healthy individuals is between 61%-62%, Protein binding – approximately 60%, Half life -1.5hrs to 2.8 hrs for single doses ,Excretion - renal and biliary
pKa (Strongest Acidic)	6.86
pKa (Strongest Basic)	-1.7
Log p	4.82

Drug name	MONTELUKAST
IUPAC name	2-[1-({[(1R)-1-{3-[(E)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl}-3-[2-(2-hydroxypropan-2-yl)phenyl]propyl]sulfanyl}methyl)cyclopropyl]acetic acid
Chemical structure	
Molecular formula	$C_{35}H_{36}ClNO_3S$
Molecular weight	586.183
Solubility	Soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (approximately 30 mg/ml). Soluble in water (10 mg/ml).
Mechanism of action	Montelukast selectively antagonizes leukotriene D_4 (LTD_4) at the cysteinyl leukotriene receptor, $CysLT_1$, in the human airway. Montelukast inhibits the actions of LTD_4 at the $CysLT_1$ receptor, preventing airway edema, smooth muscle contraction, and enhanced secretion of thick, viscous mucus.
Adverse effects	Side effects include headache, abdominal or stomach pain, cough, dental pain, dizziness, fever, heartburn, skin rash, stuffy nose, weakness or unusual tiredness.
Indication & Dosage	For the treatment of asthma (10 mg)
Physical state	Off-White to Pale Yellow Solid
Long Term Storage	-20°C Freezer, Under Inert Atmosphere
Melting point	108-110°C

Pharmacokinetics data	Metabolism: Hepatic Bioavailability: Rapidly absorbed following oral administration (bioavailability is 64%) Protein binding: 99% Half life: 2.7-5.5 hours
pKa (Strongest Acidic)	4.4
pKa (Strongest Basic)	3.12
Log p	7.9

Drug name	ZAFIRLUKAST
IUPAC name	cyclopentyl N-[3-({2-methoxy-4-[(2-methylbenzenesulfonyl)carbamoyl]phenyl}methyl)-1-methyl-1H-indol-5-yl]carbamate
Chemical structure	
Molecular formula	$C_{31}H_{33}N_3O_6S$
Molecular weight	575.68 g/mol
Solubility	practically insoluble in water
Mechanism of action	Systemic: Zafirlukast is a selective and competitive receptor antagonist of the cysteinyl leukotrienes D 4 and E 4. The cysteinyl leukotrienes, originally described as slow-reacting substances of anaphylaxis, produce airway edema, smooth muscle constriction, and altered cellular activity associated with the inflammatory process, all of which are associated with the pathophysiology of asthma
Adverse effects	Common :- neurological (Hearache) Serious :- CV-allergic granulomatousisangiitis Hepatic – hepatitis , liver failure
Indication & Dosage	ASTHMA (20mg ORALLY)
Physical state	Solid
Long Term Storage	Store at controlled room temperature between 20 and 25 degrees C (68 and 77 degrees F); protect from light and moisture
Melting point	139 °C
Pharmacokinetics data	Metabolism: in liver via CYP2C9 pathway Bioavailability: Rapidly absorbed following oral administration, reduced following a high-fat or high-protein meal. Protein binding: >99% Half life 10Hrs
pKa (Strongest Acidic)	4.29
pKa (Strongest Basic)	-1.1
Log p	5.4

BIOANALYSIS-DEFINITION [3,7]

Bioanalysis is a term generally used to describe the quantitative measurement of a compound (drug) or their metabolite in biological fluids, primarily blood, plasma, serum, urine or tissue extracts. A bioanalytical method consists of two main components.

Detection of the compound:

The common techniques that can be used in quantitative bioanalysis is high performance liquid chromatography coupled with UV detector or tandem mass spectrometry (HPLC-MS/MS) using either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) techniques. The triple quadrupole (QqQ) mass spectrometer (MS), when operated in the selected reaction monitoring (SRM) mode, offers a unique combination of sensitivity, specificity and dynamic range. Consequently, the QqQ MS has become the instrument of choice for quantitation within the pharmaceutical industry. Since ESI and APCI can be operated at flow rates as high as 1 and 2 mL/min, respectively, most of the convenience columns (e.g., C18, C8, C4, phenyl, cyanopropyl) are compatible. Recent technological advances have made 1.7 μm particle size packing material available. Coupling with high pressure pump and high-speed acquisition MS, (UPLC) offers unique high throughput and resolving power to obtain maximum chromatographic performance and superior assay sensitivity.

