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Chiral Chromatography In Analytical Chemistry: Advancements And Applications In Enantiomeric Analysis

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Abstract:

Classifications of chiral chromatography, focusing on normal phase and reversed phase methodologies. The criteria for selecting chiral stationary phases (CSPs), mobile phase compositions, and different chromatographic techniques such as HPLC, GC, SFC, CE, and SMB are thoroughly examined. Recent progress in CSP development, multidimensional chromatography, and environmentally friendly chromatography practices are highlighted. Moreover, the review addresses the challenges and future prospects in chiral chromatography, emphasizing the need for enhanced chiral selectivity, sustainability, automation, and integration with cutting-edge detection techniques. Practical applications across pharmaceuticals, environmental monitoring, food industry, forensic science, and biomedical research are discussed to underscore the versatility and significance of chiral chromatography in scientific and industrial settings. Overall, this review provides a comprehensive analysis of chiral chromatography's pivotal role in advancing enantiomeric analysis, ensuring product quality, safety, regulatory compliance, and fostering scientific innovations across diverse sectors.

Keywords: chiral chromatography, CE, HPLC, Enantiomer, SFC, GC, CSPs.

Introduction:

Chiral Chromatography

Chiral chromatography constitutes a sophisticated analytical technique devised for the resolution and examination of chiral compounds, specifically those existing as enantiomers—molecules possessing mirror-image symmetry but lacking superimposable. [1] The intricacies of chirality hold paramount importance in biological and pharmaceutical realms, where distinct biological activities are associated with individual enantiomers.

There exist two principal classifications of chiral chromatography methodologies: normal phase chiral chromatography and reversed phase chiral chromatography.[2]

Chiral chromatography emerges as a highly efficient and indispensable analytical tool for the meticulous examination of enantiomers, particularly within the realms of pharmaceuticals and related scientific domains. [3] This methodology capitalizes on the principles of stereoselective interactions between chiral entities, culminating in the distinct separation of enantiomers—stereo chemically unique isomers with divergent biological, pharmacological, and chemical attributes. The historical development of chiral chromatography stems from the initial understanding of stereochemistry by Louis Pasteur in the mid-19th century, highlighting the optical activity and enantiomeric nature of molecules. Early resolution methods such as manual picking and fractional crystallization paved the way for the emergence of modern chromatographic techniques. [4] Mikhail Tsvet's work in chromatography in the early 20th century laid foundational principles, leading to the invention of liquid chromatography (LC) and gas chromatography (GC) in subsequent decades. However, it was not until the 1970s that chiral stationary phases (CSPs) were introduced by Calvin Giddings, revolutionizing chiral separations and enabling the commercialization and widespread application of chiral chromatography in various scientific fields. Ongoing advancements in instrumentation, automation, and coupling with mass spectrometry continue to shape the future of chiral chromatography, driving towards improved resolution, sensitivity, and sustainability in enantiomeric analysis.[5] Enantiomeric analysis is crucial in various scientific and industrial sectors due to its role in ensuring drug safety, environmental impact assessment, and quality control in manufacturing processes. It enables the identification of active enantiomers, ensuring drug efficacy and regulatory compliance. Additionally, enantiomeric analysis aids in evaluating chiral compound behaviour in the environment, controlling chemical synthesis for desired stereochemical purity, assessing agricultural chemical effectiveness, and verifying food product quality.[6] In forensic and analytical chemistry, it helps identify substances and elucidate complex mixtures. Overall, enantiomeric analysis is indispensable for maintaining standards and safety across diverse applications. Enantiomers represent a pair of molecules possessing identical chemical formulas and bonding patterns, yet they exhibit non-superimposable mirror image configurations. This phenomenon is central to stereochemistry, elucidating molecular interactions and their diverse biological effects. For instance, in pharmacology, enantiomers of a drug can display disparate therapeutic outcomes, with one enantiomer eliciting beneficial effects while the other may remain inert or exert adverse reactions.[7]

Normal Phase Chiral Chromatography:

In the normal phase chiral chromatography paradigm, the stationary phase manifests polarity, whereas the mobile phase assumes a nonpolar character. The chiral stationary phase typically comprises a polar solid substrate, such as silica, which is adorned with a chiral selector. This selector, a molecular entity with chirality, engages in selective interactions with specific enantiomers. The differential strengths of interaction result in disparate migration rates through the chromatographic column, leading to effective enantiomeric separation.[8]

