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Hplc principle procedure and applications pdf

Principle and applications of hplc.

High-performance liquid chromatography (HPLC) is an analytical technique that separates, identifies, and quantifies individual components in a mixture. This process relies on the principle of column chromatography, where the mixture is separated using a stationary phase and a mobile phase. The HPLC system consists of a pump, injector, column, and detector. The pump generates a flow of eluent from the solvent reservoir into the system, while the injector introduces the sample into the mobile phase flow. The column separates the individual components based on their interactions with the stationary phase, and the detector identifies these components by analyzing the signal passed to the HPLC software. The resulting chromatogram in the software allows for the identification and quantification of the different substances within the mixture. High-performance liquid chromatography (HPLC) equipment involves various components that work together for accurate results. The column is where the separation takes place, utilizing a stationary phase and mobile phase with different properties depending on the type of analysis required. A detector measures changes in the composition of the eluent, indicating the presence or absence of analytes. Different types of detectors are available, including computer-based data processors that can perform tasks like peak-fitting, baseline correction, and concentration calculation. Additionally, HPLC equipment includes components such as a degasser to remove gases from the eluent, which can cause an unstable baseline. The column heater is crucial for maintaining consistent temperature conditions, especially for temperature-sensitive compounds or analyses requiring higher temperatures. Various types of columns are used depending on the type of analysis, including those for water-sensitive compounds, polar and non-polar samples, ions, and biomolecules. HPLC has become a versatile method applicable in various fields like chemistry, biochemistry, pharmacy, environmental analytics, drug analysis, and product quality control. Its accuracy, efficiency, speed, and precision make it an essential tool in many research and industrial settings. High-performance liquid chromatography (HPLC) is a versatile technique that enables the separation, identification, and quantification of chemical components in a mixture. This method involves injecting a small volume of sample into a tube packed with tiny particles, where individual components are separated based on their molecular structure and composition. While HPLC offers several advantages, it can be costly due to the requirement of expensive organics and may have low sensitivity for certain compounds. Some substances cannot be detected as they become irreversibly adsorbed, making gas chromatography a more suitable method for volatile substances. High-performance liquid chromatography is widely used in various aspects of drug manufacturing and research. The technique involves a column holding packing material (stationary phase), a pump moving the mobile phase through the column, and a detector showing the retention times of the molecules. The interactions between the stationary phase, molecules being analyzed, and solvent(s) used influence the retention time. The most common solvents used in HPLC are methanol and acetonitrile. Michael Tswett is credited as the father of chromatography due to his demonstration of liquid chromatography. Thin-layer chromatography (TLC) was introduced later, followed by paper chromatography in the mid-1940s and significant advancements in TLC by Egon Stahl in 1956. High-performance liquid chromatography was developed in the 1970s, involving the injection of a small volume of liquid sample into a tube packed with tiny particles. The separated components are then detected at the exit, allowing for various chemical and/or physical interactions between their molecules and the packing particles. High-performance liquid chromatography (HPLC) is a powerful analytical technique that excels in sensitivity, resolution, and sample recovery compared to traditional low-pressure column liquid chromatography. This method separates compounds based on their interactions with the stationary phase, which can be categorized into normal-phase or absorption chromatography, reverse-phase or hydrophobic interaction chromatography, size-exclusion chromatography, ion-exchange chromatography, and affinity chromatography. Normal-phase chromatography involves a polar stationary phase and a non-polar mobile phase. It is ideal for separating water-sensitive compounds, geometric isomers, cis-trans isomers, chiral compounds, and class separations. This method works on the principle of hydrophobic interactions, where more nonpolar materials are retained longer. Reverse-phase chromatography employs a non-polar stationary phase and an aqueous moderate polar mobile phase. It operates based on hydrophobic interactions, separating compounds according to their polarity levels. This technique is versatile for analyzing non-polar, polar, ionizable, and ionic molecules. Size-exclusion chromatography separates particles based on molecular size. Larger molecules are quickly eluted through the column, while smaller molecules penetrate the porous packing particles and emerge later. This method is useful in determining the tertiary and quaternary structure of proteins, amino acids, and polysaccharides by providing insight into their molecular weights. Ion-exchange chromatography involves retention based on interactions between solute ions and charged sites bound to the stationary phase. It is utilized for purifying water, ligand, and ion-exchange chromatography of proteins, high-pH anion-exchange chromatography of carbohydrates, and oligosaccharides. Affinity chromatography separates compounds through reversible interactions with specific ligands. The basic HPLC system consists of a pump, injector, column, detector, and integrator or acquisition display system. The HPLC system relies on a glass reservoir containing the stationary phase, which interacts with the mobile phase or solvent. This mixture typically consists of polar and non-polar components whose concentrations are adjusted based on the sample composition. The pump draws the mobile phase from the reservoir and forces it through the column and detector, generating pressures up to 42000 kPa (about 6000 psi) depending on factors like column dimensions, stationary phase particle size, flow rate, and mobile phase composition. The injector can be either manual or automated, providing an injection volume of 0.1-100 mL with high reproducibility under pressure up to 4000 psi. Columns are typically made of polished stainless steel, ranging from 50 to 300 mm in length and 2 to 5 mm in internal diameter. They are usually filled with a stationary phase having a particle size of 3–10 µm. To ensure optimal performance, the mobile phase and column temperatures should be kept constant during analysis. The detector at the column's end detects analytes as they elute from the chromatographic column. Common detectors include UV-spectroscopy, fluorescence, mass-spectrometric, and electrochemical detectors. Detector signals are processed by chart recorders or electronic integrators that can store and reprocess data. HPLC provides information on resolution, identification, and quantification of compounds, as well as chemical separation and purification. Its applications include pharmaceutical quality control, detection of pollutants in drinking water, and analysis of steroids in biological samples. However, HPLC has limitations, including high costs due to the need for expensive organics, potential low sensitivity or undetectability of certain compounds, and complexity. Additionally, volatile substances are often better separated using gas chromatography. High Performance Liquid Chromatography (HPLC) is a technique used to separate, identify, and quantify the components of a mixture. It involves the transport of individual sample components along a column by a liquid moved with gravity, which separates and collects the components at the exit of the column. The principle of HPLC is based on the distribution of analytes between the mobile and stationary phases. The type of HPLC used depends on the properties of the analyte, with four primary types: Normal Phase HPLC for separating phospholipid classes, Reverse Phase HPLC for compounds with hydrophobic moieties, Size-exclusion HPLC/molecular sieve chromatography for large molecules/macromolecular complexes, and Ion-exchange HPLC for separating ions and polar molecules. HPLC can be run in different ways, including isocratic and gradient modes. Isocratic mode uses a consistent mobile phase mixture over the testing time, while gradient mode changes the compounding of the eluent mixture during measurement, affecting analyte retention. The use of solvents such as aqueous solvent (water) and organic solvent (methanol, acetonitrile, and propanol), as well as acids like acetic acid and trifluoroacetic acid, can improve chromatographic peak shape. Detectors such as PDA and UV absorbance detectors provide sensitivity for light-absorbing compounds. The advantages of HPLC include its ability to test raw materials and finished products, reverse engineer formulations, solve product failure problems, detect contaminants and impurities, perform competitor product analysis, determine product stability and shelf life, and enable testing with small sample sizes. In identifying components, chromatographers utilise RF values, which represent the ratio between a component's movement distance to that of the solvent front in chromatography. This characteristic helps in distinguishing between different compounds. There are two main types of chromatography: liquid and gas chromatography. The process involves observing when an analyte emerges from the column, marking the endpoint of the separation.