eBook

Analytical sample preparation

Smart filtration for reliable HPLC performance

cytiva

Contents

Introduction

Serious problems in high performance liquid chromatography (HPLC) analysis can be avoided by being alert to preliminary warning signs and performing routine maintenance.

Most HPLC part replacement tasks such as changing pump seals are readily recognized as necessary maintenance; however, mobile phase and sample filtration are also highly important maintenance practices.

Sample and mobile phase filtration are simple, economical practices that extend the life of consumable parts on an HPLC system, decrease system wear and tear, protect chromatography columns, and preserve the integrity of the analytical system.

By reviewing the consequences of improper filtration practices, analysts can become familiar with the early warning signs of filtration-related problems and avoid the expense and downtime related to lengthy maintenance repairs and replacement costs.

Protecting HPLC systems and results

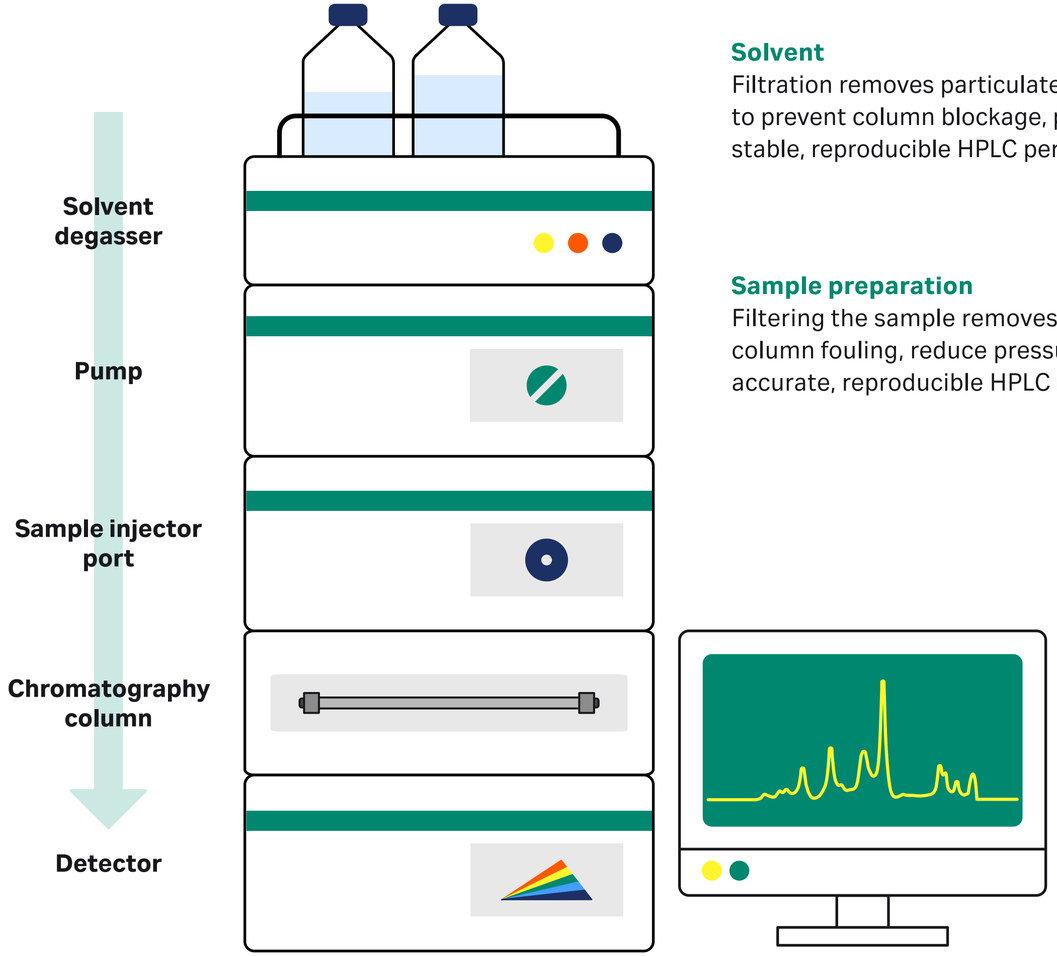
Basic HPLC system components

Regardless of the technical intricacies and cost of the system, all HPLC systems have the same basic components: a solvent reservoir, pump, injector port, chromatography column, detector, and data recording system.

In a basic HPLC system, solvent from the reservoir first passes through a solvent degasser to remove dissolved gases that could disrupt flow, then is pumped toward the sample injection port where the sample is introduced into the high pressure stream.

The mixture is carried into the chromatography column, where its components separate based on their interactions with the stationary phase.

As each component elutes, it passes through the detector, which measures a characteristic signal, and these signals are transmitted to the data system, where chromatograms are generated and analyzed.



Solvent

Filtration removes particulates from the mobile phase to prevent column blockage, protect the pump, and for stable, reproducible HPLC performance.

Sample preparation

Filtering the sample removes particulates to prevent column fouling, reduce pressure spikes, and to provide accurate, reproducible HPLC results.

Mobile phase filtration and degassing

In analytical liquid chromatography techniques such as HPLC, the mobile phase is the solvent that moves the sample through the column. It transports analytes and controls their interaction with the stationary phase, determining how fast each compound elutes. Adjusting its composition, polarity, pH, and flow rate improves separation, resolution, and retention.

The HPLC mobile phase solvent reservoir typically includes an inert bottle, vented cap, PTFE inlet line, and a 10 µm sinker frit. Frequent changing and cleaning of the reservoir bottle and sinker frit help to reduce contamination problems. More importantly, the mobile phase solvent should be filtered to remove gases and particulate.

