PALL CORPORATION

Analytical Sample Preparation

Critical for Analytical E-book

Overview: Sample and Mobile Phase Filtration

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Application Note

Why Sample and Mobile Phase Filtration is Essential for HPLC Analysis

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, and preserve the integrity of the 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.

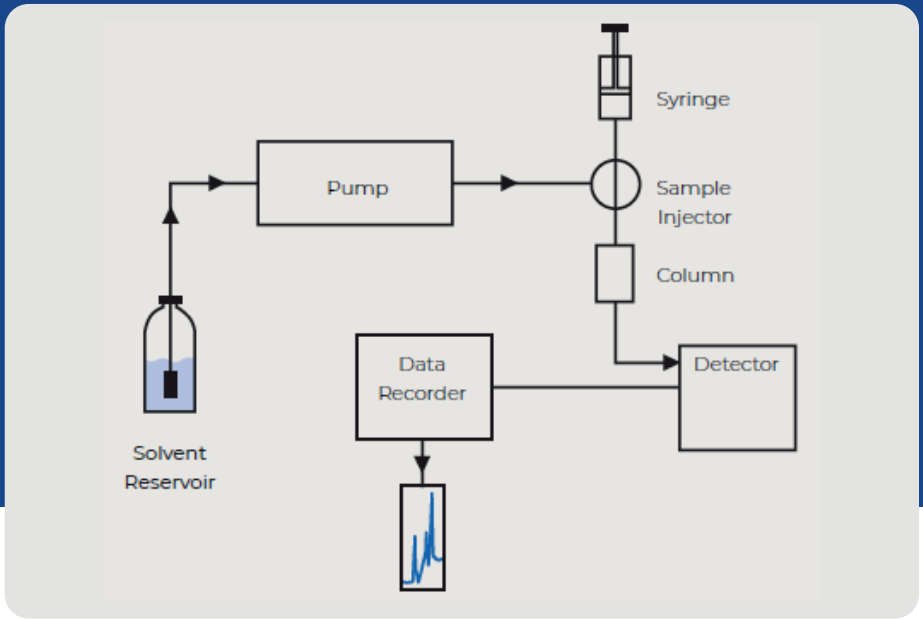


Figure 1: Basic HPLC system

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, column, detector, and data recording system (see Figure 1). Particles, dissolved gasses and microbial growth not removed by filtration can interfere with nearly every system component.

Reservoir/solvent degassing

The solvent reservoir traditionally includes an inert container, vented cap, PTFE solvent inlet line, and a 10 µm gross inlet sinker frit. The solvent reservoir is generally equipped to degas solvents by removing dissolved air. Frequent mobile phase degassing reduces erratic pump delivery of the solvent due to pressure fluctuations, and hence reduces detector noise. Degassing removes dissolved oxygen that can result in oxidative degradation of the sample and mobile phases, which reduces the sensitivity and operating stability of ultraviolet, refractive index, electrochemical and fluorescence detectors. By filtering the mobile phase, analysts can reduce debris capable of plugging the sinker and column frits, causing contamination, damaging pump valves, blocking capillaries, causing poor peak performance, and contributing to extra peaks and excessive chromatographic noise.

Mobile phase filtration is performed prior to placing the solvent into the solvent reservoir. Buffered mobile phase solvents require daily filtration with a 0.2 µm filter to eliminate microbial growth that could increase the baseline.

A typical solvent filtration apparatus is depicted in Figure 2. Contamination concerns from the filtration apparatus deter many analysts from filtering solvents. Analysts who opt to filter will often dedicate a reservoir to each solvent, and each reservoir must have a dedicated funnel and flask to eliminate cross contamination. Frequently changing and cleaning the reservoir bottle and sinker frit also reduce contamination problems. Most typically, a glass filter funnel and flask assembly are used. The solvent bottle itself must also be lifted high above the lab bench to fill the top of the funnel assembly, creating a potential health and safety hazard.

Figure 2: Glass mobile phase filtration apparatus

The SolVac® filter holder

Concerns when using a glass funnel filter assembly can be alleviated by using Pall's SolVac filter holder, which fits directly on to top of the solvent reservoir. The SolVac filter holder device depicted in Figure 3 eliminates the need to have dedicated filtration apparatuses by minimizing cross contamination when filtering multiple solvents. SolVac filter holder devices also prevent the hazards associated with elevating solvent bottles high above the lab bench. Using the SolVac filter holder device eliminates the need to pour and wait for the sample to filter, continually refilling the funnel as space allows.

The primary concern when choosing a solvent filter is solvent compatibility with the filter material. Pall offers several filters to accommodate the various types of HPLC solvents. Pall's wwPTFE membrane is a universal membrane that is chemically compatible with the majority of common mobile phase solutions and solvents, thus eliminating confusion with filter selection. Typical solvent filters range in size from 25 to 90 mm in diameter and are available in a 0.2 or 0.45 µm membrane pore size.

Figure 3: The SolVac filter holder

The SolVac filter holder 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.

