Emerging Materials for Technological Applications

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Edited by

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CHAPTER ONE

CHARACTERISTICS, PROPERTIES, ANALYTICAL AND BIOANALYTICAL PROFILE OF FIROCOXIB

K VARAPRASADA RAO[1*], K ANUSHA[1], K SAI SWETHA, CHALLA. GANGU NAIDU[2], AND Y. SRINIVASA RAO[1]

Abstract

Firocoxib is a non-steroidal anti-inflammatory drug (NSAID) that specifically targets the COX-2 enzyme. Similar to other medications within its class, Firocoxib functions by inhibiting the production of prostaglandins, thereby reducing inflammation and alleviating discomfort. Recently, a novel drug named Firocoxib has received approval for use in horses and dogs, particularly for the treatment of pain and swelling associated with osteoarthritis. This approval pertains to its oral formulation, indicating its efficacy in managing pain and inflammation caused by osteoarthritis in dogs. Notably, clinical evidence supports the improvement of limb function in dogs suffering from osteoarthritis when treated with Firocoxib. Furthermore, studies suggest potential advantages of Firocoxib over carprofen and etodolac, as observed in subjective assessments by both owners and veterinarians regarding the resolution of lameness in dogs with osteoarthritis.

Keywords: Firocoxib; Ultra Violet detection; osteoarthritis; swelling.

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1.0 Introduction

Firocoxib, identified by its chemical name "3-(cyclopropylmethoxy)-4-(4-(methyl-sulfonyl) phenyl)-5, 5-dimethylfuranone," is a member of the coxib class of anti-inflammatory drugs. It has obtained official approval for use in both dogs and horses [1]. Firocoxib functions as a Cyclo-Oxygenase (COX) inhibitor, with a primary focus on inhibiting COX-2 enzymes and minimal impact on COX-1 enzymes. COX plays a crucial role in the synthesis of prostaglandins and thromboxanes. [1]. Inhibiting COX prevents the synthesis of prostaglandins and the associated inflammatory effects, as illustrated in Figure 1. The drug exhibited minimal solubility in deionized water after two hours (4.77 ± 0.55 mg/L), and its dissolution rate was notably sluggish (with an equilibrium solubility of 19.58 ± 0.35 mg/L at 24 hours).

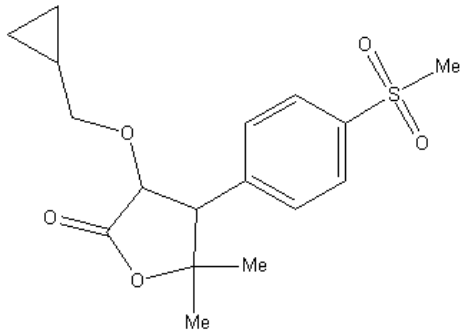

Fig. 1: Chemical structure of Firocoxib

Commonly used nonsteroidal anti-inflammatory drugs (NSAIDs) include Ibuprofen, Aspirin, Naproxen, Phenylbutazone, Carprofen, and Meloxicam. These medications act by blocking both COX-1 and COX-2 enzymes. While their associated toxicity is believed to stem from inhibiting the constitutive COX-1 isozyme, their therapeutic effectiveness is primarily attributed to the inhibition of the inducible COX-2 isozyme. In contrast to traditional nonselective therapies, selective COX-2 inhibitors, often referred to as "Coxibs," represent an advanced class of NSAIDs. They effectively reduce edema, discomfort, and fever while minimizing the risk of toxicities. Kvaternick, Malinski, Wortmann, and Fischer have developed a method for quantifying the concentration of Firocoxib in the plasma of horses and dogs. [1-8].

2.0 Characteristics of Firocoxib

NSAIDs, a category of therapeutic medications, work to alleviate pain, reduce inflammation, lower fever, and inhibit blood clot formation. The use of these medications often raises the likelihood of complications such as heart attacks, kidney issues, gastrointestinal ulcers, and bleeding. However, the specific adverse effects can vary based on the type of medication, dosage, and duration of use. In dogs, Ferocoxib has a half-life of 7.8 hours, in cats it ranges from 9 to 12 hours, and in calves, it is approximately 6.7 hours.

