LC TROUBLESHOOTING

Preventive Maintenance — Just Three Things

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Filter, degas, and flush. Practice these three to stay trouble-free.

I teach several liquid chromatography (LC) training courses each year. Recently, I was discussing my classes with a colleague who has made it a point to study the learning process. He told me that typical students retain only three central ideas from a short course. I'm not sure that I believe this theory, but I decided to try to apply it to the LC troubleshooting course I teach. Wow, seven hours of lecture condensed into three central ideas — now that's a challenge!

My three magic words for preventive maintenance are filter, degas, and flush. Certainly they don't cover all of LC troubleshooting, not by a long shot, but if you put these ideas into regular practice, you'll go a long way toward preventing problems with your LC system.

FILTER

The principle of filtration involves preventing unwanted contaminants from entering the LC system. Filtration applies to four specific areas: solvent filtration, sample filtration, in-line filtration, and guard columns. Let's look at each of these in turn.

Solvent filtration: As a general rule, every mobile-phase component that goes into an LC instrument should be filtered through a 0.5-μm (or smaller) porosity filter. For most mobile-phase systems this means filtering the solvents just before you pour them into the mobile-phase reservoirs. When you use pure HPLC-grade solvents or laboratory-prepared HPLC-grade water, you can skip this step because the solvents are filtered through a 0.2-μm filter before bottling. Commercial HPLC water-purification systems also include a final 0.2-μm filter. Because these liquids have already been filtered through such a fine filter, it doesn't make much sense to filter them through a 0.5-μm filter — in fact, there's potential for contamination if your filtration system contains particulate matter in the 0.2–0.5 μm range.

If you use any reagents other than the pure solvents mentioned above, however, filter the reagent mixture before placing it in the mobile-phase reservoir. Some reagents contain fine particulate matter that must be filtered out. Other reagents, especially buffers, may form precipitates when mixed with organic solvents.

And, just to mention the obvious, make sure that the mobile-phase reservoirs are clean. If you use laboratory glassware or commercial LC reservoirs, wash the reservoirs every week or so. If you use brown-glass solvent jugs as reservoirs, throw them away on a similar schedule. Reservoirs can become contaminated as dust settles in them, mobile phases can selectively evaporate, and microbes can grow. A regular cleaning or replacement schedule will help prevent problems.

In addition to filtering the reagents before transferring them to reservoirs, each reservoir should be fitted with an inlet-line filter on the end of each pump-inlet tube. This frit serves two functions: it filters out large, inadvertent contaminants, and it holds the inlet line at the bottom of the reservoir so that no air is drawn into the pump. Inlet-line filters are available in a variety of sizes, including 10-μm, 5-μm, and smaller porosity frits. The filters are most often made of stainless steel, but a variety of plastic units are available if you need to avoid metal in your LC system. Because these filters are designed to trap the occasional dust particle, I recommend using the 10-μm porosity frits. They do not get blocked as quickly as the smaller frits and are less likely to create a flow resistance in the inlet line, which can lead to pump cavitation. When filter blockage is suspected, remove the filter and replace it with a new one. A simple test for blockage is to siphon mobile phase through the inlet line with the filter attached. The mobile phase should flow freely if the filter is functioning properly.

Why filter solvents? The primary reason is to remove particulate matter that can block the frits downstream and accelerate wear in the moving parts of the system (primarily the pump and injector). A secondary reason for filtration is to remove microbial contamination in systems sensitive to microbial growth. This problem can occur when mobile phases with high water content are used, especially with growth-supporting buffers such as acetate. A 0.2-μm filter will effectively remove biological contaminants.

