LC TROUBLESHOOTING

Only Three Things

Preventive maintenance compressed.

John W. Dolan
LC Troubleshooting Editor

I remember reading a study on learning in the all-day short-course format. Because teaching liquid chromatography (LC) classes is a significant part of my work, my attention was captured. The writers claimed that in a 6–8 h class, only three points would be remembered. One of my LC troubleshooting classes has approximately 200 slides — what does this say about how effective a short course is at conveying critical knowledge? As the saying goes, I have tried to make lemonade out of these lemons, and use the "only three things" concept to help reinforce what I think are the key points. So, this month's "LC Troubleshooting" installment will use these points to form the core of a preventive maintenance program for your LC system.

Degassing

Mobile phase degassing is the single most effective way to avoid problems with an LC system. Liquid chromatographs and air just weren't meant to be together! LC pumps are very effective at pumping liquids, but introduce an air bubble and in the best circumstances you will observe a momentary reduction in the flow rate and a drop in the system back pressure. If the bubble is large enough, the pump will not deliver any solvent, and if the pressure drops below a preset low-pressure limit, the pump will stop. Some pump designs will pass bubbles fairly well, whereas other designs will fail to operate when a bubble is present.

Once a bubble passes through the pump, it usually will stay in solution due to the system pressure as it passes through the column. But on arrival at the detector, the system pressure returns to atmospheric pressure and the bubble might reappear in the detector flow cell, causing spikes in the chromatogram. This problem can be minimized by the use of a back-pressure restrictor on the detector outlet to provide sufficient pressure to keep bubbles in solution until they exit the detector. Of course, care needs to be taken not to exceed the pressure limits of the flow cell, or detector damage can occur.

Although noise spikes are the most common symptom of bubbles going through the flow cell, such as with UV detection, some detectors can be very sensitive to the presence of air. For example, dissolved oxygen has been reported to quench the fluorescence of some compounds when the fluorescence detector is used (1). In the reductive mode, the electrochemical detector is extremely sensitive to dissolved oxygen. Care must be taken to eliminate oxygen from the mobile phase and avoid oxygen-permeable tubing (such as PTFE) in the flow stream.

All of these problems related to dissolved air in the mobile phase can be avoided if proper care is taken to degas the mobile phase before it is used. For many years, the gold standard for degassing has been helium sparging. This simply involves bubbling helium through a frit placed in the mobile phase reservoir. Helium sparging is the most effective way to remove dissolved air from the mobile

phase, removing approximately 80% of the oxygen (2). With a well-distributed sparging stream, one volume of helium will remove almost all the gas that can be displaced from an equal volume of mobile phase (3). This means that 1 L of helium bubbled through 1 L of mobile phase will do the job.

Liquid chromatographs and air just weren't meant to be together!

Helium is the only effective way to remove sufficient oxygen from the mobile phase to avoid problems specific to dissolved oxygen, such as the fluorescence quenching or electrochemical detector problems mentioned earlier. However, if the main objective is to remove sufficient dissolved air so that bubble formation is not a problem, vacuum degassing is also effective as a degassing technique. Most of today's LC systems come with an in-line vacuum degasser either as a standard feature or an optional one. In-line degassing is simple to use, trouble-free, and effective. I give it credit for the huge reduction in bubble-related complaints that I have heard in the last few years.

Filter

Unless special precautions are taken, any particulate matter that enters the LC system will end up on the inlet frit of the column, eventually blocking the column, increasing the system back pressure, and distorting peaks in the chromatogram. As a consequence, any effort made to reduce the particulate load of the system will pay back in reliability. There are three major sources of particulate matter in the LC: the mobile phase, the sample, and the wear of internal components.

If the mobile phase comprises only high performance liquid chromatography (HPLC)-grade solvents, there is no need to filter the mobile phase. This is because organic solvents, such as acetonitrile or methanol, are filtered through 0.2-µm porosity filters during the manufacturing process. Similarly, whether you buy HPLC-grade water or generate it in the laboratory with a purification system, the last step is filtration through a 0.2-µm filter. However, if there are any additives that were once solids, such as phosphate buffer, filtration of the mobile phase is a wise step to take. Although a buffer salt might be of high purity, it can contain particulate matter, such as bits of plastic generated when the lid of the bottle rubs on the edge of the bottle. In some cases, a solid additive might not dissolve completely, leaving bits of debris in the mobile phase. Any particulate matter from the mobile phase also can cause check valve leakage if it gets trapped in the check valves. Filtration of the mobile phase through a 0.5-µm porosity filter is an effective way to remove any particles from the mobile phase. 0.2-µm filters can be used, but they are not much more effective than 0.5 µm ones for this application and they filter much more slowly. Some laboratories write their mobile phase preparation standard operating procedures (SOPs) so that mobile phases prepared only from HPLC-grade liquids do not need to be filtered, whereas all other mobile phase compositions require filtration before use. It also is important to use a sinker frit at the inlet end of the tubing connecting the reservoir and pump. This frit typically is ≥ 10 µm porosity, so it is not a substitute for a mobile phase filtration step, but it does keep dust out of the system and it holds the tubing in the bottom of the reservoir for operational reliability.