Degassing by filtration removes dissolved air, preventing pump pressure fluctuations and detector noise, while also eliminating oxygen that can degrade samples and reduce detector stability. Filtering the mobile phase of particulate before use prevents debris from clogging frits and capillaries, protecting the pump and improving peak quality. Buffered solvents should be filtered daily through a 0.2 µm filter to limit microbial growth and baseline drift.

Selecting a mobile phase filtration system

Selecting the mobile phase filtration system best suited to your needs may include factors such as laboratory space to store equipment, safety concerns such as breakable glass or lifting solvent above shoulder or head height to pour, or desire to filter and degas directly into the solvent reservoir used with your analytical system.

Glass filter funnel assembly

A typical mobile phase solvent filtration apparatus is depicted in Figure 1. It is common practice to dedicate a reservoir to each solvent, and each reservoir must have a dedicated funnel and flask to eliminate cross contamination. The size and fragile nature of glass make this practice a concern when selecting mobile phase filtration equipment. Additionally, the height of this type of filtration apparatus means the solvent bottle must be lifted high above the lab bench to fill the top of the funnel assembly, creating a potential health and safety hazard.

The SolVac™ filter holder

The SolVac filter holder (Fig 2) simplifies clean-up and degassing of mobile phase solvents and other solutions. The versatile design fits most HPLC bottles, flasks, and containers, and eliminates the added steps of washing flasks and transferring mobile phase solvent from flask to reservoir. The reusable, chemically resistant PP construction is resistant to common HPLC mobile phase solvents such as methanol, acetonitrile, and tetrahydrofuran.

The compact size of SolVac filter holder provides additional convenience when dedicating a holder for each solvent filtered. It is also simple to use—place a membrane filter, replace the cap, and apply vacuum.

Fig 1. Glass mobile phase filtration apparatus

Fig 2. SolVac filter holder

Selecting a mobile phase filtration system

The primary concern when choosing a membrane filter is solvent compatibility with the filter material. We offer several filters to accommodate the various types of HPLC solvents. Our regenerated cellulose membrane is chemically compatible with the majority of common mobile phase solutions and solvents, thus eliminating confusion with filter selection. However, when working with organic solvents (or aqueous/organic solvent mixtures), PTFE, wwPTFE, or Nylon may be used. RC, Nylon and wwPTFE can also be suitable for highly polar mobile phases. All exhibit low extractables to minimize sample contamination, and whilst all have broad chemical compatibility, PTFE and wwPTFE are specifically recommended for applications involving aggressive organic solvents, strong acids and alkalis, as they are chemically inert.

Most membranes fall into two groups, unsupported or supported, meaning the polymer may be too flimsy and pliable for easy handling, so it is either cast onto or laminated to a support material. A good example is PTFE, the film by itself is too pliable and flexible so it is usually laminated to a supportive woven or non-woven mesh of polypropylene. This fundamental difference can affect the use of SolVac filter holder, see Table 1.

Membrane characteristics	SolVac device	Glass filter funnel assembly
Unsupported membranes (e.g., regenerated cellulose, nylon, polyethersulfone, and nitrocellulose/mixed cellulose ester)	Recommended	Recommended
Particularly thin, unsupported membrane (e.g., polycarbonate)	Recommended with membrane seal gasket	Recommended
Anopore membrane	Not recommended	Recommended
Supported membranes (e.g., PTFE, hydrophilic PTFE, and Versapor)	Recommended with membrane seal gasket	Recommended
Depth filter media (e.g., glass fiber)	Recommended with membrane seal gasket	Recommended

Table 1. Recommended use of the SolVac device and glass filter funnel assembly based on filter type

HPLC systems and sample preparation

HPLC pump

The pump is the single most important component in the HPLC system. Reliable pump operation requires attention to system cleanliness, solvent and reagent quality, mobile phase filtration, and mobile phase degassing. The four most common pump problems involve check valves, pump seals, blockages, and air bubbles. Incorrect pump functioning results in increased baseline noise, irreproducible retention times, and increased operating pressures.

Most common HPLC pumps deliver flow rates between 10 µL/min and 10 mL/min. Pumping fluid at 10 mL/min against a small particle column generates considerable pressure. Unfiltered mobile phases can carry particles that accumulate in inlet frits or the packed bed, gradually restricting flow. Monitoring pressure changes allows for quick assessment of blocked frits, or columns, through exaggerated pressures. Retention times also may be affected by changes in system pressure.

Bubbles form in the pump when mobile phase mixtures become air saturated. Bubbles interfere with piston and check valve operations, causing erratic flow and pressure fluctuations. To resolve blockage or bubbles, contact your system manufacturer for the best preventive maintenance procedures.

Check valves control the solvent flow direction through the pump head and ensure steady pressures when sealed properly. Particulate in check valves can cause a leak or stick causing flow and/or pressure problems.

Check valve leakage is prevented by filtering HPLC grade solvents, using a solvent line sinker frit, flushing the system daily with non-buffered mobile phase, and regularly replacing pump seals to remove particles and entrapped air causing leakage and pump pulsation noise. Pump pulsation noise is the flow change sensed by the detector from piston movement and check valve operation. Filtering the mobile phase solvent aids in decreasing this contribution to noise. A series of increasing polarity solvent flushes should be sufficient to remove problems due to sticking and particulates.

A pump seal facilitates piston movement in the pump head. Pump seals wear more quickly than other pump parts and therefore require changing every three to six months. A failing pump seal is evident from an inability to pump at high pressures, leakage behind the pump head and change in sample retention. Pump seal wear can result in sloughing seals and contamination from this material. Crystallized buffer built up from evaporated mobile phase also accelerates wear.

