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## "Brief Study On High Performance Liquid Chromatography"

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### ABSTRACT

High-Performance Liquid Chromatography (HPLC) is a versatile and widely used analytical technique in the field of chemistry. This demonstration provides an overview of HPLC principles, instrumentation, and practical applications. Participants will gain insights into the separation and quantification of compounds in complex mixtures using HPLC. The demonstration covers the key components of an HPLC system, including columns, detectors, and mobile phases, and how they influence separation efficiency. Practical examples showcase the application of HPLC in pharmaceutical analysis, environmental monitoring, and food safety. Attendees will leave with a fundamental understanding of HPLC and its significance in analytical chemistry.

### INTRODUCTION

High-Performance Liquid Chromatography, commonly known as HPLC, is a cornerstone in the realm of analytical chemistry. It is a powerful and versatile technique used for separating, identifying, and quantifying compounds in complex mixtures. HPLC has found its application in various fields, including pharmaceuticals, environmental analysis, food safety, and more. As we embark on this demonstration, we will delve into the fundamental principles, instrumentation, and practical applications of HPLC. Our journey will encompass the intricacies of how HPLC separates compounds based on their chemical properties, and how this technique plays a vital role in ensuring the quality and

safety of products we encounter in our daily lives. Join us in this exploration of HPLC, and you will gain valuable insights into a technique that has revolutionized the world of analytical chemistry.

## HISTORY

High-Performance Liquid Chromatography (HPLC) is a widely used analytical technique in chemistry, biochemistry, and pharmaceutical sciences. Here's a brief history of its demonstration:

### **1. Invention of Liquid Chromatography:**

- The concept of liquid chromatography dates back to the early 20th century when Mikhail Tsvet, a Russian-Italian botanist, first described the separation of plant pigments using a glass column packed with calcium carbonate in 1900.

#### **- Development of Early Liquid Chromatography:**

- Throughout the early to mid-20th century, various researchers improved upon Tsvet's work, including Richard Laurence Millington Synge and Archer Martin in the 1940s. They introduced techniques like paper chromatography and column chromatography.

### **2. Introduction of Modern HPLC:**

- The true development of what we now know as HPLC began in the 1960s.
- In 1965, two researchers, R. L. Wixom and L. R. E. Snyder, coined the term "High-Performance Liquid Chromatography" to describe their improved liquid chromatography methods.
- The main breakthrough came with the introduction of smaller particle sizes for the column packing material and higher pressure pumps in the 1970s. This allowed for faster and more efficient separations.

### **3. Advancements and Widespread Adoption:**

- Throughout the 1970s and 1980s, HPLC became more popular and widely adopted, particularly in the pharmaceutical and analytical chemistry fields.
- Innovations in detectors, including UV-Vis and fluorescence detectors, enhanced its analytical capabilities.

### **4. Evolution and Applications:**

- Over the years, HPLC has continued to evolve with the introduction of various modes of chromatography, including reverse-phase, normal-phase, ion-exchange, and size-exclusion HPLC.
- HPLC is now used in a broad range of applications, including drug analysis, environmental

monitoring, food analysis, and more.

The demonstration and development of HPLC have been crucial in advancing analytical chemistry, and it remains a fundamental tool for chemical analysis and research today.

### Definition

The term "demonstration of HPLC" typically refers to the practical display or presentation of how High-Performance Liquid Chromatography (HPLC) works and is used in a laboratory or analytical setting. This can include:

- 1. Instrument Operation:** Demonstrating how to set up and operate an HPLC instrument, including the proper installation of columns, mobile phase preparation, and system calibration.
- 2. Sample Analysis:** Showing how samples are prepared and injected into the HPLC system for analysis. This may involve explaining sample preparation techniques, such as dilution or filtration.
- 3. Chromatographic Separation:** Explaining the chromatographic separation process, which involves the movement of sample components through the column and their detection based on their properties (e.g., UV absorption, fluorescence).
- 4. Data Interpretation:** Discussing how to interpret the chromatograms generated by the HPLC system, including the identification and quantification of analytes.
- 5. Troubleshooting:** Addressing common issues and problems that can arise during HPLC analysis and how to troubleshoot and resolve them.