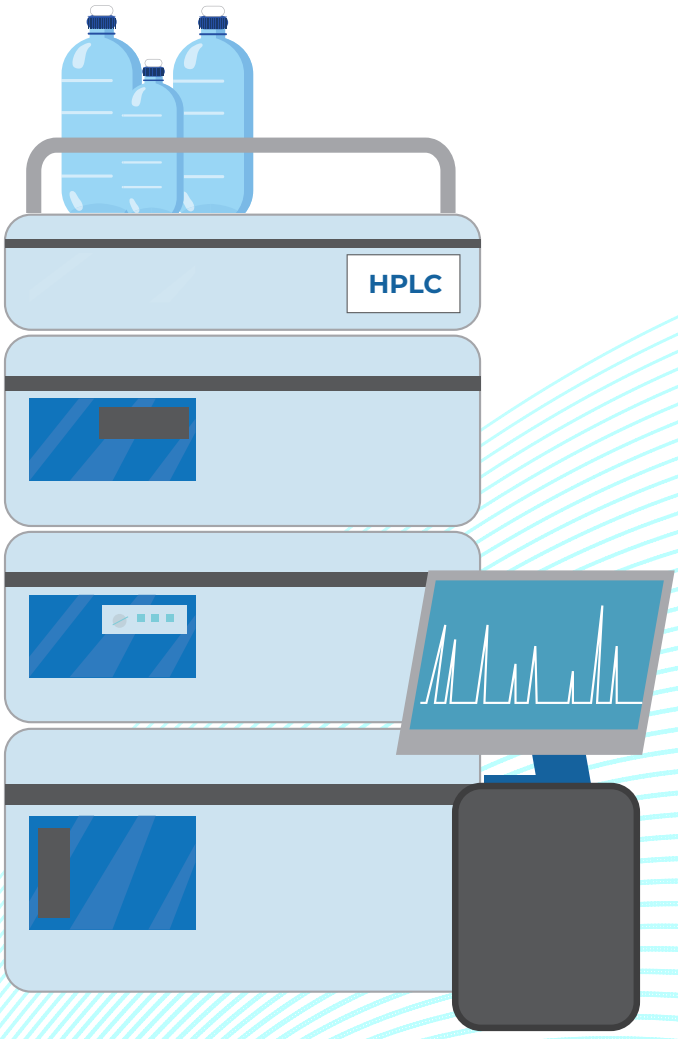
HPLC pump

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 1) check valves, 2) pump seals, 3) blockage, and 4) air bubbles. Incorrect pump functioning results in increased baseline noise, irreproducible retention times, and increased operating pressures.

A pump delivers flow rates between 10 µL/min and 10 mL/min. Pumping fluid at 10 mL/min against a small particle column generates considerable pressure. If the pump is circulating unfiltered mobile phase through the small interstitial spaces within the packed column, particulate build up is possible. 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 HPLCgrade 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. Crystalized 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 syringe filters. Disposable filters range in size (4-25 mm) 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 1 lists various types of membrane filters incorporated in syringe housings, housing material, and prefilter materials.

To reduce physical and chemical variability among manufacturers, sample and mobile phase filtration products should be purchased from the same manufacturer.

Choosing the proper filter requires knowledge of filter/solvent compatibility and the chemical/physical char-acteristics 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. A 0.45 µm pore size filter is typically selected for HPLC applications, while for bacterial removal or UHPLC applications a 0.2 µm filter is chosen. For particulate-laden samples, Pall incorporates 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 ensure 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 back pressure 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 compo-nents, 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. 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.

Table 1: Standard materials incorporate in Pall syringe filters

Membrane Material: wwPTFE, PTFE, Nylon, Polyvinylidene Fluoride (PVDF), Polyesthersulfone (PES), Acrylic Copolymer
Housing Material: Polypropylene (PP), Polyethylene (PE), Modified Acrylic
Prefilter Material: Glass Fiber, Polypropylene (PP)

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 back-pressure 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. 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.

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 from instrument manufacturers to prevent physical changes:

- Filter solvents through a 0.2 or 0.45 µm filter, such as a Pall Acrodisc® syringe filter
- Prefilter mobile phase buffers daily with a 0.2 µm filter to remove bacterial growth
- Filter samples through a 0.2 or 0.45 µm filter
- Use a 0.5 µm in-line filter to trap injector and pump particulates

Prior to any action, ensure that the problem is from blockage or a void volume and not from a change in solvent strength, pH, temperature, or mobile phase additives, such as an ion-pairing reagent, which show the same effects.

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 in-dependent 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. To address the special requirements of this technology, Pall has developed the Acrodisc MS, which utilizes a special unsupported wwPTFE membrane in a high-density polyethylene housing. These materials minimize the potential for low-level extractables that would be detected by this highly sensitive technology.

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 Pall 0.2 or 0.45 µm membrane filter, and using HPLC-grade solvents.

Tubing

Tubing length and internal diameter require careful selection to prevent system degradation. The internal diameter is dictated by pressure requirements and can vary from 0.18 to 1.0 mm depending on flow requirements. Injector-to-column and column-to-detector tubing, typically stainless steel or Teflon is generally 0.25 mm. Applications where small peak volumes are required use microbore tubing. Small I.D. tubing blocks faster, but tube blockage is rare. More commonly, blockage occurs at in-line filters or frits. Effects of 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.

1. Is your filter application automated or manual?

All of Pall's sample prep devices can be used in manual operation, however, our AcroPrep filter plates and Acrodisc PSF syringe filters have been optimized for automated operation.

2. What is the filter's chemical compatibility?

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

Aqueous samples: Hydrophilic membranes have an affinity for water and are preferable when filtering aqueous samples. Use Pall Acrodisc filters with wwPTFE, PES, Nylon, 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 Pall filters with PTFE membrane.

Aqueous and organic solvent solutions: Polymeric membranes have different chemical compatibilities. Based on the application and chemical compatibility, there may be one or several membranes and Acrodisc syringe filter possibilities. Generally, one filter type will not function for all applications due to limitations in hydrophobicity/hydrophilicity and chemical compatibility. However, Pall hydrophilic wwPTFE membrane is a universal membrane for 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. Pall specifically selects the highest grade of materials and performs rigorous extraction methods on membrane products to reduce undesired artifacts.

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. What effective filtration area (EFA) is needed for your filtration?