Sample filtration: Another likely source of particulate matter is the injected sample. Particulate matter from the sample can damage the injection valve or block the in-line filter or column inlet frit. In extreme cases, connecting tubing can become blocked. The simple preventive measure of filtering each sample will go a long way toward eliminating problems with sample-related particulates. Most workers prefer to use the self-contained disposable filters that fit on a Luer-lock syringe. Sample filters are expensive, however, at $1 or more per filter. In many cases, samples are clean enough that they don't require filtration, so you may be wasting money and adding complexity to the method by filtering every sample. The criterion that I use is a visual one: I hold the sample vial up to a light and look for cloudiness, opalescence, and visible particulate matter. If any of these symptoms are present, I filter the sample. Finally, be sure that the injection matrix is compatible with the mobile phase. A high-salt injection matrix, for example, may precipitate when injected into an acetonitrile-based mobile phase. In cases like this, sample filtration will not prevent precipitation. You can test for precipitation by adding a drop or two of the sample matrix to a test tube of mobile phase. If you see any cloudiness when the sample contacts the mobile phase, you are likely to encounter precipitation problems.

In-line filtration: I recommend using a 0.5-μm porosity in-line filter just downstream from the sample injector. This filter will trap particulates from the occasional sample that should have been filtered but was not. It will also trap debris from worn pump seals, valve rotors, and other worn parts upstream from the filter. Because of the fine porosity of the in-line filter, it will become blocked before the larger 2.0-μm filter at the head of the guard column or analytical column. The in-line filter takes only a minute or so to change, so downtime is minimal. In addition, use of this filter eliminates the need to change the column frit, which sometimes causes column damage.

Guard columns: You might not think of guard columns as filtration devices, but they provide filtration in three ways. First, the frit on the guard column acts as a physical filter. These frits typically have 2.0-μm porosity, so

they trap any material that might otherwise collect on the frit at the head of the analytical column.

Second, the guard column acts as a chemical filter to remove strongly adsorbing materials from the sample before they reach the analytical column. If you remove the frit from the head of a used column, you often see discolored column packing. The yellow, green, or brown color you see is protein, plant pigments, or other strongly retained materials. As these contaminants build up on the column, the packing surface available for normal chromatography is reduced, and gradually the retention characteristics of the column change. If a guard column is used, these contaminants will build up on the guard column instead. The guard column should be discarded before the contaminants bleed through to the analytical column. Thus, by replacing the guard column in a timely manner, the life of the analytical column can be greatly extended.

The final filtration function of the guard column is to pretreat the mobile phase before it reaches the analytical column. Most mobile phases have little or no adverse effect on the column packing material, but some mobile phases are more damaging than others. For example, most silica-based columns are recommended for use in the pH 1.5–7.5 range. When the mobile-phase pH is outside this range, the silica will dissolve or the bonded phase will be cleaved from the surface. Because the guard column is the first column the mobile phase reaches, it acts as a sacrificial column, protecting the analytical column from attack when inappropriate mobile-phase conditions are used. Note, however, that this function should not be the primary reason for using a guard column — it is important to make sure that the mobile phases are chemically compatible with the analytical column.

DEGAS

A thorough degassing of all solvents is one of the easiest ways to increase an LC system's reliability. Although some systems do not require degassing, I have yet to see a system that would not benefit from routine solvent degassing. Degassed solvents are less likely to cause problems such as cavitation, check-valve failure, and noise spikes from bubbles passing through the detector.

I prefer helium sparging for degassing solvents. In this technique, either a homemade or commercial degassing apparatus is used to sparge helium through the contents of each reservoir. Vigorous sparging for 4–5 min removes most of the dissolved air from a bottle of solvent. The helium flow then is turned down to a trickle to maintain a degassed solvent system. For LC systems that are relatively immune to bubble problems, many workers degas solvents on a batchwide basis at the beginning of each day. The degassed mobile phases are then used throughout the day with no further degassing. Helium sparging is a fast and effective way to remove excess air from the solvents. If vigorous sparging is prolonged, however, some risk of changing the composition of mixed solvents exists as the more volatile components evaporate selectively. This is not a common problem, but if you avoid prolonged sparging you minimize the risk.

