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Stationary phases used in LC: A Detailed Review

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ABSTRACT :

High-Performance Liquid Chromatography (HPLC) is a widely employed analytical technique for separating and quantifying complex mixtures. The efficiency of HPLC depends significantly on the stationary phase used in the chromatographic column, which plays a pivotal role in the interaction with the analyte and the separation process. Stationary phases are a critical component in High-Performance Liquid Chromatography (HPLC), directly influencing the separation, resolution, and efficiency of chromatographic methods. This article provides an overview of the various stationary phases used in HPLC, including silica-based, polymeric, and hybrid materials. Each type of stationary phase offers distinct advantages depending on factors such as the chemical properties of the analytes, the desired separation, and operational conditions. Selectivity of different stationary phases highlights their impact on improving chromatographic performance. Additionally, advancements in specialized stationary phases such as chiral columns, monolithic materials, and functionalized surfaces are discussed, focusing on their applications in complex sample analyses. The article aims to provide a deeper understanding of stationary phase selection and its role in enhancing HPLC methodology for diverse analytical tasks.

KEY WORDS:HPLC,Stationary phases, zicronic, Monolithic column, reverse phase stationary phase.

INTRODUCTION

High-Performance Liquid Chromatography (HPLC) is a sophisticated analytical technique for separating, identifying, and quantifying components in a mixture. It utilizes a liquid mobile phase pumped through a column packed with a stationary phase. As the mixture passes through the column, its components interact differently with the stationary phase, causing them to separate. This separation is based on the varying affinities of the component toward the stationary phase. It is an analytical technique used to separate, identify, and quantify components in a mixture known as HPLC Developed in 1970s [1]

CHROMATOGRAPHY

Chromatography is a technique that separates components in a mixture due to the differing time taken for each element to travel through a stationary phase when carried through it by a mobile phase .Chromatography is a widely used laboratory technique for separating mixtures of substances into their components. It works by passing the mixture through a medium where the components travel at different rates, leading to their separation. The term "chromatography" comes from the Greek words chroma (color) and graph (to write), because it was originally used to separate colored compounds, though it can be applied to any kind of mixture[1].

STATIONARYPHASE

In High-Performance Liquid Chromatography (HPLC), the stationary phase is the material packed inside the column that interacts with the sample components, causing them to separate based on their different affinities for the stationary phase. There are two main types of stationary phases used in HPLC

Normal Phase Stationary Phase:This type typically consists of silica (SiO_2) or modified silica. In normal phase HPLC, the stationary phase is polar, and the mobile phase is usually non-polar or less polar.

Reverse Phase Stationary Phase: This is the most commonly used stationary phase in modern HPLC. It is typically made of silica been chemically modified with non-polar groups[2].

TYPES:

1. Hybrid organic/ inorganic packing

Hybrid organic-inorganic packing in High-Performance Liquid Chromatography (HPLC) stationary phases combines the advantages of both organic and inorganic materials to create a more versatile and efficient packing material.

Inorganic component:The inorganic component, usually silica, provides high mechanical strength and stability, as well as a high surface area, which for the separation of analytes. Silica is commonly used because of its well-established properties in chromatography.

Organic Component: The organic component, typically a bonded phase (such as alkyl chains or polar functional groups), enhances selectivity and retention characteristics for a broad range of analytes. The organic groups are typically bonded to the silica surface through silane chemistry, modifying the surface properties of the silica[3].

2.Zicronicbasedpacking

Zicronic -based packing stationary phases in HPLC typically refer to Zicronic (ZrO2), based materials , which are utilized for their unique properties such as high chemical stability, mechanical strength, and ability to interact with polar compounds These phases are engineered using zirconium dioxide particles as the core material, which are then sometimes modified with specific functional groups to enhance their performances in chromatography.

Zicronic (ZrO2): The main component of Zicronic-based packing materials is zicronic dioxide, a highly stable ceramic compound. Its properties give the stationary phase exceptional stability phases in extreme pH conditions, both acidic and basic, compared to traditional silica-based phases.

Surface modification: To tailor the materials for different separation ,Zicronic phases can be modified with various chemical groups such as hydrophilic (e.g,C18, C8),hydrophilic(e.g amino or diol) or even charged groups (e.gsulfonic acid groups), which enable specific interaction with analytes[6].