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" fluids. Increasing the EFA can lengthen the life of a filter.

Filters come in a variety of sizes ranging from the area within a single well of a 96-well plate, to spin filters and syringe filters. 25 mm Acrodisc PSF syringe filters, as well as 13 mm diameters for smaller sample volumes, are available in a variety of membrane and pore size choices.

Hold-up volume: Another aspect of choosing the right filter size is the hold-up volume. This is the volume of liquid remaining in the filter after use. A filter with a low hold-up volume is recommended for use with expensive fluids or those with limited availability. Pall offers a broad range of device sizes. The minispike outlet, available on our 13 mm syringe filter, allows for minimal sample hold-up and easy dispensing into autosampler vials. Additional options include the Nanosep® MF centrifugal device, or AcroPrep™ Advance filter plates. Table 2 outlines general guidelines to the appropriate filter size for different volumes of fluid 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 Acrodisc PSF syringe filter with GxF multi-layered glass fiber prefilter is the best option for extremely particulate laden samples (see Figure 4).

Easy identification: 13 and 25 mm Acrodisc syringe filters and their packaging have color-coded printing with membrane type and pore size on each filter: wwPTFE, Nylon, PTFE, Glass Fiber, PVDF, and Poly-ethersulfone (PES).

Table 2: Selecting filter size for different volumes of fluid

Volume to be filtered	Device type	Typical hold-up volume
<500 µL	Nanosep MF Device	<2 µL
<900 µL	AcroPrep 96 1 mL Filter Plate	<18 µL per well
<2 mL	4 mm Acrodisc Syringe Filter	<10 µL
<10 mL	13 mm Acrodisc Syringe Filter (Minispike)	<14 µL
<10 mL	13 mm Acrodisc Syringe Filter	<30 µL
<125 mL	25 mm Acrodisc PSF Syringe Filter	<200 µL

The Acrodisc PSF GxF syringe filter has a serial glass fiber (GxF) prefilter to allow for maximum throughput and faster flow rates than standard glass fiber prefilter devices.

The multi-layered prefilter, rated from >40 to 1 µm, traps particulate, thereby extending filter life.

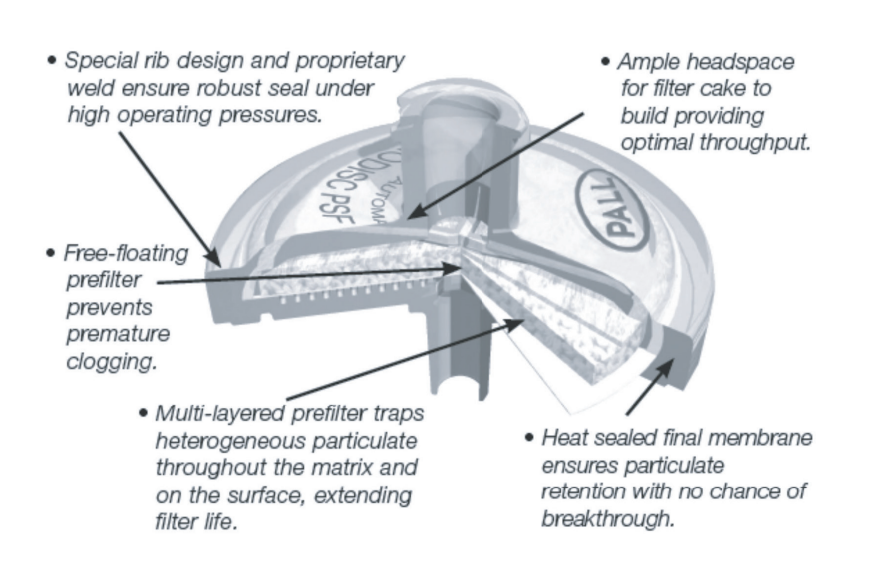


Figure 4: Acrodisc PSF GxF syringe filter

4. What pore size should be used for an effective filtration?

What pore size rating is optimal for sample clean-up? Choosing the proper pore size for your filter can extend the life of your column and reduce maintenance due to particulate in the pumping system. Poor size should be determined based on the column packing size. As you can see in Figure 5a, the column packing particles touch each other. Ideally, you would not want contamination to fit into the space between the particles of packing. This space (labeled Flow path) is identified in Figure 5a. The idea is to find out how large that space is and remove particles that size.

Figure 5b shows how one could calculate the size of the space using a series of equilateral triangles. So, if the column packing is 3 µm in diameter, the flow path is 0.43 µm. When an HPLC column has a packing size of 3 µm or smaller, you should use a 0.2 µm Acrodisc One syringe filter because a 0.45 µm syringe filter may let particles through that will plug the column.

For liquid chromatography systems using columns with larger than 3 µm packings, the filtration industry standard is 0.45 µm for syringe filters and mobile phase membranes. For columns with 3 µm or smaller packings, including ultra high pressure liquid chroma-tography (UHPLC) microbore columns, or when concerned about microbial growth, a 0.2 µm filter is recommended.

Once the best pore size rating is chosen for the application, you must rely on the filter manufacturer to provide an accurate pore size rating.

Summary

Sample and mobile phase filtration are simple economical practices that can extend the life of consumable HPLC parts. With a basic understanding of how proper filtration benefits various components of an HPLC system, and by selecting the proper filter, laboratories can avoid the time and expense of unexpected part repair and replacement.