In a broader sense, the demonstration of HPLC encompasses educational or training sessions designed to teach students, researchers, or technicians how to use HPLC for analytical purposes. These demonstrations help individuals understand the principles and practical aspects of HPLC, ensuring accurate and reliable results in their work.

## Purpose of Demonstration of HPLC

The demonstration of High-Performance Liquid Chromatography (HPLC) serves several important purposes:

- 1. Education and Training:** HPLC demonstrations are essential for educating and training students, laboratory technicians, and researchers on the principles, operation, and applications of HPLC technology.
- 2. Skill Development:** They help individuals acquire hands-on experience in operating HPLC instruments, which is crucial for developing the skills necessary for analytical chemistry and research.
- 3. Quality Control:** Demonstrations are used in quality control and assurance programs to ensure that laboratory personnel can accurately and consistently use HPLC for quality testing and analysis of products, such as pharmaceuticals, food, and environmental samples.
- 4. Method Validation:** Demonstrating HPLC methods helps validate analytical procedures, ensuring that they are suitable for their intended purpose and meet regulatory standards.
- 5. Troubleshooting:** Demonstrations often include guidance on identifying and addressing common issues and problems that can arise during HPLC analysis. This knowledge is essential for maintaining instrument performance and obtaining reliable results.
- 6. Research and Development:** For researchers, HPLC demonstrations are a way to learn how to apply this technique to their specific research projects, allowing them to separate, quantify, and analyze compounds of interest accurately.
- 7. Technological Advancements:** Demonstrations of the latest HPLC technologies and innovations help scientists and professionals stay up-to-date with the field's advancements.
- 8. Compliance with Regulations:** In regulated industries like pharmaceuticals, demonstrating HPLC proficiency is critical for ensuring compliance with industry standards and regulations.
- 9. Efficiency and Cost Savings:** Proper HPLC training and demonstrations can lead to more efficient use of resources, reduced downtime, and cost savings by preventing errors and instrument downtime.

In summary, the purpose of demonstrating HPLC is to equip individuals with the knowledge and skills required to use this powerful analytical technique effectively, whether for research, quality control, or regulatory compliance in various industries.

## OBJECTIVES

- Certainly, here are some objectives for a demonstration of High-Performance Liquid Chromatography (HPLC):
  - Introduction to HPLC Principles:
    - To provide with a foundational understanding of HPLC principles, including the theory of separation, components of an HPLC system, and the role of mobile phases and detectors.
  - Instrumentation Familiarization,
    - To familiarize attendees with the key components of an HPLC system, such as the column, pump, injector, and detector, and how they work together to achieve efficient compound separation.
  - Sample Preparation Techniques.
    - To demonstrate sample preparation techniques, including sample extraction and purification, which are crucial for obtaining accurate and reliable HPLC results.
  - Chromatographic Separation.
    - To explain the role of detectors in HPLC and demonstrate how they detect and quantify compounds in the eluent, and how to analyze the resulting data.
  - Applications in Various Fields.
    - To showcase practical applications of HPLC in areas such as pharmaceutical analysis, environmental monitoring, and food safety, highlighting its significance in these industries.
  - Troubleshooting and Maintenance.
    - To emphasize safety protocols and best practices in handling chemicals, operating HPLC equipment, and managing waste in the laboratory.
  - Q&A and Hands-On Interaction
    - To encourage questions and hands-on interaction with HPLC equipment to reinforce the understanding of the demonstrated concepts.
  - Appreciation of Analytical Chemistry.
    - To instill an appreciation for the role of HPLC in analytical chemistry and its impact on various industries, as well as its contribution to scientific research and quality control.

## PRINCIPLE

The principles behind a demonstration of High-Performance Liquid Chromatography (HPLC) involve explaining the fundamental concepts and processes that underlie this analytical technique. Here are the key principles to cover: Chromatographic Separation.