NEED OF BIOANALYTICAL METHOD DEVELOPMENT:

Bioanalytical Method is specifically to determine the concentration of drug or its metabolites or both in biological samples like [5] serum, plasma, urine etc.,

- Bioanalytical information can be helpful in Clinical pharmacology, Bioavailability and Bioequivalence studies as part of Pharmacokinetic studies.
- Bioanalytical methods of various drugs can be used in non-human Pharmacology or toxicology for conducting pre-clinical studies.

STEPS IN METHOD DEVELOPMENT:

1. Literature search for drugs.
2. Identification of analytical techniques which are available or to newly create methods and optimization of the method.
3. Selection of drug
4. Study physical, chemical and pharmacokinetic properties of the drugs selected.
5. Internal standard selection

6. Standard and sample preparation by using different extraction process. (Sample pre- treatment) & sample storage to perform further validation studies.
7. Set the detection wavelength. (Run a UV-spectra to determine λmax.) as part of chromatographic condition.
8. Fix chromatographic conditions.
9. Optimize the chromatographic method.
10. Check for retention times.

The parameters to be considered while selecting a drug for Bioanalytical work(Fig.1). This is very important aspect in the literature survey.

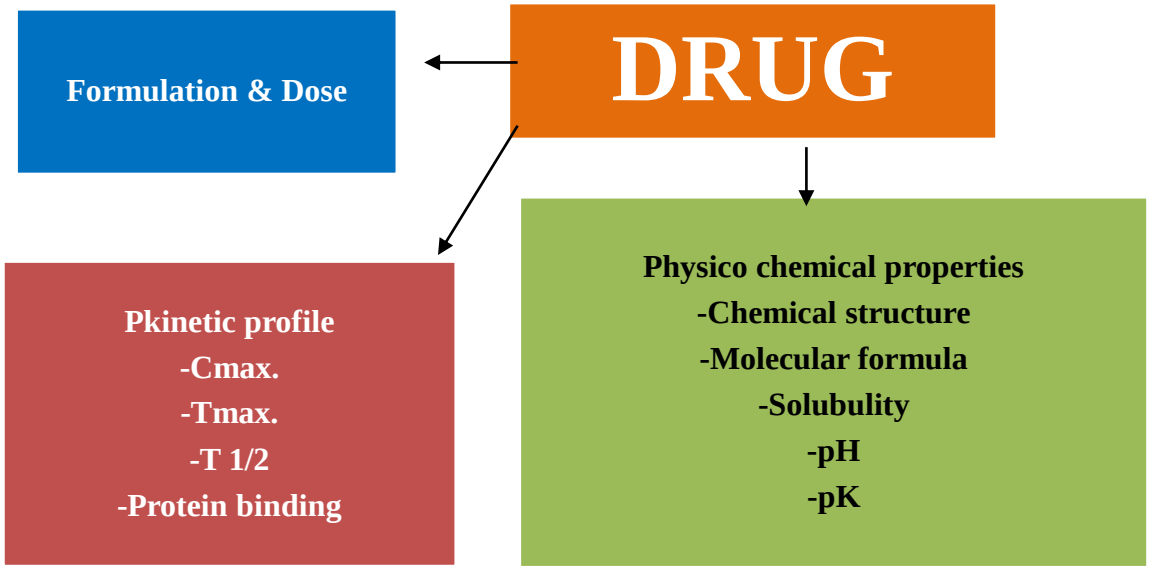


FIGURE 1 Parameters to Be Considered While Selecting A Drug For Bioanalytical Work

