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HPLC Basics: Background & Overview



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Beyond HPLC: The Pros and Cons of 5 Preparative Liquid Chromatographic Techniques



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# HPLC Basics: Background & Overview

igh-performance liquid chromatography (HPLC) is a popular and well-established technique used in analytical chemistry. With its pumps, columns, solvents, and detectors, it enables highly sensitive analysis, measurement, or isolation of target compounds. Advances in chromatography have established HPLC as the recognized and accepted standard for purification across several scientific and industrial environments, including research and pharmaceutical settings.<sup>1</sup>

# **HPLC Mechanics**

HPLC uses complex fluid dynamics to separate components in a heterogeneous mixture by various properties which include size, polarity, electric charge, and the ability to bind an antibody. The mixture is first dissolved in a mobile phase, which can be a blend of polar and non-polar solvents. This fluid is then forced through a stationary phase (comprising a column containing either a solid or liquid) which separates incoming materials based on particle size and the selective adhesion of molecules to its surface (adsorption). These two factors cause elements of the mixture to travel at different speeds. Most common HPLC systems have a solid stationary phase made up of spherical silica beads.<sup>2</sup> These beads can be of various sizes, offering different surface area and pore sizes for reaction mixtures.

HPLC separations are categorized based on the polarity of the mobile and stationary phases. When the stationary phase is more polar than the mobile phase (e.g., acetonitrile as the mobile phase and a silica column as the stationary phase), it is classified as normal-phase HPLC. Alternatively, when the stationary phase is more polar (e.g., water as the mobile phase and a pentafluorophenyl column as the stationary phase), the process can be identified as reversed-phase HPLC. Column capacities are determined by column length and inner diameter. Longer columns with wide inner diameters allow more fluid to pass through.3 HPLC systems utilize pressure to process samples, and pressures are considerably higher when using thinner columns, smaller volumes, or less tubing. Fraction collectors are necessary when the retention of analyzed samples is required for future work. Otherwise, fractions are discarded following signal detection.



# **HPLC Applications**

Several commonly used products such as soaps, mouthwash, shampoo, and toothpaste are subjected to HPLC analysis for quality assessment prior to appearing on the shelf.<sup>4</sup> The United States Pharmacopeia (USP) has guidelines for HPLC columns used for the production of drugs (prescription and over-thecounter) and dietary supplements.<sup>5</sup> The USP also has established standards for food ingredients which manufacturers must adhere to by verification through HPLC analysis to comply with the Food Chemicals Codex (FCC). All enforcement in the food, drug, and manufacturing industry is enforced by the Food and Drug Administration (FDA) in the USA and similar authorities elsewhere.

HPLC has developed into a valuable tool used for quality control and producing good yield with reproducible purity in several manufacturing arenas.<sup>6</sup> HPLC is considered the accepted purification standard in various industries. There are other techniques that can be used in conjunction with a basic system, including HPLC-MS (mass spectrometry) and HPLC-NMR (nuclear magnetic resonance). More information on HPLC's many applications can be found in Article 3: "<u>A Whole New World:</u> <u>Applications of HPLC</u>".

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# Against All Odds: Analytical vs. **Preparative HPLC**

PLC is a valuable tool used in several fields for technical analysis and compound purification. HPLC systems can be classified by their ability to separate particles using polarity, size or affinity. However, all categories of HPLC can be sorted into two broad classes that serve distinct functions: analytical and preparative.

HPLC is used to either identify or purify samples. Analytical HPLC is used to detect and quantify a specific compound. This is commonly employed as a quality control measure for purity analysis. Preparative HPLC isolates and purifies target constituents from a mixture. Drug development pipelines rely heavily on preparative HPLC for raw material purification.<sup>1</sup> The two modes do not have to be mutually exclusive, but most systems are set up to fulfill one function.<sup>2,3</sup>

## Similarities

Both analytical and preparative modes have a general setup consisting of an injection system, a degasser, a solvent manager, a column chamber, a pump, and a detector. The injection system introduces samples either manually or automatically. A degasser removes dissolved air from the liquid phase before it enters the HPLC. The solvent manager houses and distributes the polar and non-polar solvents used as the mobile phase for dissolving and transporting samples to the column chamber.

The pumping system regulates the pressure and flow of mixtures transported to the column chamber. HPLC columns are tubes filled with the stationary phase component responsible for separating mixture elements based on polarity, surface chemistry properties, and size. The detector registers the signal of the segregated particles as they move through the columns at different speeds. This signal is then plotted visually on a chromatogram with intensity on the y-axis and time on the x-axis. The visual patterns displaying various components of a separated mixture are called peaks, which are used for either identification or isolation.

## Differences

The differences between analytical and preparative HPLC are related to their functions. The main purpose of analytical HPLC is to identify or quantify unknown mixtures, whereas preparative



HPLC isolates compounds with high purity and yield. With these considerations in mind, the following equipment and functional differences between the two modes exist.

# **Analytical HPLC**

Analytical HPLC requires minute volumes of sample for analysis which are typically discarded after processing. This application does not require a fraction collector. Analytical systems have shorter and less tubing in general. They also have thinner and shorter columns and thus have considerably higher system pressures.