Explain the primary principle of HPLC, which is the separation of compounds in a mixture based on their interactions with a stationary phase (column) and a mobile phase. Discuss how compounds with different properties (e.g., polarity) move through the column at different rates. Mobile Phase: Describe the role of the mobile phase, typically a solvent or mixture of solvents, in carrying the sample through the column. Emphasize how the choice of the mobile phase affects the separation. Stationary Phase.

Explain the stationary phase, which can be a packed column filled with a solid adsorbent material or a capillary column coated with a stationary phase. Discuss how the stationary phase interacts with analytes to facilitate separation. Sample Injection: Demonstrate how the sample is introduced into the HPLC system, emphasizing the need for precise and reproducible injections. Column Efficiency.

Discuss factors that influence column efficiency, such as column length, particle size, and column temperature, and how these affect the resolution of peaks. Detection: Explain the role of the detector in HPLC, which detects and quantifies the separated compounds. Discuss common types of detectors, such as UV-Vis, fluorescence, and mass spectrometry. Data Analysis. Highlight the importance of data analysis in interpreting HPLC results, including how retention times, peak shapes, and peak areas are used for compound identification and quantification. Sample Preparation: Touch on the significance of proper sample preparation, including extraction, filtration, and dilution.

Discuss the wide range of applications for HPLC, including pharmaceutical analysis, environmental monitoring, food quality control, and more. Quality Control: Emphasize the role of HPLC in quality control and regulatory compliance, particularly in industries like pharmaceuticals and food. Instrumentation: Provide an overview of the key components of an HPLC system, such as the pump, injector, column, detector, and data acquisition system. Troubleshooting: Briefly cover common issues that may arise during HPLC analysis and how to troubleshoot them.

## INSTRUMENTATION

In a demonstration of High-Performance Liquid Chromatography (HPLC), it's important to introduce and explain the key components of an HPLC system.

**Pump:** The pump is responsible for delivering the mobile phase at a constant flow rate to the HPLC

column. Explain how it maintains the pressure required for efficient chromatographic separation.

**Injector:** Describe the injector, which is used to introduce the sample into the HPLC system. Explain the importance of precise and reproducible sample injection.

**Column:** Discuss the HPLC column, which is packed with a stationary phase that interacts with analytes to separate them. Explain the role of column length, diameter, and particle size in separation efficiency. **Column Oven:** If applicable, mention the column oven, which controls the temperature of the column. Temperature control can influence separation and peak shape.

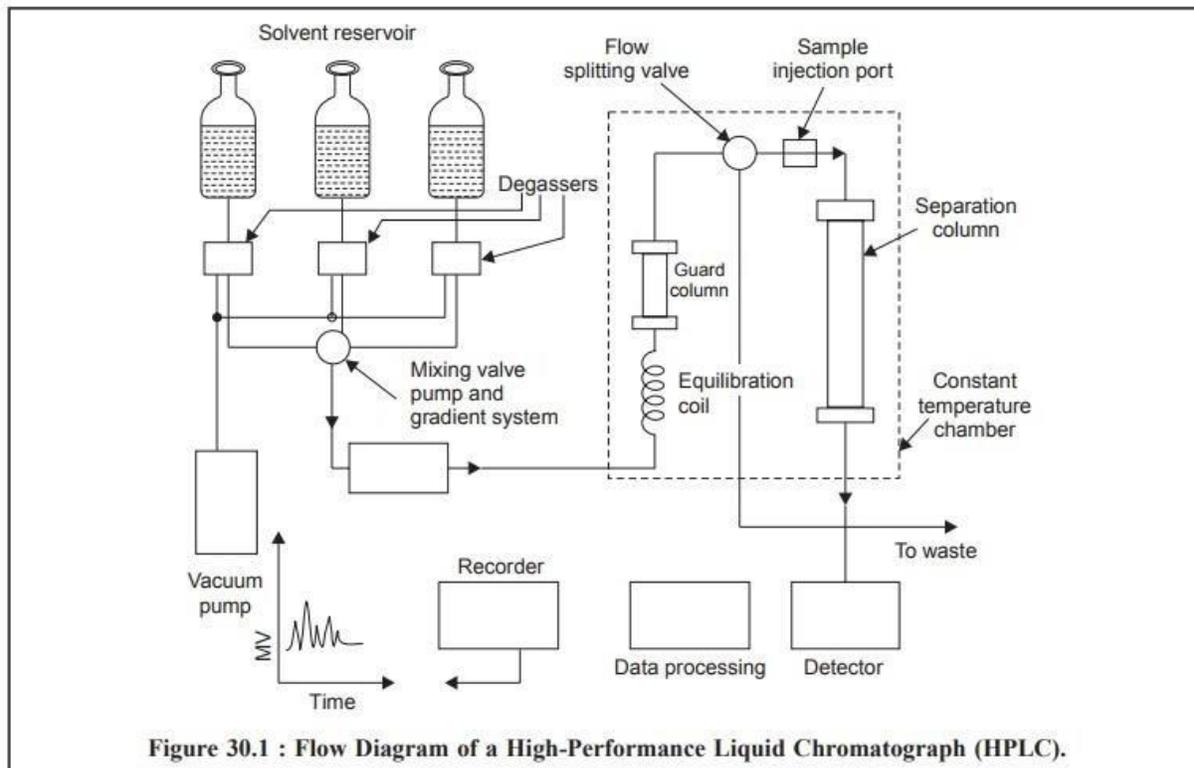
**Detector:** Explain the role of the detector in HPLC, which monitors the eluent as it exits the column and provides data for compound identification and quantification. Common detectors include UV-Vis, fluorescence, and mass spectrometry. **Autosampler:** If available, introduce the autosampler, which automates sample introduction and allows for high-throughput analysis.

