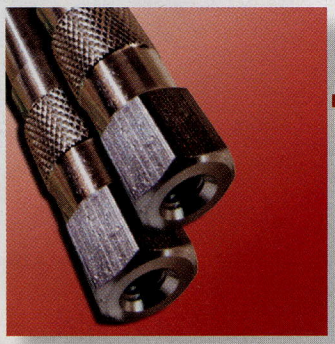


LC TROUBLESHOOTING

LC Troubleshooting Strategies

How to quickly get to the root of the problem.

John W. Dolan
LC Troubleshooting Editor

I had a telephone call the other day from a chromatographer who spends a large part of his time transferring liquid chromatography (LC) methods to other laboratories. He was inquiring if there was a way to quickly isolate the source of problems he encounters so that he can correct them. Troubleshooting is a normal part of my daily job, whether it is writing this column, teaching troubleshooting techniques, or consulting. For me, troubleshooting is second nature, as it is for many of you. However, we all have to build troubleshooting skills from some starting point. This month, I'd like to review some of the techniques that I find most powerful when trying to isolate problems.

Divide and Conquer

This is perhaps the most powerful strategy for problem isolation. I borrowed the term years ago from John Hinshaw, who writes the "GC Connections" column for *LCGC*. My partner, Tom Jupille, calls it the Binary Search. Whatever term you like to use, the divide-and-conquer process helps you to efficiently get to the root cause of a problem. It is very simple — just figure out a test that divides all possible problem sources into two large parts. Make the test and the results should allow you to eliminate half the potential sources. Repeat this process with the remaining possibilities until you have found the root cause of problem. This technique comes so naturally after a while that it seems trivial, yet I am regularly surprised to find it ignored in the troubleshooting process.

Let's take an example of inconsistent retention times observed when examining the results of an overnight run. Three possible problem sources come quickly to mind. Could it be associated with the column, the mobile phase, or the instrument? First, we should take a closer look at the data — is the variation observed only with the samples or also with the standards? Let's say it happens with both. Now we need to determine if the method is behaving properly under controlled conditions. The easiest way to do this is to repeat the system suitability test. We observe that system suitability fails due to retention time variation, but the peak area, peak shape, and resolution requirements are met.

At this point, we could shift to the most obvious cause (see below) — pump problems — but we will continue with the divide-and-conquer strategy. You might wonder if we have a problem with the instrument or with the method itself. My next step in divide-and-conquer might be to determine which of these major areas is at fault. So I put a new C18 column (or one I've dedicated to this process) in the system and run the column manufacturer's column test. I'll inject the test sample six times so that I can calculate retention-time precision. If I can get results that are close to what the column manufacturer

obtained in terms of column plate number, peak tailing, and retention time, this suggests that the instrumentation is working correctly. Don't expect exactly the same values the manufacturer quotes, but you should be within 5–10%. Remember that the column manufacturer uses an LC system that has been optimized for column testing, so it will show off their columns in the best light. If the retention time precision of the test mixture meets your method-suitability requirements, the results point to a problem related to the method itself — the column and mobile phase would be the next items to examine. If retention precision is poor, look next for problems associated with flow rate or on-line mixing. Three major influences on flow rate are check-valve performance, pump-seal wear, and bubbles. On-line mixing problems can be isolated by comparing results with hand-mixed mobile phases.

Check the Obvious

When we encounter problems with our LC methods, sometimes we assume the worst and ignore simple solutions to the problem. We all joke about "Is it plugged in?" but it is surprising how often a simple operator error is the root cause of a problem. Did you install the correct column? Is the flow rate set properly? Did you put sample in the vial? The list goes on and on. You can use the divide-and-conquer strategy in some cases to group problems and think of solutions, but a very simple way to reduce operator errors is to use a checklist. I like a checklist that is at a fairly high level — perhaps it lists 5–10 steps in the process of setting up the method. This may not be used in detail for every step, but it should be scanned to make sure something obvious was not missed. For example, I have such a list that contains items that I need to check when I do the preflight checks on my airplane. I've done the preflight so many times that it is second nature, yet before I start the plane I always pull out the list and read through it to be sure I didn't miss something. It is also a good idea to keep a more detailed list of settings that you might seldom change. For example, it is likely that the detector has several pages of settings in the setup program that you never adjust on a daily basis, things like the time constant or slit width. But if you suspect a detector problem, perhaps after a power outage, you can pull out the detector settings list and verify that everything is set correctly.

Sometimes you can design the experimental setup so that the problems will be even more obvious than normal. For example, I recommend using an in-line filter between the autosampler and guard column or analytical column on every LC system. This should contain a filter with a porosity less than or equal to that of the column inlet frit. Thus, a 0.5-µm porosity in-line frit would be used for a 5-µm or 3-µm particle size column packing. These columns commonly use 2-µm or 0.5-µm inlet frits, respectively (although some 3-µm particle columns use 2-µm inlet frits). For ultrahigh-pressure LC (UHPLC) applications, where sub-2-µm column packings are used, 0.2-µm frits are used on the column, so frits ≤0.2-µm in porosity should be used in the in-line filter.