Reversed Phase Chiral Chromatography:

Contrarily, reversed phase chiral chromatography entails a nonpolar stationary phase, while the mobile phase adopts a polar nature. The chiral selector in this configuration is covalently bonded to the nonpolar support, imparting a reversed polarity to the chiral stationary phase. Separation is attained through variances in the interactions between the enantiomers and the chiral selector embedded within the stationary phase. This method offers an alternative approach to chiral resolution, allowing for distinct analytical capabilities in chiral compound separations.[9]

Principle:

principle involves utilizing a chiral stationary phase in the chromatographic column, which selectively interacts with one enantiomer over the other. The stationary phase, known as a Chiral Stationary Phase (CSP), can be a chiral molecule or a chiral derivative of a non-chiral molecule. This interaction between the analyte enantiomers and the CSP results in differential retention, allowing for their separation. Enantioselectivity is a critical aspect of chiral chromatography, stemming from specific molecular interactions like hydrogen bonding, vander Waals forces, or dipole-dipole interactions between the chiral stationary phase and the enantiomers. The mobile phase, typically a solvent or a solvent mixture, carries the analyte through the chromatographic column. The choice of the mobile phase needs to be compatible with the chiral stationary phase.[10] Chiral chromatography can be implemented using various techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), or supercritical fluid chromatography (SFC). The detection of enantiomers often involves specialized chiral detectors like circular dichroism (CD) detectors, polarimetric detectors, or chiral mass spectrometers. Achieving optimal separation may require experimenting with different chiral stationary phases, a process known as column screening. This involves testing multiple columns with different chiral selectors to identify the most suitable one for a particular separation.[11] Method development in chiral chromatography includes optimizing parameters like column temperature, mobile phase composition, flow rate, and detection conditions to achieve the best separation of enantiomers .Widely employed in the pharmaceutical, food, and chemical industries, chiral chromatography ensures the analysis and purification of chiral compounds, playing a crucial role in producing enantiomerically pure substances, particularly important in pharmaceuticals where the stereochemistry of components can significantly influence drug activity and safety.[12]

Mechanism:

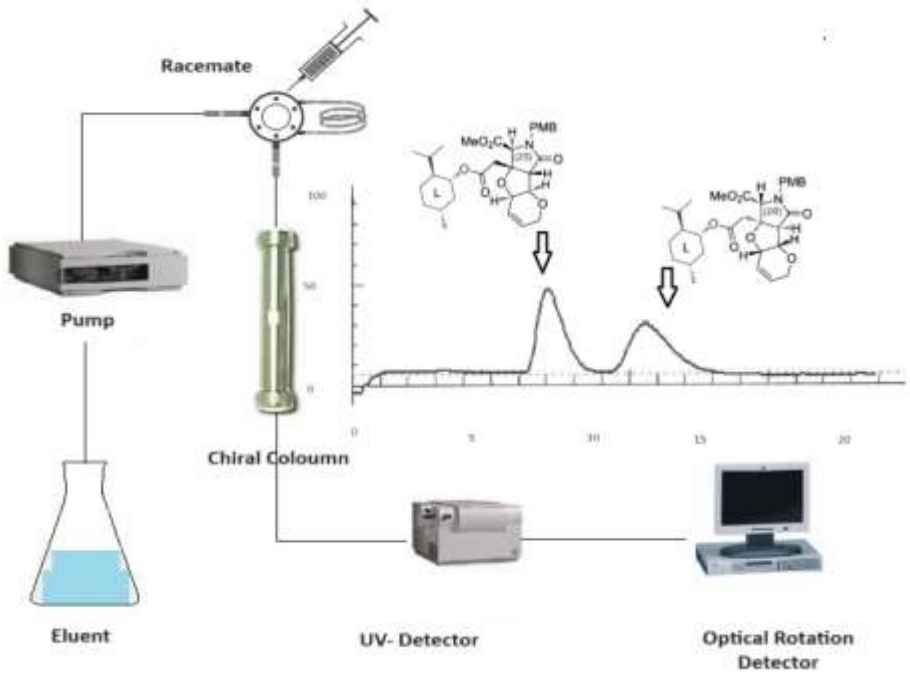

Fig 1. Mechanism of chiral chromatography

Types of Chiral stationary phases:

Chiral chromatography employs diverse chiral stationary phases (CSPs) to facilitate the separation of enantiomers, addressing their distinct stereochemical characteristics. These CSPs encompass various categories, each distinguished by unique compositional attributes:[13]

Polysaccharide-Based CSPs:

Originating from natural polysaccharides like cellulose or amylose, these CSPs manifest robust enantioselectivity, particularly in normal-phase HPLC. Application for the separation of diverse enantiomers, notably pharmaceutical compounds.[14]

Cyclodextrin-Based CSPs:

Derived from modified cyclic oligosaccharides known as cyclodextrins, these CSPs offer favourable enantioselectivity and solubility in organic solvents, rendering versatility across various chromatographic techniques. Widespread utilization in both normal-phase and reversed-phase chromatography for segregating pharmaceuticals, agrochemicals, and natural products.[15]

Protein-Based CSPs:

CSPs grounded in proteins, such as bovine serum albumin (BSA) or human serum albumin (HSA), demonstrate heightened enantioselectivity across a spectrum of chiral compounds. Prevalent in the separation of pharmaceuticals, amino acids, and biologically active compounds.[16]

Macrocyclic Glycopeptide CSPs:

Comprising macrocyclic glycopeptides like vancomycin, these CSPs furnish potent enantioselectivity for a diverse array of chiral analytes. Valuable for the resolution of antibiotics and other chiral compounds.[17]

Immobilized CSPs:

Immobilized CSPs entail the fixation of chiral selectors, such as polysaccharides or cyclodextrins, onto solid supports like silica. This immobilization enhances stability and allows for stationary phase reusability. Conventional in HPLC for a myriad of chiral separations.[18]

Pirkle-Type CSPs:

Pioneered by William H. Pirkle, these CSPs hinge on a tris-(3,5-dimethylphenyl) carbamate derivative, delivering elevated enantioselectivity and extensive applicability in chiral chromatography. Suited for diverse chiral compounds in both normal-phase and reversed-phase chromatography.[19]

Ionic Liquid-Based CSPs:

CSPs integrating ionic liquids as chiral selectors. Ionic liquids confer distinctive properties, including heightened thermal stability and modifiable selectivity. Beneficial for challenging separations in both HPLC and gas chromatography (GC).[20]

Selection criteria for chiral stationary phase

Selecting a suitable chiral stationary phase (CSP) for chiral chromatography involves considering several critical criteria. Chiral selectivity is fundamental, requiring the CSP to effectively differentiate between enantiomers and provide high resolution. Understanding the retention mechanism is crucial as well, ensuring that the CSP interacts specifically with analytes through mechanisms like hydrogen bonding or steric effects.[21] Compatibility with the chosen mobile phase, whether liquid or gas, is essential, encompassing factors such as solvent strength and carrier gas composition. The CSP must also demonstrate chemical stability under chromatographic conditions to maintain consistent performance.[22] Commercial availability from reputable suppliers is important for reliable results, along with considering the physicochemical properties of the analytes and the application-specific requirements. Practical aspects like method development considerations, ease of column conditioning, and scalability for preparative chromatography are also taken into account when selecting a CSP. These criteria collectively contribute to the successful and efficient separation of enantiomers in chiral chromatography.[23]

Advancement in CSP development

Recent advancements in chiral stationary phase (CSP) developments have significantly improved the efficiency and versatility of chiral chromatography techniques. These advancements include the introduction of novel CSPs such as polysaccharide-based, protein-based (e.g., chiral antibodies and enzymes), and synthetic CSPs (e.g., crown ethers, cyclodextrins), which offer enhanced chiral selectivity and broader applicability to diverse chiral compounds. Multimodal CSPs have also emerged, combining different chiral

recognition mechanisms within a single stationary phase to achieve synergistic effects and better separation of complex mixtures.[24] Additionally, there has been progress in immobilized CSPs, where chiral selectors are immobilized onto solid supports, providing improved stability, reproducibility, and suitability for automated systems. Functionalized CSPs with additional ligands or groups have been developed to enhance chiral recognition, while monolithic CSPs with continuous porous structures offer advantages in terms of efficiency and rapid mass transfer.[25] Coupling chiral chromatography with advanced techniques like LC-MS and LC-NMR has further expanded the capabilities of CSPs for comprehensive structural characterization of chiral compounds. These collective advancements drive innovation in chiral chromatography, facilitating more efficient and selective separations of enantiomers across various scientific and industrial applications. [26]