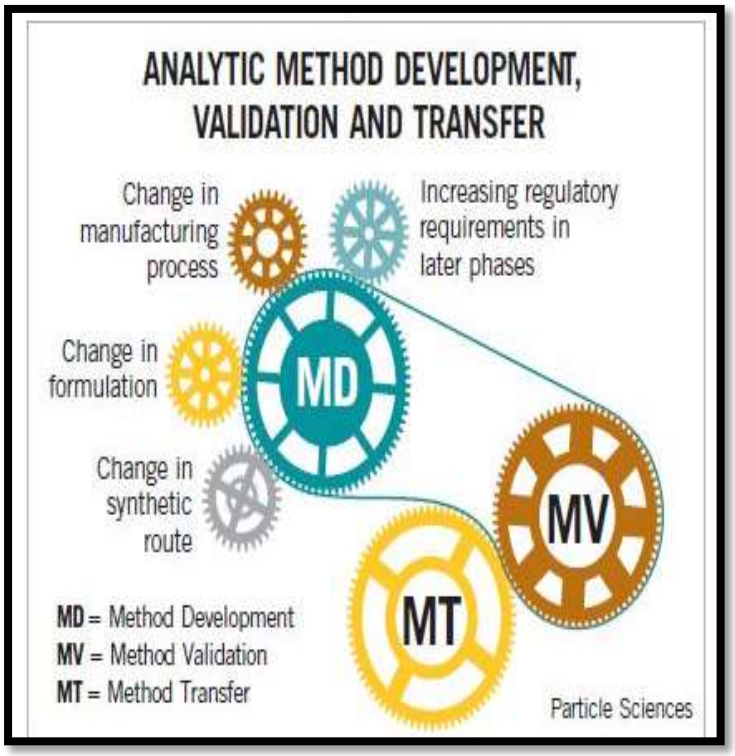


FIGURE 2: Analytical Method Development, Validation And Transfer

HOW TO CHOOSE A METHOD: [8]

Duly utilizing the information available from the literature, methodology is evolved since the methods are changed wherever required. Occasionally it is imperative to get additional instrumentation to develop, modify or reproduce and validate existing procedures for analytes and samples.

If there are no past suitable methods available to analyze the analyte to be examined.

STUDY PHYSICAL, CHEMICAL AND PHARMACOKINETIC PROPERTIES OF THE DRUGS:

Physical & Chemical Properties:

- Check for the sample polarity, whether the compound is polar or non polar. If the compound is non polar, choose a non polar mobile phase solvent system, which is said to be Normal Phase Chromatography.
- If the compound is polar in nature, select an appropriate polar solvent as mobile phase which you can analyse by using Reverse Phase Chromatography.
- Based upon the above characteristics in this way one can select the solvent to achieve greater solubility of the sample.

List of experiments to assess Pharmacokinetic characters of drugs refer Table 2.

Table 2: List of experiments to assess Pharmacokinetic characters of drug[3]

Parameter examined	**Typical experiments**
Absorption	Caco-2 cells, MDCK cells, PgP transport In vivo PK profiling
Distribution	In vitro protein binding In vivo tissue distribution studies
Metabolism	Metabolic stability Microsomes subcellular fractions, hepatocytes P450 inhibition studies Microsomes P450 induction studies Gene chips, multiple dosing
Elimination	Quantitation of drugs and metabolites in biological fluids.

SELECTION OF INTERNAL STANDARD:

An internal standard in analytical chemistry is a chemical substance that is added in a constant amount to samples, the blank and calibration standards in a chemical analysis which can then be used for calibration by plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentration of the standards. This is done to correct for the loss of analyte during sample preparation or sample inlet. IS is a compound that is very similar, but not identical to the chemical species of interest in the samples, as the effects of sample preparation should, relative to the amount of each species, be the same for the signal from the internal standard as for the signal(s) from the species of interest in the ideal case. Adding known quantities of analyte(s) of interest is a distinct technique called standard addition, which is performed to correct for matrix effects.

Sample collection and preparation

The biological samples that contain the analyte usually are blood, plasma, urine, serum, etc. Blood is usually collected from human volunteers/ subjects by vein puncture with a hypodermic syringe up to 5-7 ml. The venous blood is withdrawn into tubes with an anticoagulant, generally EDTA, heparin is used. Plasma is obtained by centrifugation at 4000 rpm for 15 minutes. Around 30-50% of the volume is collected.