Pump seal life can be extended by filtering the mobile phase solvents to remove the particles responsible for accelerated seal wear.

Sample injector

Injecting clean samples prolongs injector and column life. Samples are cleared of particulate and bacteria with disposable, multi-well filter plates, syringe filters, or all in one filtration devices such as our Mini-UniPrep™ syringeless filters. Disposable filters range in size and pore size (0.2-1.0 µm).

Syringe filters are membranes enclosed in plastic housings that attach to a syringe with a luer fitting. Samples are filtered by drawing fluid into the syringe, attaching the filter, and dispensing the sample through the filter into a vial. Table 2 lists various types of membrane filters incorporated in syringe housings, housing material, and prefilter materials.

Many laboratories standardize mobile phase filtration on chemically robust membranes while selecting application-specific syringe-filter membranes for sample preparation. To further support lot-to-lot consistency, it is often recommended to source all filtration devices from a single manufacturer to minimize variability in membrane composition and construction.

Choosing the proper filter requires knowledge of filter solvent compatibility and the chemical and physical characteristics of the filter. These characteristics include pore size, pore distribution, filter thickness, extractables, hydrophobic/hydrophilic character, binding properties, pyrogenicity, gas and liquid flow rate, burst strength, autoclavability, pore size, and nominal particulate retention.

For routine HPLC, a 0.45 µm filter is commonly used for general clarification. For UHPLC or small-particle columns, a 0.2 µm filter is recommended to better protect the flow path. For particulate-laden samples, we incorporate large pore size prefilters in one device with smaller pore size membranes. Low protein binding and sterile filters are also available.

HPLC injectors are available in several styles including a septum, septum-less stop-flow device, and a manual or automated valve system. A valve injector is most typical. An injector should provide reproducible sample introduction. Sample and solvent filtration prevents low volume injector fittings from blocking, scratching, and leakage. Loop or waste line blockage results in high backpressure and loop filling difficulty. Low dead volume fittings, located between the valve injector and column to decrease band broadening, also are subject to blockage. Other contributors to HPLC problems include mismatched or damaged injector components, variable sample volumes, leaks, and increased system pressure. With filtration, properly adjusted and clean injectors should last 5,000 injections.

Autosamplers run unattended, so clean filtered samples will decrease malfunction. New, clean sample vials, free of dust and other particulates, also contribute to clean samples. Particulate-free samples are essential to decrease blocked sample needles, connection tubing, and injectors. Connection tube blockage results from sample particulate, septum fragments, or small internal diameter tubing. Sample, mobile phase, and in-line filtration products deter these situations. For blockage at the injector's low-pressure side, the needle and needle valve tubing should be checked. Symptoms include smaller than expected peak heights and peak absence. On the high-pressure side, find the location by loosening the connection fitting, starting at the column head and working upstream. Once the blockage is located, backflush with a clean filtered solvent.

In-line filters and guard columns

In-line filters and guard columns can remove particulate before the main column. These two filters are configured into the HPLC system as follows: sample injector — in-line filter — guard column —main column. They are not intended to replace sample pre-treatment, or sample and solvent filtration. Particulate-laden samples will quickly overload the in-line filter and guard column allowing particles to enter the main column.

In-line filters are important because it is impossible to avoid particulate from system wear, such as polymeric seal wear from the pump and sample injector, except with an in-line filter. In-line filters function to reduce blockage of the column frit and the backpressure restrictor. The in-line filters should have removable frits of 0.45 to 2.0 µm for frequent replacement and low dead volume housings.

Guard columns can collect chemical and physical waste that block the main column inlet, cause column voids, and degrade performance. The guard column retains irreversible and strongly bound components that degrade the column and decrease its lifetime, providing an inexpensive alternative to frequent column replacement. The frits of a guard column are typically 2.0 µm, which is not sufficient for particulate removal.

Sample and mobile phase filtration will preserve the capacity of the guard column for its intended use: chemical contamination removal.

Standard materials incorporated in our filtration devices

Filter material	wwPTFE, H-PTFE, PTFE, nylon, polyvinylidene fluoride (PVDF), polyethersulfone (PES), acrylic copolymer, regenerated cellulose (RC), cellulose acetate (CA), Anopore (ANP), glass microfiber (GMF), depth polypropylene (DpPP)
Prefilter material	Glass fiber, polypropylene (PP)
Housing material	Polypropylene (PP), polycarbonate (PC), modified acrylic

Table 2. Materials used in the construction of our analytical sample preparation devices

Columns

Proper HPLC column selection is crucial for efficient compound separation and identification. High performance columns are composed of small particles of narrow size distribution. Optimal peak profiles depend on column operating characteristics and should be instrument independent. Columns, depending on sample type, sample preparation, and operator filtration practices, can handle a few to several thousand injections.

Two significant problems with HPLC columns are chemical and physical changes. Chemical changes are prevented with guard columns. Physical changes involve blocked frits and channel voids. Voids are created by particulate matter and pressure shock.

If poor peak shapes become evident through badly tailing, splitting, and non-Gaussian bands, without a change in retention time, blocked frits or a column void has occurred.