Mobile phase composition in chiral chromatography:

Mobile phase composition is a crucial aspect of chiral chromatography, influencing the separation and resolution of enantiomers on a chiral stationary phase (CSP). The mobile phase is a solvent or solvent mixture that elutes the analytes through the chromatographic column. The mobile phase serves multiple functions in chiral chromatography. It facilitates the elution of analytes from the chiral stationary phase, influences the interactions between analytes and the CSP, and affects the overall chromatographic performance. [27] The selection of solvents in the mobile phase is critical. Common solvents include polar organic solvents such as methanol, ethanol, acetonitrile, and isopropanol. These solvents are chosen for their compatibility with the chiral stationary phase and their ability to dissolve a wide range of chiral compounds. Solvent choice is often based on the nature of the analyte, with consideration given to factors such as solubility, stability, and the specific interactions desired during the separation.[28] Modifiers or additives are often introduced into the mobile phase to enhance enantioselectivity or improve separation efficiency. These may include acids (e.g., formic acid or acetic acid) or chiral additives like cyclodextrins. Modifiers can influence the retention and separation of enantiomers by altering the mobile phase properties. [29] The choice and concentration of modifiers depend on the specific requirements of the separation and the type of chiral stationary phase used. In some instances, the mobile phase itself may be chiral, containing chiral additives or chiral solvents. This approach enhances the enantioselectivity of the separation and is particularly relevant for certain chiral compounds.[30] Chiral mobile phases can provide additional selectivity in the separation of enantiomers, but careful optimization is necessary to avoid complications and achieve optimal results. The pH of the mobile phase can impact the ionization state of chiral compounds and influence their interactions with the chiral stationary phase. pH adjustment is especially relevant when analysing chiral compounds with acidic or basic functional groups. [31] Optimal pH conditions are determined by the nature of the analyte and the specific chiral stationary phase being used. Gradient elution involves changing the composition of the mobile phase during the chromatographic run. This technique allows for enhanced separation of complex mixtures by adjusting the elution strength over time. Gradient elution is particularly useful when dealing with challenging separations or when analysing complex samples.[32]

Chromatographic Techniques for Chiral Separation

HPLC

High-Performance Liquid Chromatography (HPLC) stands as a cornerstone technique for chiral separations within scientific endeavours, celebrated for its unparalleled resolution, sensitivity, and adaptability. The method employs specialized Chiral Stationary Phases (CSPs), which may encompass polysaccharide-derived or protein-derived phases, meticulously integrated into HPLC columns.[33] These CSPs act as the crux of selective retention and separation of enantiomers, relying on nuanced interactions with the stationary phase to achieve the desired chiral separation. The versatility of HPLC in accommodating various types of CSPs underscores its significance as a go-to method for precise and efficient chiral analyses in diverse research and industrial applications.[34]

Example	Objective	Chiral Stationary Phase	Mobile Phase	Detection	Method Details
Ibuprofen Enantiomers	Separation and quantification of R- and S-Ibuprofen	Chiralpak AD column	Isocratic mixture of methanol and water (80:20 v/v) with 0.1% formic acid	UV detector at 220 nm	Isocratic elution at 1.0 mL/min, 25°C column temp.
Ketoprofen Enantiomers	Enantiomeric separation and quantification of R- and S-Ketoprofen	Chiralcel OJ-H column	Gradient elution with acetonitrile and water with 0.1% trifluoroacetic acid	UV detector at 254 nm	Gradient elution from 20% to 60% acetonitrile over 20 min at 1.2 mL/min
Flurbiprofen Enantiomers	Separation and analysis of (R)- and (S)-Flurbiprofen	Chiralpak IA column	Isocratic elution with ethanol and water (80:20 v/v) containing 0.1% phosphoric acid	UV detector at 230 nm	Isocratic elution at 0.8 mL/min, 30°C column temp.
Naproxen Enantiomers	Enantiomeric separation of (R)- and (S)-Naproxen for pharmacokinetic studies	Chiralpak AD-RH column	Isocratic elution with methanol and water (70:30 v/v) with 0.1% acetic acid	UV detector at 220 nm	Isocratic elution at 1.0 mL/min, 35°C column temp.

