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EDITION

How to Buy HPLC Technology

A guide to all the key considerations to help you find the best HPLC technology for your lab's needs

★★★★★

eBook

SelectScience® 20 YEARS

Expert Insight to Help You Make the Best Choice

In this HPLC buying guide, discover the key factors and application considerations when looking to buy new HPLC technology. Plus, read impartial reviews to gain insights from industry leading professionals.

Contents

1. Introduction to High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is one of the most powerful analytical tools available today and works by separating individual compounds based on their polarity. HPLC separation is distinct from liquid chromatography (LC), with solvent passing through the column under high pressure, making separations much faster and, in turn, reducing solvent consumption.

Figure 1 : Discover how scientists are bridging the gap between HPLC and UPLC with the groundbreaking ACQUITY Arc System f rom Waters Corporation

The higher pressure allows for the use of a smaller particle size for the column packing material. Columns with smaller particles generate sharper peaks with increased resolution, due to reduced diffusion distances for analytes.

By comparison, ultra-high-performance liquid chromatography (UHPLC) uses an even smaller particle size than HPLC for the packing material. This helps to produce increased resolution and sensitivity. UHPLC requires even higher pressures than HPLC, which means systems capable of handling

TOP TIP

When choosing your HPLC column, consider seeking advice from chromatography experts such as Restek, who have helped analysts around the world with the innovative tools they need.

Figure 2 : Find out how Adam Rosebrock is advancing metabolomics with adaptive uses of HPLC and mass spectrometry to determine biochemical pathways

this increased pressure can be relatively expensive. It is worth noting that methods developed for HPLC do not readily transfer to UHPLC and vice versa. However, it can be noted that some systems have been developed to bridge the gap between the two, as shown in this video interview on ACQUITY Arc System from Waters Corporation.

> **"Methods developed for HPLC do not readily transfer to UHPLC."**

In selecting the most appropriate column, a number of considerations must be taken into account. These are summarized in Table 1, and the sections to follow. For insights into how one laboratory has adapted its HPLC analysis to advance metabolomics, watch this exclusive interview with Dr. Adam Rosebrock on The Scientists' Channel.

Consider Your Application	Stationary Phase	Column Dimensions
• Analyte Molecular Weight • Analyte Solubility	• Column Chemistry • Separation Mode • Particle Size • Retention Capacity	• Internal Diameter • Length

Table 1. Overview of HPLC Column Selection

2. Sample Preparation for HPLC

A sample needs to be prepared so that it can be injected onto an HPLC column.

Effective sample preparation is an important step to ensuring reproducible and specific results, with samples needing to be dissolved in the relevant solvent and free of particles which may cause interference during detection or may block the column.

The benefits of good sample preparation include:

- No blockage of your HPLC column
- Higher accuracy
- Less background noise
- Higher analysis sensitivity
- Less false-positive peaks

Sample preparation for HPLC consists of four steps, with the benefits of optimization described below:

1. Preparation of solvent

With up to 80% of problems with HPLC being linked to the quality of water, it is essential you choose a reliable source for your ultrapure water. You can learn more about water quality and its impact on your HPLC in this application note.

Choosing a reliable water system such as arium® pro VF ultrapure water system is key.

2. Preparation of standards

For any quantitative HPLC experiment, all prepared standards need to be within defined concentrations. This is reliant on accurate weighing, which is enhanced by the use of an automated system such as the Cubis II balance.

3. Pipetting

The use of accurate electronic pipettes ensures that all standards are prepared to exacting standards.

4. Filtration

Finally, clarification of your sample by filtration is a key step in ensuring sample quality – while the most common method is to use syringe filters. This can be hard, tedious work if you are running large numbers of samples on your HPLC. The Claristep® Filtration System is designed to save you both time and effort, with the ability to filter eight samples at once.

3. Top Tips for Choosing an HPLC Column — from the Experts

Here are a list of top tips, direct from the experts:

• Don't stick with the system default, be prepared to search and ask questions - it is entirely possible that the best HPLC column for your experiment won't yet have been used for your application.