3.Siliconbasedstationaryphases

In High-Performance Liquid Chromatography (HPLC), silicon-based stationary phase is widely used for its high surface area and versatility. The most common type is the silicon-based stationary phase, which can be modified in various ways to improve selectivity and separation efficiency. Here are some key types of silicon-based stationary phases used in HPLC.

Bare Silica (Unmodified Silica)

Bare silica is often used in normal-phase chromatography, where the stationary phase is polar, and the mobile phase is nonpolar or less polar. The separation is based on differences in polarity between the analytes and the silica surface.

Modified Silica (Silica-Based Reversed-Phase Columns)

In reversed-phase HPLC (the most commonly used mode), silica particles are chemically modified by attaching hydrophobic groups like alkyl chains (C18, C8, C4)[4].

4.Size-Exclusion(GelPermeation)ChromatographyPhases:

In size-exclusion chromatography (SEC), which is commonly used in High-Performance Liquid Chromatography (HPLC), the stationary phase consists of a porous material that separates molecules based on their size. The stationary phase typically contains small, spherical particles, often made of materials.

Polystyrene-divinylbenzene (PS-DVB): This is one of the most common materials for SEC columns. It provides good separation for a wide range of molecular sizes.

Silica-based particles: These are often used in SEC, although they tend to be less stable with high-pH mobile phases compared to PS-D[3].

5.ChiralStationaryPhases:

Chiral stationary phases (CSPs) are specialized materials used in High-Performance Liquid Chromatography (HPLC) for separating enantiomers, which are non-superimposable mirror image molecules. These phases are crucial when working with chiral compounds, like drugs, that may have different biological activities based on their enantiomeric form.

There are several types of chiral stationary phases, and they can be classified based on the material or the chiral selectors they contain. Some common categories include:

Protein-based CSPs: These use natural proteins like albumin or globulin as the stationary phase. They offer high selectivity and are often used in the separation of racemates (mixtures of enantiomers) of pharmaceuticals.

Cyclodextrin-based CSPs: Cyclodextrins are cyclic oligosaccharides that form inclusion complexes with chiral analytes. These CSPs are often used for separating a wide range of chiral compounds, including small organic molecules[5].

6.Ion-ExchangeStationaryPhases

Ion exchange stationary phases used in HPLC (High-Performance Liquid Chromatography) are typically made of resin materials that are functionalized with charged groups.

There are two main types of ion exchange stationary phases:

Cation-exchange stationary phases: These have negatively charged functional groups (e.g., sulfonate groups, $-SO_3^-$) that attract and retain positively charged ions (cations).

Anion-exchange stationary phases: These have positively charged functional groups (e.g.quartanary, aminegroups, $-N(CH_3)_3^+$) that attract and bind negatively charged ions anions[10].

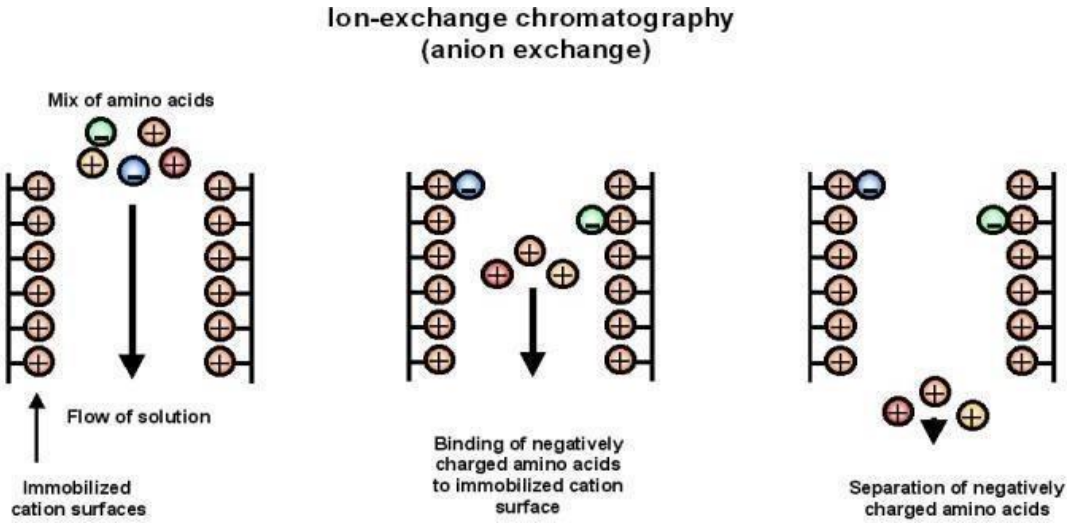

Fig6.1:Ion-Exchangestationaryphases.