The following are tips to prevent physical changes:

- Filter samples through a 0.2 or 0.45 µm filter, such as a Cytiva Acrodisc™, Puradisc™, or SPARTAN™ syringe filter, or Mini-UniPrep syringeless filter
- Filter mobile-phase solvents through a 0.2 µm or 0.45 µm membrane using Cytiva membrane discs with the SolVac filter holder when preparing them, and replace buffered mobile phases regularly to minimize particulates and prevent microbial growth.
- Buffered mobile phases should be filtered through a 0.45 or 0.2 µm membrane when prepared, and replaced regularly to prevent microbial growth.
- Use a 0.5 µm in-line filter to trap injector and pump particulates

Detectors

Detectors for HPLC are classified as bulk property or solute property detectors. A bulk property detector measures the physical property difference of the solute in the mobile phase compared to the mobile phase alone. The solute property detector responds to physical or chemical properties of the solute and is independent of the mobile phase. Examples include spectrophotometry, fluorescence, and electron capture detectors.

As detector sensitivity increases, the choice of filter media becomes more important. One of the most sensitive detector technologies available is mass spectrometry, which separates compounds by their charge to mass ratios.

Insufficient mobile phase degassing causes pressure fluctuations and/or sharp noise spikes due to bubbles. These bubbles form when the mobile phase mixture becomes saturated with air. This interferes with detector operation. The presence of oxygen in the samples can cause oxidative degradation, leading to a decrease in sensitivity. Degassing methods include mobile phase filtration followed by a continuous degassing through helium sparging, ultrasonic treatment, vacuum application, or heating with vigorous stirring. Solutions for removing the negative effects of oxygen in detectors include continuous sparging, filtering buffers through a 0.2 or 0.45 µm membrane filter and using HPLC grade solvents.

Tubing

Effects of tubing blockage include significant pressure rises, and fitting and seal leakage. Blockage, partial or complete, can be due to poorly filtered mobile phase, particles in the injected sample, pump/injector seal wear, leakage of silica particles from guard or analytical columns, precipitation of mobile phase salts, and any particulate matter in the HPLC system.

Filter selection

Analysts have four main points to consider when choosing the best filter for their application (Fig 3).

1. Analysis type

When selecting a sample preparation filtration device, the analysis method and instrumentation place critical demands on both the filter membrane and housing. For HPLC and UHPLC, low extractables, chemical compatibility, and consistent pore size are essential to protect sensitive columns and avoid pressure issues. Ion chromatography (IC) requires filters that do not leach ionic species, as even trace contamination can distort conductivity detection and compromise quantitative accuracy. For LC–MS applications, membrane selection is particularly critical, as extractables can cause ion suppression, background noise, and false positives.

2. Consider chemical compatibility of sample and filter

Chemical compatibility is a critical consideration when selecting the sample or mobile phase filter for your application.

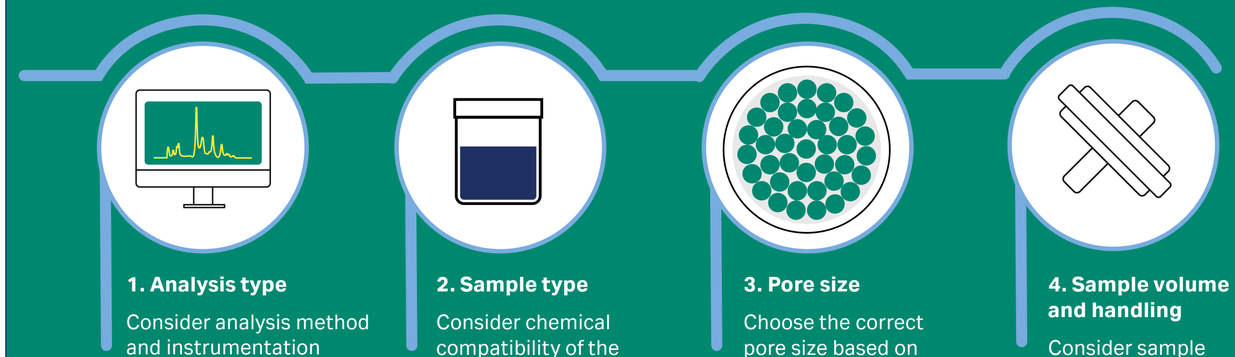
Aqueous solvents

Hydrophilic membranes have an affinity for water and are preferable when filtering aqueous samples. Use our filtration devices with wwPTFE, PES, Nylon, RC, CA, ANP, DpPP, or PVDF membranes.

Gases and aggressive organic solvents

Hydrophobic membranes repel water and are inert to aggressive organic solvents, making them ideal for gases and organic solvents. Choose our filters with PTFE membrane.

Considerations when selecting analytical sample preparation filtration devices.



1. Analysis type

Consider analysis method and instrumentation

- HPLC/UHPLC
- IC
- LCMS

2. Sample type

Consider chemical compatibility of the sample and filtration device (filter and housing)

3. Pore size

Choose the correct pore size based on application and/or analytical chromatography column packing

- HPLC: 0.45 µm
- UHPLC: 0.2 µm

4. Sample volume and handling

Consider sample volume, device effective filtration area (EFA), hold-up volume, and the use of a prefilter

Consider handling: Manual, automation or syringeless

Fig 3. Filtration considerations

Aqueous and organic solvent solutions

Different polymeric membranes have different chemical compatibilities. Based on the application and chemical compatibility, there may be one or several membranes and filter device possibilities.

Generally, one filter type will not function for all applications due to limitations in hydrophobicity/hydrophilicity and chemical compatibility. However, our hydrophilic wwPTFE, H-PTFE, and RC membranes are universal membranes compatible with both aqueous and organic applications.

Exceptionally low extractable levels

The wrong filter can be a source of contaminants in the form of extractables that elute into the sample from the filter device. These undesired artifacts can jeopardize analytical results. Some extractable concerns include coelution, false quantitation, and extraneous peaks. We select the highest grade of materials and perform rigorous extraction methods on membrane products to reduce undesired artifacts.

