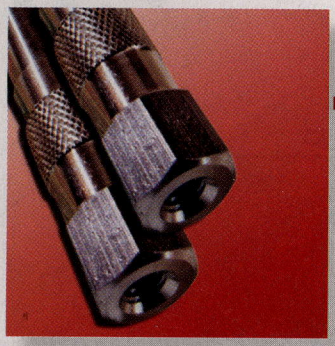

LC TROUBLESHOOTING

Troubleshooting Basics, Part I: Where to Start?

Where do you start when you encounter a new problem with your liquid chromatography system?

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LC Troubleshooting Editor

Old timers, like me, find that troubleshooting a liquid chromatography (LC) problem is instinctive. But this is not in our DNA — we had to learn these skills over time. I continually see new faces in the LC world who are not as fortunate as I was. They do not have mentors to show them the ropes. So I would like to go back to the basics of LC troubleshooting and consider some fundamental problem-solving approaches that we can apply to most LC problems. In this month's "LC Troubleshooting" we'll start with some general principles. In future installments, we'll look at specific problems in more detail.

Core Problems

If you consider all the LC problems that you are likely to encounter, they generally fall into one (or more) of five categories:

- Pressure problems
- Leaks
- Peaks that are out of place in the chromatogram
- Peak-shape problems
- Data processing problems.

One of my six troubleshooting rules of thumb is called "The Divide and Conquer Rule." The way I apply this is to make tests or observations that eliminate potential problem sources. That means I start by classifying the problem into one of the five categories listed above, so that we don't have to worry about the other categories. Let's look at each of the categories briefly.

Pressure Problems

You will notice that any LC method that you use has a normal system pressure or system-pressure range. For example, an isocratic method might normally run at approximately 150 bar (approximately 2200 psi); or a gradient method might have a pressure range during the gradient of 140–200 bar (2000–2900 psi). When the pressure increases or decreases 20% from the normal values, then perhaps the system is trying to tell you something. You may notice the pressure change when examining the system, or the high- or low-pressure-limit setting may trigger an alarm or a system shut-down. Pressure can rise, fall, or fluctuate. Each of these factors can tell you something about potential problems, such as the presence of blocked frits, leaks, bubbles, or worn parts.

Leaks

Leaks are often one of the simplest problems to isolate, but not always. Many LC systems have leak detectors consisting of simple electrical contacts that are connected by a few drops of conductive mobile phase when a leak occurs. In other cases, you may notice a puddle of mobile phase on the bench or a buildup of white crystals at a fitting.

Out-of-Place Peaks

Under normal operating conditions, the peaks in a chromatogram should have constant retention times. There will often be a little variation from run to run (generally 0.1 min or less, or perhaps a little longer for day-to-day variations), but in each case, a normal pattern of variation will occur. When the pattern is broken, it is a sign that something is wrong. Sometimes you will observe a drift for some (or all) of the peaks to longer or shorter retention times.

In other cases, peaks may be missing, or extra peaks may appear. These can be attributed to problems with the sample, the column, the mobile phase, or the LC hardware. This category of problems is perhaps the most common one and the most difficult to troubleshoot.

Peak-Shape Problems

Ideally, peaks in chromatograms will be symmetrical, but most peaks have a little bit of a tail on them. When this tailing increases or when peak fronting appears, something is going wrong. Modern columns, which use high-purity silica packings, are much less susceptible to peak tailing problems than the lower-purity columns that were standard 15–20 years ago. But you may be using a method that was developed on an older column, so tailing may be an everyday occurrence for your method. A change in peak tailing is often associated with a problem with the mobile phase or column.

Data-Processing Problems

Sometimes you will first notice a problem when you are examining the data produced from a batch of samples. Perhaps the quality-control (QC) samples are out of range; the calibration curve is no longer linear; or all the peaks are too large or too small. Problems of this nature can often be traced to sample preparation or stability, or to problems with the autosampler.

Multiple Problems

Sometimes you will find a problem that fits into more than one category. For example, a leak will cause the pressure to drop; the flow-rate will then also decrease, which will increase the retention times of the peaks in the chromatogram. Conversely, a leak may occur because of a blockage, in which case the pressure will go up. Because some problems fall into more than one of the five categories above, it is a good idea to determine if this is the case, because it may simplify the troubleshooting process. For example, if you notice increased retention times, look for leaks. This is a quick check and leaks are usually easy to fix. If you ignore the possibility of a leak and focus on mobile-phase problems, it might take much longer to find the root cause of the problem. As we look at the potential causes of the various symptoms, I'll try to show you how you can consider any one of the multiple categories for a problem and quickly find the source of the problem.

Rules of Thumb

I have six rules of thumb to help me remember some key troubleshooting techniques:

- The Rule of One
- The Rule of Two
- The Divide and Conquer Rule
- The Module Substitution Rule
- The Put It Back Rule
- The Documentation Rule

Let's look at each of these in a little more detail.

The expense of a new column and the hassle of flushing or replacing tubing might be avoided if you had a policy for pump seal replacement.