7.AffinityStationaryPhase:

High-Performance Liquid Chromatography (HPLC), Affinity stationary phases are used to selectively separate molecules based on their specific interactions with a ligand or receptor that is covalently attached to the stationary phase. These interactions are typically non-covalent, such as antigen-antibody binding, enzyme-substrate affinity, and ligand-receptor interactions. Affinity chromatography is offered for the highly selective separation of biomolecules like proteins, nucleic acids, or other biologically relevant compounds.

Some common types of affinity stationary phases used in HPLC include:

Protein A/G/L: Used for the purification of antibodies. These stationary phases are designed to bind specifically. Bind to bind specifically to the region of immunoglobulins(IgG)

Agarose-based or Separose-based matrices: These are often used for a variety of biological applications, as they provide a stable and inert surface for the attachment of ligands[13].

8.Mixed-ModeStationaryPhases:

In HPLC (High-Performance Liquid Chromatography), a mixed-mode stationary phase refers to a stationary phase that combines two or more types of interaction to separate analytes. These phases typically combine both hydrophobic and hydrophilic properties or ion-exchange and hydrophobic interactions, allowing for a broader range of applications. Mixed-mode columns are particularly useful for separating complex mixtures that may interact with different modes simultaneously.

For example, a column might combine reversed-phase (RP) interactions (hydrophobic interaction) with ion exchange (which can interact with charged molecules), enabling it to separate both hydrophobic and ionic compounds effectively[11].

9.HydrophilicInteractionChromatography(HILIC)Phases:

Hydrophilic interaction chromatography (HILIC) is a type of chromatography that is especially useful for separating polar and hydrophilic compounds. The stationary phases in HILIC are designed to interact with analytes through polar interactions, often facilitating the retention of highly polar compounds. Common stationary phases used in HILIC for HPLC include[9].

Silica-based stationary phases**: These are the most common and often modified with hydrophilic functional groups such as amino, diol, or cyano groups. The silica surface is often bonded with ethylene glycol or other hydrophilic functional groups to enhance interaction with polar analytes[9].**

Polymer-based stationary phases**:These include phases made from materials like polyacrylamide or polyethylene glycol, which are highly hydrophilic and can offer better stability under certain mobile phase conditions, such as higher organic solvent content.**

Ammonium or zwitterionic-modified stationary phases: Some HILIC columns use specialized modifications, like the incorporation of zwitterionic or ammonium functional groups,whichhelptoenhancepolarinteractions, improvingretentionandseparationofhighly polar analytes.

The mobile phase in HILIC typically consists of a high concentration of organic solvent (usuallyacetonitrile) withasmallamount ofaqueousbuffer, oftenwithhighionic strength, to balance the polar interactions between the analyte and the stationary phase[11].

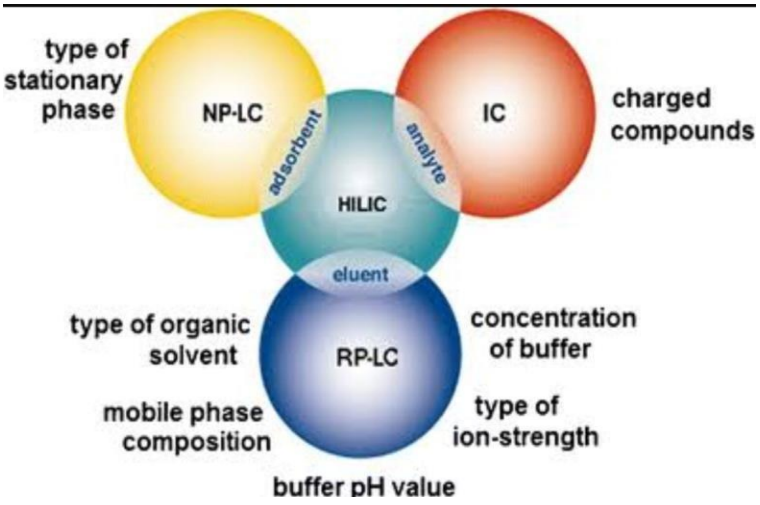

Fig9.1:HydrophilicInteractionchromatography(HILIC)phases.