For batch-to-batch consistency, the SPARTAN range of syringe filters is tested and certified with documentation for the absence of UV-absorbing substances at wavelengths of 210 and 254 nm with water, methanol, and acetonitrile.

Sample adsorption

Unwanted drug binding during routine pharmaceutical sample analysis can be a serious problem and cause out-of-specification results. No single analytical method can provide reliable information on comparative filter properties and the full range of extractables for all filters. Therefore, choose a low adsorption filter such as the Acrodisc One syringe filter with wwPTFE membrane. The wwPTFE membrane is extremely low in biomolecule and API binding. Typical binding levels are below 5%.

3. Consider filter pore size

The appropriate selection of pore size during sample filtration is critical for prolonging column lifetime and minimizing instrument maintenance. The required pore size should be determined in relation to the diameter of the column packing material.

For HPLC columns with packing sizes of 3 µm or smaller, and especially for UHPLC columns using sub-2 µm particles, a 0.2 µm syringe filter is recommended, as a 0.45 µm filter may allow particles to pass that can obstruct the column.

For liquid chromatography systems employing packings larger than 3 µm, the industry standard filtration recommendation is a 0.45 µm pore size for both syringe filters and mobile-phase membrane filters.

Once the appropriate pore size specification has been selected for the application, it is essential to rely on the filter manufacturer to provide an accurate and validated pore size rating.

4. Sample volume and handling

The particulate contained within a fluid affects the life of a filter. As particles are removed from the fluid, they block pores and reduce the usable portion of the filter.

Particulate-laden fluids generally plug a filter more quickly than “clean” or preclarified fluids. Filtration devices come in various formats and effective filtration areas (EFAs), ranging from 96-well plates to spin filters, syringeless filters, and syringe filters. Increasing the EFA can lengthen the life of a filter.

Hold-up volume

Hold-up volume is the amount of liquid remaining in the filter after use. A filter with a low hold-up volume is recommended for use with expensive samples or those with limited availability.

We offer a broad range of device sizes. The minispike outlet, available on our 13 mm syringe Acrodisc and SPARTAN filters, reduces sample hold-up and offers easy dispensing into autosampler vials. Additional options to reduce hold-up volume include the Nanosep™ MF centrifugal devices, AcroPrep™ Advance filter plates, and Mini-UniPrep syringeless filters.

Table 3 outlines general guidelines to the appropriate filter size for different volumes of sample and the typical hold-up volume of devices.

Prefiltration

For difficult-to-filter samples, it is best to use a syringe filter with a glass fiber prefilter over the membrane. The prefilter removes larger particulate contamination before the sample reaches the final membrane, helping to prevent premature clogging and extending the life and performance of the final filter. Whatman GD/X™ syringe filters and Acrodisc PSF syringe filters with GxF multi-layered glass fiber prefilters are the best options for extremely particulate-laden samples, as both devices contain built-in prefilter layers (Fig 5).

Handling

When selecting analytical sample preparation filtration devices, handling requirements are a critical consideration, as they directly impact efficiency, reproducibility, and operator safety. The volume and number of samples to be processed will influence whether syringe filters, syringeless filters, or higher-throughput multi-well filter plates are most appropriate, with lone samples favoring simple manual devices and larger batch workflows benefiting from parallel processing formats.

Volume to be filtered	Filtration device	Typical hold-up volume
<500 µL	Nanosep MF Device	<2 µL
<2 mL	Acroprep Advance 96-well filter plates (350 µL, 1 mL, and 2 mL)	<18 µL per well
<2 mL	4 mm Acrodisc syringe filter and 4 mm Puradisc syringe filters	<10 µL
<10 mL	13 mm Acrodisc syringe filter with minispike outlet	<14 µL
<10 mL	Whatman™ Puradisc syringe filters, 13 mm	<25 µL
<10 mL	13 mm Acrodisc syringe filter	<30 µL
<10 mL	Whatman SPARTAN syringe filters, 13 mm	<30 µL
<100 mL	Whatman Puradisc syringe filters, 25 mm	<100 µL
<100 mL	25 mm Acrodisc syringe filter	<125 µL
<125 mL	25 mm Acrodisc PSF syringe filter	<200 µL

Table 3. General guidelines to the appropriate filter size for different volumes of fluid and the typical hold-up volume of devices

Extractables and maintaining analytical integrity

Extractables from chemical compounds in syringe filters is a major concern especially when using liquid chromatography and mass spectrometry instruments.

Extractables

A filter extractable is an undesired artifact contributed to the sample fluid from the filter device. This material may be a membrane or housing formulation component, or a component introduced during the manufacturing or packaging process

Extractable materials may leach into the sample during sample preparation through several mechanisms (solubility, particle displacement, chemical interaction, and diffusion). The appearance of extractable materials from a filtration device depends on the solubility of device components in the sample fluid. As membrane and/or housing components become more soluble with sample fluid components, extractable materials will increase.

Filter compatibility

All sample constituents (both major and minor components) require consideration. Because solubility is dependent on temperature, concentration, and exposure time, these parameters are significant in determining chemical compatibility. Displacement can occur when residual manufacturing materials are dislodged. We perform rigorous extraction methods on their membrane products to prevent the risk of this occurrence.

Filter selection

Different polymeric membranes have different chemical compatibilities. Based on the application and chemical compatibility, there may be one or more syringe filter possibilities.

Generally, one filter type will not function for all applications due to limitations in hydrophobicity and hydrophilicity and chemical compatibility. However, our wwPTFE membrane is a universal membrane for the majority of applications. It has excellent chemical compatibility for aqueous and aggressive organic solvents. wwPTFE membrane is hydrophilic and can be used when membrane selection is difficult for complex sample matrices. The option of a built-in glass prefilter (GxF/wwPTFE) is also available for heavily particulate-laden samples in both Acrodisc and Acrodisc PSF versions.