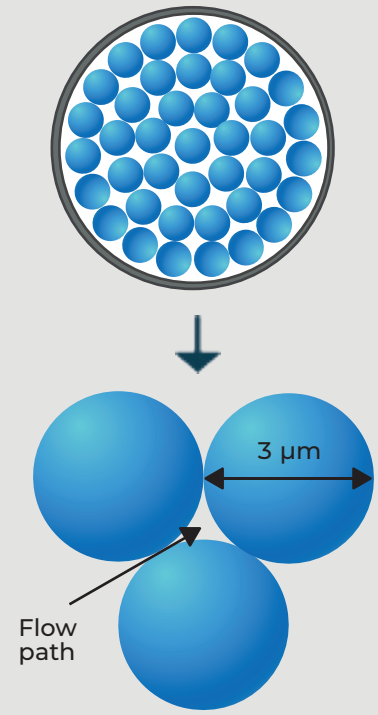


Figure 5a: Column packing flow path

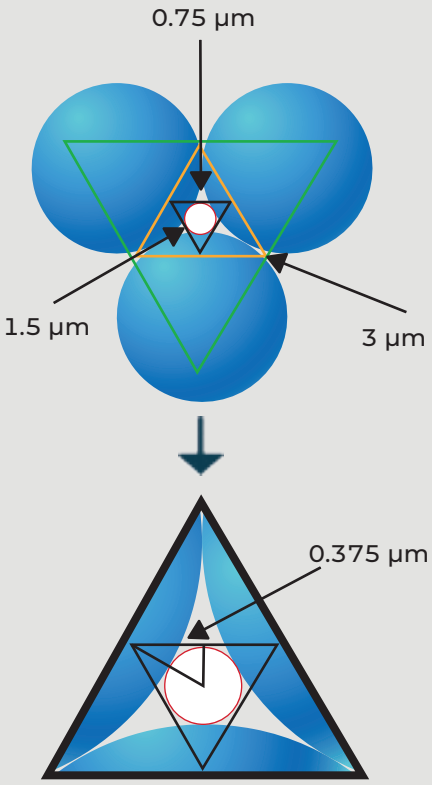


Figure 5b: Flow path size

FAQs: Extractables and Maintaining Analytical Integrity

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

What are extractables and where do they come from?

A syringe 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 syringe 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.

How do I know if my syringe filter is compatible with the sample fluid?

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. Pall performs rigorous extraction methods on their membrane products to prevent the risk of this occurrence.

“Extractable materials can jeopardize analytical results. For chromatographic analysis, scenarios resulting from extractable materials include sample absorption, coelution, and extraneous peaks”

Why are there so many different Acrodisc syringe filter types?

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

Generally, one filter type will not function for all applications due to limitations in hydrophobicity/hydrophilicity and chemical compatibility. However, Pall's wwPTFE membrane is a universal membrane for the majority of applications. It has excellent chemical compatibility for aqueous and aggressive organic solvents. Pall's wwPTFE membrane is hydrophilic and ideal when 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.

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.

How to avoid extractables' negative effects?

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 milliliters of fluid eluting from the syringe device. Generally, the amount of extractable materials eluted from the filter diminishes with the volume passed through the filter.

What contributes to an Acrodisc syringe filter's quality?

Pall's membranes and Acrodisc and Acrodisc PSF syringe filters are not the same as other look-alike products. The specific quality of raw materials, amount of quality control, membrane manufacturing procedures, and post-treatments all affect the resultant membrane properties and amount of extractable materials. Pall specifically selects the highest grade of materials and performs several extractions to ensure that the product is free from extractable materials for sample preparation. Additionally, our polypropylene housing material, is the highest grade of plastic with minimal additives, and passes United States Pharmacopeia (USP) Biological Reactivity Test, In Vivo <88> plastics testing.

What testing has Pall conducted to see the effect of the Acrodisc syringe filter on column lifetime?

Extractable experiments demonstrate the quality of the Acrodisc and Acrodisc PSF syringe filter product lines, which include wwPTFE, PVDF, nylon, and PTFE. Studies demonstrated that syringe filters, although similar in design and materials of construction can be dissimilar with regard to extractable materials. Pall uses high-quality raw materials and performs thorough post-treatment processes on membranes that will be used in its HPLC product line to minimize the occurrence of extractables. To verify low levels of UV-detectable extractables, samples of the entire HPLC Acrodisc syringe filter product line are evaluated during the manufacturing process.

Please contact Pall (www.pall.com/contact) to learn more about this testing and the HPLC certification.

Why is Filtration so Important to Method Development and Sample Preparation

Whether it is conducting R&D work or monitoring manufacturing and quality control, protecting data integrity and validity is essential. Nonetheless, it is hard to obtain accurate, reproducible data when high performance liquid chromatography (HPLC) and ultrahigh-pressure liquid chromatography (UHPLC) columns perform sub-optimally. One way to improve that performance is to filter both the sample and the mobile phases before analysis.

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The following interview was conducted between LCGC publication and Pall’s analytical sample preparation Product Manager.

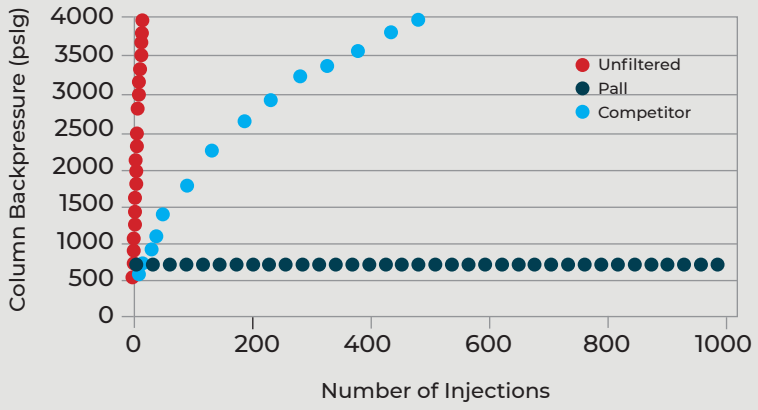
What is the importance of sample filtration?