Increased column pressure points to a restriction to flow in the system, most commonly a blocked frit due to particulate matter collected from sample injections. This debris is going to collect on the first frit that it encounters. With an in-line filter installed, the location of the problem is obvious — and easily corrected by frit replacement. Similar logic can be used when a guard column is used.

Module Substitution

Another powerful problem-isolation tool is module substitution. This is simply a matter of replacing a suspect part with one that is known to be good. We use this technique most commonly when we suspect we have a problem with the column. We remove the column that we think has problems and replace it with a new column. If the problem is corrected, we've solved it quickly. Module substitution does not need to be limited to consumable items, such as columns, guard columns, and frits. You can use it for major modules of the LC system if you have a duplicate system available. For example, an entire pump or detector can be replaced with one that is known to be good. If the problem is fixed by substituting a good module, you'll know which module was at fault.

Module substitution can influence your decisions about which spare parts to stock or even which LC system to purchase. You should always keep a good inventory of consumable parts, such as columns, filters, guard columns, tubing, fittings, pump seals, and so forth. If you have several systems of the same brand and model in your laboratory, it is much easier to justify stocking a spare detector lamp or a set of check valves and pistons, and even circuit boards. If you work for a large company with its own metrology or service staff, it might make sense to keep most of the modules for entire systems in stock as spares. Thus, when a pump fails, another pump can be substituted to put you back in business and the failed pump can then be repaired in the instrument shop and put on the shelf as a future replacement.

Isolating Pressure Problems

An increase in system pressure is one of the most common LC problems. As mentioned earlier, this often is the result of a frit accumulating debris from the sample or mobile phase until the flow is restricted. But pressure increases can result from a restriction or blockage anywhere in the system. Isolation of pressure problems is done most easily by loosening fittings with the pump flow on and observing what happens to the pressure. (Be sure to put on your safety glasses!) In the earlier example of a blocked in-line filter, if the fitting on the input side of the filter is loosened, the pressure will drop significantly. But this does not prove anything, because the pressure will drop if this procedure is repeated after a new filter is installed, as well. For this reason, it is a good idea to systematically loosen fittings on a properly working LC system, starting at the detector outlet, working upstream until you reach the fitting attached to the outlet check valves, and make a note of the pressure before and after each fitting is loosened. This will give you an idea of what the normal pressure-drop values should be, so you will spot problems more readily. For example, the components downstream from the column should have minimal pressure

drop, perhaps 50–100 psi (3–7 bar) at the most. The column normally is the highest source of resistance in the system, so when the connection at the column inlet is loosened, you should see a reduction in pressure of 1000–3000 psi (70–200 bar), depending upon the flow rate. This is the most important pressure to note, so you can assess the likelihood of column blockage. A guard column will create much less back pressure by itself and a new guard column might not make a noticeable difference in the overall pressure when it is installed.

The in-line filter should have very little resistance to flow by itself, but it is the single most common source of increased system pressure when it becomes blocked. By designing the system to fail at the in-line filter, you've created a quick, easy, inexpensive, and logical way for system failure to occur. A similar practice is used when pouring large slabs of concrete — it is very difficult to prevent cracking, so control joints are troweled into the concrete at regular intervals to ensure that when the cracks occur, they are where you want them and don't ruin the cosmetics of the floor. The same goes for the in-line filter. When pressure rises, the probability of frit blockage is so high, it usually makes the most sense to replace the frit (module substitution, check the obvious) before attempting any other problem isolation schemes.

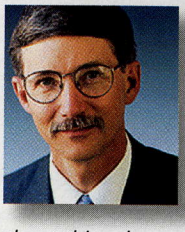
The remainder of the LC system upstream from the in-line filter will generate negligible pressure (for example, ≤100 psi, ≤7 bar) for conventional LC systems designed for operation ≤6000 psi (≤400 bar). However, this is not the case for many of the newer LC systems that are designed for use at higher pressures, such as with sub-2-μm particle columns. Because these UHPLC systems are designed for minimal extracolumn volume and maximum protection of the columns, the tubing and frits can generate 1000 psi (70 bar) or more of pressure with no column connected. You can see that knowledge of the normal pressure at each stage of the flow path will help you make decisions when trying to isolate a blockage. If you loosen the fitting going into the in-line filter and observe 500 psi (35 bar) of pressure remaining, it may signal problems upstream for a conventional LC system, but may be normal behavior of a UHPLC system.

Summary

Problem isolation techniques become second nature to experienced workers, yet for newcomers to the field, figuring out the location of an LC problem can be a daunting task. Some people are more adept at this process than others, as well — most laboratories have a go-to person who can help out for those stubborn cases in which the problem is not quickly isolated.

The techniques discussed this month should become a natural part of your troubleshooting toolbox. I suspect many readers perform these tasks without thinking about it. Are there other general processes that you find especially helpful when isolating problems? Send me an e-mail (John.Dolan@LCResources.com) and perhaps I can share these with other readers in the future.

John W. Dolan



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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.org.