When are extractables a concern?

Extractable materials can jeopardize analytical results. For chromatographic analysis, scenarios resulting from extractable materials include sample absorption, coelution, and extraneous peaks. Anomalous results are an analyst's nightmare because procedures typically require action to remedy or identify miscellaneous and unexpected peaks.

Extractable materials become even more of a concern as the amount of analyte diminishes. With recent liquid chromatography column trends utilizing smaller inner-diameter columns (<1 mm for micro LC) and smaller packing sizes, the ability to separate and detect trace quantities of material is increasing. With these improvements comes increasing concern for the effects of extractable materials.

Reducing the risks associated with extractables

Application testing preserves analysis validity. To application test, analyze the sample fluid before it is filtered. Compare these results with the results obtained after the sample is filtered. If any quantitative or qualitative differences occur, select another filter type. Another method of application testing involves evaluating the results obtained from passing the matrix solvent through the syringe filter and evaluating the results. This will demonstrate if material will extract with the neat solvent. Flushing is a third method. When excess sample fluid is available, discard the first few millilitres of fluid eluting from the filtration device.

Generally, the amount of extractable materials eluted from the filter diminishes with the volume passed through the filter.

Frequently asked questions

Whether conducting R&D activities or monitoring manufacturing and quality control, protecting data integrity and validity is essential. However, it is difficult to obtain accurate, reproducible data when HPLC columns perform suboptimally. One way to improve column performance is to filter both samples and mobile phases prior to analysis.

The following frequently asked questions address the importance of filtration in method development and sample preparation.

What is the importance of sample filtration?

Filtration extends the operational lifetime of an HPLC column by preventing particulate matter from obstructing the column bed. It also reduces overall system maintenance by minimizing excessive wear on critical components such as check valves, piston pumps, and pump seals. In addition, effective filtration reduces ghost peaks, baseline drift, and analytical interferences, thereby improving overall data quality.

When samples are not adequately filtered, particulate matter travels through the column and becomes trapped within the interstitial spaces of the stationary phase.

Furthermore, the column end frits contain even smaller pore sizes, which further retain these particles. As a result, unfiltered particulates accumulate in these regions, leading to preferential clogging at the front of the column. This uneven blockage adversely affects system pressure (Fig 4), and chromatographic performance, ultimately interrupting the analysis. Consequently, laboratories are often forced to repeat analyses and perform additional preventative maintenance that would otherwise be unnecessary.

How do I select the best filter for my analysis?

A wide range of filter types and membrane materials are available. Some are specified in regulatory methods, and laboratories that operate under these methods are often reluctant to deviate from the prescribed materials. However, for analyses in which no specific material is stipulated, laboratories should evaluate the nature of the sample solution and assess membrane compatibility with the intended application.

Solvent polarity is a key consideration in determining whether a hydrophobic membrane (e.g., PTFE) is required for organic solvents, or whether a hydrophilic membrane (e.g., nylon, PVDF, or PES) is more suitable for aqueous-based solutions. The applicable pH range and membrane chemical compatibility across that range must also be considered.

Analysts should also consider the nature of the target analytes. Nylon membranes are typically used for small-molecule applications but are less suitable for proteins and peptides due to their propensity for binding. PVDF, PES, and regenerated cellulose (RC) membranes are commonly used for proteins and peptides because of their low binding characteristics.

In addition, neither the membrane nor the housing should introduce chemical contaminants that could compromise chromatographic performance or data integrity.

We offer wwPTFE, H-PTFE, and RC membranes that are suitable for most sample preparation applications. These membranes can be used to filter organic solvents, aqueous solutions, as well as acidic and basic samples. Selecting one of these membranes allows laboratories to use a single filter type for the majority of their tests.

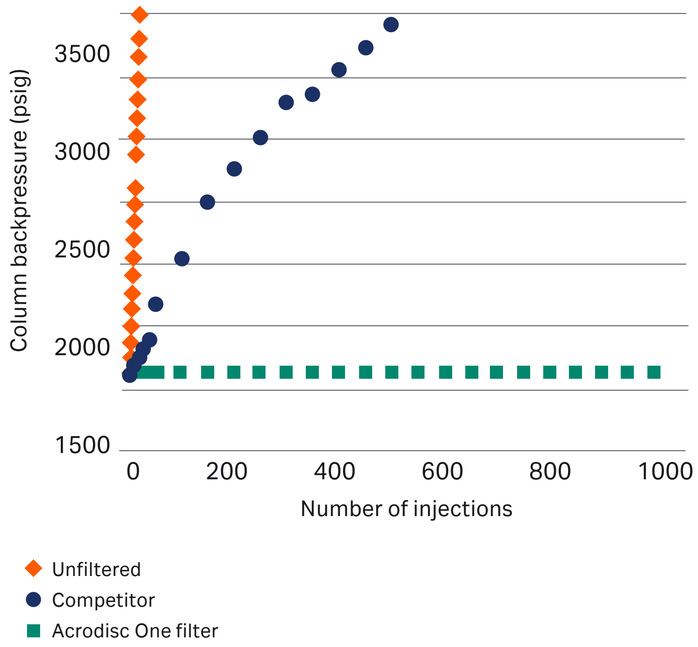


Fig 4. Effects of filters on HPLC column life following injections of unfiltered and filtered 0.05% latex sphere suspensions. With unfiltered samples, the column failed due to plugging after 19 injections. Samples passed through competitor filters plugged the columns after 500 injections. No increase in backpressure was observed after 1000 injections of samples filtered with Acrodisc One filters with wwPTFE membrane.