Table 1. Examples of enantiomeric separation by HPLC. [35-38]

GC

Gas Chromatography (GC) emerges as a crucial technique for chiral separations, especially suited for volatile and thermally stable compounds within scientific endeavours. The fundamental principle underlying GC involves the separation of enantiomers based on their distinct partitioning behaviour between a stationary phase, coated inside the chromatographic column, and a mobile gas phase. The choice of stationary phase in GC columns for chiral separations is pivotal, with options ranging from cyclodextrins and chiral derivatizing agents to immobilized chiral selectors. This strategic selection of chiral stationary phases in GC facilitates the precise and efficient separation of enantiomers, making GC an indispensable tool in the arsenal of chiral chromatographic techniques utilized in various research and industrial domains**.** [39]

Example	**Objective**	**Data (Retention Times)**	**Conclusion**
Propranolol Tablets	Determine enantiomeric purity	R-propranolol: 12.68 min	Gas chromatography successfully separates propranolol enantiomers in tablets, ensuring quality assessment.
Chiral Pesticide Residues in Soil	Separate chiral pesticide residues	S-pesticide: 8.32 min	GC aids in detecting and quantifying chiral pesticides in soil, supporting environmental monitoring.
Chiral Amino Acids in Biological Samples	Quantify chiral amino acids	R-amino acid: 9.74 min	GC enables precise analysis of chiral amino acids in biological samples, aiding in understanding physiological processes.
Enantiomeric Separation of Chiral Drugs	Evaluate drug enantiomeric purity	S-drug: 12.35 min	Gas chromatography ensures accurate separation of drug enantiomers, meeting regulatory standards and enhancing therapeutic effectiveness.
Chiral Pesticides in Water	Analyse chiral pesticide residues	R-pesticide: 7.91 min	GC plays a crucial role in detecting and quantifying chiral pesticides in water samples, supporting water quality assessment.
Chiral Volatile Compounds in Foods	Identify chiral compounds in foods	S-volatile compound: 5.42 min	Gas chromatography assists in identifying chiral volatile compounds in food products, ensuring product safety and authenticity.

Table2. This table presents a clear overview of compound categories along with their examples and corresponding clinical data, highlighting their significance in various fields such as pharmacology, environmental studies, agriculture, and sensory sciences. [40-41]

SFC

Supercritical Fluid Chromatography (SFC) represents a sophisticated chromatographic technique that amalgamates characteristics from both High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC), employing supercritical fluids like carbon dioxide as the mobile phase. SFC presents notable advantages such as expedited separations, reduced solvent usage, and compatibility across a diverse spectrum of compounds.[42] The distinctive feature of SFC lies in its utilization of specialized Chiral Stationary Phases (CSPs) tailored for interactions specific to supercritical fluids. This strategic application of CSPs in SFC facilitates precise chiral separations, contributing to its prominence as an efficacious and resource-efficient technique in chiral chromatography within scientific and industrial realms.[43]

Compound Category	**Examples**	**Clinical Data**
Chiral Pharmaceuticals	Ibuprofen, Naproxen, Ketoprofen, Fluoxetine	Enantiomeric disparities affect pharmacological activity.
Pesticides and Herbicides	Metalaxyl, Propisochlor, Fenamidone	Essential for environmental studies and regulatory compliance.
Chiral Natural Products	Terpenes, Flavonoids, Alkaloids	Demonstrates versatility in biological matrices.
Amino Acids and Peptides	Alanine, Leucine, Valine Derivatives	Key in understanding biological processes and drug actions.
Chiral Agrochemicals	2-(4-Chlorophenoxy) propionic Acid, Fenoxaprop-ethyl	Important for agricultural research and product development.
Chiral Flavor Compounds	Menthol, Limonene Derivatives	Impacts taste and olfactory properties of products.