3.0 Properties of Firocoxib

3.1 Pharmacodynamic Properties

Firocoxib belongs to the Coxib family and specifically inhibits the synthesis of prostaglandins, a process carried out by the enzyme COX-2. Cyclooxygenase is responsible for generating prostaglandins, with the isoform COX-2 playing a significant role in producing prostanoid mediators associated with pain, inflammation, and fever. This enzyme has been shown to be induced by pro-inflammatory stimuli. As a result, Coxibs, including Firocoxib, possess analgesic, anti-inflammatory, and antipyretic properties. Additionally, COX-2 is believed to be involved in various physiological processes, including ovulation, fever induction, pain perception, cognitive function, ductus arteriosus implantation and closure, and central nervous system functions. In "in vitro" testing using horse whole blood, Ferocoxib demonstrates selectivity for COX-2 up to 643 times more than COX-1.

3.2 Pharmacokinetic Properties

Upon intravenous administration of firocoxib, peak plasma concentrations were detected within one minute. These concentrations were approximately 3.7 times higher than those observed two hours after oral administration (oral Tmax-2.02 hours). Firocoxib binds to plasma proteins to a degree exceeding 97%. In horses, steady-state concentrations are achieved after 6 to 8 days of therapy due to drug accumulation resulting from repeated dose administration. The primary metabolic pathways for firocoxib involve dealkylation and glucuronidation in the liver. Elimination predominantly occurs through excretion, primarily in the feces, with some biliary excretion.

3.3 Biological Properties

Firocoxib functions as a non-narcotic analgesic, a COX-2 inhibitor, NSAID, and an anti-neoplastic agent. Classified within the cyclopropanes family, it is also identified as a butenolide, a sulfone, and an enol ether. As a selective inhibitor of cyclooxygenase-2, Firocoxib belongs to the class of non-steroidal anti-inflammatory drugs.

4.0 Mechanism of Action

Much like other NSAIDs, Firocoxib elicits analgesic and anti-inflammatory effects by impeding the formation of prostaglandins. This inhibition targets the COX enzyme, which exists in two forms: COX-1 and COX-2. COX-1 is primarily responsible for generating prostaglandins essential for maintaining the health of the gastrointestinal tract, renal function, platelet function, and other normal bodily functions. On the other hand, activated COX-2 produces prostaglandins, serving as the main mediators of pain, inflammation, and fever. Although COX-1 and COX-2 actions can sometimes overlap, it is recognized that COX-2 activity is crucial for various biotic activities. In in-vitro studies, the selective COX-2 inhibitor Firocoxib is found to be more sparing of COX-1 compared to previous NSAIDs, emphasizing its specificity towards COX-2.

Table 1: Analytical and bio analytical methods for determination of Firocoxib

S.NO	Column	Mobile phase	Range, LOQ, LOD	Method	Detection	Flow rate	References
1	Sunfire C_{18}	Water with 0.025% trifluoroacetic acid& Acetonitrile(50:50)	Range: 5-1500µg/gm LOQ: 5µg/gm	HPLC	290nm UV	1.1mL/min	[1]
2	HALO biphenyl column(30x4.6mm)	The Mobile Phase-A & B are 0.1% H_3PO_4 in H_2O& Acetonitrile	LOD: 0.12µg/ml LOQ: 0.4µg/ml	RP-HPLC	240nm UV	2.5ml/min	[3]
3	Phenyl-hexyl column C_{18}	45%Acetonitrile and 55% ammonium formate buffer in the mobile phase	Plasma LOQ: 1µg/ml Urine LOQ:5µg/ml	Automated LC-MS/MS	220nm PDA	—	[4]
4	Hydrophobic-lipophilic copolymer SPE column	—	LOQ: 25µg/ml LOD: 10µg/ml	—	—	—	[5]

5	HALO C_{18} (100x2.1mm, 2.0µm)	0.1% formic acid in water for mobile phase- A & Acetonitrile, mobile phase -B	—	HPLC	290nm UV	—	[6]

5.0 Conclusion

A precise and accurate analytical approach was formulated to assess Firocoxib in equine tissues and pharmaceutical bulk dosage forms. Various analytical techniques, such as HPLC, RP-HPLC, LC-MS, were employed in the development of this stability-indicating strategy. The method's recovery, linearity, precision, and accuracy were thoroughly validated. The results indicate that the HPLC method consistently provides reliable quantification of Firocoxib in tissues and is reproducible.