Is filtration of the mobile phase important to HPLC or UHPLC operation?

Filtering the mobile phase is a critical, yet often overlooked, step in analytical liquid chromatography workflows. While many analysts purchase HPLC-grade reagents and assume they can be used without further preparation, it is essential to filter the mobile phase just as carefully as the sample. Even if particles are not visible to the naked eye, it is unsafe to assume that the mobile phase is free of contaminants. Filtration through a 0.45 µm or 0.2 µm membrane, depending on system requirements, is strongly recommended.

As with sample filtration, mobile phase filtration reduces overall wear on the system and extends column life. It also serves to degas the mobile phase, a key factor in maintaining optimal system performance. Removing dissolved gases prevents bubble formation, which can lead to baseline fluctuations, retention time shifts, and unreliable data.

For mobile phase filtration, we recommend the SolVac filter holder used in conjunction with 47 mm membrane disc filters.

Sample requirements can differ significantly. Laboratories may handle very small-volume samples, while in others, they may process multiple samples simultaneously. Is a syringe filter the right device for all sample filtration requirements?

Syringe filters are commonly used in analytical sample preparation applications, however they may not always the right device for every sample filtration requirement, because sample volume, throughput, and workflow can vary widely across laboratories.

Small volume samples, for example, can be processed with 4 mm syringe filters, which minimize dead volume and conserve precious material, but their limited capacity makes them unsuitable for larger or more particulate laden samples. In contrast, 13, 25, and 30 mm syringe filters provide progressively larger surface areas and higher hold-up capacities, making them more appropriate for routine sample filtration. These formats work well for individual, manual sample processing, but can become inefficient when throughput demands increase.

For laboratories managing high sample numbers or requiring simplified workflows, alternative devices may offer significant advantages. Syringeless filtration devices, such as Mini-UniPrep, combine filtration and vialing in a single step, reducing handling, improving reproducibility, and saving time—ideal for busy analytical labs processing moderate sample volumes (Fig 5).

Our Nanosep MF devices with the wwPTFE membrane can also be used to process smaller sample volumes (Fig 6). This product is available in 0.2 µm and 0.45 µm pore sizes, and it is a small centrifugal device. It is appropriate for volumes as large as 500 µL and has a hold-up volume of 2 µL. When laboratories are working with ultrasmall volumes, they can spin through and recover all samples before it goes onto the instrument.

When sample numbers increase further, multi-well filter plates provide parallel processing, allowing up to 96 samples to be filtered simultaneously using a vacuum manifold or centrifuge (Fig 7). These are well suited for screening, bioanalytical workflows, or high-volume QC environments. Ultimately, the right filtration device is determined by the specific combination of sample volume, matrix, particulate load, and processing throughput, rather than relying on syringe filters alone.

Fig 5. Mini-UniPrep syringeless filters

Fig 6. Nanosep MF devices

Fig 7. AcroPrep multi-well filter plates

How do Mini-UniPrep syringeless filters work?

Chromatography sample preparation traditionally involves multiple consumables such as syringe filters, vials, and septa, which increases both cost and the time required for sample filtration.

Mini-UniPrep syringeless filters combine four products into one to provide a fast, efficient, and environmentally friendly solution for sample filtration prior to chromatography (Fig 8). These filters eliminate the need for separate disposable components and prepare samples in one-third of the time required by other methods used for HPLC.

They feature an integrated autosampler vial, a plunger with an attached filter membrane, a septum, and a cap. To use the filter, the sample is placed into the device, and the plunger is inserted and compressed. The sample is filtered rapidly and easily. The unique design allows the liquid sample to be forced into the reservoir of the plunger, while any excess air escapes through the vent hole until the seal is engaged. The Mini-UniPrep syringeless filter can then be placed directly into an autosampler.

The filters can be used manually or with a multicompressor. The multicompressor can process up to eight samples at one time, further improving sample processing time and reducing the risk of hand stress.

Mini-UniPrep syringeless filters are designed to process up to 0.4 mL of unfiltered sample. They are available in a wide range of membranes with 0.2 µm and 0.45 µm pore sizes to meet specific sample filtration requirements.

Fig 8. Mini-UniPrep syringeless filters combine four steps into one to provide a fast, efficient, and environmentally friendly solution for sample filtration prior to chromatography.

How can using Mini-UniPrep syringeless filters reduce plastic waste?

Figures 9a and 9b show the average amount of plastic waste generated during the preparation of 10 HPLC samples using two different workflows, a conventional sample preparation method using syringe filters and the Mini-UniPrep syringeless filter workflow. To model the plastic waste generated in conventional HPLC sample preparation, we used two syringe sizes (5 and 10 mL), and a range of syringe filter diameters sourced from Cytiva, Sartorius, and Merck Millipore.

All syringe filters used in the study were equipped with hydrophobic PTFE membranes. We recorded the weight of 10 syringes, syringe filters, polypropylene HPLC vials, and caps with septa.

For the Mini-UniPrep syringeless filter workflow, we weighed and recorded 10 filters along with the pipette tips (Eppendorf) used during sample preparation. The weight of the plastic packaging that the consumables were supplied in was not recorded as this was outside the scope of the study.

Compared to conventional workflows using 13 mm Acrodisc and Puradisc syringe filters, the Mini-UniPrep syringeless filter reduced plastic waste by 73% and 74%, respectively. In addition, we observed a 75% reduction compared to workflows using the 13 mm Millex-FH and the 15 mm Minisart SRP syringe filters. Consistent with previous results, we found that the mean plastic waste produced increased when the larger variants of the syringe filters were incorporated into the conventional workflow.