Table3. Data of compounds by Supercritical Fluid Chromatography technique. [44-46]

CE

Capillary Electrophoresis (CE) is a highly regarded analytical technique predicated on the distinct migration patterns of charged enantiomers within a capillary under an applied electric field. This method's efficacy in chiral separations is predicated on the intricate interactions between enantiomers and chiral selectors or additives within the separation buffer. These interactions facilitate the swift and efficient separation of charged compounds, rendering CE an esteemed choice for chiral discrimination in analytical chemistry. [47] CE's

unparalleled ability to handle charged species and exploit chiral interactions underscores its pivotal role in scientific investigations, providing a robust platform for precise and high-throughput chiral analyses. Researchers utilized trimethyl-β-cyclodextrin (TM-β-CD) as a chiral selector in Capillary Electrophoresis (CE) to analyse enantiomers of certain 2-arylpropionic acid derivatives. Their accurate methods were effective for studying pharmacokinetics, bioavailability, and optical purity. High resolution (Rs: 3.77–4.32) was achieved for Ketoprofen (KTP), while Ibuprofen (IBP) showed lower resolution (1.04–2.26) in human urine and plasma. Combining heptakis-6-sulfato-β-cyclodextrin (HS-β-CD) with TM-β-CD improved resolution for nonsteroidal anti-inflammatory drugs (NSAIDs) like IBP and KTP (Rs: 2.9–6.1). Dual cyclodextrin systems, especially those with TM-β-CD, enhanced chiral separations for substances inadequately resolved with HS-β-CDs alone.

Chiral Selector	**Description**
TM-β-CD	Trimethyl-β-cyclodextrin
DM-β-CD	Dimethyl-β-cyclodextrin
HP-β-CD	2-hydroxypropyl-β-cyclodextrin
PMMA-β-CD	Permethyl-6-monoamino-6-monodeoxy-β-CD
HxDAS	Hexakis(2,3-diacetyl-6-O-sulfo)-α-cyclodextrin
HXS	Hexakis(6-O-sulfo)-α-CD
HxDMS	Hexakis(2,3-di-O-methyl-6-O-sulfo)-αcyclodextrin
ODMS	Octakis(2,3-di-O-methyl-6-O-sulfo)-γ-CD
OS	Octakis(6-O-sulfo)-γ-cyclodextrin
HMs	Hemispherodextrins QA-β-CD Quaternary ammonium-β-CD
2-AHP-β-CD	6-monodeoxy-6-mono(2-hydroxy) propyl amino-β-cyclodextrin
6-HPTMA-β-CD	6-O-(2-hydroxy-3-trimethylammoniopropyl)-β-CD

Table 4. Data of compounds by Capillary Electrophoresis technique. [48-50]

SMB

Simulated Moving Bed Chromatography (SMB) is an innovative chromatographic technique that employs multiple columns and continuous phase movement to achieve uninterrupted chiral separations. This dynamic strategy optimizes efficiency and minimizes solvent consumption compared to conventional batch chromatography methods. SMB's superior throughput and effectiveness position it as a preferred choice for intricate chiral separations, offering significant advancements in analytical precision within scientific and industrial contexts.[51]

Pharmaceutical Examples	Scenario and Application	Outcome
Insulin Purification	Separating insulin from crude extracts using SMB Chromatography for diabetes.	High-purity insulin suitable for safe and effective diabetes management.
Antibiotic Production	Isolating and purifying antibiotics (e.g., penicillin) from fermentation broth.	Obtaining pure antibiotics, ensuring effectiveness against bacterial infections.
Anti-Cancer Compound Extraction	Purifying active anti-cancer compounds from natural sources using SMB.	Highly purified compounds ready for advanced preclinical and clinical cancer studies.
Vitamin Purification	Separating and purifying vitamins to high purity for supplements/pharmaceuticals.	Production of pure vitamins meeting regulatory standards for health-related products.
Protein Therapeutics Purification	Isolating and purifying therapeutic proteins/peptides using SMB.	Obtaining highly pure therapeutic molecules for precise medical formulations.