The number-one answer is that it extends the life of an HPLC column by preventing particles from blocking it. It also allows for reduced system maintenance by reducing excessive wear on components like check valves, piston pumps, and pump seals. From a data perspective, it reduces ghost peaks, baseline drifts, and interferences.

When we think about what happens to particles in a column, the unfiltered particles travel through the column and get trapped in the interstitial spaces

between the different parts of the stationary phase. Then, the column end caps have even smaller pore sizes to trap those particles. So, the particles that we don’t filter out get clogged in there as well.

The front part of the column gets clogged faster than the rest of the column, which affects the data and system pressures, basically, stopping the analysis. Laboratories end up repeating the work and redoing a lot of preventative maintenance they would not otherwise do.



Effects of filters on HPLC column life following injections of unfiltered and filtered 0.05% latex sphere suspensions(1). 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 Pall Acrodisc® One syringe filters.

How do I select the best filter for my analysis?

Several different filter types and materials are available. Some are specified in regulatory methods, so laboratories that follow these methods are hesitant to diverge from them.

But when we think about analyses that are not specified for a certain material type, laboratories should consider the sample solution and whether the filter is compatible with what they're trying to process and analyze. Solvent polarity will help determine whether a hydrophobic filter (e.g. PTFE) is needed for things like organic solvents or a hydrophilic filter (e.g. nylon, PVDF, or PES) is needed for aqueous-based solutions. One must also consider things like the pH range and which membranes are compatible with which pH.

Laboratories must also consider the analytes they are trying to detect. Nylon is typically used with small molecules, but less often with proteins and peptides because it binds them. PVDF, by contrast, is used with proteins and peptides because of its low binding nature.

In addition, neither the membrane nor the housing should add any chemical contaminants to the chromatograms or the data.

Pall offers the Acrodisc One syringe filter, which is suitable for all these applications. It can filter organics, aqueous solutions, and acids and bases. It has a hydrophilic, water-wettable PTFE (wwPTFE) membrane that allows laboratories to use one filter type for all analyses and sample analytes. For the analyst, it reduces method validation time and simpli-fies the method development process. It also eliminates the need to have multiple filters on-hand.

The Acrodisc One syringe filter has high particle retention, which protects the column and extends its life as much as 52 times longer than if it was used with unfiltered samples. That can translate into $26,000–$30,000 worth of material cost savings alone.

The filter is low-drug and low-protein binding. It will not bias data by removing anything from the sample, and it contributes minimal extractables. This ultraclean product is suitable for numerous applications in various laboratory settings.

How does the wwPTFE membrane help me simplify method development?

It's all about method development and simplifying the process and method validation.

When developing and validating methods, analysts need to account for chemical interactions between their samples and filter, and how they affect the data. Their samples can be in a range of different conditions, along with different analyte concentrations. From there, analysts filter samples through the different membrane chemistries that they could potentially use and see how they affect the data.

The Pall Acrodisc One syringe filter simplifies that process with only one product to validate against. Regardless of the sample polarity, the pH, or the target analytes, only one membrane chemistry requires validation when using the Acrodisc One with wwPTFE membrane.

Analysts don't have to worry about mixing and matching sample conditions to membranes and validating each outcome. They can take any sample condition they might find in their laboratory, filter it through the Acrodisc One syringe filter, know that it is an inert chemistry that will not affect the sample, and then validate through that process.

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

It is, and is often overlooked. Most people purchase HPLC-grade reagents and assume they can be used as is. Filtering the mobile phase, however, is just as important as filtering the sample. It is never safe to assume that a mobile phase is free of waste particles, even if they cannot be seen with the naked eye. So, it is always a good idea to filter through either a 0.45 µm or 0.2 µm filter, depending on the needs of the system.

Similar to filtering samples, this process reduces overall wear and tear on the system and extends column life. It also degasses samples, which is a key part of keeping an HPLC or UHPLC system running optimally. Removing dissolved gases prevents bubble formation that could shift baselines, create shifts in retention times, and give questionable data.

For filtering mobile phases, Pall recommends the SolVac filter holder along with 47 mm membrane disc filters. This takes the place of traditional glass funnels, which is a significant improvement in safety and ease-of-use for analysts. With traditional glass funnels, the analyst must hold a full liter-bottle of solvent over the laboratory bench, pour, and wait for it to filter through before transferring the solvent into the HPLC mobile phase reservoir.

The SolVac filter holder sits right atop the mobile phase reservoir and filters directly out of the solvent bottle. Then, the reservoir can be put directly onto the HPLC system, which reduces cross-contamination and improves safety for the analyst. Because the filter holder is made of hard polypropylene, analysts don't have to worry about it breaking.

One more thing that's important to remember is that because we went through the method development and validation on the sample with the wwPTFE mem-brane and the Acrodisc One syringe filter, we also want to use that same membrane material type to filter the mobile phase. It reduces the time it takes to streamline the process and do method development and sample prep. then validate through that process.

The SolVac filter holder

The needs of a laboratory vary. In some cases, it may process very small-volume samples, and in others, it may process multiple samples at once. Is a syringe filter the right device for whatever samples a laboratory is working with?

