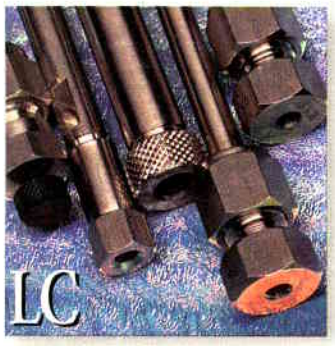

LC Troubleshooting

Divide and Conquer

The author presents a simple isolation technique for identifying problems with LC methods and equipment.

John W. Dolan
LC Troubleshooting Editor

I call my favorite liquid chromatographic (LC) troubleshooting technique "divide and conquer." Somewhere early in my career, I found that I was happiest when I could put items in separate categories. This has extended to my teaching style, my lab work, and the way I approach problem isolation. This month's installment of "LC Troubleshooting" will focus on this very simple but powerful tool.

The basis of the divide-and-conquer approach is to devise a test whose outcome will eliminate a large portion of the possible problem sources. From there, a second test will be used to eliminate another group of causes, and so forth until you find the problem source. Let's look at some examples.

System or Method?

Perhaps the biggest question that arises when an LC problem occurs is whether the source is related to the LC system (the hardware) or the method. Because this is such a universal and important question, we need a simple test to determine which category is the culprit. A simple test that we use in my laboratory for this purpose is repetition of the manufacturer's new column test. We install a new column (actually a column that is dedicated to this test) and run the column manufacturer's test. This usually is something like 60–70% methanol in water with a sample containing uracil, methyl benzoate, and toluene, or a similar sample. Simply run this test and see if you can get the same plate number and retention the manufacturer obtained with the new column. You should be able to get within about 10% of the manufacturer's results if everything is working well with your LC system. If the test passes, the system is all right. If it doesn't, the problem is most likely with the LC system itself.

But what happens if the LC system cannot duplicate the column manufacturer's results? This is a big "if" and one that you don't want to have to answer. The easy way around this is to run the test on a system that you know is working well and record the results. If this is part of the installation process as the operational qualification, you'll have a point of reference for future use. We feel that this evaluation of system performance is so important, we include it in our semiannual LC system test suite. At best, it will assure us that the system is working properly. At worst, it will provide early warning that something needs attention.

So the bottom line is to run the system check when the hardware is new and then periodically to ensure that it still is operating as expected. When a problem arises that you can't pinpoint to the system or method, run the system check and the classification of the problem should be obvious. Not only is this an efficient way to make that first major division of the problem source, it could save you an expensive false-alarm service call if you suspect the system and the problem turns out to be the method.

Where's the Blockage?

High system back pressure is a common symptom of a problem. It often is the first sign of column failure, but it could be the result of a blockage somewhere else in the system. How do you know if the pressure is too high? If you record the system pressure at the beginning of each batch of samples or day of work, you'll have a record for the column that you can track over time. Of course, a pressure over-limit shutdown is a no-nonsense signal that something is wrong.

One approach to isolating a pressure problem is to start with the flow on and progressively loosen fittings, starting at the detector and working your way toward the pump. This is a systematic and effective approach, but most of us suspect where the problem is located, so it might waste time. Why not divide and conquer? The most likely location of a pressure restriction is at the 0.5-μm in-line frit just after the autosampler (you *are* using an in-line frit, aren't you?). Loosen the fitting at the outlet of the frit holder. If the pressure drops somewhat (because you've removed the column) but is still high, the problem is

upstream from the fitting you loosened. You've eliminated everything downstream without loosening all those fittings. Now take off the fitting at the inlet of the frit holder. If the pressure drops, the frit is blocked. Not only have you isolated the problem, you have the fittings loose already, so replacement is easier. If the pressure still is high, continue working upstream until the blockage is located.

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Pressure Pulses

Pressure pulses can originate from many causes, including air bubbles, bad check valves, leaky pump seals, mobile-phase mixing, and pump starvation. Enter the divide-and-conquer strategy. If you have a two-pump system, change the settings so that only one pump is pumping and observe the result. Then change to the other pump. This should allow you to isolate the problem pump.