Aim of sample preparation [3,9]

- Sample preparation is a technique used to clean up a sample before analysis and/or to concentrate a sample to improve its detection. . Material in biological samples that can affect with analysis, the chromatographic column or the detector includes endogenous macromolecules, proteins, salts, small molecules, and metabolic by products.

- When samples are biological fluids such as plasma, serum or urine, this technique is described as bioanalytical sample preparation. The determination of drug concentrations in biological fluids yields the data used to understand the time course of drug action, or PK, in animals and man and is an essential component of the drug discovery and development process.
- Most bioanalytical assays have a sample preparation step to remove the proteins from the sample. Protein precipitation, liquid–liquid extraction and solid phase extraction (SPE) are routinely used.

EXTRACTION PROCEDURES FOR BIOSAMPLES:

General procedures for sample preparation like liquid/liquid extraction, solid-phase extraction (SPE) and protein precipitation. [6,9]

Liquid – Liquid extraction:

It is based on the principles of differential solubility and partitioning equilibrium of analyte molecules between aqueous (the original sample) and the organic phases. Liquid – Liquid extraction generally involves the extraction of a substance from one liquid phase to another liquid phase. Improved LLE techniques like liquid phase micro extraction, single drop liquid phase micro extraction and supported membrane extraction.

Solid Phase Extraction (SPE):

Solid phase extraction is selective method for sample preparation where the analyte is bound onto a solid support, interferences are washed off and the analyte is selectively eluted. Due to many different choices of sorbents, solid phase extraction is a very powerful technique. Solid phase consists of four steps; conditioning, sample loading, washing and elution.

Conditioning: The column is activated with an organic solvent that acts as a wetting agent on the packing material and solvates the functional groups of the sorbent. Water or aqueous buffer is added to activate the column for proper adsorption mechanisms. [10]

Sample Loading: After adjustment of pH, the sample is loaded on the column by gravity feed, pumping or aspirating by vacuum.

Washing: Interferences from the matrix are removed while retaining the analyte.

Elution: Distribution of analyte – sorbent interactions by appropriate solvent, removing as little of the remaining interferences as possible. Typically, sorbents used in SPE consists of 40 µm diameter silica gel with approximately 60 A0 pore diameters.

What type of extraction to be followed for your drug?

When sensitivity of the drug is more, prefer protein precipitation and check for recovery, precision and interferences. When sensitivity of the drug is less, prefer liquid-liquid extraction and check for recovery, precision and interferences. When the recovery and reproducibility is less in liquid-liquid extraction, prefer solid phase extraction for better sensitivity, recovery, precision and low interferences.

Checking the analytical method in biological matrix: [11,12]

Checking the developed bioanalytical method with matrix samples for accuracy, precision and recovery is essential before finalizing the method for pre-validation. Minimum three aliquots each of HQC and LQC and LLOQ matrix samples are analysed with one set of extracted calibration curve standards including matrix blank and zero standard (blank with only internal standard) and the results shall be compared for recovery with aqueous quality control samples of equivalent concentration. The method is accepted if it meets the criteria of accuracy, precision and recovery. If needed, the method shall be considered for modification.

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 analyte peak.

The following are the parameters to be considered during selection and optimization of mobile phase.

- Buffer
- pH of the buffer
- Mobile phase composition

Buffer and its role: Buffer and its strength play an important role in deciding the peak symmetries and separations. The retention time depends on molar strength of buffer. Molar strength is proportional to retention time. [11]In order to achieve better separation the strength of the buffer can be increased.

Commonly used buffers are- Acetic buffers includes ammonium acetate, sodium acetate. Acetic acid buffers are prepared using acetic acid. Another important component is the influence of the pH since this can change the hydrophobicity of the analyte. For this reason most methods use a buffering agent, such as sodium phosphate, to control the pH. The buffers serve multiple purposes: they control pH, neutralize the charge on any residual exposed silica on the stationary phase and act as ion pairing agents to neutralize charge on the analyte. Ammonium formate is commonly added in mass spectrometry to improve detection of certain analytes by the formation of

ammonium adducts. A volatile organic acid such as acetic acid, or most commonly formic acid, is often added to the mobile phase if mass spectrometry is used to analyze the column eluent. Trifluoroacetic acid is used infrequently in mass spectrometry applications due to its persistence in the detector and solvent delivery system, but can be effective in improving retention of analytes such as carboxylic acids in applications utilizing other detectors, as it is one of the strongest organic acids. The effects of acids and buffers vary by application but generally improve the chromatography.

