LC TROUBLESHOOTING

Column and Detector Problems

John W. Dolan

Readers ask for advice on stubborn problems with column blockage and noise. Here's help, too, with setting up problem-isolation steps.

This month's "LC Troubleshooting" addresses readers' questions relating to column blockage and isolation of detector noise. The reader experiencing column blockage has performed all the standard corrective procedures, yet the column continues to become blocked. In isolating a detector-noise problem, the second reader is concerned that, without proper problem-isolation techniques, the problem source may not be identified even after the problem has been corrected.

COLUMN BLOCKAGE

Q: We are having a puzzling problem with our liquid chromatographic (LC) system. Our method uses a C18 column with a methanol/water mobile phase buffered to pH 2.95 with phosphate. If the system is left running continuously, there are no problems. However, if we shut off the system overnight, the pressure jumps by as much as 1000 psi when we restart the system the next day; this pressure increase does not disappear. The problem can be corrected only by replacing the frit, or often the column itself is blocked.

We use a guard column and change it regularly. At the end of each day we flush the system with nonbuffered mobile phase for at least an hour before shutting off the system. Flushing with pure water does not help. We filter all mobile phases through a 0.2-μm filter and use frits on the solvent inlet lines. We are not using an autosampler. What have we overlooked?

JWD: You seem to be doing all the obvious things to correct a problem with the mobile phase. The problem certainly is not caused by buffer precipitation in the column. By changing to nonbuffered mobile phase or water, you should be able to wash the residual buffer from the column in just a few column volumes. Complete flushing is expected in 10–15 column volumes. I think you have eliminated the mobile phase as a point of concern. I assume that you have taken appropriate precautions to avoid introducing particulate matter with the sample.

One additional precaution I would take is to add a 0.5-μm porosity in-line filter directly after the injector. This filter should prevent particulates from reaching the column. The frit at the head of the column or guard column typically has 2-μm porosity and may not provide sufficient protection against particulates. Furthermore, when this filter becomes blocked — as indicated by an increase in system pressure — it can be replaced without risk of disturbing the column or guard column.

I can think of only one other cause for the failure pattern that you observe: perhaps turning the pump off and on is disturbing some "reservoir" of particulate matter somewhere in the system. If there is a poorly swept cavity in a misassembled fitting or in a mixer or pulse dampener, particles might settle, only to be disturbed when the pump is restarted. The most likely source of these particles is worn pump seals. First, replace the pump seals, inspecting the seal area for any contamination. Look for gray deposits that may resemble poorly erased pencil marks. Clean the head(s) and piston(s) carefully, install new seals, and reassemble the pump. Disconnect the column and flush the plumbing between the pump and the column for several minutes at the maximum flow rate (for example, 10 mL/min). You might tap (gently!) on the mixers, pulse dampeners, and transfer tubing to see whether you can displace any particulates. It might be interesting to run the waste stream through a 0.2-μm membrane filter to see whether any visible particulates are discharged. As a final precaution, disassemble, rinse, and reassemble all the fittings. If there are any spurious particles in the system, this flushing procedure should remove them. Reconnect the column and cross your fingers.

If these procedures do not correct the problem, you have me stumped. I would appreciate the views of any readers on this issue.

NOISE PROBLEMS

Q: When I get noise spikes in the chromatogram, I have a hard time figuring out the source of the problem. I try a number of fixes, and often correct the problem, but then I'm not sure whether I've fixed the problem or whether it has just disappeared. What are the tricks for fixing my problem in a logical manner?

JWD: Some simple diagnostic tools will help you locate noise problems. Once you know the source of the problem, you can work at correcting it. First of all, remember two of the "rules of thumb" that were discussed in an earlier column (1). The "Rule of Two" says to make sure that a problem appears at least twice before attempting to correct it, so that you don't spend your time chasing problems that happen only occasionally. The "Rule of One" states that you should change only one thing at a time. Although the shotgun approach may fix the problem, it won't tell you what the problem source was. By changing one thing at a time, you will gain knowledge so that you can fix the problem more quickly next time. Now let's look at isolating the noise problem.

The first step is to eliminate the most likely source of noise spikes: bubbles. If you shut off the pump flow, the noise spikes should stop if they are the result of bubbles. If a bubble is lodged in the detector, the baseline will stay at full scale because the bubble will be blocking the light path. If the bubble is not trapped in the detector, the baseline should be normal because no bubble interference is present. If bubbles are not the source of the problem, the noise spikes should continue, even with the flow shut off. If bubbles are determined to be the problem, degas the mobile phase (continuous helium sparging is preferred). Also, be sure that you have a back-pressure restrictor installed after the detector to keep bubbles in solution until after they exit the detector. (Remember that excessive pressure may cause detector cell leakage, so don't exceed the detector pressure limits.) If you have a lot of air trapped in the system, it may take an hour or more of pumping degassed mobile phase to remove all the bubbles. If bubble problems persist, even when you are pumping degassed mobile phase, check for leaks on the low-pressure side of the pump; it is likely that a loose fitting is allowing air to be drawn into the system.