**Data Acquisition System:** Mention the data acquisition system, which collects and processes data from the detector, converting it into chromatograms for analysis.

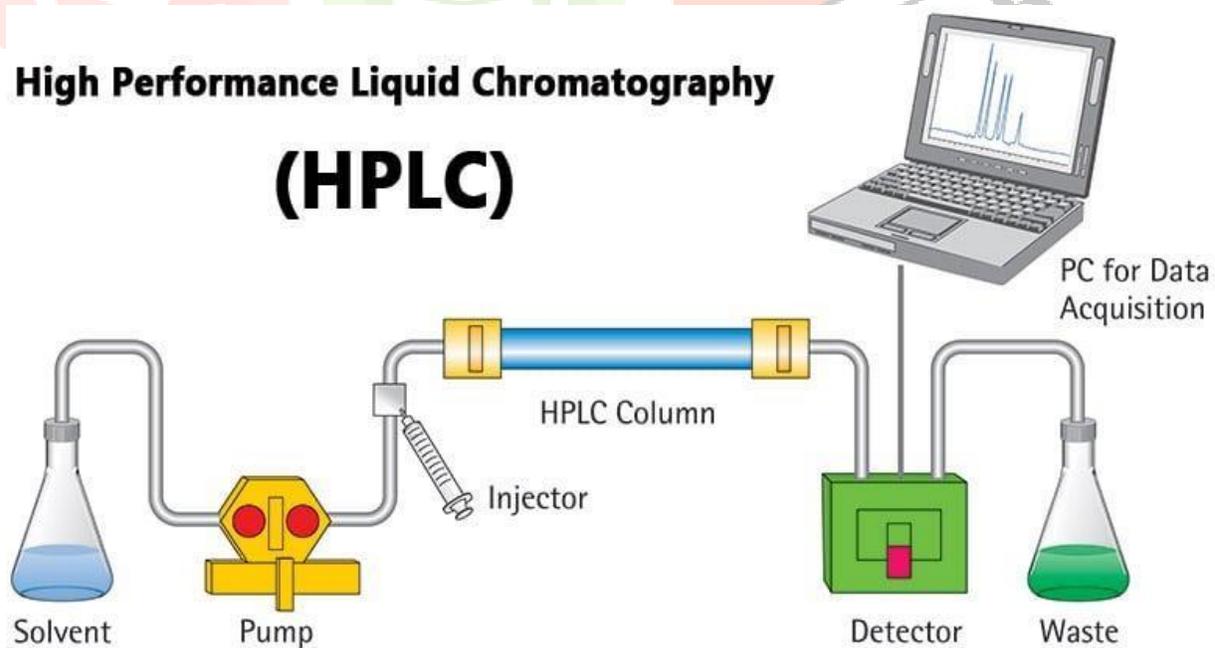
**Mobile Phase Reservoirs:** Show the containers that hold the mobile phase solvents and discuss the importance of solvent selection and preparation.

