L C T R O U B L E S H O O T I N G

Isolating and Correcting LC System Problems

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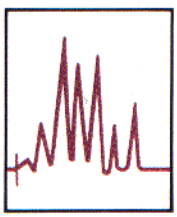

"LC Troubleshooting" usually covers specific problems that are common to liquid chromatographic (LC) systems, but in response to readers' questions, this installment reviews general troubleshooting techniques.

ON THE LOOKOUT

LC system problems result in downtime, which means that you get behind in your work. Using good preventive maintenance practices will go a long way toward avoiding LC problems. But no matter how well you maintain your LC system, unexpected problems occur. For this reason, you always should be on the lookout for problems or potential problems. The system readouts (including the pressure and lamp meters), the system's outward appearance, and the chromatogram are the key indicators of problems.

If you take a moment a couple of times a day to make a quick visual inspection of the system, you may be able to spot leaks before they cause many problems. This visual check is especially important when you make plumbing changes or swap columns regularly. Just follow the mobile phase flow path from the reservoir to the pump, autosampler, column, and detector. Take extra care inspecting fittings that have recently been changed. Also look for moisture underneath the pump heads, an indication of pump seal failure. Generally, leaky fittings can be fixed by tightening them slightly. During your inspection, make sure that there is sufficient solvent in the mobile phase reservoir(s) for the samples that you plan to analyze, and that the waste container has room for the used mobile phase. If you make regular inspections so that you spot a leaking pump seal early, you can usually continue running the system until the end of the shift before replacing the seal. If it takes a big puddle on the bench for you to notice that the pump seal is leaking, you may have to abort your present analyses to replace the seal.

SYSTEM READOUTS

Most LC systems have analog or digital readouts indicating the system pressure and the condition of the detector lamp. Many liquid chromatographs have sophisticated electronic monitoring systems that can warn you of failure of the internal components. The pressure monitor is the most useful system readout for diagnosis of system problems. Each day you should make a note of the system operating pressure for reference purposes.

Changes in the back pressure indicate problems or potential problems. A gradual, day-by-day increase in pressure is common during routine operation and tells us that something is restricting the flow of mobile phase through the system. The most likely cause of a gradual pressure increase is blockage of the first frit after the sample injector — that is, blockage of an in-line filter or guard column, if you use one. Otherwise, the blockage is at the head of the analytical column. Replacement of the problem frit usually corrects the problem. If such pressure increases occur regularly, you should consider additional preventive measures such as using sample filtration or adding in-line filters.

When the pressure drops but then remains steady, usually a leak exists somewhere in the system. By carefully tracing the plumbing — paying special attention to the connecting fittings — you should quickly find the leak. Correct the problem by tightening, reassembling, or replacing the fitting.

Fluctuating pressures suggest problems with the pump. An air bubble in the pump head, a check-valve malfunction, or pump seal failure are the most probable causes, in descending order of likelihood. Use degassed mobile phase to prevent air bubble problems. To remove bubbles from the pump, you may need to bleed the check valves by loosening them slightly while pumping mobile phase. Sometimes you can remove bubbles by opening the purge valve and increasing the flow rate, allowing several milliliters of solvent to flush the bubbles from the pump heads. If degassing and flushing do not solve the problem, check-valve failure is likely. Clean or replace any suspect check valves. Finally, if degassing and check valve replacement are ineffective, replace the pump seals to correct the fluctuating pressure.

The UV lamp meter can help you isolate detector problems. Again, if you note the lamp readout when the system is operating correctly, you can quickly spot problems when they occur. The most obvious indicator — no energy on the lamp meter — tells us that the lamp is not on, because of either operator oversight or total lamp failure. Correct this problem by turning on or replacing the lamp. Low lamp energy often is a precursor of lamp failure. The detector operation manual should give the range of energy over which you can expect good lamp performance. When you observe low lamp energy, be especially watchful for other indications of lamp failure, such as changes in the chromatographic signal or in the lamp readout. Finally, erratic lamp meter readings, which may be accompanied by sharp noise spikes in the chromatogram, indicate lamp failure. Replace the lamp.

CLUES FROM THE CHROMATOGRAM

The chromatogram often gives us the first evidence of a problem with the LC system; it also can give us many clues to help pinpoint the source of the problem. As with other aspects of the system, you should have a reference chromatogram from a run made under controlled conditions, most commonly an assay of a standard, control, or calibrator that is analyzed each day before samples are run. Some of the diagnostic characteristics of the reference chromatogram are discussed below.

Retention shifts result from a change in the column, the mobile phase, or the flow rate. If the capacity factors [$k' = (t_R - t_0)/t_0$, where t_R = retention time and t_0 = column dead time] of all peaks stay constant, but the retention time shifts, a change in the flow rate has occurred. Check the pump flow setting and make sure that the pump is delivering properly (no bubbles or check-valve problems). You can verify the flow rate by making a timed collection of mobile phase at the detector outlet. If the capacity factors for one or more peaks shift, problems with the mobile phase or column are likely. Pin down the cause by using a new batch of mobile phase or a new column. Don't confuse a gradual deterioration of the column with a problem of sudden onset caused by a bad batch of mobile phase or a contaminated sample. Checking the chromatograms for several days or weeks should help you establish which changes are to be expected because of normal column aging.

Peak shape changes are another symptom of problems that need corrective action. When shoulders or double peaks are ob-

served for all the peaks, a blocked frit or a column void is the most likely cause. Remember that the most likely suspect is the frit or column closest to the injector. Verify that a problem originates with a guard column (or in-line filter) by bypassing this unit. When you replace a column frit, check to be sure that there is no depression or void beneath the frit. Although some workers successfully fill such voids, it is best to discard a column that contains one.