• Look for a phase chemistry that is similar to your analyte. For example, aromatic compounds with multiple rings work well on a biphenyl phase, whereas hydrophobic compounds with alkyl chains are better suited for a C8 or C18 phase.

• Always use the largest particle size you can whilst still achieving resolution and sensitivity. This will be less prone to column blocking and high back pressure.

• Consider the type of detector and sample amount when selecting a column ID. With UV detectors and "large" sample injection volumes (5-10µL or higher,) a 4.6mm ID column is common. For "small" samples (< 5µL) and MS/MS detection, a 2.1mm ID column is preferred.

• Experiment with orthogonal selectivities and not just different brands of the same phase. Screen HILIC phases similar to how you would reversed phases. Not all HILIC columns were created equally.

4. Column Selection Based on the Application

In order to select the correct column, you will have to consider your application — there are a few simple steps to work through, to match your application to a column.

The first of these is to calculate the molecular weight of your analyte. In general, compounds are divided into two groups: low molecular weight, <5000 daltons, and high molecular weight, >5000 daltons. Assigning an analyte to one of these should be relatively easy. Secondly, you'll need to find out if the analyte is soluble in water (polar) or an organic (non-polar) solvent. Lastly, the mode of separation can be determined, to further narrow your column choice.

The process is summarized in Table 2 and works as follows: if your analyte is <5000 g/mol then you should start at the

Molecular Weight	Analyte	Column Dimension
Low MW (<5000 g/mol)	Water Soluble	• Ion Exchange • Reversed Phase • HILIC
	Organic Soluble	• Normal Phase • Reversed Phase • Non-Aqueous Size Exclusion
High MW (>5000 g/mol)	Water Soluble	• Aqueous Size Exclusion • Ion Exchange • Hydrophobic Interaction • Reversed Phase
	Organic Soluble	• Non-Aqueous Size Exclusion

Table 2. Determination of Separation Mode

low molecular weight section; if it is water soluble, move on and select your required separation mode. Further details on this are given later in this guide.

HPLC and UHPLC are both routinely used within analytical research, from pure chromatographic studies to life science research and drug discovery. When the technique is used in drug discovery, for example, it has been shown to significantly improve the success of the process. It can prevent poor drug candidates from progressing through the discovery process by monitoring factors such as metabolic stability and toxic metabolite production. The technique has also been successfully applied to the separation and analysis of challenging samples such as the analysis of petroleum and the environmental impact of oil spills, as seen in Figure 3 with Professor Sunghwan Kim, of Kyungpook National University, South Korea. The usefulness of HPLC and UHPLC can be further enhanced by linking to other systems such as mass spectrometers.

In this video interview with Ivana Gavrilovic, find out how HPLC can be used in sample preparation, during steroid analysis. She discusses how this has been used for drug control in Olympic athletes

There are many different columns on the market that have been designed for specific applications, such as: chirality; enantiomers; vitamins; pesticides; carbohydrates. It is worth investigating if there are already columns available that have been optimized for your application.

Figure 3 : Discover how separation technology is being used for the analysis of petroleum and the impact of oil spills on the natural environment

5. Column Selection Based on the Stationary Phase

Once the application has been identified, choosing the best column for your application is based on two considerations, stationary phase and column dimensions.

The first and most important of these is the type of stationary phase. The factors to be considered in this regard are the column chemistry, the separation mode, the particle size and the retention capacity.

4.1 Column Chemistry Considerations

Columns are usually filled with porous particles coated with a material that interacts with the injected sample. HPLC uses what is called a true stationary phase: column 'chemistries' are bonded tightly to the packing material and do not bleed off.

There are various ways to categorize the types of stationary phase available, but the most widely accepted of these is the USP 'L' system, a list of more than 60 column classifications ordered according to the type of bonded phase (C18, C8...), packing material and particle size. Table 3 shows some of the most common types.