Once you have eliminated bubbles as the source of the noise problem, the next most likely cause is a failing detector lamp. I don't know any simple way to isolate the lamp as the problem source. Today, the life expectancy of a deuterium lamp is about 1000

hours of operation. Most manufacturers use lamps that have a use meter mounted on one of the lamp wires. This meter looks like a small thermometer about 2 cm long and is calibrated to 1000 hours. If the meter reads at or near 1000 hours, the lamp is the likely cause of trouble; replace it, and you should be back in business. If the lamp has less than 500 hours of use, it probably is OK. If you are uncertain, replace it. If it turns out that the old lamp is still good, reinstall it — if you don't, you are just throwing away good use of an expensive part. Remember, when installing a lamp, never touch the glass portion with your fingers because fingerprints can etch the surface when the lamp is turned on. If you inadvertently touch the lamp, wipe it clean with a soft cloth before turning it on.

Electronic problems can be classified into four categories: LC module problems, cabling problems, line-voltage problems, and radio-frequency interference (RFI).

When you have eliminated bubbles and the lamp as problem sources, the remaining possibilities are electronic. An earlier column (2) dealt with this area in detail, so refer to it if you need more information. Electronic problems can be classified into four categories: LC module problems, cabling problems, line-voltage problems, and radio-frequency interference (RFI). Correcting electronic problems inside the detector or data system is beyond the capabilities of most of us, but we can isolate these problems. First, check the system's diagnostic aids and self-tests to see whether the system itself can locate the problem. Alternatively, substitute known working modules for those in question. Once you have isolated the problem module, consult the service manual or request a service call to correct the problem.

The connecting cables supplied by the module manufacturer should provide satisfactory performance because they are assembled with proper shielding and grounding. Inspect these cables for improper connections and any visible damage or kinking (which might indicate internal damage). Again, substitution of a known good cable will help to isolate cable problems. If homemade or "universal" signal cables are used, be sure that they are shielded (they should have a grounded wire-mesh sleeve around the signal wires) and that they are grounded only at one end. Poor shielding or poor grounding is often the cause of RFI problems.

Finally, look at line-voltage fluctuation. Any electric device that draws much current can cause a line-voltage change when it is turned on or off. The most common problem sources in the lab are heaters (for example, water baths), motors (for example, vacuum pumps), or building utilities such as air conditioners, compressors, or elevators. If you have a line-voltage monitor, you may be able to correlate a particular piece of equipment with the chromatographic noise you observe. Another option is to disconnect any suspect equipment and see whether the problem disappears. If you cannot eliminate or identify the source of line-voltage problems, either install a "clean" circuit for LC use only or use a line-voltage conditioner to filter out the problems.

Electronic problems can be the most frustrating LC problems to deal with, partly because they are difficult to isolate and partly because most chromatographers don't know much about electronics. Don't hesitate to ask for help from a more knowledgeable colleague in your facility or from the LC instrument manufacturer.

REFERENCES

(1) J.W. Dolan, *LC•GC* **6,** 304–308 (1988).
(2) L.H. Fleming, J.P. Milsap, and N.C. Reynolds, Jr., *LC•GC* **6,** 978–985 (1988).

"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Lafayette, California, USA, and is a member of the Editorial Advisory Board of LC•GC. *Correspondence concerning this column can be sent to "LC Troubleshooting,"* LC•GC, *P.O. Box 10460, Eugene, OR 97440, USA.*

Bulletin

Burris receives Kenneth A. Spencer Award. The Kansas City Section of the American Chemical Society (ACS) presented the Kenneth A. Spencer Award for outstanding achievement in agricultural chemistry to Dr. Robert H. Burris of the University of Wisconsin (Madison). The award recognizes meritorious contributions in the field of agricultural and food chemistry and promotes further progress in research, education, and industry. Burris's work has included developing analytical procedures using GC and MS to measure nitrogen fixation — a factor in renewable fertilization — in crop fields, forests, lakes, and oceans.

Request for nominations for the 1991 Spencer Award are due 1 September 1990; a jury will select the award recipient in December 1990. For more information, contact Susan I. Tripp, Marion Laboratories, Marion Park Drive, Park A, Kansas City, MO 64137, tel. (816) 966-5000.