Vacuum degassing, either alone or in combination with sonication, is another popular method for degassing solvents. A water aspirator or simple vacuum pump draws the more volatile gases from the mobile phase. When bubbles stop appearing, the vacuum is released, and the mobile phase is used. Vacuum degassing is not as effective as helium sparging, but it is satisfactory for many LC systems, especially those that use high-pressure mixing.

Recently, degassing systems using semipermeable membranes have become widely available. A vacuum is drawn on the outside of the membrane, and dissolved gasses pass through it, leaving a degassed mobile-phase stream. The product literature claims that these devices are as effective as helium sparging, less expensive to operate, and more environmentally sound. The users I've talked to are happy with the performance of these devices.

FLUSH

No matter what sample pretreatment and filtration method you use, contaminants will build up in the LC system. If you flush the system periodically with appropriate solvents, you can remove most of these contaminants and mobile-phase components that can cause problems when the system is out of service.

Mobile phase: The first step in system flushing is to remove mobile-phase buffer or salt components that can precipitate under strong solvent conditions. (Buffer precipitates can cause system blockage and greatly accelerate pump and injector wear.) The safest approach is to flush the system first with nonbuffered mobile phase. For example, if the mobile phase is 60:40 methanol–buffer, flush with 60:40 methanol–water. This will remove the buffer without changing the organic content. Five column volumes of solvent should be sufficient for this step. The column volume (in milliliters) can be estimated for standard 0.46-cm i.d. columns by multiplying the length (in centimeters) by 0.1. Thus, a 25-cm column has a volume of ~2.5 mL. So, for the 25-cm column, five column volumes would be ~12.5 mL. Remember that with any flushing or equilibration step the volume, not the time, is important. This means that you would get the same flushing effect in 12 min at 1 mL/min as you would get in 6 min at 2 mL/min. Avoid excessive pressure; we like to keep the pressure below ~2500 psi in our laboratory.

After the buffer has been removed from the system, switch to 100% strong solvent such as the methanol used in the present example. Ten column volumes (~25 mL for the 25-cm column) should be sufficient for flushing. Many workers use this as an end-of-day procedure:

just flush to strong solvent and leave the system until morning, and then reequilibrate the column with mobile phase and proceed with analysis.

If you suspect that the strong-solvent flush has failed to remove the contaminants, you can use an even stronger solvent to flush the column. This can be the case if you see an undulating baseline resulting from elution of very strongly retained peaks or if you know that you have introduced contaminants that need to be washed from the system. In the present reversed-phase example, methylene chloride is my next solvent of choice. Flush the system with 10–20 column volumes of methylene chloride, then return to the strong mobile-phase solvent (methanol). Be sure to use miscible solvents in each step of the flushing procedure or you may end up with an immiscible aqueous and organic phase in the column (remove this by flushing with isopropanol). Use your knowledge of the sample and sample matrix to choose the appropriate flushing conditions. For example, ethylenediaminetetraacetic acid (EDTA) will remove metal contaminants, and chaotropes such as 4 M guanidine will help remove protein residues.

Pump: For most applications, the mobile-phase flush described above will be satisfactory for the pump. When high buffer–content mobile phases (for example, >100 mM) are used, pump-seal wear can be accelerated. When this occurs, flushing the pump head behind the pump seal can extend seal life greatly. Many of today's LC systems are equipped with a flushing channel just behind the check valves on each pump head. Flush ~10 mL of warm water through the channel to remove any buffer leakage, and follow that with another 10 mL of isopropanol to remove the water. Workers who routinely use this procedure find that the pump seals last indefinitely.

Injector: For the most part, injectors and autosamplers are self-flushing. Be sure to use a wash solvent that is compatible with both the mobile phase and the sample matrix. With manual injectors and some autosamplers, it is necessary to flush the waste line occasionally because solvent evaporation can leave buffer and sample residues that can block the waste line.

SUMMARY

That is preventive maintenance in a nutshell. Prevent contaminants from entering the LC system and remove those that do get in. Good preventive maintenance practices, such as those described here, will help you avoid most common LC problems.

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