USP	Stationary Phase	Details
L1	C18 - Octadecyl silane	Octadecyl silane bonded to porous silica, 1.5-10 µm
L7	C8 - Octyl silane	Octyl silane bonded to totally porous silica, 1.5-10 µm
L8	NH2 - Aminopropyl silane	Aminopropyl silane bonded to totally porous silica, 3-10 µm
L10	CN - Nitrile group	Nitrile groups bonded to porous silica, 3-10 µm
L11	Phenyl	Phenyl groups bonded to porous silica, 1.5-10 µm
L43	PFP - Pentafluorophenyl	PFP groups bonded to porous silica, 5-10 µm

Table 3. Common USP 'L' Classifications

4.2 Considering the separation mode

The column chemistry selected is also dependent upon the separation mode that you are going to use.

In general, three primary characteristics of chemical compounds can be used to create **HPLC separations:** 1) Polarity, 2) Electrical charge, 3) Molecular size

The following table (Table 4) gives an overview of the most common separation modes, and the primary characteristics associated with each.

Separation Mode	Key Separation Characteristic	Separation is based on	Application
Normal Phase HPLC	Polarity	The polar stationary phases and non-polar mobile phases during chromatography	Separation of analytes with low to intermediate polarity and high solubility in low-polarity solvents
Reversed Phase HPLC	Polarity	Partition equilibrium between stationary and mobile phase	Most organic analytes can be analyzed
Hydrophilic Interaction Chromatography (HILIC)	Polarity	Hydrophilic interaction	Used for the separation of highly polar compounds, which would be unretained in Reversed Phase HPLC
Hydrophobic Interaction Chromatography (HIC)	Polarity	Hydrophobic interaction	Separation of large biomolecules such as proteins
Ion-Exchange Chromatography (IEC or IEX)	Charge	Electrostatic interaction between ionic exchanger and ionic solutes	Typically used for the analysis of biological samples such as proteins, peptides, amino acids, nucleic acids and glycoproteins
Size-Exclusion HPLC (SEC)	Size	A network of pores on the surfacing of the packing materials, which works as a molecular sieve	Usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers

Table 4. Separation Mode Considerations

Figure 4. Raptor™ Biphenyl LC Columns from Restek Corp

4.3 Particle Size Considerations

The next considerations are size, shape and chirality of the particles that make up the column packing material.

The majority of new HPLC methods are performed on spherical shaped or spheroidal (almost spherical) particles. Spherical particles provide higher efficiency, better column stability and lower back-pressures compared to irregularly shaped particles.

Phenyl columns (USP L11) are an alternative to C18 columns and provide a unique selectivity for aromatic compounds through pi-pi interactions. Biphenyl columns, such as the Raptor™ Biphenyl LC columns by Restek (Figure 4), are the next generation of phenyl columns. They offer both aromatic selectivity and an increase in hydrophobic retention. These phenyl phases are of particular interest when analyzing drug compounds and metabolites, such as fentanyl and its analogues.

If all other factors are constant, the smaller the particles are, the more efficient the separation. The major problem with a decrease in particle size, from 10-, 7-, 5-, and 3-micron diameters, is that the backpressure increases exponentially. This roughly means a column using 3-micron particles is about twice as efficient as a 5-micron column, but pressures are three times as high.

Although separation efficiencies can be increased by reducing particle size even more, to below 2 microns, the hardware required becomes more expensive, as it needs to handle the extremely high pressures. UHPLC systems have been designed to overcome these limitations and UHPLC columns can deliver shorter run times, cleaner separations, sharper/taller peaks, and improved detection limits.

Some applications also benefit from end-capped HPLC columns. A reversed-phase HPLC column that is end-capped has gone through a secondary bonding step to cover unreacted silanols on the silica surface. End-capped packing materials can eliminate unpredictable secondary interactions.

