Glossary

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Adjusted Retention Time (t_R') – The adjusted retention time (t_R') adjusts for the column void volume or the t_0 time: $t_R' = t_R - t_0$

Analyte - The compound of interest to be analyzed.

Analytical Column - An HPLC column used for separation of a mixture of components and used for both qualitative and quantitative analysis. Typical column dimensions are 50–250 mm × 4.6 mm. The column is usually made of stainless steel.

Back Pressure – Pressure displayed in the software resulting from resistance downstream from the pump. Most of this resistance is caused by the small particles in the HPLC column.

Bar – A unit of pressure measurement in HPLC equal to 1 atmosphere ~15 lb/in.^{2} (psi).

Baseline – The baseline represents the signal from the detector when only the mobile phase is passing through.

Buffer – A solution that maintains a constant pH by resisting changes in pH from addition of small amounts of acids and bases.

C18 – octadecylsilane. A stationary phase consisting of 18 carbon atoms bonded together. The most common stationary phase used in Reversed Phase HPLC.

Capacity Factor (k') – A superseded term used to described the degree of retention. Now replaced by the retention factor (k).

Carrier Gas – The mobile phase in gas chromatography (GC).

Check Valve – A device inserted in the flow path of the mobile phase that allows flow in only one direction. Used on the inlet and outlet sides of an HPLC pump.

Chromatogram - A plot of detector signal output versus time. A component in the mixture is represented by a peak.

Chromatograph – The hardware / instrument used for a chromatographic separation.

Column Packing – The solid material (usually a porous solid) with or without a chemically interactive surface (stationary phase) placed inside of the HPLC.

Data Acquisition Rate – A term referring to the rate of sampling of a detector output. This is the rate at which the analog signal is converted to a digital signal. To characterize a chromatographic peak at least 20–30 data points must be collected across the peak to sufficiently define the peak. The data acquisition rate, usually measured in hertz, defines how many data points per second are collected while the peak is moving through the detector. Modern detectors have data rates up to 80 Hz; also known as data rate and sampling rate.

Degasser – A part of the HPLC hardware which can execute degassing of the mobile phase before the liquid is drawn into the pump.

Degassing - The process of removing dissolved gas from the mobile phase before or during use. Degassing can be carried out by heating the solvent, helium sparging, or using vacuum (in a vacuum flask) or using an on-line degasser.

Dwell Time - The time a component takes to move from the mixing point of the mobile phases to the start of the column.

Dwell Volume - The volume between the mixing point of the mobile phases and the head of an HPLC column. Important in gradient elution or in isocratic elution situations when changes in solvent composition are made so that the column experiences the composition change in the shortest possible time. Low-pressure mixing systems generally have larger dwell volumes than high-pressure mixing systems.

Efficiency (N) - A measure typically determined by the number of theoretical plates (N) calculated from the equation $N = 16 (t_R/w_b)2$, where w_b is the peak width measured at the base. If the peak width is measured at half height, the following equation is used $N = 5.545 (t_R/w_{1/2})$. The plate height (H) or HETP is determined by $H = L/N$.

Eluent - The mobile phase used to perform a separation.

Elution - Analytes carried along by the mobile phase and eventually exiting out the end of the column.

Endcapping - A technique used to remove / reduce silica gel silanol groups that may remain after reaction with a large silylating agent such as octadecyltrichlorosilane (C18). The column is said to be endcapped when a small silylating reagent (such as trimethyl-chlorosilane or dichlorodimethylsilane) is used to bond residual silanol groups on a silica-gel–based packing surface. Endcapping is often used with reversed-phase packings to minimize undesirable adsorption of basic, ionizable, and ionic compounds.

Flow Rate - The volumetric rate of flow of a mobile phase through an HPLC column. Typical flow rates are 1–2 mL/min for a conventional 4.6-mm i.d. HPLC column.

Frit - The porous element / filter at either end of a column that prevents the column packing extruding out the ends of the column. Frits can be stainless steel or other inert metal or plastic such as porous PTFE or polypropylene. The frit porosity must be less than the smallest particle in the HPLC column to prevent particles passing through the frit.

Gradient - A process to change solvent strength as a function of time (normally solvent strength increases) thereby eluting progressively more highly retained analytes. Typically gradients can be binary, ternary, and quaternary solvent mixtures in which solvents are blended to achieve the proper strength.

Gradient Elution - Technique for decreasing separation time by increasing the mobile-phase strength during an analysis. Also known as solvent programming. Gradients can be continuous or stepwise. Binary, ternary, and quaternary solvent gradients have been used routinely in HPLC.

Guard Column - A small column located between the injector and the analytical column. It protects the analytical column from contamination by sample particulates and strongly retained species. The guard column usually is packed with the same packing material as that in the analytical column and is often of the same inner diameter. It is much shorter, costs less, and usually is discarded when it becomes contaminated.

High Performance Liquid Chromatography (HPLC) - The modern form of liquid phase chromatography technique that uses small particles and high pressures and sophisticated hardware.

High Pressure Mixing - A configuration of a gradient HPLC system where the solvents are mixed on the high pressure side of multiple pumps (usually 2, binary). Such a system offers a lower dwell volume than low pressure mixing systems where the solvents are mixed by proportioning valves before a single pump.

HPLC Hardware – The actual HPLC system is often referred to by this term.

Hydrophilic - Greek word for water loving.

Hydrophobic - Greek word for water hating.