10. Core-Shell particles:

Core-shell particles are widely used as stationary phases in High-Performance Liquids. Chromatography (HPLC) to improve separation efficiency, reduce backpressure, and increase analysis speed. The structure of core-shell particles typically consists of a solid core material (often silica) that is a thin shell layer, which can also be made of silica or other materials. The shell is responsible for providing the surface properties necessary for interaction with analytes.

Benefits of Core-Shell Particles in HPLC:

Improved Efficiency: The core-shell design reduces the diffusion path for the analyte, leading to sharper peaks and better resolution.

Lower Backpressure: Since the particles are larger than traditional fully porous particles but still have a thin shell, the backpressure is much lower, making them suitable for higher flow rates and faster separations.

Faster Separations: Reduced diffusion distances and improved packing density help achieve faster separations, which is ideal for high-throughput analysis.

Better Peak Shape: The core-shell particles improve peak symmetry and reduce tailing, which is important for accurate quantification and analysis.

Common Types of Core-Shell Materials Used:

Silica-based core-shell: The most common type, often used in reverse-phase HPLC and other separation techniques. The core provides mechanical strength, while the shell provides the active surface for interaction with analytes[6].

11. Monolithic columns:

Monolithic columns in stationary phases have application in UPLC (Ultra-Performance Liquid Chromatography) because they offer several advantages, particularly in terms of speed and efficiency.

Monolithic columns are characterized by a single, continuous piece of porous material that forms the stationary phase rather than the traditional packed bed of small particles. These columns typically have large pore sizes and fewer restrictions for solvent flow, leading to:

Lower Backpressure: Due to the continuous structure of the stationary phase the flow resistance is lower allowing for faster analyses without the high backpressure typically associated with packed columns in UPLC[14].

High Efficiency: The large surface area and open channel structure allow for high mass transfer rates, which can lead to improved resolution and separation efficiency in UPLC.

Faster Analysis: Monolithic columns can be used with higher flow rates due to the lower back pressure, resulting in shorter run times compared to conventional packed columns, which is ideal for UPLC's focus on rapid analysis.

Enhanced Robustness: The solid structure is less prone to column packing issues like Those seen with packed columns, resulting in longer column lifetimes and more consistent performance over time.

Because UPLC typically operates at pressures much higher than traditional HPLC, the benefits of monolithic columns become even more apparent, offering higher throughput and potentially better performance. However, they may not be suitable for all types of separations, particularly those requiring high resolution or specialized selectivity. Monolithic columns in HPLC represent a distinct advancement over traditional packed columns. They use a single, continuous piece of porous material, rather than packed particulate materials, to form the stationary phase. This architecture results in several differences that offer advantages in chromatography. Here's a deeper dive into their properties, benefits, and applications:

Structure and Composition

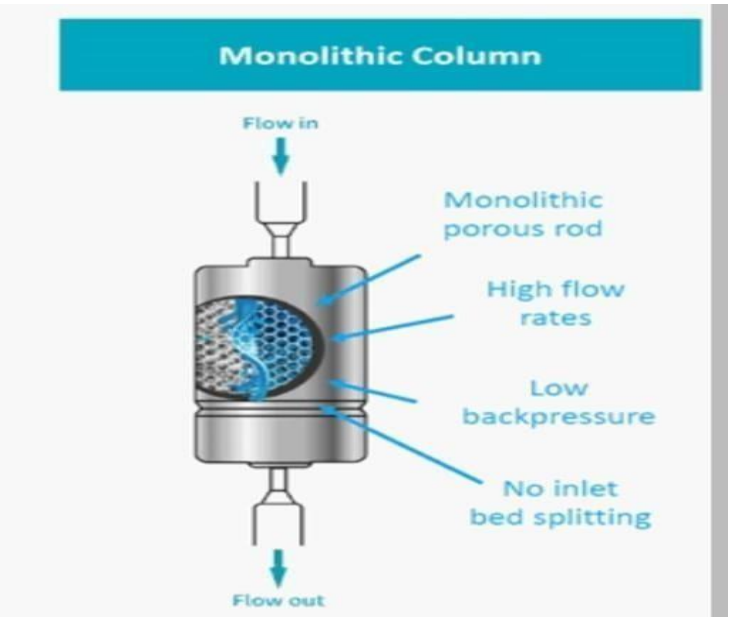

Fig11.1:Monolithiccolumn.