## **Preparative HPLC**

Preparative HPLC systems are commonly used to refine samples for subsequent usage and therefore require a fraction collector. They process significantly larger volumes requiring longer and wider tubes with more complex setups.<sup>4</sup> Larger volumes of solvents are also needed for the mobile phase. Finally, the columns used are longer and wider, correlating with lower system pressures.5

HPLC continues to be an important tool across research laboratories, the biopharmaceutical industry, and manufacturing operations. The core differences between analytical and preparative HPLC are attributed to the two central tenets of chromatography: identification and isolation. Although not the only option for preparative LC, HPLC is highly popular and widely used by academic, industrial, and government scientists alike.

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Good preparative liquid chromatography (LC) applications are judged on purity, recovery, and runtime. Each of these LC techniques has its advantages and disadvantages.

		Pros	Cons
<b>F</b> Au fr	Flash A rapid form of preparative LC using low-pressure apparatus or quick and straightforward eparation and purification	<ul> <li>System is easy to maintain and clean</li> <li>Fast method development</li> </ul>	<ul> <li>Low efficiency and separation power</li> <li>Prepacked columns can be reused only a few times</li> </ul>
	Centrifugal Partition Chromatography (CPC) Jses two immiscible liquid olvents as the mobile and tationary phases for simple to complex purifications	<ul> <li>High injection capacity and scalability</li> <li>Not necessary to denature samples or replace columns</li> </ul>	<ul> <li>Longer method development</li> <li>Electrical device in lieu of 'inert' column</li> </ul>
	Supercritical Fluid Chromatography (SFC) Uses supercritical fluids such as trarbon dioxide as the mobile shase, which is heated before it eaches the column	<ul> <li>Cheaper mobile phase and less solvent used</li> <li>Faster drying times, faster run times</li> </ul>	<ul> <li>Requires specialized personnel for operation</li> <li>Incompatible for separating several compounds</li> </ul>
	<b>Simulated Moving</b> <b>Bed (SMB)</b> Continuous process using HPLC columns; olid phase (silica)	<ul> <li>High productivity and yield</li> <li>Lower production costs; fewer solvents needed to elute compounds</li> </ul>	<ul> <li>Costly to on-board and maintain</li> <li>Limited to separating binary mixtures</li> </ul>
	True Moving Bed (TMB) Continuous process using CPC columns	<ul> <li>No solid stationary-phase maintenance required</li> <li>High flow rate, yield, and productivity with low reagent consumption</li> </ul>	<ul> <li>Complex method development process</li> <li>Requires specialized personnel for operation</li> </ul>

# A Whole New World: Applications of HPLC

PLC offers convenient and reliable separation or quantification of complex mixtures. This revolutionary technique is no longer limited to only specialists in analytical chemistry. Innovations in column separation, ultra high-pressure liquid chromatography (UHPLC), and pairing with applications such as mass spectrometry (HPLC-MS) has driven HPLC to be aggressively incorporated in different fields of research and discovery, separation science, and manufacturing industries.<sup>1</sup> The versatility and customization afforded by modifying columns or mobile phase solvents have allowed HPLC to be commonly used for products across several different markets.<sup>2</sup>

# Life Sciences

Novices in academia and research are routinely introduced to HPLC as a standard laboratory technique. An increasing number of graduate students and research scientists have begun adopting this powerful application for investigations, and this growth is reflected in the prevalence of HPLC usage in published journal articles. Despite the complexity of HPLC modules and operating guidelines, HPLC-automated data report generation after merely loading samples makes the learning curve simple for anyone with a proper background in chromatographic principles and scientific judgment. Scientists with experience in HPLC applications consider it a definite asset when pursuing industries outside of academic research.

## **Pharmaceutical Industry**

Pharmaceutical laboratories in the US routinely use HPLC to assess drug products for impurities and degradants using standardized protocols to meet United States Pharmacopeia (USP) guidelines.<sup>3</sup> USP-established HPLC standards are used by regulatory agencies such as the Food and Drug Administration (FDA) to enforce product quality by verifying consistency and purity. Another typical application for HPLC is evaluating drug active pharmaceutical ingredient (API) three-month stability to determine shelf life. HPLC has boosted drug discovery since its incorporation into the field, separating and analyzing novel medical candidate agents post-chemical synthesis.

# Food and Manufacturing

FDA regulations necessitate highly precise and consistent



qualitative analysis when testing for pesticide residue and other contaminants. HPLC is well suited for production-scale industries involved in extracting vitamins, fish oils, and different flavors.<sup>4</sup> Since HPLC operations are automated, flexible, and customizable with a series of detectors, it is ideal for FDA analysis and monitoring. Preparative HPLC is also commonly used for the production of a wide range of polymers that are commonly used daily.

## **Clinical Settings**

HPLC combines analytical chemistry with biology to offer novel and personalized solutions for pressing problems such as biomarker analysis for cancer and clinical diagnoses for other medical disorders. Isolating structure-specific proteins to enable a targeted proteomics approach is gaining significant attention in several medical and pharmaceutical niches. The rise of monoclonal antibodies (mAbs) in research projects and therapeutics in recent times has driven mAb sales to the same heights as many top-selling drugs.<sup>5</sup> HPLC is also regularly used to test Olympic athletes for usage of banned substances.<sup>6</sup>

Everyday products that we interact with have been subjected to chromatography over the course of production. From dyed fabrics used to make our clothes to food items such as milk and cereal, the quality analysis of these products all involve chromatographic analysis. HPLC continues to be incorporated into a variety of pharmaceutical and manufacturing settings and has grown into the industry standard choice for separation. HPLC is commonplace in research and applied scientific fields, and further innovations in HPLC will present users with a plethora of applications in the coming years.

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