Suspect air bubbles in the pump? Switch to 100% methanol that has been thoroughly degassed. Purge the pump and then pump at 1 mL/min. Is the pressure steady? If it is, you know that the pump is capable of a pulse-free flow. Change back to mobile phase and if operation remains normal, you probably just had a bubble in the pump. Degas the mobile phase in the future for more reliable operation.

Check-valve problems can be difficult to isolate. Some systems come with directions about how to isolate a particular check valve as problematic. Most of us, however, will have to resort to substitution of known good check valves for the suspect ones.

If you suspect pump starvation, rather than individually checking each component upstream from the pump, test everything at once. Remove the fitting where the solvent is delivered to the mixing manifold in a low-pressure mixing system or at the pump inlet on a high-pressure mixing system. When the reservoir is above the fitting, you should observe siphon action delivering solvent at several times the required flow rate. I like to see at least 10 times the required solvent delivery. For example, if you typically run at 1 mL/min, make sure that at least 10 mL/min is delivered via siphon pressure. Just put the solvent line in a graduated cylinder and time how long it takes to deliver 10 mL. This will tell you if there is a restriction on the inlet side of the pump. Prime suspects are the frit in the mobile-phase reservoir or a crimped delivery line. Again, divide and conquer — remove the frit and restart the siphon. If it works now, the frit needs to be replaced. If it still doesn't work, replace the tubing.

System Suitability

Let's look at a few examples of using our divide-and-conquer technique to isolate method problems. The system-suitability test is a major divide-and-conquer tool. If you have suspect results with a sample set, you need to know if the method is working well when it is fully under your control. The system-suitability test offers just this information. System-suitability testing should be designed to assure you that the method is working as it should. One convenience of this test is that it can be run without switching back to the system test when you want to determine whether the problem is with the method or the system.

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The system-suitability test often includes several replicate injections of a mock sample. If the retention time, response, and area reproducibility are within the accep-

tance criteria, you know that the LC system is working properly, the mobile phase was formulated correctly, and the column still is all right.

If the system-suitability test does not pass, it can give you sufficient information to correct the problem without reverting to the system test. Did you just make up a new batch of mobile phase? Maybe it was a formulation error. Has the pressure been gradually rising over the last week? Maybe the column is due for replacement. Poor retention reproducibility? Look for bubbles in the pump or a bad check valve. Area reproducibility problems? Focus on the autosampler.

Questionable Results?

Maybe the system-suitability test passes, but you suspect that the analytical results aren't right. Divide the results into known versus unknown samples. Does the standard curve behave as expected? Look closely at linearity, low-concentration samples, and any bias in the values. Are you using quality control (QC) samples interspersed with the unknowns? QC samples are mock samples used to check that the method is working properly throughout a batch of samples. When checked against the standard curve and against their expected values, QC samples can give valuable information about method stability, drift, or other problems. A sudden change from good QC results to poor ones in the middle of a run can point to some kind of system failure — a proportioning valve, check valve, detector lamp, or some other problem.

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Is it an individual sample, a small group of samples, or the entire batch giving unexpected results? If the entire batch is off, check for causes common to all the samples, such as sample preparation errors. If it is an individual sample or group of samples, reinjection might help illuminate the situation. (You should have a standard operating procedure in place that describes the procedure for reinjection and how to report the results of multiple assays of a sample.) If replicate injections of the sample give differing results, perhaps the autosampler is at fault. Next, check replicate injections of a controlled sample, such as a QC sample or a system-suitability sample. If the controlled sample is not the problem, something is wrong with the unknown. Was it properly mixed? Was it completely thawed? Was the vial so full that a vacuum formed when sample was withdrawn? Did the injection solvent evaporate?

Be Prepared

"Be prepared" is the Boy Scout motto — it also should be the chromatographer's motto. The divide-and-conquer technique is a powerful one and simple to use, but if you haven't gathered baseline data about your LC system and your method, you have given up much of its power. Prepare for future problems by testing the system when you know it works well. Keep the performance data for future comparison. Make sure you use a system-suitability test that has been designed to demonstrate acceptable performance of the method.

Once you have performance data for the system and the method when they are working properly, you can use the divide-and-conquer testing strategy to help isolate a problem.

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