TOP TIP

Many leading manufacturers have LC experts on hand to offer assistance when it comes to selecting the right column for you regarding problematic separations (e.g. ColumnMatch.com or Hamilton application index). By informing the manufacturer what type of separation you require, whether chiral, achiral or aromatic, they can suggest the best column for you, or even design one that fits your needs. This will ensure fast and efficient analysis of your compounds.

4.4 Retention Capacity Considerations

The retention capacity is the time samples spend in the column. It is of importance to both the quality and speed of separations.

The retention capacity is influenced by surface area and carbon load (the percentage of carbon in the packing material). The pore size and volume of the packing material is also important in determining retention capacity. This is because surface area is inversely proportional to pore size. A larger pore size results in lower retention. The particle pore size is measured in angstroms and generally ranges between 100-1000 angstroms; 300 angstroms is the most popular pore size for proteins and peptides and 100 angstroms is the most common for small molecules.

> **The pore size and volume of the packing material is also important.**

Depending on your requirements, HPLC columns can be quite expensive and you will therefore want to ensure they last as long as possible. Guard columns can be used to protect your analytical column and prolong its lifetime. HPLC guard columns (or cartridges) are installed in front of an analytical column and protect it from strongly retained impurities. The SecurityGuard™ Universal HPLC Guard Cartridge System by Phenomenex Inc offers a convenient and economical way to extend HPLC column lifetimes. This column guard is also universal so can effectively protect all analytical columns without effecting results. An alternative to using a guard system is the use of an in-line filter. Filters are a simple, cost-effective means of providing a quick, efficient clean-up for occasional 'dirty' samples.

Status: Reviewer★
Member since: 2012
Organization: LabCorp

Ease of use	★★★★★
After sales service	★★★★★
Value for money	★★★★☆

"SecurityGuard offers a convenient and economical way to extend HPLC column lifetimes. It is effectively designed to eliminate carryover contamination which is the common cause of high backpressure, split peaks, broad peak, baseline noise and loss of valuable resolution. Such system works to trap any contamination from passing to the HPLC column; thus it can extend the lifetime of HPLC columns and result in better chromatography. It is very easy and simple to install and handle."

Review date: 02 Jun 2014 | SecurityGuard™ Universal HPLC Guard Cartridge System

6. Column Selection Based on Column Dimensions

The physical dimensions of the HPLC column hardware should also be optimized for the desired separation. Larger columns are useful in the scale-up process, but smaller columns generally offer greater sensitivity and are therefore more useful in analytical applications.

The column length is determined by how many – and what type – of compounds you have to separate. Long columns provide better resolution and sensitivity but require longer retention times and higher pressure. It is best to choose the shortest column possible for your application, without affecting resolution.

> "It is best to choose the shortest column possible for your application."

The internal diameter (ID) of an HPLC column influences both detection sensitivity and separation selectivity (resolution). The most commonly used ID for HPLC columns is 4.6mm. Small column diameters provide higher sensitivity than larger column diameters for the same injected mass, because the concentration of the analyte in the mobile phase is greater. Smaller diameter columns also use less mobile phase per analysis, because a slower flow rate is required to achieve the same linear velocity through the column. The major disadvantage associated with smaller diameter columns is that the sample loading capacity is reduced.

In summary:

• **Larger ID columns (over 10 mm)** are mainly used to purify large-scale amounts of material because of their relatively large loading capacity.

• **Analytical scale columns (4.6 mm)** are the most common type. They are most often used in quantitative analysis.

• **Narrow-bore columns (1–2 mm)** are used for applications where more sensitivity is required.

• **Capillary columns (under 0.3 mm)** are used almost exclusively with alternative types of detection, such as mass spectrometry.

Choosing the physical dimensions of your column will therefore depend on your application and throughput requirements.

7. Current Hot Areas of Application

The number of uses for HPLC is constantly growing and, like any other analytical tool, the realms of application are vast.

Current hot topics can be seen on SelectScience in the HPLC/UHPLC section of the website. Key topics to keep an eye on over the coming months are indicated below, with some excellent examples of their applications.