6.0 Acknowledgements

We are thankful to the management of Vignan Institute of Pharmaceutical Technology for giving support to complete this work.

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CHAPTER TWO

A RECENT LC AND LC-MS METHODS FOR IONIC LIQUID BASED DLLME EXTRACTIONS FOR THE ENRICHMENT OF FLUOROQUINOLINE ANTIBIOTICS: A REVIEW

K VARAPRASADA RAO[1], B LAVANYA[1], G. SAI PRASANNA[1], CHALLA. GANGU NAIDU[2]*, AND Y. SRINIVASA RAO[1]

Abstract

A non-toxic liquid-liquid micro-extraction method utilizing an efficient vortex was employed for the identification of fluoroquinolone-containing antibiotics in biological matrices or tissues. To make the extraction process environmentally friendly, an ionic liquid was utilized as one of the solvents. The study also investigated the impact of salt content using ionic solutions. Utilizing the ionic liquid "1-butyl-3-methylimidazoline hexa phosphate", the research established a dispersive liquid-liquid micro-extraction technique for extracting four fluoroquinolone pharmaceuticals from biological matrices or tissues. In the optimal conditions, the four fluoroquinolines (norfloxacin, ciprofloxacin, lomefloxacin, enrofloxacin) exhibited varying enrichment factors. Residues of norfloxacin, ciprofloxacin, and enrofloxacin were detected in seven out of 60 authentic

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meat samples, although their concentrations were below the permissible upper limit for residual levels.

Keywords: Keywords: Fluoroquinolones, Dispersive liquid-liquid microextraction-DLLME, Ionic liquids, HPLC.

1.0 Introduction:

Discovered in the 1970s and 1980s, Fluoroquinolines (FQ) antibiotics exhibit activity against a wide spectrum of bacteria, encompassing both Gram-positive and Gram-negative strains, in both human and veterinary medicine. The mechanism of action of fluoroquinolones involves inhibiting two DNA topoisomerases, which are absent in eukaryotic cells but essential for bacterial DNA replication. This class of antibiotics operates through a selective and bactericidal mode of action.

Ionic liquids (ILs) have the potential to serve as more environmentally friendly solvents compared to traditional organic solvents, owing to their low volatility, flammability, and toxicity. They are frequently employed as alternatives to organic solvents in various analytical applications. The liquid-like behavior of ionic compounds is attributed to their long molecular structure, close intermolecular connections, and ion packing. Ionic liquids are increasingly used in separation processes, particularly due to their capability to solvate molecules with different polarities. Analytical separations involving ILs encompass techniques such as liquid-liquid extraction, DLLME (dispersive liquid-liquid microextraction), solid-phase extraction, and gas chromatography employing IL-based stationary phases.

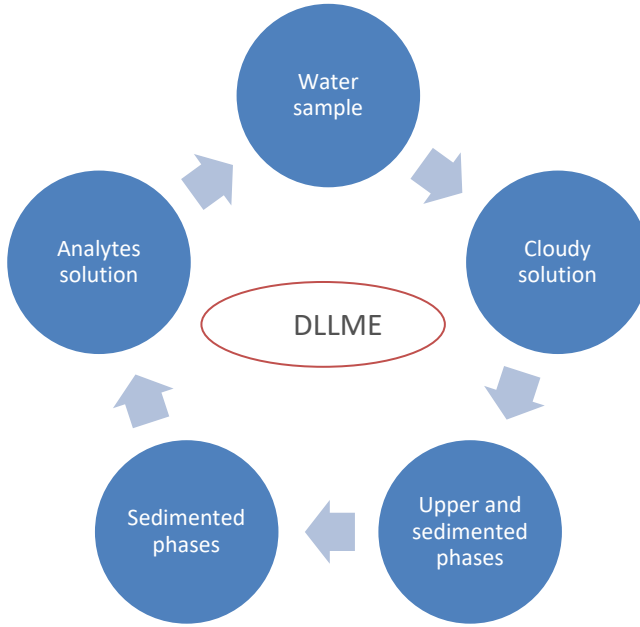
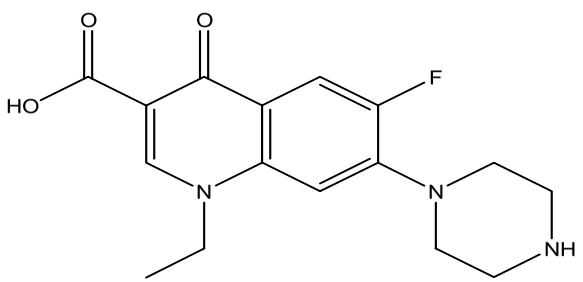
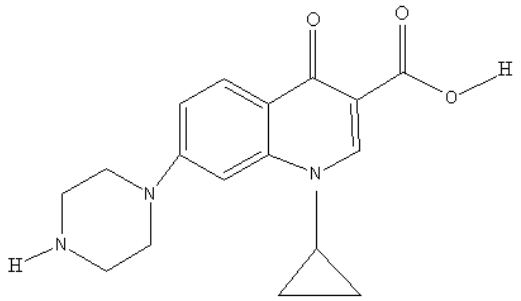