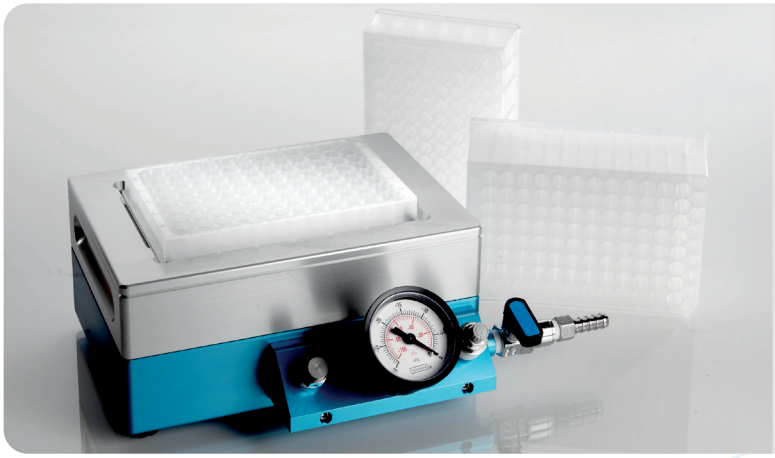
Pall recommends syringe filters for volumes of approximately 2–125 mL. For lower volumes, we recommend our Nanosep MF with the wwPTFE membrane. It has the same membrane chemistry, and making that available across product formats makes it easy to adjust volumes without having to re validate against different material types.

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 about 500 µL and has a hold-up volume of about 2 µL. When laboratories are working with ultrasmall volumes, they can spin through and recover all samples before it goes onto the instrument.

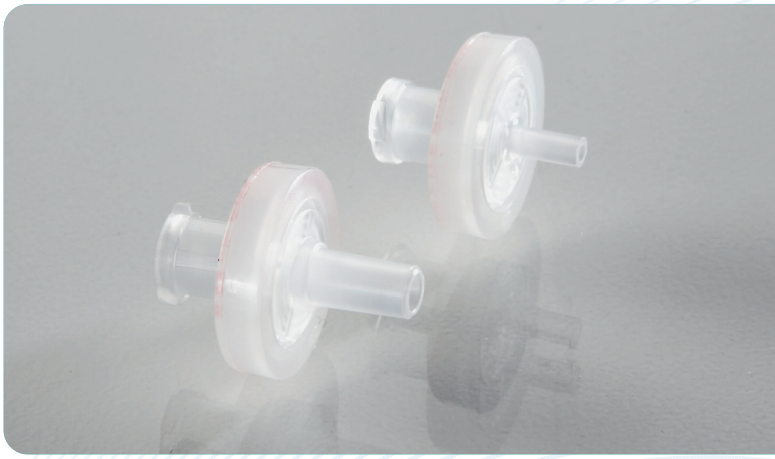
If laboratories are working with more than one sample or trying to design an experiment or set up a screening study, Pall offers the AcroPrep Advance 96-well filter plates. With 96 wells, they are appropriate for automated liquid handling and high-throughput analysis. Customers can also set up screenings in each of the wells to determine how best to move forward with research.

To summarize: For small volumes, use the Nanosep MF. For mid volumes, use either a 13 mm Acrodisc syringe filter or the Acrodisc One. And for multiple samples, filter them through the AcroPrep Advance 96-well filter plate. Pall has a filter option to suit all analytical needs.

Nanosep MF centrifugal devices



AcroPrep multi-well filter plates



13 mm Acrodisc syringe filters, Luer outlet and minispike outlet

APPLICATION NOTE: Suitability of Various Filters for Sample Preparation in Dissolution Testing, Based on Drug Binding

Introduction

Filtration is a common method of sample preparation in dissolution testing before an HPLC injection. Non-dissolved solids interfere with the resulting chromatography by continuing to dissolve throughout the period of the analysis and by plugging the HPLC column.

Sample preparation using filtration is a well-known method that results in more reproducible chromatography and longer column life, but has several potential drawbacks. The first is that the filter may adsorb active pharmaceutical ingredient (API) from the drug mixture, leaving the concentration in the filtrate too low and out of specification (OOS).

This study will evaluate filters for the adsorption of API. The drug product selection and product formulations in this study represent a wide variety of compounds that differ in chemical structures, ionization properties, and molecular weights, and therefore, differ in binding propensity. In addition, a broad range of media for the sample preparation is matrixed into the study to evaluate elution profiles of each filter. All experiments are designed based on well-characterized (validated) USP methods.

“Unwanted drug adsorption, as well as the presence of possible extractables eluted from the filter during routine pharmaceutical sample analysis, can be a serious problem and cause OOS results”

Pharmaceutical Products

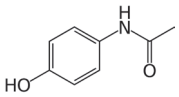
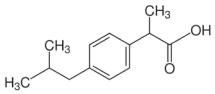
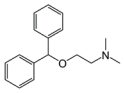
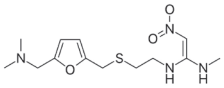
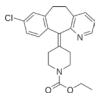
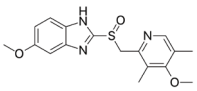
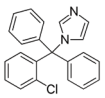
Drug Product (Brand Name)	Molecule Type	Molecular Structure	HPLC Mobile Phase
Acetaminophen (Tylenolu) Tablets	Acetamide MW 151.16		Mixture of organic (MeOH) and water (25:75)
Ibuprofen (Motrinu) Tablets	Phenylpropionic acid MW 206.28		Mixture of organic (ACN), and aqueous chloroacetic acid buffer (60:40), pH 3.0
Diphenhydramine (Benadrylu) Tablets	2-(Diphenylmethoxy)-N N-dimethylethylamine MW 291.82		Mixture of organic (ACN), and aqueous buffer, pH 3.0
Ranitidine (Zantacu) Tablets	Hydrochloric salt MW 350.87		Mixture of organic (ACN), and aqueous phosphate buffer, pH 7.1
Loratadine (Claritinu) Tablets	Pyperidine carboxylate MW 382.88		Mixture of organic (ACN and MeOH), and aqueous phosphate buffer (60:60:70),
Omeprazole (Prilosecu) Tablets	Benzimidazole MW 345.42		Mixture of organic (ACN and MeOH), and aqueous glycine buffer, pH 9.0
Clotrimazole (Lotrimin) Tablets	1-[(2-chlorophenyl)-diphenylmethyl] imidazole		Mixture of organic (ACN) and aqueous phosphate buffer (75:25) MW 344.84

Table 1: Chemical structures vary for pharmaceutical products

Methodology

Experimental Materials. IHPLC-grade syringe filters tested for this experiment include the Acrodisc One syringe filter with 0.45 µm wwPTFE membrane and the Acrodisc One syringe filter with GxF prefilter and 0.45 µm wwPTFE membrane.