Industry professionals highlight glycan mapping and biotherapeutic applications as two of the most hotly discussed applications currently. Read this article to find out how UHPLC has been used accelerate glycobiology. Other examples include the use of HPLC for the characterization of biosimilars and analysis of mAbs, ADCS and impurities.

We are also seeing increasing interest in the use of HPLC columns for the analysis of persistent environmental pollutants, such as PFOA and PFOS. Find out how scientists in California have developed systems for efficient sediment analysis using Kinetex Core-Shell HPLC/UHPLC Columns from Phenomenex in this article.

> **"Chromatography is also finding itself at the heart of cannabis analysis laboratories."**

Canabis Testing

We have seen enormous growth in the cannabis market, particularly detailed cannabinoid profile analysis, to ensure accurate potency testing. This application note highlights a LC-UV method for the resolution of 16 cannabinoids, using Restek columns.

In this webinar (Figure 5), produced in partnership with Restek, discover how both HPLC and gas chromatography are being used to ensure product quality and consumer safety.

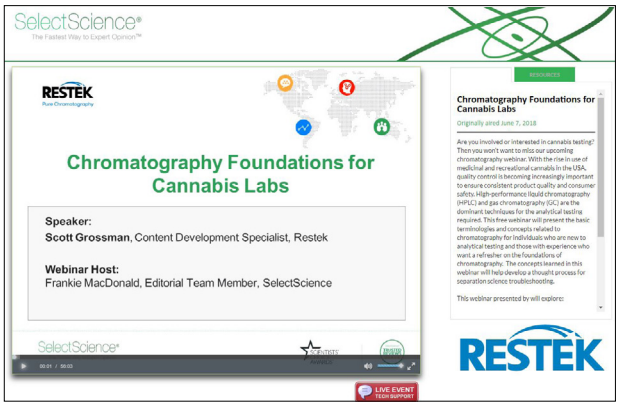
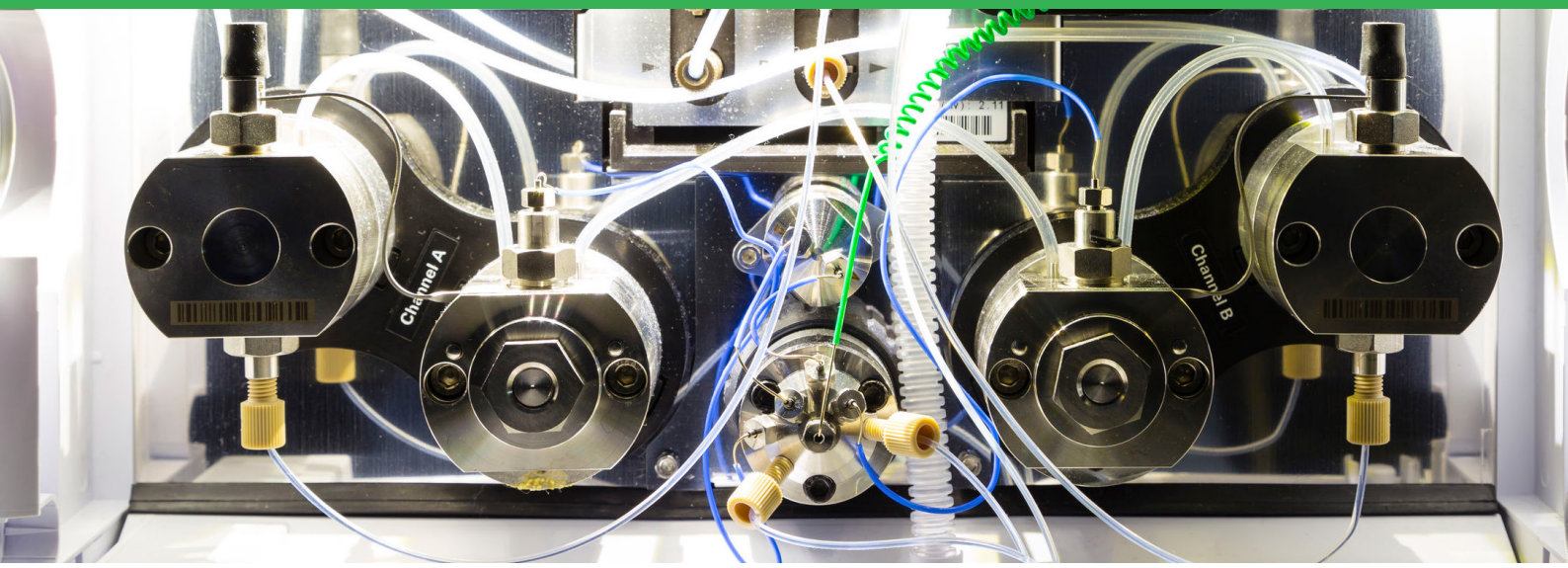