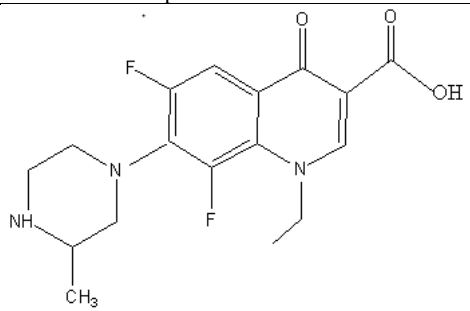
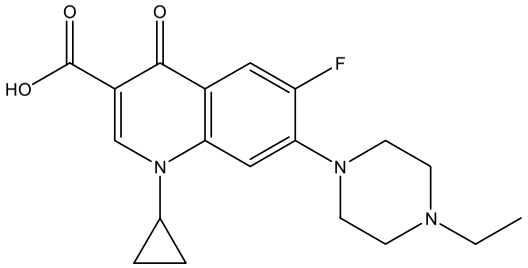
Fig.1: Procedure for DLLME

2.0 Role of Ionic Liquid Based DLLME Extraction of Flouroquinoline Antibiotics:

The compact liquid-liquid extraction (LLE) approach known as DLLME has gained attention among analysts for its effectiveness in extracting various analytes from diverse matrices like water, tissues, biological fluids, and food matrices. In DLLME, the ternary component solvent system consists of the aqueous phase, the disperser solvent, and the extraction solvent. Conventional DLLME typically involves using an extraction solvent that has a higher density than water. When the extraction solvent and disperser solvent are combined and promptly injected into the aqueous phase, it results in a turbid or hazy solution. The most often employed extraction solvents (CF) in conventional DLLME are hazardous chlorinated solvents like carbon tetrachloride (CCl4), trichloroethylene (TCE), and chloroform. A modified version of DLLME called DLLME-SFO, which is conditional on compaction of floated organic droplets, was created by Xu et al. to get around this restriction. Low detection solvents with room-temperature melting temperatures were used in this iteration of DLLME.

Table

Name	Iupac name	Structure	Uses
Norfloxacin	"1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline- 3-carboxylic acid"		Infections caused by bacteria are treated with it.
Ciprofloxacin	"1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid"		Several bacterial diseases, including pneumonia and gonorrhea, are treated with it.

Lomefloxacin	"(RS)-1-Ethyl-6,8-difluoro-7-(3-methylpiperazin-1-yl)-4-oxo-quinoline-3-carboxylic acid"		It is used to treat bacterial illnesses like bronchitis and urinary tract infections.
Enrofloxacin	"1-cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid"		Infections caused by bacteria such as urinary tract, tonsils, sinus, nose, throat, are treated with it.

3.0 Methodology

Table: Analytical and bio analytical profile of fluoroquinolones

S.no	DRUG NAME	Column	Mobile phase	Range, LOQ,LOD	Method	Detection(nm)	Flow rate (ml/min)	Reference
1.	NORFLOXACIN	Eclipse plus zorbax C18	Acetic acid: methanol (80:20)	LOQ: 0.10µg/ml LOD: 0.32µg/ml	HPLC	277nm	1.0ml/min	[9]
2.	CIPROFLOXACIN	Hypersil C18(250X4.6mm	Acetonitrile:0.25M H3PO4 (60:40v/v)	LOQ: 0.5 ng/ml LOD: 1 ng/ml	HPLC	275nm Uv detector	1ml/min	[10]
3.	LOMEFLOXACIN	C8,C18	Trichloro acetic acid: acetonitrile	LOQ: 0.2 µg/ml	Bioanalytical	—	1.2ml/min	[11]
4.	ENROFLOXACIN	(RP)C18e (250x4.0 mm)	Acetonitrile: methanol	LOQ: 0.10µg/ml LOD: 0.03µg/ml	Hplc	267 Uv detection	0.8ml/min	[12]