Table6. Applications of SMB Chromatography. [52-55]

Method Development and Optimization

Method development and optimization in chiral chromatography are pivotal stages focused on tailoring chromatographic conditions for precise and selective enantiomeric separation. This intricate process encompasses several key elements, including the meticulous optimization of mobile phase conditions by selecting suitable solvents, additives, and pH levels to enhance chiral selectivity and resolution. [56-57] Moreover, the choice of chiral selectors and additives, such as polysaccharides or proteins, plays a crucial role in influencing the specificity and efficiency of enantiomeric separation. Various strategies, such as adjusting temperature, flow rate, and column dimensions, are employed to enhance resolution, often utilizing factorial design experiments or response surface methodologies for optimization.[58] Additionally, rigorous method validation and robustness testing ensure the reliability, accuracy, and reproducibility of the developed method, adhering to regulatory guidelines and considering scale-up considerations for preparative chromatography applications. This comprehensive approach aims to achieve robust and efficient separations of enantiomers, ensuring the practical applicability and reliability of chiral chromatographic methods in scientific and industrial settings.[59]

Applications of Chiral Chromatography

Chiral chromatography exhibits a wide array of applications across scientific and industrial sectors, owing to its exceptional ability to separate enantiomers with high precision and efficacy. In the pharmaceutical realm, chiral chromatography is instrumental in analysing chiral drugs, assessing enantiomeric purity, studying pharmacokinetics, and developing separation methods crucial for drug development and quality assurance.[60] Moreover, its utility extends to natural product analysis, where it aids in identifying chiral components, quantifying bioactive compounds, and ensuring the authenticity and quality of natural products like plant extracts and herbal supplements. In the food and beverage industry, chiral chromatography plays a vital role in determining enantiomeric compositions of amino acids, sugars, and flavor compounds, ensuring compliance with regulatory standards and safeguarding food safety. Environmental monitoring benefits from chiral chromatography as well, facilitating the study of chiral pollutants' fate, behaviour, and environmental impact.[61] Furthermore, in biomedical research, chiral chromatography aids in characterizing chiral biomolecules, investigating biomarkers, and studying enantioselective interactions within biological systems. These applications underscore the versatility and significance of chiral chromatography in advancing scientific understanding, industrial processes, and regulatory compliance across diverse domains.[62]

Recent Advances and Innovations

Recent advancements and innovations in chiral chromatography have led to significant improvements in the field, particularly in terms of efficiency, sensitivity, and versatility. Novel Chiral Stationary Phases (CSPs) such as zwitterionic phases, molecularly imprinted polymers (MIPs), and hybrid materials have expanded the scope of chiral selectivity, allowing for more precise separations.[63] Multidimensional chromatography techniques, including two-dimensional liquid chromatography (2D-LC) and comprehensive two-dimensional gas chromatography (GCxGC), have significantly enhanced resolution and peak capacity, especially for complex chiral mixtures. [64] Progress in chiral ion exchange chromatography has facilitated the separation of ionizable chiral compounds, offering valuable insights in pharmaceutical and environmental analyses. Automation technologies and high-throughput screening methods have expedited chiral method development, streamlining chromatographic conditions and sample analysis processes.[65] Enantioselective detectors such as circular dichroism (CD) detectors and chiral mass spectrometers (CMS) have markedly improved specificity and sensitivity in chiral analysis, especially in intricate matrices. Integration of chiral chromatography with advanced techniques like nuclear magnetic resonance (NMR), mass spectrometry (MS), and molecular modeling has enabled comprehensive structural elucidation and in-depth mechanistic studies of chiral interactions.[66] The advent of green chromatography methods, including supercritical fluid chromatography (SFC) and chiral capillary electrophoresis (CE), promotes sustainable chiral separations by minimizing solvent consumption and waste generation. Bioanalytical chiral separations have seen significant advancements with the introduction of microfluidic devices, chiral stationary phases tailored for proteins and peptides, and chiral biosensors, enhancing sensitivity and specificity in biomolecule and pharmaceutical analyses. These collective advancements underscore the evolving landscape of chiral chromatography,

providing researchers and industries with advanced tools and methodologies to address complex chiral separation challenges across various scientific and industrial sectors.[67]