Figure 5: Discover how HPLC columns are being used in cannabis analysis in this webinar



8. Future Chromatography Trends

Over the next few years, we can expect more advancements to emerge rapidly in the HPLC column market.

Most notably, we expect to see increasing variation in column chemistries and pore sizes; as well as more columns which are designed to be short and narrow, to fit mass spectrometry requirements.

We can also expect to see increasingly small particle sizes and better packed columns, aimed at increasing sensitivity and efficiency of detection.

Ask the Experts: What future trends do you expect to see around HPLC columns?

- **Polymer-based supports for high pH use (above 8.0)**
- **Mixed mode stationary phases for multiple retention mechanisms in the same run**
- **Application-specific columns (EtG/EtS, sugars, organic acids, etc.)**
- **Bio-ranges for HPLC, including size exclusion as the number of biotherapeutics on the market increases**

9. Summary and Acknowledgements

There are many different types of HPLC column on the market and finding the correct one for your application may seem daunting, but by applying a few simple considerations, selecting the correct column can be made an easier task.

HPLC columns will continue to develop and new technologies will emerge. Visit the SelectScience product library to find out about the latest HPLC columns from leading manufacturers and read user reviews. Use the SelectScience application note library to keep up-to-date with the latest HPLC methods.

Acknowledgements

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- **Petra Lewits, Global Product Manager Analytical Chromatography, Special HPLC Columns – Merck KGaA**
- **Jason Wrigley, Senior Product Manager HPLC – Merck KGaA**
- **Tim Liddicoat, Senior Manager, Product Management Chromatography Consumables – Thermo Fisher Scientific**

10. Editor's Picks

Cubis® II
Sartorius Group
★★★★★
"The results of weighing are consistent from day to day which gives us confidence and reliability in the measurements."
Leonard Chay,
Sciex

Raptor™ Biphenyl LC Column
Restek Corp.
★★★★★
"This column has been a very useful alternative to C18 columns for the separation of polar compounds and difficult to separate isomers."
Jacqui Adcock,
Deakin University

SecurityGuard Standard HPLC
Phenomenex Inc.
★★★★★
"These SecurityGuard cartridges are an essential in our lab. They extend the life of our columns and are easy to install. It is as simple as popping it in and out!"
Sharita Ellison,
Edgewell Personal Care Analyst

Thermo Scientific™ Vanquish™ Duo UHPLC System for Dual LC
Thermo Fisher Scientific
★★★★★
"This is a whole new level for potency assays by HPLC for the dietary supplement industry, who needed to stay in cGMP compliance."
Angel Gutierrez Cajiao,
Enzyme Process Intl

Agilent Polaris C18-A HPLC Columns
Agilent Technologies
★★★★☆
"Good column packing and consistency. Good customer service. Great, amazing instruments, not to mention Chromeleon, the best software!"
Yen-Huei Lin,
Novavax

Waters Alliance® HPLC System
Waters
★★★★★
"Waters' Alliance is the best high pressure chromatograph on the market. I have been using its older and newer versions for almost 20 years."
Jacek Stadnik,
BIOTON S.A.