**Waste Collection System:** Briefly mention the waste collection system for collecting and disposing of waste solvents and samples.

**Control Software:** Explain the software used to control and monitor the HPLC system, including setting up the method, controlling the pump, and collecting and analyzing data. During the demonstration, consider showing how these components are assembled and connected in a typical HPLC system. You can also discuss the importance of proper maintenance and calibration of these components for accurate and reliable results. Practical demonstrations or animations can help participants visualize how the instrumentation works together in an HPLC analysis.



## High Performance Liquid Chromatography (HPLC)



## WORKING

The working of a demonstration of High-Performance Liquid Chromatography (HPLC) involves explaining the step-by-step process of how an HPLC analysis is conducted. Here's a simplified overview of the working process for an HPLC demonstration:

**1. Sample Preparation:** Begin by explaining the importance of sample preparation, including steps like extraction, filtration, and dilution. Show how a properly prepared sample is essential for accurate HPLC analysis.

**2. Mobile Phase:** Introduce the mobile phase, which is a solvent or solvent mixture used to carry the sample through the column. Discuss the significance of choosing the appropriate mobile phase for the specific separation.

**3. Sample Injection:** Demonstrate how a small volume of the prepared sample is precisely injected into the HPLC system using an autosampler or manual injection. Highlight the need for consistency and precision in sample injection.

**4. Column Separation:** Explain how the sample travels through the HPLC column, which is packed with a stationary phase. Discuss how compounds are separated based on their interactions with the stationary phase and mobile phase, resulting in distinct peaks in the chromatogram.

**5. Detector Function:** Describe the role of the detector, which continuously monitors the eluent as it exits the column. Discuss how different detectors work (e.g., UV-Vis or mass spectrometry) and how they measure compound concentration and produce signals.

**6. Data Acquisition:** Show how the detector's signals are collected and processed by the data acquisition system. Explain how these signals are converted into chromatograms that display peaks representing separated compounds.

**7. Data Analysis:** Discuss how the chromatogram is analyzed, including determining retention times, peak areas, and peak heights. Emphasize that data analysis is crucial for compound identification and quantification.

**8. Sample Waste Collection:** Mention the disposal of waste solvents and samples, which is an important part of the process to maintain a clean and safe working environment.

**9. Results Interpretation:** Present and interpret the chromatogram, showing how it can be used to identify and quantify compounds in the sample. Discuss the significance of peak shape, retention times, and peak areas.

**10. Maintenance and Troubleshooting:** Explain that proper maintenance of the HPLC system is essential for consistent results. Mention common issues and how to troubleshoot them.

Throughout the demonstration, consider using visual aids, such as diagrams and real HPLC equipment, to illustrate each step of the process. Additionally, a practical demonstration with a simple sample can provide hands-on experience for participants to understand how HPLC works in practice.

### NEW ADVANCEMENTS

As of my last knowledge update in January 2022, several advancements and new technologies had emerged in the field of High-Performance Liquid Chromatography (HPLC). While I can't provide the most up-to-date information beyond that date, I can give you an idea of some trends and advancements that were underway at that time. Be sure to check for the latest developments in the field since then:

**1. UHPLC (Ultra-High-Performance Liquid Chromatography):** UHPLC systems were becoming more prevalent. These systems operate at much higher pressures and offer improved resolution and speed compared to traditional HPLC.

**2. Column Technologies:** Advances in column materials and design were enhancing separation efficiency and selectivity. New types of stationary phases, such as superficially porous particles and monolithic columns, were being developed.

**3. Miniaturization and Microfluidics:** HPLC systems were getting smaller and more portable, allowing for on-site and in-field analyses. Microfluidic HPLC chips were becoming popular for their rapid separations.

**4. 2D-LC (Two-Dimensional Liquid Chromatography):** This technique was gaining traction for complex sample analysis by providing improved separation power through the use of two different

columns.