pH of buffer:

pH plays an important role in achieving the chromatographic separation as it controls the elution properties by controlling the ionization characteristics. [11] A different concentration of buffer was chosen to achieve required separations. It is important to maintain the pH of mobile phase in the range of 2.0 to 8.0 as most of the columns does not withstand out of this range 12. [11] As Siloxane linkages are cleaved below pH 2 and at above pH 8 silica dissolves.

In Table 3 overall methods suggested for Montelukast, Zafirlukast & Pranlukast has been represented.

Table No.3 : Representing Overall Methods Suggested For Montelukast, Zafirlukast & Pranlukast

Name of the drug	Type of analytical method & instrument name	Mode of chromatography	Elution technique followed	Column used	Mobile phase	Flow rate	Detection	Retention time & linearity
Montelukast	Standard Analytical & Shimadzu LC2010c HT	RP-HPLC	Isocratic-gradient	Phenomenex C8, 5 µm, 25 cm x 4.6 mm i.d.	ACN: Acetate buffer= 6.5:3.5 of pH 3	1 mL/min	UV-Visible detector (SPD-IOA)- wavelength (222 nm)	3.08 min 10-100 µg/mL
Montelukast	Bioanalytical & Agilent technologies, CA.	HPLC	Isocratic	Zorbax Eclipse-XDB C18, 150 x 4.6 mm, 5 µm column	Methanol:ACN:0.04M disodium hydrogen ortho phosphate (22:22:56v/v), PH-4.9	1 mL/min	Spectroflourimetric detector- 350nm for excitation & 450nm for emission	5-1000ng/ml
Montelukast	Bioanalytical & HPLC system (1200 series model, Agilent Technologies, Waldbronn, Germany), Mass spectrometry API 4000 triple quadrupole instrument	LC-ESI–MS/MS method	Isocratic	YMC-pack pro C18, 50 x 4.6 mm, S-3 µm column	10mM ammonium formate (pH 4.0):acetonitrile (20:80 v/v)	0.8 mL/min	m/z 586.2→568.2	1.0–800.0 ng mL−1
Montelukast Sodium in combination	Standard Analytical & Shimadzu	RP-HPLC	Isocratic	C18 column (Phenomenex C18, 5µ,	methanol: acetonitrile : 1% trichloroacetic acid in the ratio of	1.0ml/min	UV detection at 220 nm	3.17min 0.5-10 µg/ml

with Bambuterol	(Columbia, MD) RP-HPLC instrument (LC-2010CHT) equipped with PDA detector			250mm x 4.6mm)	80:10:10 v/v/v			
Zafirlukast (valdecoxib as an internal standard (IS)).	Bioanalytical & An Agilent (Agilent Technologies, Waldbronn, Germany) 1100 series LC system	LC-MS/MS method with electrospray ionization	Isocratic	Hypersil BDS C18 column	10 mM ammonium acetate (pH 6.4):acetonitrile (20:80, v/v)	0.4 mL/min	574.2 → 462.1 for ZFK and 313.3 → 118.1 for IS	ZFK and IS 1.11min and 1.58 min respectively. 0.15–600 ng/mL
Zafirlukast	Standard Analytical & Agilent 1100 series	LC method	Gradient	Zodiac 100, C18,250 mm x 4.6 mm, with a 5 µ m particle size	10 mM potassium buffer, 5 mM of 1-decane sulphonic sodium salt at pH 4.0	0.8 mL/min	UV detection at 220 nm	LOQ of 1.5 g mL-1
Pranlukast	Bioanalytical	LC/MS/MS method	Gradient	30 mm 2 mm i.d., 3 µ m particle size, Hypersil BDS C-18 HPLC column	20 mM ammonium acetate-methanol system	300 µ L/ min	MS	10.0 to 2000 ng ml—1