In-line Filter - A filter that prevents particulate matter from damaging the column. The filter is located between the injector and the column. A filter in this position prevents sample particles from entering the packed bed or column inlet frit.

Injection Solvent - Solvent used to inject sample into an HPLC column. The solvent should be of equal or lower strength than the mobile phase to prevent premature movement down the column due to the presence of a stronger solvent.

k' - An outdated term that has been replaced by the IUPAC-approved term, retention factor (*k*)

Mobile Phase - The liquid that moves a component through the column.

Normal-phase HPLC - A mode of chromatography performed when the stationary phase is more hydrophilic than the mobile phase. A typical normal-phase system would be adsorption chromatography on silica gel or alumina using mixtures of less hydrophilic eluents such as hexane–diethyl ether as a mobile phase.

Octadecylsilane - The most popular reversed phase in HPLC. Octadecylsilane also called C18 or ODS) phases are bonded to silica or polymeric packings.

Organic Modifier - Water-miscible organic solvent added to an aqueous mobile phase to change the degree of hydrophobicity in reversed-phase HPLC. Common organic modifiers are acetonitrile, methanol, isopropanol, and tetrahydrofuran.

Packing - The solid support used in an HPLC column. Most modern analytical HPLC packings are less than 10µm in average diameter and 5µm is the most popular.

Particle Size - The average particle size of the packing in the HPLC column. The standard particle size is 5µm but for UHPLC, sub two micron (STM) particle size columns are often used.

Partition Chromatography - Separation process in which one of two liquid phases is held stationary on a solid support (stationary phase) while the other is allowed to flow freely down the column (mobile phase). Solutes partition themselves between the two phases based on their individual partition coefficients.

Peak - The graphical representation of an analyte compound as it elutes from a column through a detector.

Peak Area - The area of a chromatographic peak as measured from the baseline from the left side of the peak drawn to the baseline on the right side of the peak. The peak area increases as the concentration of the component in the original mixture increases.

Peak Height - The height of a chromatographic peak as measured from the baseline to the peak apex. The peak height increases as the concentration of the component in the original mixture increases.

Peak Width – The width of the peak measured in time (minutes).

Residual Silanols - The silanol (–Si–OH) groups that remain on the surface of a packing after chemically bonding a phase onto its surface. These silanol groups, which may be present in very small pores, may be inaccessible to a reacting bulky organosilane such as octadecydimethyl-chlorosilane) but may be accessible to small polar compounds. Often they are removed by endcapping with a small organosilane such as trimethylchlorosilane.

Resolution - A measure of the degree of separation of two chromatographic peaks. $R = (t_{RA} - t_{RB})/0.5[(w_A + w_B)]$, where t_{RA} and t_{RB} are the retention times of the two peaks and w_A and w_B are the baseline widths of the two peaks.

Retention Factor (k) – The time that the sample component interacts with the stationary phase relative to the time it resides in the mobile phase. It is calculated from the adjusted retention time divided by the unretained time or holdup time; $k = (t_R - t_0)/t_0$, where t_R is retention time for the sample peak and t_0 is the retention time for an unretained peak. (Formerly, k' was used, and it was called the capacity factor or the capacity ratio.)

Retention Time (t_R) - The time taken from the injection of the mixture into the system to the exit from the system.

Reversed-phase Chromatography - The most frequently used mode in HPLC. Uses low-polarity (hydrophobic) packings such as octadecyl- or octylsilane phases bonded to silica or neutral polymeric beads. The mobile phase usually is water or water-miscible organic solvents such as methanol or acetonitrile (organic modifier). Elution usually occurs based on the relative hydrophobicity of the analytes. The more hydrophobic, the stronger the retention. The greater the water solubility (more hydrophilic) of the analyte, the less it is retained. The technique has many variations in which various mobile-phase additives impart a different selectivity. For example, adding a buffer and a tetraalkylammonium salt to an anion analysis would allow ion-pairing to occur and generate separations that rival those of ion-exchange chromatography. More than 90% of HPLC analysts use reversed-phase chromatography.

Selectivity Factor / Separation Factor (α) - A ratio of the two retention factors of two adjacent peaks. The relative retention; $\alpha = t_{R2}'/t_{R1}' = k_2/k_1$, where t_{R2}' and t_{R1}' are the adjusted retention times of peaks 2 and 1, respectively, and k_2 and k_1 are the corresponding retention factors.

Stationary Phase - The material in the column that causes retention of the components in the mixture. The more interaction with the stationary phase, the longer the retention time and the higher the retention factor (k).

Support - Refers to solid particles in the HPLC column. A support can be naked, coated, or have a chemically bonded phase in HPLC. Normally the solid support doesn't contribute to the chromatographic process.

Theoretical Plate (N) - Relates chromatographic separation to the theory of distillation. Plates are calculated as $N = 16\ (t_R/w_b)2$ where w_b is the width at the peak base and t_R is the retention time.

UHPLC - Ultra High Pressure Liquid Chromatography. This often refers to any separation performed over the pressures of conventional pumps (400 Bar).

Unretained Time (t_0) - The elution time of an unretained peak. Also called the dead time and the holdup time (t_M) and the void time. The void volume is determined by multiplying the void time by the flow rate.

Void - The formation of a space or gap, usually at the head of the column, caused by a settling of the column packing. A void in the column leads to decreased efficiency and loss of resolution.

Void Volume (V_M) - The total volume of mobile phase in the column. This volume does not include the volume occupied by the packing material (stationary phase and support material).