Challenges and Future Perspectives

Challenges and future perspectives in chiral chromatography encompass several critical areas that researchers and industries are actively addressing to propel the field forward. Key challenges include the resolution of complex chiral mixtures containing multiple enantiomers or closely related stereoisomers, which necessitates advancements in chiral selectivity, optimization of chromatographic conditions, and utilization of multidimensional chromatography techniques for enhanced resolution. Method development for novel classes of chiral compounds remains a challenge, with a focus on exploring innovative chiral selectors, developing specific Chiral Stationary Phases (CSPs), and leveraging computational tools for method optimization.[68] Sustainability is also a pressing concern, leading to the exploration of green chromatography practices such as reduced solvent usage, minimized waste generation, and eco-friendly mobile phases. [69] Automation and high-throughput screening methods are integral to streamlining chiral method development and analysis, with potential future integration of artificial intelligence (AI), machine learning (ML), and robotics for enhanced efficiency. [70] The rise of chiral biomolecules and biopharmaceuticals necessitates specialized chiral separation techniques and bioanalytical methods, driving advancements in chiral stationary phases for biomolecules, chiral CE, and chiral biosensors. Miniaturization and portable chiral separation devices are on the horizon, including microfluidic platforms and handheld analyzers for on-site and rapid screening applications.[71] Lastly, the integration of chiral chromatography with advanced detection techniques like MS, NMR, and vibrational spectroscopy offers comprehensive structural elucidation and stereochemical analysis capabilities, indicating a promising future for chiral characterization and mechanistic studies.[72]

Case Studies and Practical Examples

Chiral chromatography finds extensive applications across diverse industries, underscoring its versatility and scientific significance. In pharmaceutical sciences, it plays a critical role in ensuring drug safety and efficacy, as exemplified by its application in the separation and analysis of enantiomers in thalidomide, where chiral chromatography identified and separated the enantiomers responsible for therapeutic effects and teratogenicity.[73] Environmental monitoring relies on chiral chromatography for the precise analysis of chiral pollutants, such as the chiral pesticide metalaxyl, pharmaceutical residues like fluoxetine in water samples, and other contaminants like polychlorinated biphenyls (PCBs) in soil samples, contributing to accurate environmental impact assessments.[74] Within the food industry, chiral chromatography serves as a pivotal tool for authentication and characterization of chiral compounds, such as amino acids in protein supplements or chiral flavors in natural products, ensuring product quality and regulatory compliance.[75] Forensic science benefits from the ability of chiral chromatography to distinguish between drug enantiomers, such as amphetamine enantiomers in drug abuse cases or chiral herbicides in forensic toxicology, aiding in legal investigations and evidence-based conclusions.[76] In biomedical research, chiral chromatography facilitates the study of chiral biomarkers, such as chiral amino acids in urine samples as potential biomarkers for metabolic disorders like phenylketonuria (PKU), offering valuable insights into disease diagnostics and

therapeutic monitoring.[77] Furthermore, chiral chromatography optimizes synthesis processes and enables the characterization of stereoisomers in the chemical and petrochemical sectors, such as chiral hydrocarbons in gasoline or chiral intermediates in pharmaceutical synthesis, contributing to advancements in materials science and industrial applications. [78]

Conclusion:

This comprehensive review underscores the pivotal role of chiral chromatography as an indispensable analytical tool for the precise analysis and separation of enantiomers. The historical development of chiral chromatography, from Louis Pasteur's foundational work to modern advancements in chromatographic techniques and stationary phases, is elucidated. Key aspects such as chiral stationary phase (CSP) selection criteria, mobile phase composition, and chromatographic techniques (HPLC, GC, SFC, CE, SMB) are discussed, alongside recent advances in CSP development, multidimensional chromatography, automation technologies, and green chromatography practices. Challenges and future perspectives in chiral chromatography, including enhanced chiral selectivity, sustainability, automation, and integration with advanced detection techniques, are also addressed. Practical examples across various sectors underscore the wide-ranging applications of chiral chromatography in ensuring product quality, safety, regulatory compliance, and scientific advancements. Overall, this review encapsulates the critical role of chiral chromatography in driving advancements in enantiomeric analysis and separation, contributing to the evolution of analytical techniques in diverse scientific and industrial domains.

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