4.0 Isolation of FQs from Biological Fluids

In recent literature reviews, there has been a frequent application of the IL-DLLME (ionic liquid-dispersive liquid-liquid microextraction) method for the direct extraction of target analytes from water samples. Numerous studies utilizing the IL-DLLME technique, also referred to as homogeneous liquid-liquid microextraction, have been reported. These studies assert that the development of IL-DLLME technology necessitates the incorporation of an aqueous phase. In the current experiment, the initial stage involved transferring the residual fluoroquinolones and meat through the aqueous phase. Early investigations commonly employed organic solvents and aqueous buffers to recover fluoroquinolones residues from animal-derived products. The mentioned buffer, utilized as the aqueous phase in the extraction solvents, was intentionally not specified in the present research to avoid potential interference from salt ions in such buffers on the outcomes of the IL-DLLME technique. Acetonitrile was employed to transform the residual fluoroquinolones in the meat into a liquid phase. This liquid phase was then concentrated to a small volume and diluted to 5.0 mL with water for the subsequent IL-DLLME method.

5.0 Conclusion

The article asserts that an emerging category of solvents known as "Ionic Liquids" is increasingly being utilized for extracting various analytes from diverse materials. This study specifically devised a dispersive liquid-liquid microextraction technique incorporating High-Performance Liquid Chromatography (HPLC) for the identification of four fluoroquinolines medications in meat, employing the ionic liquid [C4MIM] [PF6]. The method showcased exceptional sensitivity, enrichment capability, and extraction efficiency. Through the examination of 40 samples comprising real and counterfeit meat, the developed approach proves to be a sensitive and accurate method for detecting the presence of residues from the four fluoroquinolines drugs in meat.

6.0 Acknowledgements

The Authors wish to thank the Principal, Vignan’s Institute of Pharmaceutical Technology for constant encouragement for Publication**.**

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CHAPTER THREE

ANALYTICAL METHODS USED FOR THE SEPARATION OF ENANTIOMERS OF LINAGLIPTIN: A MINI REVIEW

PALLA AMARENDRA[1], MUSINANA RESHMA[1], CHALLA. GANGU NAIDU[2], D. VASUDHA[1*], G. ANUSHA[1], K VARAPRASADA RAO[1], AND Y. SRINIVASA RAO[1]

Abstract

Analytical and clinical uses, as well as the assessment of pharmacological or hazardous effects on human health, depend critically on the isolation and identification of specific enantiomeric drug molecules. There is a growing market for racemic variants of pharmaceutical drugs that are given. One family of oral hypoglycemic medications used to treat type II diabetes mellitus includes DPP-4 inhibitors. Through the proper regulation of blood glucose levels, these drugs function by preventing the action of the dipeptidyl peptidase-4 enzyme. Enantiomeric separation of linagliptin (R-LINA and S-LINA) is reported in the literature as an entity. The present review also explores various methods for liquid chromatography and capillary electrophoresis. Crownpak CR (+), CR-I (+), AD-H, Lux cellulose-4, IA-3, and other chiral column types are examples of direct approach techniques for enantiomer separation. Chromatomethyl-β-

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cyclodextrin (CM-β-CD) is employed in capillary electrophoresis as a chiral selector.

Keywords: Linagliptin, Capillary electrophoresis, Methodology, Liquid chromatographic separation.

1.0 Introduction

Linagliptin's chemical name is "8-[(3R)-3-amino-1-piperidinyl]." [(4-methylquinazolin-2-yl) methyl] -7-(but-2-yn-1-yl) "3,7 di hydro 3 methyl 1H-purine-2,6-dione" is one of them. Blood sugar levels are controlled and type 2 diabetes is treated with lina [1]. Linagliptin is a member of the class of medications known as dipeptidyl peptidase-4 (DPP-4) inhibitors [2,3]. Incretin hormones, such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are degraded into through the enzyme dipeptidyl peptidase-4, which controls blood glucose levels in humans [4]. Linagliptin is distinct from other DPP-4 inhibitors in two key pharmacological properties. Because of its inconsistent pharmacokinetic behavior, people with poor liver or kidney function can use it without changing the dosage. [5.6, 7].

