Filtration: Preventative Maintenance for HPLC

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by Analytical Technical Service

Introduction

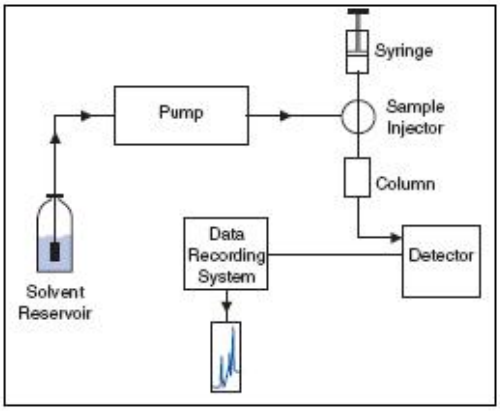
Sample and mobile phase filtration are simple, economical practices that serve to extend the life of consumable HPLC parts, decrease system wear and tear, and preserve the integrity of the HPLC system. The adverse effects of improper filtration practices that occur to each component of the HPLC system are systematically and thoroughly explored in this section. By reviewing these consequences, the analyst 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.

Sample and mobile phase filtration are simple, economical practices that serve to extend the life of consumable HPLC parts.

Serious problems in HPLC 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 maintenance practices. Routine sample and mobile phase filtration are simple, inexpensive and convenient ways to decrease HPLC problems. Regardless of the technical intricacies and cost of the system chosen, all HPLC systems have the same basic components, indicated in Figure 1.

A basic HPLC system consists of a solvent reservoir, pump, injector, column, detector, and data recording system. Particles and microbial growth not removed by filtration interfere with nearly every system component. This paper serves as a troubleshooting guide to problems associated with inefficient filtration. It is ordered in sections corresponding to the specific HPLC component affected.

Figure 1
A Basic HPLC System Configuration



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Solvent 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 also removes dissolved oxygen that can result in oxidative degradation of the sample and mobile phases, and reduces the sensitivity and operating stability of ultra-violet, refractive index, electrochemical and fluorescence detectors. By filtering 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 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. By dedicating a reservoir to each solvent, and frequently changing and cleaning the reservoir bottle and sinker frit, contamination problems are reduced.

The primary concern when choosing a solvent filter is solvent compatibility with the filter material. Pall offers several filters to accomodate the various types of HPLC solvents. Pall's GHP (hydrophilic polypropylene) membrane is a universal solvent membrane to reduce 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 2
Mobile Phase Filtration Apparatus

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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. When a pump will not deliver degassed solvent, particulate build-up is possible. Monitoring pressure changes allows quick assessment of blocked frits, or columns, through exaggerated pressures. Retention times also may be affected. 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 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.

Pump seal life can be extended by filtering the mobile phase solvents.

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. Buffer crystals built up from evaporated mobile phase also will accelerate wear. Pump seal life can be extended by filtering the mobile phase solvents to remove the particles responsible for accelerated seal wear.

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Injector

Injecting clean samples prolongs injector and column life. Samples are cleared of particulate and bacteria with disposable syringe tip filters. Disposable filters range in size (3-25 mm) and pore size (0.2 to 1.0 µm). Syringe tip filters are membranes configured 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 tip 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.

Filtering your sample prior to injection can prolong your injector and column life.

Table 1
Standard Materials Incorporated in Syringe Tip Filters

Membrane Material	Nylon, PTFE, Polyvinylidene fluoride (PVDF), Cellulose (mixed esters, acetate), Nitrocellulose, Polysulfone, Polyethersulfone, Acrylic Copolymer, Polypropylene
Housing Material	Polypropylene (PP), Polyethylene (PE), Polyvinylchloride (PVC), Modified Acrylic, Nylon
Prefilter Material	Glass, Polypropylene, Cellulose Nitrate, Polyethersulfone

Choosing the proper filter requires a knowledge of filter/solvent compatibility and the chemical/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, absolute pore size, and nominal particulate retention. Typically for HPLC applications, the 0.45 µm pore size filter is selected, or the 0.2 µm filter for bacterial removal from buffers. For particulate-laden samples, Pall incorporates large pore size prefilters in one device with smaller pore size membranes. Low protein binding and sterile filters also are available.

Sample and solvent filtration deters low-volume injector fittings from blockage, scratching and leakage.

HPLC injectors are available in several styles including a septum, septumless stop-flow device, and a valve system, either manual or automated. A valve injector is most typical. An injector should ensure reproducible sample introduction. Sample and solvent filtration deters low-volume injector fittings from blockage, 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, are also subject to blockage. Other contributors to HPLC problems include mismatched or damaged injector components, variable sample volumes, leaks, and increased system pressure. Backflushing is recommended prior to disassembly. 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.

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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 pretreatment, 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 ideal 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 retained 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.

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

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Column

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 by badly tailing, splitting, and non-gaussian bands, without a change in retention time, blocked frits or a column void has occurred.

Tips from instrument manufacturers to prevent physical changes include:

- filtering solvents through a 0.2 or 0.45 µm filter, such as Pall Acrodisc® syringe filter
- prefiltering mobile phase buffers daily with a 0.2 µm filter to remove bacterial growth,
- filtering samples through a 0.2 or 0.45 µm filter,
- utilizing 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.

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Detector

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.

Solutions for removing the negative effects of oxygen in detectors include filtering buffers through a Pall 0.2 or 0.45 µm membrane filter.

Insufficient mobile phase degassing causes pressure fluctuations and/or sharp noise spikes due to bubbles. Bubbles form when the mobile phase mixture becomes saturated with air. This interferes with detector operation. Degassing methods include mobile phase filtration followed by a continuous degassing through helium sparging, ultrasonic treatment, vacuum application, or heating with vigorous stirring.

Air bubbles cause problems with detector response due to excessive oxygen interference. Solvent degassing removes dissolved oxygen from the mobile phase which may result in oxidative degradation of the sample, a reduction in sensitivity, and decreased operating stability of UV/VIS, fluorescence, refractive index, and electrochemical detectors.

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.

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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 phases, articles 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.

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