12.Fluorinatedstationaryphases:

Fluorinated stationary phases in High-Performance Liquid Chromatography (HPLC) are used to enhance selectivity and improve separation in certain analytical applications. These phases typically have a high degree of fluorine substitution, which introduces hydrophobicity, increases chemical stability, and can improve interactions with specific analytes.

Hydrophobicity: The presence of fluorine atoms increases the non-polar nature of the stationary phase, which can make it particularly useful for separating hydrophobic compounds.

Selectivity: Fluorinated phases can provide unique selectivity, often being able to resolv compounds that may not separate well on traditional C18 phases.

Chemical Stability: Fluorine atoms are highly electronegative, which can improve the chemical stability of the stationary phase, particularly in the presence of harsh solvents or high temperatures[13].

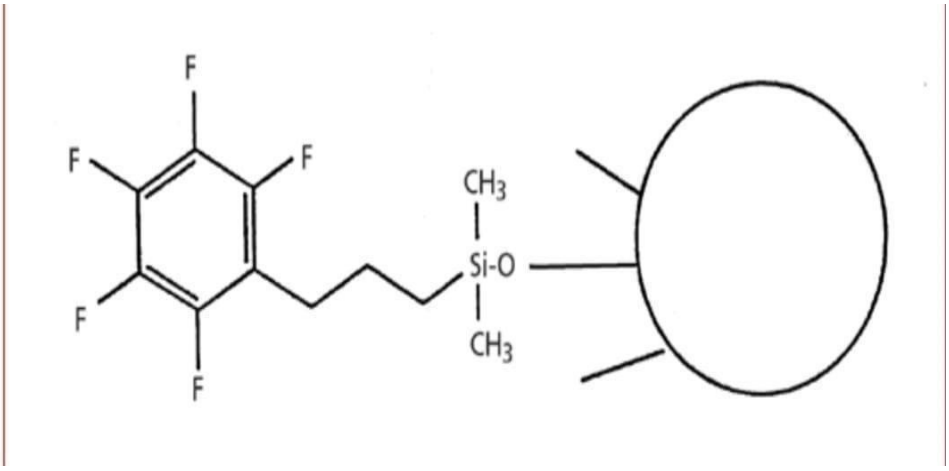

Fig12.1:Fluorinatedstationary phases.

13.Non-poroussilica-basedphases:

Non-porous silica-based phases in HPLC (High-Performance Liquid Chromatography) are used for certain types of separations, typically in applications where faster analysis times or lower back pressure are desired. These stationary phases are made of silica that is not porous, meaning they

do not have the internal surface area typical of porous silica materials.
Here's a breakdown of their key characteristics: Structure: Non-porous silica particles are compact and smooth, with a uniform size distribution. They typically have a high surface area, but it's all on the external surface of the particle.
Advantages:
Reduced Backpressure: Due to the lack of pores, the flow of the mobile phase through the stationary phase is more straightforward, resulting in lower backpressure compared to porous phases.
Fast Separations: These phases can allow for faster analysis times because of the reduced diffusion path and lower resistance to flow[12].

14. Nanomaterial-Based Stationary Phases

Nanomaterial-based stationary phases in High-Performance Liquid Chromatography (HPLC) are a cutting-edge innovation that significantly improves the efficiency, selectivity, and sensitivity of chromatographic separations. The use of nanomaterials in HPLC stationary phases has gained attention due to their unique properties, such as high surface area, enhanced interaction capabilities, and tunable chemical functionalities. Here's a detailed overview:
Nanomaterials in HPLC:
Nanoparticles (NPs):
These are ultra-fine particles, typically in the range of 1-100 nm in diameter. They can be used to modify the surface properties of traditional silica-based materials or be utilized as the stationary phase them selves.
Examples include silica nanoparticles, carbon nanotubes, and metal nanoparticles.
Nanostructured Materials:
These include materials with ordered nanoscale structures like mesoporous silica (such as SBA-15), which has well-defined pores that can provide better separation efficiency.