When the shape of only one peak changes, several things could be happening. Often a second peak may be interfering with the peak of interest because of sample deterioration, new components in the sample, elution of a late peak, or a change in the column. Sample reinjection, rerunning standard compounds, and column substitution are tools that can isolate the specific cause.

You may observe another problem: peak broadening that is more pronounced early in the chromatogram. Two common causes are possible. First, if too much strong solvent is injected with the sample, early peaks tend to broaden. To avoid this problem, inject less than ~ 25 µL of sample–solvent mixture or use an injection solvent that is less than about half the mobile phase strength (for example, prepare the sample for injection in <35% methanol if the mobile phase contains 70% methanol). The other cause of early-peak broadening is extracolumn effects. These effects usually are caused by using tubing that is too large or too long between the injector (or autosampler) and the column. Thus, extracolumn effects don't occur suddenly unless a plumbing change has been made (with the rare exception of a fitting that slips apart).

Peak broadening throughout the chromatogram results from plate number loss caused by deterioration of the column (or guard column). You should be able to verify the source of the problem by replacing the suspect column.

When you observe an increase in chromatographic noise, the appearance of the noise can help diagnose the problem source. Very short-term noise with a cycle of <1 s usually results from electrical interference and can be corrected by using increased electrical shielding and verifying that the signal cables to the data system are connected properly. Short-term periodic noise (having, for example, a cycle < 30 s) can result from pump bubbles, mixing problems, or other pump malfunctions. Verify that the noise is pump-related by correlating the frequency of the noise with the piston cycle on the pump. Long-term noise (cycle > 30 s) can result from mobile phase mixing problems, or it may be just a buildup of late-eluting materials on the column. Flushing the column with a strong solvent should help identify the source of the problem. Sharp noise spikes can result from electrical problems or from bubbles passing through the detector. Electrical noise spikes tend to be very sharp, like a line drawn by a ruler across the chromatogram. Electrical noise can result from failure or near failure of a system component (especially the lamp) or from the cycling of another device on the same electric circuit (for example, an air conditioner or motor). Bubbles in the detector often show up as a peak with a ramped front (almost like a chromatographic peak) followed by a sharp tail. Use of degassed mobile phase and a postdetector back-pressure regulator should eliminate bubble problems.

MODULE SUBSTITUTION

The most powerful tool for isolating LC problems is module substitution. Just replace the suspect system module with one that you know works well. For many workers, it is second nature to replace a suspect column, but this technique can also be applied to the other LC modules: pumps, detectors, and so forth. This practice greatly speeds troubleshooting, especially if the cause of the problem is internal, such as failure of an electronic component. When the failed module is replaced, the system should behave normally.

You can take substitution one step further by isolating problems within a module. You may save yourself a service call by swapping circuit boards until you find the problem board (be sure to mark the boards with a felt pen so that you don't mistake one for another).

One caveat: when you use the substitution technique, reinstall all the good parts after you've located the bad one. If you don't, you'll end up with a lot of parts of questionable quality. Later, you or others in your lab may avoid using any of these parts for fear of installing someone else's problems.

WHEN IN DOUBT, GET HELP

When the solution to your LC system problem is not obvious, you should get some help. Asking a co-worker for assistance is a good first step. The most neglected source of troubleshooting help is the operation and service manual, which contains a wealth of information specific to your system. Or you can call the manufacturer for advice (ask for "technical support") or request a service call. A number of third-party tools can also help you isolate and correct problems with LC systems. Most economical are the free troubleshooting guides supplied by equipment or column manufacturers (1,2). Other resources include books (3,4), a video course (5), and short courses taught at scientific meetings or offered by consulting firms. Finally, previous installments of "LC Troubleshooting" and "Column Watch" provide detailed help in the diagnosis and correction of many LC problems. These various resources can help guide you through the steps of confirming, isolating, and correcting problems with your LC system.

SUMMARY

When you are isolating LC problems, it is important to proceed in a logical stepwise manner and to use all the information available to solve the problem quickly. Often more than one symptom is present to help confirm that a problem exists. For example, when you suspect that a drop in flow rate is causing increased retention times, your hunch should be substantiated by a corresponding drop in system back pressure.

Good troubleshooting practice can be summarized in the five rules of thumb discussed in an earlier "LC Troubleshooting" column (6):

- Rule of One
- Rule of Two
- Put It Back
- Throw It Away
- Write It Down

The "Rule of One" reminds you to change only one thing at a time when substituting parts. In this way the problem can be quickly and positively identified. The "Rule of Two" suggests that you make sure that a problem really exists by confirming that it occurs at least twice. Too much time can be lost chasing problems, such as an occasional air bubble, that don't occur often enough to justify their correction. To practice the "Put It Back" rule, reinstall good parts that you replaced during module substitution. This avoids collecting a drawer full of parts of questionable quality. Once you have found a defective part, "Throw It Away" so that it will not be confused with good parts and cause more problems in the future. Finally, "Write It Down" so that you have a record of the repair and maintenance history of each instrument. This record will help you find out which components fail regularly with your system so that you can take the proper preventive maintenance precautions to avoid as many system problems as possible.

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"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.

Bulletin

Upcoming Meeting:
HPLC '90, the 14th International Symposium on Column Liquid Chromatography, will be held 20–25 May 1990 at the Boston Park Plaza Hotel in Boston, Massachusetts. The five-day meeting will include invited lectures, contributed presentations, posters, discussion sessions, and an instrument exhibition. For more information, contact Shirley E. Schlessinger, Symposium Manager, HPLC '90, 400 East Randolf Drive, Suite 1015, Chicago, IL 60601, USA, tel. (312) 527-2011.