High Pressure Liquid Chromatography

Introduction

High Pressure Liquid Chromatography (HPLC) is used in analytical chemistry or biochemistry to separate chemical compounds in mixtures for analysis or purification.

Components in a mixture are separated on a column packed with silica-based particles (referred to as stationary phase) by pumping a solvent (referred to as mobile phase) through the column. Depending on the unique affinity of each component (referred to as the analyte) between the mobile phase and the stationary phase, each analyte migrates along the column at different speeds and emerges from the column at different times, thus establishing a separation of the mixture. Analytes with higher affinity for the mobile phase migrate faster down the column, whereas those with higher affinity for the stationary phase migrate slower. This migration time (referred to as retention time) is unique for each analyte and can be used in its identification. With the appropriate use of a detection method after the column, each analyte can also be quantified for analysis.

Smaller column particle size can improve chromatographic resolution, but increased solvent delivery pressure is needed. Further reduction of column particle size can allow for higher solvent flow rates, reducing analysis time without sacrificing resolution. This is what gives Ultra High Pressure Liquid Chromatography its advantage over other LC techniques.

☑ *Note*

Refer to Syringe Pump Application Note AN4 Ultra HPLC System Configuration for additional information about Ultra High Pressure Liquid Chromatography applications.

Basic Types of HPLC Column Chemistries:
- Normal Phase Chromatography, e.g. Silica particles
- Reverse Phase Chromatography, e.g. C-18 coated Silica

Basic Types of Mobile-Phase Delivery Systems:
- Isocratic – constant solvent mixture, e.g. 10% Water in Methanol throughout the entire separation run.
- Gradient – time-varying solvent mixture, e.g. starting a run with pure Hexane followed by a slow increase of Ethyl Acetate concentration until pure Ethyl Acetate at the end.

Basic components of an HPLC system are a solvent delivery pump, sample injection port, column, and detector. HPLC performance requires instruments with characteristics not found in typical LC systems. Solvent delivery must be pulseless and accurately calibrated at flow rates in the 1 mL/minute to 100 μL/minute range. Syringe pumps are generally acknowledged to be superior to piston pumps for HPLC applications where low, stable flow rates are required.

Teledyne ISCO Syringe Pumps are excellent HPLC pumps for these high performance applications.
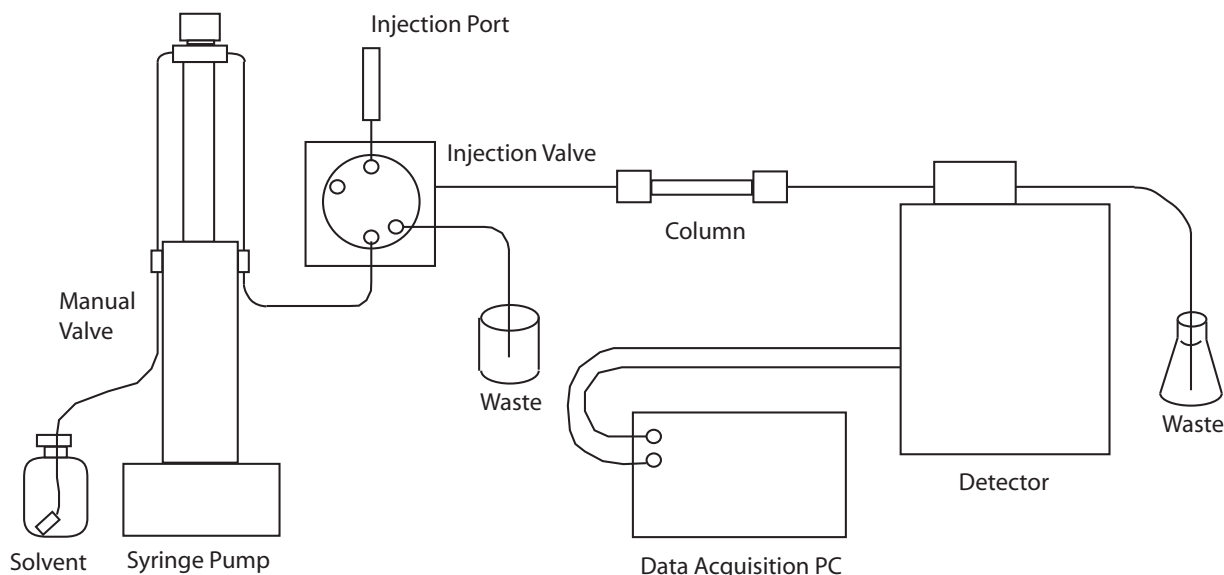

Figure 1: Isocratic Solvent Delivery System

Theory

The Van Deemter equation is the key to understanding the fundamentals of chromatography. According to the Van Deemter equation:

$$H = A + B/\mu + C\mu$$

where: H is the column's plate height, and μ is the mobile phase linear flow rate. A, B, and C are constants. A smaller plate height H indicates higher separation resolution.[2]

The constant A is the eddy diffusion term. Eddy diffusion results from multiple flow paths in the column and is independent of mobile phase flow rate. Due to the packing particles, analyte molecules can follow multiple pathways of differing path lengths. These multiple pathways of differing length spread the analyte molecules apart and cause peak broadening. The A term depends on the compactness of the stationary phase. Voids in the stationary phase contribute to peak broadening due to channeling. In contrast, smaller packing particles offer smaller differences in path length, thus reducing peak broadening.