Nanocomposites:
These are hybrid materials that combine nanoparticles with other materials (organic or inorganic) to enhance the chromatographic properties.
Carbon-Based Nanomaterials:
These include carbon nanotubes (CNTs) and graphene, which offer high surface area, chemical stability, and the ability to modify their surface for various interactions (hydrophobic, π-π interactions, etc)[7].

15.electrostaticstationaryphase

In High-Performance Liquid Chromatography (HPLC), electrostatic stationary phases refer to materials that interact with analytes through electrostatic forces, such as ion exchange or dipole-dipole interactions, rather than purely hydrophobic or Van der Waals interactions. These stationary phases are often used for the separation of charged or polar compounds.

Some examples of electrostatic stationary phases include:

1. Ion-exchange columns: These are commonly used for separating ionic compounds based on their charge. The stationary phase in these columns consists of an ion-exchange resin that can either be positively or negatively charged, allowing the retention and separation of analytes based on their charge.

2. Amino or cyanopropyl bonded phases: These are polar stationary phases where the functional groups (amino or cyano groups) interact with analytes through electrostatic or hydrogen bonding interactions. These phases can be used for separating polar compounds and can exhibit some degree of electrostatic interaction with analytes[13]

16.Reversed–phasedstationaryphase
In reversed-phase HPLC, the stationary phase is typically made of silica particles that are modified by attaching hydrophobic groups. The mobile phase, in contrast, is usually a polar solvent, often water or a water-methanol or water-acetonitrile mixture. The separation in reversed-phase chromatography occurs because the analytes interact with the hydrophobic stationary phase.
Hydrophobic interactions: The stationary phase (with hydrophobic groups) interacts with non-polar or less-polar compounds, slowing their movement through the column.
Polar compounds: On the other hand, more polar compounds are less attracted to the hydrophobic stationary phase and thus travel through the column more quickly.
Reversed-phase chromatography is one of the most common modes in HPLC due to its versatility, efficiency,
To separate a wide range of compounds, especially non-polar and moderately polar substances[14].

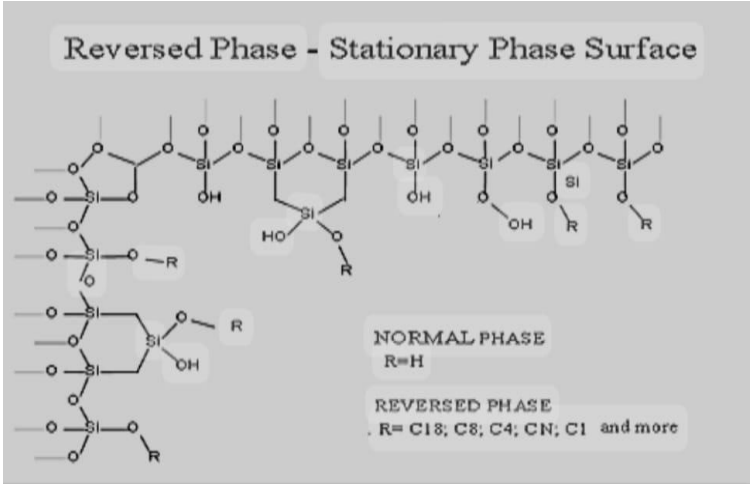

Fig16.1:Reversedphasestationaryphase.