The two enantiomers of linagliptin are R and S, respectively. The pharmaceutically active part of lina is its R-enantiomer; on the other hand, its S-enantiomer is an unwanted isomeric impurity that is not active. Regulatory agencies such as the USFDA, EMEA, and International Conference on Harmonization (ICH) have developed guidelines for the manufacturing of chiral pharmaceuticals with the goal of improving quality, safety, and efficacy [11]. It is recommended by the ICH, Q6A [12], to control the intended enantiomer. Less than 0.15% of the active ingredient in linagliptin should have the undesired S-enantiomer. Thus, developing an analytical technique to determine the enantiomeric integrity of linagliptin is essential [13, 14].

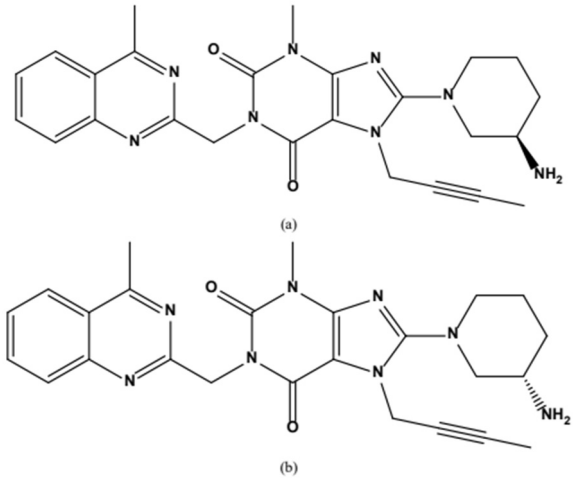

Figure (a) represents the chemical composition of the R-enantiomers of Lina gliptin, Figure (b) depicts the molecular structure of the S-enantiomers of Lina gliptin, denoted as S-LINA.

2.0 Liquid Chromatographic Separation

When it comes to separating enantiomers, chiral direct separation using CSP is preferred since it removes the requirement for intermediary processes such isomer derivatization or adding mobile phase to the chiral additives are required. Precise selection of the appropriate chiral selector and organic modifier, column temperature, pH, and mobile phase composition are necessary for successful enantiomer separation. To separate the two compounds by enantiomer, high-performance liquid chromatography (HPLC) is frequently employed with columns that have distinct CSPs. Capillary electrophoresis can also be used for this in some circumstances.

2.1 Separation of Enantiomers by Polysaccharide Based Stationary Phase

2.1.1 Using Amylose Coated Stationary Phase:

A) Polar mode:

- LINA samples (1 mg/mL) are prepared in methanol. In order to make the system suitable, R-LINA and S-LINA were mixed in an equal ratio with a concentration of 0.2 mg/mL in methanol.

- The primary goal of technique development was to differentiate between S-LINA and R-LINA. Chiralcel OJ-H, Chiralpak-IA, Chiralcel OD-H, Chiralpak-IC, Chiralpak-IB, and Chiralpak-IE chiral columns from Diacel Japan were used in the development of the method. Several experiments were carried out to determine the stationary and mobile phases that would give S-LINA from R-LINA the maximum resolution. A mobile phase containing different ratios of n-hexane, 2-propanol, and ethanol was used in this experiment. Diethylamine is used as an M.P modifier to improve the peak shape of linagliptin because it has a basic functional group.

B) Reverse phase mode:

The linagliptin S-isomer (LGP) and metformin hydrochloride (MET HCl) tablets were intended to be identified using a new high-performance liquid chromatography (RP-HPLC) technique called LGP-MET HCL.

2.1.2 Standard Solution:

An electronic balance was used to weigh 4.50 mg of the S-isomer in 170.0 ml of MP to create the stock standard solution. After filling a 200 ml VF with the solution, the volume was adjusted with MP until the target volume was reached. It was made sure to mix everything well when diluting. Next, 5.0 ml of the solution was taken and added to a 250 ml VF. To get the desired volume, this was followed by dilution with mobile phase. A final concentration of 0.45 µg/ml for the S-isomer in the standard stock solution was achieved by properly mixing during the final dilution phase.