The sample is a second source of particulate matter in the LC system. Some laboratories filter all their samples through a 0.5-µm filter before loading them in the autosampler tray. This is an effective way to remove sample-related particles, but I have several concerns about this procedure that cause me to avoid sample filtration in most cases. First, it is expensive — $1 or more per filter — which can add a significant amount to sample processing costs. Also, for a vali-

dated method, you need to validate the filtration process and use filters for every sample, not just the ones you think need filtration. You never get 100% of the sample through the filter — there are always a few microliters left behind. Is there any adsorptive loss on the filter or contaminants leached from the filter? If there is loss, is it the same at all sample concentrations? If filtration is to be used, all of these issues must be addressed in the validation process, which can add work and expense to the validation procedure. I have found that an equally effective procedure for most samples is to centrifuge the sample in a bench-top centrifuge for a few minutes to settle out any particles, then transfer the supernatant to the autosampler tray.

The final major source of particulate matter in the LC system is wear of pump seals and injection valve rotors. Pump seals generally will last six months to a year in a normal laboratory. I recommend replacing these on a semiannual or annual basis as part of a preventive maintenance session. The cost is low compared with the expense of a column blocked by pump seal particles. Some pumps have frits or screens in the flow path to trap wear debris from the pump seals before it works its way further downstream. Consult your pump operation manual to find the recommended cleaning or replacement intervals for such filters. Autosampler rotor seals also wear over time, but in my experience, it takes several years of intense use before the rotor seals wear out. If your system has a function that counts injection valve cycles, you might be able to set an alarm to notify you when a specific number of valve cycles have occurred. I have heard quotes that the injector should last for 20,000 cycles, but this is only 10,000 injections — not much of a lifetime for a laboratory involved in regular sample analysis. I think they last much longer — several years in my experience. Of course, pump seal and rotor seal wear will increase in the presence of more abrasive mobile phases. Thus, if you routinely run ion-exchange gradients, such as 0–1 M sodium chloride, I would expect the seals to wear more quickly than if you use reversed-phase conditions with 10 mM phosphate buffer.

No matter what source of particulate matter I am trying to eliminate, I always use an in-line 0.5-µm porosity filter between the autosampler and the column on every system, even if a guard column is in use. This in-line filter will become blocked instead of the 2-µm filter at the head of the column, and it can be replaced in a few minutes with an inexpensive replacement frit. Just check the system pressure at the beginning of each batch of samples. When the pressure rises to a trigger point, such as a 25% or 500-psi increase, just replace the frit and you should be back in service in a few minutes.

Flush

My third key practice for reliable LC system operation is to keep it clean. If you follow the flow path through the system, you will notice several areas that can benefit from regular flushing. First, the mobile phase reservoirs

should be washed or replaced with each new batch of mobile phase. A dirty reservoir can contaminate an otherwise pure mobile phase. I don't like to use buffers longer than about a week and organic solvents for more than a month. Rather than refilling a reservoir, get in the habit of filling the reservoir, using the solvent, then replacing the reservoir when a new batch of solvent is made rather than topping it off.

Next in line is the pump. I don't like to shut off a pump for more than a few minutes if it contains a nonvolatile buffer, such as phosphate. When mobile phase evaporates, such as on the back side of the piston seal, nonvolatile materials will leave a solid deposit. This is one of the major causes of pump seal wear. If buffered mobile phases are left in the system for extended periods, especially if acetonitrile is used, they can form precipitates, which can cause seal wear and check-valve leakage. So flush the pump with nonbuffered mobile phase before shutting it off for any extended period.

The autosampler should be cleaned on a regular basis, too. I've never seen an autosampler that was not subject to leaks and drips. These can leave deposits of buffer or sample, contaminating the system. The wash solvent in the autosampler should be treated in the same manner as the mobile phase in terms of expiration dates and regular washing or replacement of the wash reservoir.

Contaminants build up on the column over time, often being eluted as additional background noise in future chromatograms. This problem can be minimized by flushing the column with the strong solvent of the mobile phase (for example, methanol or acetonitrile) at the end of each batch of samples or whenever the column is removed from the system.

In my experience, it is easier to damage the detector than to improve it with routine cleaning efforts. For this reason, I rely on the column and system flushing procedures to remove contaminants from the detector flow cell. Only if there is some compelling reason do I take specific action to clean the flow cell for detectors that operate in the liquid state, such as UV or fluorescence detectors. Evaporative detectors, such as evaporative light scattering detectors or mass spectrometers are a different story. These detectors eventually build up a film of nonvolatile contaminants and require cleaning on a regular basis.

Summary

So there it is — degas, filter, and flush. Now you've received all the knowledge that you would have acquired in a one-day short course on preventive maintenance. Of course, it isn't quite that simple, but these three practices will go a long way toward more reliable LC system operation. Good luck!

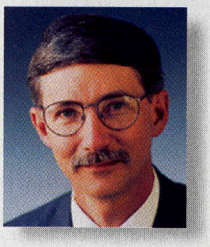
References

(1) S.R. Bakalyar, M.P.T. Bradley, and R. Honganen, J. Chromatogr. 158, 177 (1978).

(2) J.N. Brown, M. Hewins, J.H.M. van der Linden, and J.H.M. Lynch, J. Chromatogr. 204, 115 (1981).

(3) L.R. Snyder, J. Chromatogr. Sci. 21, 65 (1983).

For more information on this topic, please visit www.chromatographyonline.com/dolan



John W. Dolan

"LC Troubleshooting" Editor John W. Dolan is Vice-President of LC Resources, Walnut Creek, California; and a member of LCGC's editorial advisory board.

Direct correspondence about this column to "LC Troubleshooting," LCGC, Woodbridge Corporate Plaza, 485 Route 1 South, Building F, First Floor, Iselin, NJ 08830, e-mail John.Dolan@LCResources.com.

For an ongoing discussion of LC troubleshooting with John Dolan and other chromatographers, visit the Chromatography Forum discussion group at http://www.chromforum.com.