B is the longitudinal diffusion coefficient. It depends on the diffusion coefficient of the analyte molecules in the mobile phase. Faster mobile phase flow rates reduce resident time, and shorter resident time of the analyte molecules in the column reduces the effects of longitudinal diffusion. This reduction contributes to better separation efficiencies since the analyte molecules have less opportunity to spread out through diffusion, thus explaining the 1/v factor.

C is the analyte mass transfer coefficient. It depends on the time needed for the analyte molecules to equilibrate between the mobile and stationary phases. If this equilibration is too slow, then some of the analyte molecules, which did not have enough time to bond to the stationary phase, will flow down the column with the mobile phase; whereas, the other molecules, which did not have enough time to detach from the stationary phase, are left behind. Higher mobile phase flow rates will contribute to the spreading out of the analyte molecules, thus explaining the v factor. Smaller stationary phase particles are expected to have shorter equilibration time.

As stated above, smaller stationary phase particles or beads should contribute to smaller A and C values. The A term will contribute less to H and allow for higher resolution. As the C term becomes less significant to the value of H, increasing mobile phase flow rates will not sacrifice separation performance as much. This would allow for faster separations with the same resolution. Even though smaller column beads may not directly affect the B value, the higher flow rates reduce its contribution to H. Smaller column beads would allow for faster separations with higher resolution.[3]

Smaller beads will pack with smaller interstitial spaces, thus reducing the A value, but offer higher resistance to solvent flow. In turn, this requires higher solvent pressures. With higher optimal separation flow rates, column back pressure increases as an inverse cube of the stationary phase particle size; i.e., $P\alpha 1/d^3$.[4] Typically, pressures of 1,000 psi are required for HPLC with 5 μm column beads, whereas 20,000 psi or above is required for Ultra-HPLC (UHPLC) with 1.7μm beads.[1,4]
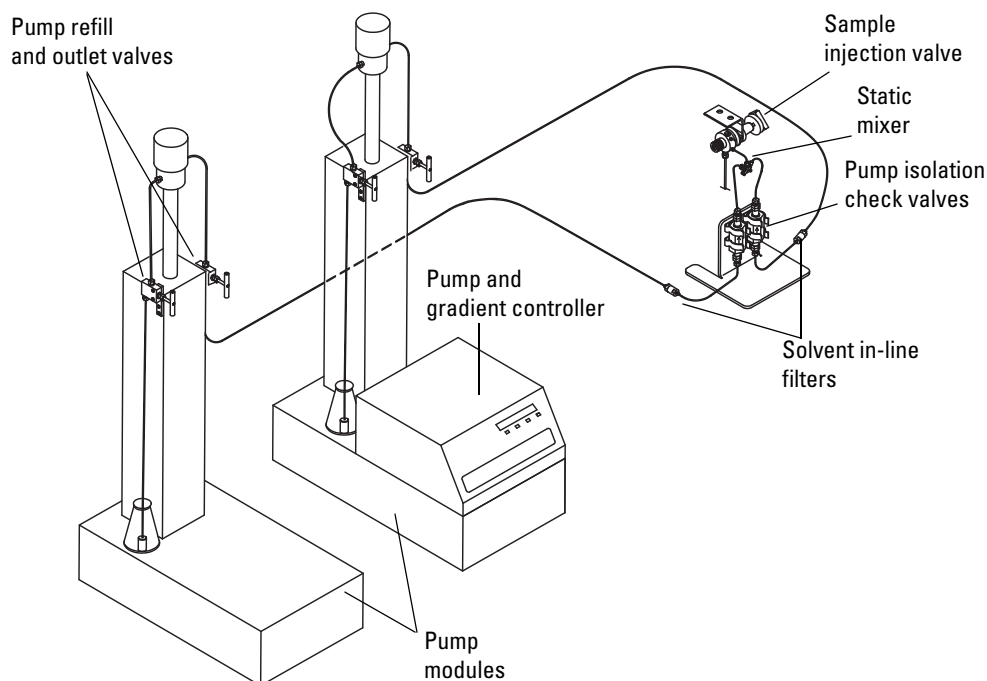

Figure 2: Gradient Solvent Delivery System

HPLC PUMPS

Reciprocating pumps are usually available with single or dual pistons. Piston swept volumes are typically small, around 100 μL. Small volumes require frequent refills, creating periodic pressure drops. Dual piston pumps produce smoother flows than single piston pumps, but are not always ideal.

Syringe Pumps are most suitable for high performance applications requiring high pressures with pulseless and accurate flow. Syringe pumps have much larger cylinder volumes, up to 1 L, and usually do not have to refill during runs. Therefore, baselines are much more stable, without periodic pressure drops.

The ideal pumps should be able to operate in isocratic or gradient mode.

Table 1: Commonly Recommended HPLC Pumps

	1000x	500x	260x	65x
Flow Range (mL/min)	0.100 - 408	0.001 - 204	0.001 - 107	0.00001 - 25
Pressure Range (psi)	0 - 2,000	0 - 5,000	0 - 9,500	0 - 20,000

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Product model names have been updated in this
document to reflect current pump offerings.

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