The APIs used in this evaluation represent a range of different functionalities and structures and should, therefore, demonstrate a range of adsorption to membrane filters. As seen in Table I, the chemical structures vary from single aromatic rings to multiple aromatic rings to a non-aromatic, polycyclic structure. Included in the study are an acid, a base, an amide, a urethane, an ester, and a lactone structure. The physical structures vary from a more flat and planar structure like that of acetaminophen to the flat but flexible structure of ibuprofen and ranitidine HCl to the more rigid and distinct three-dimensional structures of omeprazole and clotrimazole.

Fraction Collected	Acetaminophen Tylenol		Ibuprofen Motrin		Diphenhydramine HCl Benadryl		Ranitidine Zantac		Loratadine Claritin		Omeprazole Prilosec		Clotrimazole Lotrimin	
	% LC_F	% $LC_{\Delta FC}$	% LC_F	% $LC_{\Delta FC}$	% LC_F	% $LC_{\Delta FC}$	% LC_F	% $LC_{\Delta FC}$	% LC_F	% $LC_{\Delta FC}$	% LC_F	% $LC_{\Delta FC}$	% LC_F	%$LC_{\Delta FC}$
%LCC	101.3		101.2		101.6		99.0		96.0		99.6		100.8	
1st mL	100.7	-0.5	101.2	0.0	93.2	-8.3	99.4	0.3	99.5	3.7	100.5	0.9	100.3	-0.5
2nd mL	100.2	-1.1	101.2	0.0	100.8	-0.7	99.3	0.3	99.1	3.2	100.5	0.9	100.2	-0.6
3rd mL	101.6	0.3	101.5	0.4	101.2	-0.3	99.3	0.3	99.7	3.9	100.3	0.7	100.3	-0.5
5th mL	99.9	-1.3	101.4	0.3	100.9	-0.7	99.3	0.3	99.0	3.2	100.4	0.8	100.2	-0.6
10th mL	100.2	-1.0	101.1	-0.1	100.7	-0.9	99.3	0.3	99.0	3.2	100.4	0.8	100.1	-0.7
15th mL	100.8	-0.4	101.6	0.4	99.4	-2.1	99.1	0.1	98.1	2.2	100.4	0.8	99.8	-1.0
20th mL	100.5	-0.7	101.3	0.1	100.7	-0.9	99.0	0.0	98.1	2.2	100.3	0.7	100.9	0.1

Table 2: Amount of API expressed as percentage of label claim in centrifuged samples (%LCC) and in samples filtered with Acrodisc One syringe filter GxF/0.45 µm wwPTFE (%LCF). The difference in the magnitude in recovery of each drug following filtration or centrifugation is shown as %LCΔFC. API concentrations were determined by HPLC analysis with UV detection at 243 nm for Acetaminophen, 254 nm for Ibuprofen, 230 nm for Ranitidine HCl, 254 nm for Loratidine, 305 nm for Omeprazole, and 206 nm for Clotrimazole according to USP methods.

Methods. USP methods , such as USP <711>, are intended for drug testing, so slight modifications to the sample handling in the methods are needed for filter comparison.

Well-characterized pharmaceutical samples are analyzed in duplicate and triplicate followed by statistical evaluation, which allows for increased reliability of the drawn conclusions on filter suitability.

Results are obtained by HPLC analysis with UV detection. All calculations are performed according to each specific USP procedure against the appropriate, well-characterized (certified), corresponding USP reference standard.

- Label claim percentage (% LC) of each drug is calculated as a ratio of the amount of drug that is found during analysis in each filtrate to the amount known (or claimed) to be present in the tested solution, and expressed as a percentage.
- Recovery of each drug upon filtration (i.e., % LC to centrifuged) is calculated as a ratio of the amount that is found during analysis in each filtrate to the amount that is found in the centrifuged sample, and expressed as a percentage.

Specifications for the filtered samples are set to meet wider 97–103% interval of acceptability. This criterion is set based on the assumption that handling of the filtering process should not add more than 1% error to the sample analysis regardless of the individual filter compatibility.This assumption was validated in the filter study (see Tables 2 and 3).

The data spread (% relative standard deviation [RSD]), which is caused solely by filtration, is less than 1% for all filters. Therefore, results outside the 97-103% interval are indicating OOS results and signaling potential filter incompatibility.

Filter Evaluation Methodology. The flush volume required for consistent sample analysis (flush study) is determined in three steps:

1. Centrifuged samples of each drug are prepared in duplicate and analyzed in duplicate for average percent recovery of the active compound against the label claim. All testing follows USP methodology (Tables 2 and 3).

2. 20 mL of the sample solution are run through each filter. The 1st, 2nd, 3rd, 5th, 10th, 15th and 20th 1 mL aliquots are collected and analyzed. The drug concentration is measured after filtration. Duplicate HPLC injections of the seven 1 mL aliquots are performed for each filter, with each drug evaluated (280 samples total). The flush volume evaluation from step two is determined as sufficient when the recovery value for the filtered sample is within 97–103% of the centrifuged sample.