2.1.3 Test Solution:

The average weight of the twenty LGP-MET HCL tablets was calculated after they were weighed. Using the mill and crusher to coarsely powder the 7.5 mg equal to LGP, the material was then transferred to a 25 ml volumetric flask. Once the flask was fully filled with mobile phase, 15 ml of the mixture was vibrated for roughly 20 minutes while being shaken periodically. With the sonicator bath kept at a temperature between 20°C and 25°C, the mobile phase was properly diluted and well mixed. For about ten minutes, a portion of the solution was centrifuged at 4000 rpm, or 2683 g. To get a clear solution, the resultant supernatant solution was collected and centrifuged again for five minutes at four thousand rpm (around 2683.0 g).

2.2 Method Development

- A mixture of ethanol, methanol, and monoethanolamine (MEA) with a volumetric ratio of (50.0:50.0:0.2, v/v/v) made up the first mobile phase used for the study. MeOH was used because of its solubility with the chemical, which allowed it to dissolve LGP effectively. The MP was kept in place as a diluent in order to avoid negative peaks. Since alcohols were considered required for this purpose, ethanol was added to the mobile phase to improve selectivity and provide a homogenous mixture of solvents.

- Using this method, the analysis time was reduced from 110 minutes to 60 minutes while maintaining the same chromatographic conditions. To enhance the separation between LGP and S-isomer, a single column was selected rather than two Chiralpak IA columns with smaller particle sizes. The column that was chosen was the Chiralpak IA-3, which was 250 x 4.6 mm and included particles of a size of 5µm. As to the current investigation, the column consisted of immobilized amylose tris (3,5-dimethylphenylcarbamate) with silica gel. The MP used for the separation of linagliptin was a combination of EtOH: MeOH: MEA (60.0:40.0:0.20) v/v/v.

- The temperature of the column oven was maintained at 25°C, and the PDA detector wavelength was adjusted to 296 nm. The linagliptin elution flow rate was 0.5 mL/min. The entire runtime required was about 110 minutes, even though the Met HCl, S-isomer, and LGP were precisely separated. One column with the same HPLC settings as those listed in [19] was utilized in order to limit the mobile

phase consumption, lower column expenses, and shorten the total time to sixty minutes.

2.3 Using Cellulose Based Stationary Phase

- The sample was prepared by dissolving at a rate of 750 µg/mL in the solvent. The S-enantiomer of linagliptin, 300 µg/mL, was utilized to development. The pH was adjusted to 6.5 by adding orthophosphoric acid (OPA) to water that had a suitable salt dissolved in it at a concentration of 6.6 µg/L. As a result, 50 mM ammonium hydrogen phosphate (DAHP) buffer solution was made. The buffer solution was then filtered using a 0.45 µm porosity membrane filter.

- To separate enantiomers using RP HPLC, careful consideration was given to selecting the most suitable chiral reversed-phase columns. A number of columns, including Crown pack CR (+), Chira Dex, Lux Cellulose-2, Chiralpak-IA, and Lux Cellulose-4, were examined using mobile phases consisting of 20.0 mM "phosphate (pH 3.0)", "0.20% per chloric acid," 50 mM ammonium acetate (pH 6.60), and acetonitrile. Using a Lux Cellulose-4 column and an ammonium acetate and acetonitrile 40:60 v/v ratio, enantiomers were satisfactorily separated. The tailing of the resultant peaks, however, suggests that there is still a need for improved resolution between the enantiomers. To address this concern, the pH of the mobile phase was raised. It's important to keep in mind that the pH of the mobile phase greatly affects the separation of the R and S enantiomers. Diethylamine (DEA) added to the mobile phase had no effect on the peak shape or resolution, as reported in [20]. In HPLC systems operating in normal phase and polar organic modes, alkylamines, a type of basic additives, are commonly added to the mobile phase to improve peak shape for basic compounds [21, 24].

2.4 Capillary Electrophoresis Method for Determination of Enantiomers

- Methanol and water were mixed in a 10 mL container at a 30% (v/v) ratio to create a stock solution of lina with a concentration of 2000 g/mL (20 mg). Similarly, to this, 5 mg of S-Lina was dissolved in 5 mL of 30% (v/v) methanol to create a stock solution with a concentration of 1000 µg/mL. After that, the concentrations of this stock solution were diluted to 100 µg/mL for the standard