**5. Hyphenated Techniques:** HPLC was increasingly being combined with other analytical techniques, such as mass spectrometry (LC-MS), nuclear magnetic resonance (LC-NMR), and infrared spectroscopy (LC-IR), to provide comprehensive characterization of compounds.

**6. Automation and Robotics:** Automation in sample handling, injection, and analysis was becoming more sophisticated, reducing human error and increasing throughput.

**7. Green HPLC:** There was a growing emphasis on eco-friendly or "green" HPLC, with a focus on reducing solvent consumption and waste generation.

**8. Data Handling and Software:** Improved software solutions for data analysis, integration, and visualization were enhancing the usability of HPLC systems.

**9. Chiral Separations:** Advancements in chiral stationary phases and chiral HPLC methods were aiding in the separation and analysis of enantiomers.

**10. Bioanalytical HPLC:** HPLC was playing a vital role in the analysis of biomolecules, such as proteins, peptides, and nucleic acids, with specific columns and methods developed for these applications.

Remember to consult recent literature and industry news to get the most current information on advancements in HPLC, as the field continues to evolve and innovate.

## MOBILE PHASE

In a demonstration of High-Performance Liquid Chromatography (HPLC), it's important to explain the role of the mobile phase and its selection. The mobile phase is a crucial component of the chromatographic process. Here's how to address its use in the demonstration:

**1. Introduction to Mobile Phase:** Start by introducing the concept of the mobile phase. Explain that it is a liquid or a mixture of liquids used to carry the sample through the HPLC column.

**2. Purpose of the Mobile Phase:** Emphasize that the mobile phase is essential for the chromatographic separation of compounds in the sample. It facilitates the movement of analytes through the column.

**3. Mobile Phase Composition:** Discuss the different types of solvents commonly used as mobile phases, such as water, acetonitrile, methanol, and various combinations thereof. Explain that the choice of mobile phase depends on the compounds being analyzed, considering factors like polarity and solubility.

**4. Mobile Phase Purity:** Stress the importance of using high-purity solvents to prevent contamination and ensure the accuracy of results.

**5. Gradient Elution:** Mention the use of gradient elution, a technique where the composition of the mobile phase changes during the analysis to improve separation. Explain its benefits in complex sample analysis.

**6. Buffer Solutions:** Discuss the use of buffer solutions in the mobile phase for controlling pH, particularly in the analysis of ionizable compounds.

**7. Preparation and Handling:** Explain the proper preparation and handling of the mobile phase, including degassing to remove dissolved gases, filtration to remove particulate matter, and the use of solvent reservoirs.

**8. Flow Rate Control:** Describe how the flow rate of the mobile phase is controlled by the HPLC pump and why it is critical for reproducible results.

**9. Mobile Phase Selection for Specific Applications:** Give examples of how different mobile phases are chosen for specific applications, such as reversed-phase HPLC, normal-phase HPLC, or ion-exchange chromatography.

**10. Safety Considerations:** Mention safety considerations when working with mobile phases, including handling flammable solvents and the need for proper ventilation.

During the demonstration, you may want to show the participants how to prepare a mobile phase, set up the HPLC system with the chosen mobile phase, and emphasize the significance of mobile phase selection in achieving successful separations. Additionally, visuals or diagrams can help participants understand the role of the mobile phase in the chromatographic process.

## STATIONARY PHASE

In a demonstration of High-Performance Liquid Chromatography (HPLC), it's essential to explain the role of the stationary phase and its significance in the chromatographic process. Here's a detailed breakdown of how to discuss the use of the stationary phase:

- 1. Introduction to Stationary Phase:** Start by introducing the stationary phase as a critical component in HPLC. Emphasize its role in separating compounds within the sample.
- 2. Purpose of the Stationary Phase:** Explain that the stationary phase is the fixed, immobile part of the HPLC column, and it interacts with the sample components as they pass through the column. This interaction is what leads to the separation of compounds.
- 3. Types of Stationary Phases:** Discuss the various types of stationary phases commonly used in HPLC, including reversed-phase, normal-phase, and ion-exchange. Explain that the choice of stationary phase depends on the nature of the sample and the analytes to be separated.
- 4. Reversed-Phase HPLC:** If applicable to your demonstration, explain in more detail the concept of reversed-phase HPLC, where a nonpolar stationary phase is used to separate polar compounds.
- 5. Normal-Phase HPLC:** Similarly, describe normal-phase HPLC, where a polar stationary phase is used to separate nonpolar compounds.
- 6. Selectivity and Separation:** Discuss how the choice of stationary phase significantly affects the selectivity and efficiency of the separation. Different stationary phases interact differently with analytes, leading to distinct separation patterns.
- 7. Particle Size and Pore Size:** Mention that stationary phases are available in different particle sizes and pore sizes, and these properties can influence the separation efficiency and resolution.

**8. Column Dimensions:** Explain that the length and diameter of the column can also impact separation, with longer columns offering higher resolution but longer analysis times.

**9. Packing Material:** Discuss the packing material used for stationary phases, which can be silica-based, polymer-based, or other materials. Mention that modern stationary phases often include chemically bonded phases for enhanced selectivity.

**10. Column Regeneration:** Mention that some columns can be regenerated and reused, while others are disposable and for single use.

**11. Storage and Care:** Emphasize proper storage and care of HPLC columns, including the need to prevent column drying and contamination.

**12. Method Development:** Explain that optimizing the stationary phase and column conditions is a crucial part of method development to achieve the desired separation.

During the demonstration, consider showing participants how to choose and set up the appropriate stationary phase for their specific application. Visual aids, such as images of different column types and stationary phases, can help participants better understand the role of the stationary phase in HPLC separations.

## APPLICATIONS

In a demonstration of High-Performance Liquid Chromatography (HPLC), it's important to showcase practical applications to illustrate the versatility and relevance of this analytical technique. Here are some common applications you can discuss during your demonstration:

### 1. Pharmaceutical Analysis:

- Quantification of active pharmaceutical ingredients (APIs) in drug formulations.
- Detection of impurities and degradation products in pharmaceuticals.
- Bioavailability studies to assess drug absorption.

### 2. Environmental Monitoring:

- Analysis of water samples for pollutants, such as pesticides, heavy metals, and organic compounds.
- Monitoring of air quality by analyzing volatile organic compounds (VOCs) and particulate matter.

### 3. Food and Beverage Analysis:

- Determination of food additives, preservatives, and contaminants in food products.
- Identification of flavor compounds in beverages and food items.
- Detection of mycotoxins in agricultural products.

### 4. Clinical Diagnostics:

- Quantification of biomarkers and metabolites in biological samples.
- Analysis of proteins and peptides in proteomic studies.
- Drug testing in clinical toxicology.

### 5. Environmental Chemistry:

- Soil analysis for pesticides and contaminants.
- Analysis of groundwater for pollutants.
- Monitoring of soil and water quality in agricultural and environmental studies.

### 6. Chemical Research:

- Separation and identification of organic compounds in chemical synthesis.
- Investigating reaction kinetics by monitoring reactants and products.
- Determination of the purity of chemical compounds.

### 7. Pharmacokinetics and Pharmacodynamics:

- Assessment of drug absorption, distribution, metabolism, and excretion (ADME) in pharmacological studies.
- Monitoring drug levels in blood and tissues to understand drug efficacy.

### 8. Forensic Science:

- Drug testing in forensic toxicology.
- Detection of illicit substances and their metabolites in biological samples.
- Analysis of trace evidence in criminal investigations.

### 9. Biotechnology and Biopharmaceuticals:

- Analysis of biopharmaceuticals, including monoclonal antibodies and proteins.
- Characterization of gene products and recombinant DNA products.

## 10. Materials Science:

- Analysis of polymer composition and molecular weight distribution.
- Assessment of material degradation and stability over time.
- Identification of components in complex materials, such as paints and coatings.

During your demonstration, you can pick one or more of these applications to highlight and explain how HPLC is used in each scenario. Consider providing practical examples and real-world case studies to make the applications more relatable and engaging for the participants.

## REFERENCE

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