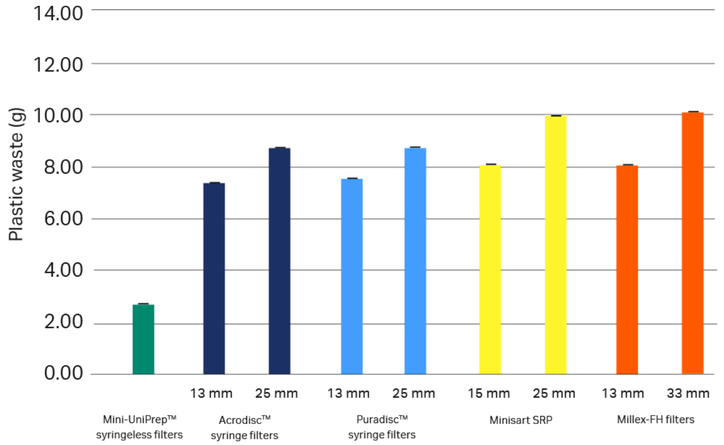


Fig 9a. Mean plastic waste generated in the Mini-UniPrep syringeless filter vs conventional HPLC sample preparation workflows using a 5 mL syringe.

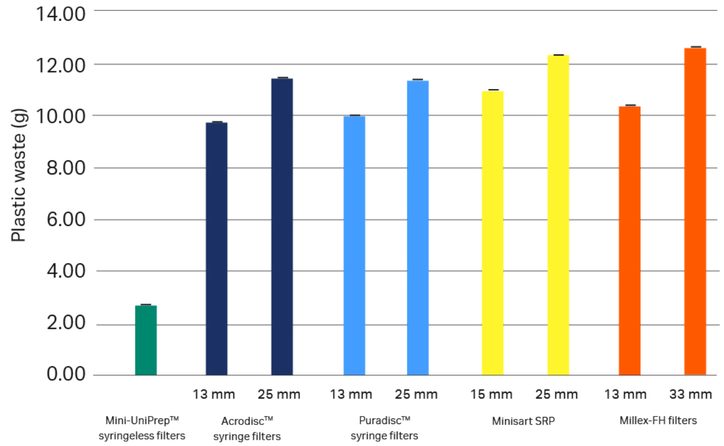


Fig 9b. Mean plastic waste generated in the Mini-UniPrep syringeless filter vs conventional HPLC sample preparation workflows using a 10 mL syringe.

Do Cytiva provide devices for filtering larger volumes of solvents?

For larger filtration needs we provide a range of inline disc filters and capsule devices.

When selecting a larger filtration device for solvent filtration, it is important to consider the chemical compatibility of the filter housing. Our disposable disc and capsules filters for solvent filtration use polypropylene housings, as polypropylene is chemically resistant to many organic compounds, lightweight, durable, and cost-effective.

Our PTFE filter capsules are available in a variety of sizes and pore sizes to meet different solvent filtration requirements (Fig 10).

Fig 10. Polydisc inline filters and the AcroPak 300 capsule filter

Are there other sustainability solutions Cytiva offers for analytical sample preparation?

As part of providing additional solutions for sustainability, we are advancing the use of certified biobased plastics in laboratory filtration products.

Biobased materials are derived from renewable feedstocks that are not suitable for human consumption, such as waste and residues from vegetable oil refining and used cooking oil collected from the food industry and restaurants. By taking steps like replacing traditional fossil fuel–based plastics with certified biobased polypropylene resins, we can help reduce carbon footprint by at least 120% and decrease fossil fuel depletion by approximately 70%*, while also lowering overall greenhouse gas emissions. These materials support broader sustainability goals, including Scope 3 emissions reductions, without compromising product quality or performance.

Certified biobased plastics are available in Whatman syringe filters:

- Whatman Puradisc 25 mm high performance filters
- Whatman GD/X 25 mm designed for difficult to filter samples
- Whatman GD/XP 25 mm designed for inorganic ion analysis

These filters offer the same high level of performance. They are verified for sustainability and full traceability throughout the supply chain.

Learn more about our sustainability solutions here.

*vs. fossil fuel-based in terms of GWP and abiotic resource depletion/LCA based on ISO 14040, ISO 14044, ISO 14067 critically reviewed by third party panel/percentage includes credit for biogenic uptake which cannot be accounted as carbon reduction when reporting to SBTi.

Summary

Reliable analytical liquid chromatography results depend on effective sample and mobile phase filtration. This eBook highlights filtration as a fundamental part of analytical sample preparation and routine system maintenance, rather than a secondary or optional step. By removing particulate matter, potential contaminants, and microorganisms before analysis, filtration protects critical analytical instrument components, extends column lifetime, and improves data quality and reproducibility.

Inadequate filtration of both samples and mobile phases contributes to common HPLC issues such as increased backpressure, column blockage, pump seal wear, baseline noise, ghost peaks, and inconsistent retention times.

Selecting the appropriate sample preparation devices based on analysis type, sample chemistry, pore size requirements, and sample volume is critical to ensuring reliable and accurate analytical results. The impact of extractables, analyte adsorption, and drug binding must also be considered.

Through practical recommendations, application examples, and comparative studies, this eBook demonstrates how correct filtration choices improve instrument performance, reduce downtime, and support accurate, reproducible analytical results across research, quality control, and pharmaceutical workflows.

Explore our analytical chemistry sample preparation product range here.

Learn more about our analytical solutions and talk to filtration specialist

cytiva.com

For local office contact information, visit cytiva.com/contact

CY57675-30MAR26-EB