APPLICATIONS

1. Separationofdrugs,metabolites, andimpurities[2]
2. Detectionofpesticides,herbicides, and pollutants.
3. It is usefulforseparatingchargedcompoundssuchasproteins, amino acids, and inorganic ions based on their charge interactions[1].
4. Primarily used for biomolecules, polymers, and macromolecules where separation is based on molecular size (e.g., proteins, polymers, and polysaccharides).
5. It is critical for the separation of enantiomers, such as in the pharmaceutical industry forchiral drugs, pesticides, and amino acids.
6. Used for the separation of amino acids, carbohydrates, and polar compounds through hydrophilic interactions[1].
7. Employedforseparatingpositivelychargedionsorcationslikemetals,drugs,and other cationic species.
8. Used for separating negatively charged ions (anions) such as chloride, sulfate, phosphate, and other anionic compound.
9. Commonly used for separating a variety of organic compounds such as alcohols, aldehydes, ketones, and small organic molecules in both normal-phase and reversed- phase applications[7].
10. Suitable for thermally unstable compounds or where silica columns may not be appropriate (e.g., separating small organic molecules and pharmaceuticals.
11. Designforbiomolecularseparationssuchasisolatingproteins, enzymes, antibodies, and nucleic acids based on selective binding[4].
12. Ideal for the separating ofpolar and hydrophilic compounds, such as peptides, sugars, and polar metabolites.
13. Used to isolate specific proteins or antibodies by exploiting antigen-antibody interactions (e.g., separating biomarkers in clinical diagnostics[5].
14. Used for complexseparationswhere multiple interactionsare required, like separating compounds with varying polarity, charge, or size (e.g., in pharmaceutical and environmental analysis).
15. PharmaceuticalsC18columnsareusedextensivelyforanalysisofpharmaceuticals and biologically active compounds.
16. Environmentalanalysis:Usedintheanalysisoforganiccontaminantsinwater,soil,and air samples[11].
17. Biologicalsamples:C18columnsarepopular forseparatingpeptides,proteins,nucleic acids, and metabolites.
18. Food and beverage testing: C18 columns are used to analyze preservatives, additives, and natural compounds in food products[7].
19. Less hydrophobic compounds: C8 phases are used when the target analytes are less hydrophobiccomparedtothosetypicallyanalyzedwithC18columnsmakingthem suitable for separating compounds like small peptides or water-soluble molecules[9].
20. Drug analysisC8 phases are also effective in analyzing various pharmaceutical compounds that have strong hydrophobic properties.
21. Polarandioniccompounds:Usefulforseparatingpolarandioniccompounds,including sugars, nucleotides, and amino acids[13]

CONCLUSION

In conclusion, the stationary phase is a critical component of HPLC, and the choice of phase can significantly impact the success of the separation. By understanding the properties and applications of different stationary phases, analysts can optimize their HPLC methods and achieve high-quality separations. The selection of stationary phases in High-Performance Liquid Chromatography (HPLC) is a critical determinant of the technique's effectiveness in achieving efficient separations. The stationary phase plays an essential role in the chromatographic process by interacting with the analytes, influencing their retention times and separation. Given the wide variety of compounds analyzed through HPLC, different stationary phases are tailored to suit specific needs, choosing phase a vital aspect of method development. The most commonly used stationary phases, including silica-based, reverse-phase, ion-exchange, size-exclusion, and chiral phases, each serve distinct purposes based on the chemical characteristics of the target analytes.

In reverse-phase HPLC, for example, C18 and C8 bonded phases are ideal for separating non-volatile, polar compounds, offering high efficiency and versatility for a broad range of applications. Conversely, normal-phase HPLC with silica-based stationary phases is often used for separating non-polar analytes, particularly when polarity differences are the key separation mechanism. Ion-exchange stationary phases offer an excellent resolution for charged molecules, making them invaluable in the analysis of biomolecules, while size-exclusion chromatography is crucial for separating molecules based on their molecular weight employed in protein and polymer analyses.

Chiral stationary phases are specifically designed for the separation of enantiomers, which is essential in the pharmaceutical industry for the analysis of stereochemically active compounds. These highly specialized phases provide significant advancements in the analysis of complex compounds, where the ability to distinguish between isomers can have profound implications for drug development and regulatory standards.

Theevolutionofstationaryphasematerials,suchasmodifiedsilicasandpolymer-basedphases, has further broadened the scope of HPLC, allowing for more selective, efficient, and reproducible separations. The selection of an appropriate stationary phase requires a comprehensive understanding of the physicochemical properties of both the stationary phase and the analyte, including polarity, charge, size, and interaction mechanisms. Advances in HPLC technology have made it possible to fine-tune these interactions, leading to improved resolution, faster analysis times, and more reliable results.

The diverse range of stationary phases available for HPLC provides analysts with a powerful toolkit to tackle a range of separation challenges. The correct choice of stationary phase not only ensures high-quality results but also allows for the optimization of analytical processes, making HPLC a versatile and indispensable tool in various industries such as pharmaceuticals, environmental testing, food safety, and biotechnology. As HPLC technology continues to evolve, further innovations in stationary phase materials will likely offer greater selectivity, efficiency, and versatility, pushing the boundaries of chromatographic separations even further.

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