Table 4: US FDA Guidelines for Bioanalytical Method Validation

SELECTIVITY (SPECIFICITY)	Analyses of blank samples of the appropriate biological matrix (plasma, urine or other matrix) should be obtained from at least six sources. Each blank should be tested for interference and selectivity should be ensured at LLOQ
ACCURACY	Should be measured using a minimum of six determinations per concentration. A minimum of three concentrations in range of expected concentrations is recommended for determination of accuracy. The mean should be ±15% of the actual value except at LLOQ, where it should not deviate by ±20%. This deviation of mean from the true values serves as the measure of accuracy
PRECISION	Should be measured using a minimum of fi ve determinations per concentrations. A minimum of three concentrations in the range of expected concentrations is recommended. The precision determined at each concentration level should not exceed 15% of the coeffi cient of variation (CV) except for the LLOQ, where it should not exceed 20% of the CV
RECOVERY	Recovery experiments should be performed at three concentrations (low, medium and high) with unextracted standards that represent 100% recovery
CALIBRATION CURVE	Should consist of a blank sample (matrix sample processed without internal standard), a zero sample (matrix sample processed with internal standard) and six to eight non-zero samples covering the expected range, including LLOQ
LLOQ	Analyte response should be five times the response compared to blank response. Analyte peak should be identifiable, discrete and reproducible with a precision of 20% and an accuracy of 80–120%
FREEZE–THAW STABILITY	Analyte stability should be determined after three freeze–thaw cycles. At least three aliquots at each of the low and high concentrations should be stored at the intended storage temperature for 24 hours and thawed at room temperature. When completely thawed, refreeze again for 12–24 hours under same conditions. This cycle should be repeated two more times, then analyze on third cycle. Standard deviation of error should be <15%. If the analyte is unstable, freeze at –70°C for three freeze–thaw cycles

SHORT-TERM STABILITY	Three aliquots of each of the low and high concentrations should be thawed at room temperature and kept at this temperature for 4–24 hours and analyzed. % Deviation should be <15%
LONG-TERM STABILITY	At least three aliquots of each of low and high concentrations at same conditions as study samples. Analyze on three separate occasions. Storage time should exceed the time between the date of fi rst sample collection and the date of last sample analysis
STOCK-SOLUTION STABILITY	Stability of stock solutions of drug and the internal standard should be evaluated at room temperature for at least 6 hours. % Deviation should be <15%
QUALITY CONTROL (QC) SAMPLES	QC samples in duplicates at three concentration levels (one near the 3× LLOQ, one in mid-range, one close to high end) should be incorporated at each assay run. At least four out of every six should be within 15% of respective nominal value. Two of six may be outside 15% but not both at the same concentration. Minimum number QCs should be at least 5% of total number of unknown samples or six total QCs, whichever is greater

CONCLUSION:

Herein, an effort was made to review all the suggested works which are carried out for this class of drugs (Leukotriene receptor anatagonists). Only few analytical studies are reported related to Montelukast & Zafirlukast. A new Bioanalytical method development and validation by HPLC for the drug Pranlukast are yet to be conducted and are assumed to contribute in the significant fields for advanced research is lacking. There is significant active contribution is ongoing in the field of Bioanalytical method development and validation. Thus, it is hoped that all these lines of ongoing research, combined, should lead to a deeper understanding. Thus, we conclude that these categories of drugs discussed in this review can be potentially utilized for research and development for the treatment of Bronchial asthma.

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Abbrevations

1.

DMSO	Dimethyl sulfoxide
DMF	Dimethyl formamide
HPLC)	High performance liquid chromatography
LOQ	Limit of quantitation
LOD	Limit of detection
CysLT	Cysteinyl leukotriene
COPD	*Chronic obstructive pulmonary disease*
FLAP	Lipoxygenase activating protein
LOX inhibitors	Lipoxygenase inhibitors
FDA	Food and Drug Administration
NDA	New drug application
HQC	Higher Quality Control
LQC	Lower Quality Control
LLOQ	Lower Limit of Quantification
SPE	Solid-phase extraction
LLE	Liquid/liquid extraction
UPLC	Ultra-high pressure liquid chromatography
IS	Internal standard