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Commentary

Short Note on High Performance Liquid Chromatography (HPLC)

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DESCRIPTION

High Performance Liquid Chromatography (HPLC) is a method in analytical chemistry used to isolate the components in a mixture and to identify and quantify each component. It was first discovered as an analytical technique in the initial twentieth century and was used to isolate coloured compounds in a mixture. The word "chromatography" refers to "colour writing." He was the botanist M. S. Tswett who invented this technique in around 1900 to study leaf pigments (mainly chlorophyll). He isolated the pigments based on their interaction with a stationary phase. In 1906, Tswett invited two fundamental documents describing the various characteristics of liquid-adsorption chromatography in detail. He also pointed out that, in spite of its name, other substances can also be separated by chromatography. Recently high performance liquid chromatography has developed from the separation efficiency, versatility, and speed has been improved significantly. The molecular species subjected to separation exist in a sample that is composed of analytes and matrix. The analytes are the molecular species of concentration, and the matrix is the rest of the components in the sample.

For chromatographic separation, the sample is introduced along with a mobile phase that passes through a stationary phase. The mobile phase is a moving liquid and is characterized by its composition, solubility, UV transparency, viscosity, and miscibility with other solvents. A stationary phase is a stationary complex that can be a stagnant bulk liquid, a liquid layer on the solid phase, or an interfacial layer between liquid and solid. In HPLC, the stationary phase is usually in the form of a column filled with very small porous particles, and the liquid mobile phase is moved through the column by a pump. The advance of HPLC is mainly the development of the new columns, which need new particles, new stationary phases (particle coatings), and improved procedures for filling the column. The pump plays an important role to force a liquid (mobile phase) through at a specific flow rate (milliliters per minute). The injector aids to introduce the liquid sample into the flow stream of the mobile phase. The column is present in the center of instrument and vital component of HPLC, and the column's stationary phase divides the sample components of concentration using various physical and chemical parameters. In order to detect the individual molecules that elute from the column the detector is used. The computer usually functions as the data system, and the computer not only controls all the elements of the HPLC instrument, but it also receives the signal from the detector that used to determine the retention time, the sample components, and quantitative analysis. Different separation mechanisms were used based on the different properties of the stationary phase of the column. There are two main types include normal phase chromatography, reverse phase chromatography, ion exchange, size exclusion chromatography, and affinity chromatography. In this method, the columns are packed with polar inorganic particles and a nonpolar mobile phase is used to run through the stationary phase. Normal phase chromatography is mostly used for the refining of crude samples, the separation of polar samples, or analytical separations by thin layer chromatography.

Reverse Phase (RP) chromatography

In Reverse Phase (RP) chromatography, the stationary phase has a hydrophobic appeal while the mobile phase has a polar character. This process is the opposite of normal-phase chromatography. The interactions in RP-HPLC are measured to be hydrophobic forces, and these forces are initiated by the energies resulting from the disturbance of the dipolar structure of the solvent. The separation is usually depends on the partition of the analytic between the stationary phase and the mobile phase. The solute molecules are in balance between the partially polar mobile phase and the hydrophobic stationary phase. The more hydrophobic molecules have a longer retention time, while the ionized organic compounds, inorganic ions, and polar metal molecules show little or no retention time.

Ion exchange chromatography

The ion exchange instrument is based on electrostatic interactions among hydrated ions from a sample and oppositely charged functional groups in the stationary phase. There are two kinds of mechanisms are used for the separation in one process, the elution consumes a mobile phase that contains opposing

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ions that would replace the analytic ions and drive them off the column, another process is to add a complexing reagent in the mobile phase and modify the sample species from their initial form. This alteration to the molecules will lead them to elution. In addition to the exchange of ions, ion-exchange stationary phases are able to maintain specific neutral molecules. This procedure is related to the retention of specific ions based on the formation of complexes, and specific ions such as transition metals can be retained on a cation-exchange resin.