3. Filtered aliquots (from step two) are compared with centrifuged samples (from step one). The recovery of each drug preparation is determined as a percentage of label claim and as a ratio of percentage of label claim to the centrifuged sample, according to USP methodologies (see Tables 2 and 3).

Fraction Collected	Acetaminophen Tylenol		Ibuprofen Motrin		Diphenhydramine HCl Benadryl		Ranitidine Zantac		Loratadine Claritin		Omeprazole Prilosec		Clotrimazole Lotrimin	
	% LC_F	% $LC_{\Delta FC}$	% LC_F	% $LC_{\Delta FC}$	% LC_F	% $LC_{\Delta FC}$	% LC_F	% $LC_{\Delta FC}$	% LC_F	% $LC_{\Delta FC}$	% LC_F	% $LC_{\Delta FC}$	% LC_F	% $LC_{\Delta FC}$
%LCC	101.3		101.2		101.6		99.0		96.0		99.6		100.8	
1st mL	100.4	-0.8	100.9	-0.2	102.2	0.6	99.5	0.4	98.0	2.1	99.7	0.1	101.0	0.2
2nd mL	100.5	-0.8	100.7	-0.4	101.7	0.2	99.2	0.2	97.7	1.9	99.7	0.1	101.7	0.9
3rd mL	100.6	-0.6	101.2	0.1	101.8	0.3	99.2	0.1	97.9	2.1	99.7	0.1	100.9	0.1
5th mL	100.7	-0.5	100.8	-0.4	102.0	0.4	99.2	0.2	97.9	2.0	99.7	0.1	101.0	0.2
10th mL	100.9	-0.4	100.9	-0.2	102.0	0.4	99.1	0.1	97.4	1.4	99.7	0.1	101.1	0.3
15th mL	101.0	-0.3	101.2	0.1	102.0	0.4	99.3	0.3	98.1	2.2	100.0	0.4	100.5	-0.3
20th mL	101.1	-0.1	101.0	-0.2	102.0	0.4	99.2	0.2	98.0	2.2	99.9	0.3	100.3	-0.5

Table 3: Amount of API expressed as percentage of label claim in centrifuged samples (%LCC) and in samples filtered with Acrodisc syringe filter 0.45 µm wwPTFE (%LCF). The difference in the magnitude in recovery of each drug following filtration or centrifugation is shown as %LCΔFC. API concentrations were determined by HPLC analysis with UV detection at 243 nm for Acetaminophen, 254 nm for Ibuprofen, 230 nm for Ranitidine HCl, 254 nm for Loratidine,305 nm for Omeprazole, and 206 nm for Clotrimazole according to USP methods.

The instrumentation was:

1. Waters HPLC 1525 with Waters 2487 UV Detector

2. HPLC columns (as directed in each applied USP method)

3. General laboratory equipment and Class A analytical glassware

Results

Both tested filter types perform comparably for the tested drugs. In this study, the adsorption of an active ingredient on a filter is evaluated in successive aliquots of filtrate and compared to centrifuged samples.

These experiments reveal feasibility of the applied methodology and allowed for determination of the filtration conditions with the least risk of handling error. Subsequently, filter performance is compared in conditions resembling routine finished product analysis at the preferred handling conditions.

Unlike previous studies showing that a flush volume of up to 3 mL can be required to compensate for OOS results when filtering API, the data obtained using the Acrodisc One syringe filter without a prefilter show no such requirement for any of the drugs tested. The Acrodisc One syringe filter with GxF prefilter required a 1 mL flush for only one of the tested drugs, diphenhydramine. Each aliquot tested, from the first to the twentieth milliliter of sample was within specification. The ultra-low binding nature of the wwPTFE membrane used in the Acrodisc One syringe filter simplifies testing procedures and methods.

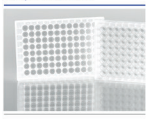
Since there is no longer a requirement for an initial flush volume when using the Acrodisc One syringe filter, the analyst can be comfortable knowing that there is no loss of API due to binding even across a wide variety of drug types and chromatographic requirements.

The results confirm that filtration does not affect the finished drug product assay numerically and all tested filters are suitable for achieving 97-103% accuracy with the data spread (precision) less than 1%.

Conclusions

It is accepted that membrane and drug chemistry can affect the amount of adsorption of drug products in a negative manner. If the filter membrane adsorbs too much API, the results may be OOS. Choosing and using filters correctly (to reduce the amount of adsorption) is critical for accurate HPLC in dissolution testing. This investigation demonstrates that Acrodisc One syringe filters with wwPTFE membrane, with and without glass prefilter layers have acceptable drug binding performance in a wide variety of API structures and chemistries.

Analytical sample preparation product selection table

Product	AcroPrep Advance 350 µL Filter Plate	Nanosep MF Device	AcroPrep Advance 1 mL Filter Plate	AcroPrep Advance 2 mL Filter Plate	4 mm Acrodisc Syringe Filter	13 mm Acrodisc Syringe Filter	25 mm Acrodisc Syringe Filter	25 mm Acrodisc PSF Syringe Filter
								
Sample Volume	≤ 300 µL	≤ 500 µL	≤ 900 µL	≤ 1.9 mL	≤ 2 mL	≤ 10 mL	≤ 100 mL	≤ 125 mL
Membrane Choice								
wwPTFE	●	●	●	●		●	●	●
PTFE	●		●	●	●	●	●	●
Nylon						●	●	●
PVDF						●	●	●
PES (IC)						●	●	●
Versapor						●		●
Supor						●	●	●
Glass Fiber								●

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