The Rule of One. "The Rule of One" says to change just one thing at a time. This is the scientific method, which we use in most of our work in the laboratory, but we seem to forget it when we are troubleshooting an LC problem. For example, if peak shape is poor, you might change the column, the guard column, and the mobile phase to fix the problem. But if you change all three of these at once, you don't gather information that will help you to identify the problem the next time it occurs and shorten the next troubleshooting cycle. So change one thing, then another, until you find the root cause of the problem.

Of course, there are times when it may be appropriate to ignore the "The Rule of One," but those should be the exception, rather than the rule. For example, if it is 4 p.m. on Friday and you have the problem mentioned above, it may be faster to change several things to help ensure that you can get the system operating for a long run over the weekend.

The Rule of Two. "The Rule of Two" tells us to make sure that we have a problem that is reproducible, that is, it occurs at least two times. Unfortunately, some problems with an LC system or method may happen once in a while, but not regularly enough to easily isolate and correct. For example, when examining the data after a batch of samples, we notice that a QC sample has an area that is unexpectedly low. Is this a real problem that should be fixed, or did something happen to that one injection that is a random error? The easiest way to apply "The Rule of Two" is to repeat an injection (or inspect duplicate injections that are part of an existing data set). Do you get the same result twice? If the result is reproducible, you now have a change that you can focus on. Does the same low peak area occur for all QC samples or just some? How about other types of samples such as test samples or calibration samples? You can use "The Rule of Two" to help you begin to find failure patterns, and after a pattern is observed, tracing its origin is much easier.

The Divide and Conquer Rule. "The Divide and Conquer Rule" rule was briefly mentioned earlier. I try to make an observation or experiment that allows me to discard a large number of possible root causes of a problem. Then I divide the territory again and again until I find the problem source. Does the problem occur only with samples, but not with standards? Does the problem occur more often with a column that has had 1000 samples through it than with a fairly new column? Does it show up at the beginning of a sample

batch, but disappear by the end? Is it specific to one model of LC system, one operator or one method? By eliminating the things we know are not the cause of the problem, we can more easily identify those that are.

The Module Substitution Rule. "The Module Substitution Rule" is perhaps the most powerful way to isolate the source of a problem. This simply involves replacing a suspect part with a good one. The most common application of module substitution is exchanging one column for another, or replacing a batch of mobile phase with a fresh one. Module substitution can be practiced at any level, from exchanging an entire LC system to a circuit board or component on a circuit board. In the context of hardware troubleshooting, the power of module substitution may help justify certain instrument purchase decisions. For example, if you are budgeted to purchase a new LC system, seriously consider getting another system just like the one you have. Then you can exchange parts — pumps, autosamplers, detectors, and so forth — to isolate problems much more easily than if the two systems were different brands. You will also be able to transfer specific troubleshooting and maintenance skills more readily.

The Put-It-Back Rule. "The Put-It-Back Rule" is closely related to "The Module Substitution Rule." It reminds us to put a good part back into service if it was replaced during module substitution, but was not found to be faulty. Although this process may seem obvious, it is amazing how many columns are discarded in this manner. You may replace the column as part of the problem-isolation process, but later realize that the problem was related to the mobile-phase pH, not the column. But you are in a hurry to get going, so you start the next sample batch with the new column. The original column, although perfectly good, gets set aside, and is often forgotten. How likely are you to pick up that column and use it as a replacement for another column in the future? Usually a used part is suspect and we'd rather not risk using it, so we discard it. A good column-tracking procedure, where the sample history for each column is recorded, might help to avoid this problem. But as a general practice, if a new part substituted for an old one doesn't fix the problem, reinstall the old part.

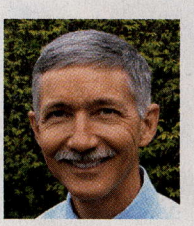
The Documentation Rule. One of my friends who works in quality assurance (QA) has a favorite saying: "If it isn't documented, it didn't happen." Most of the readers of this column work in the pharmaceutical industry, where good recordkeeping is mandated by the regulatory agencies. But besides being a good defense to use during an audit, troubleshooting and maintenance records can be invaluable tools to help avoid future problems. One of the uses of these records is to establish failure patterns or lifetime expectations for various components of an LC system or method. For example, if we wait until the pump seals fail, there can be a number of secondary problems, such as having to reanalyze a batch of samples that did not pass because of flow variations. In addition, the frit at the top of the column may get blocked from the bits of seal material that are sloughed off during the wear process. For those of you using ultrahigh-pressure liquid chromatography (UHPLC) systems, you might consider column frit blockage a lucky occurrence: The extremely small-diameter tubing in the system may get blocked before the pump seal fragments reach the column. The expense of a new column and the hassle of flushing or replacing tubing might be avoided if you had a policy for pump seal replacement. For example, after several failure cycles, you may find that seals tend to fail between 6 and 12 months of use. Why not replace the seals proactively every 6 months and avoid the problem completely? Although pump seals are not inexpensive, they are less than 10% of the cost of a column — and all it takes is one failed seal to ruin an otherwise good column.

Whether you decide to keep your LC maintenance and troubleshooting records electronically or on paper, be sure the process is simple and easy to use. Otherwise, good intentions may get ignored because of the hassle factor. My favorite recordkeeping system is still a looseleaf notebook, one for each LC system. With separate sections in the notebook for maintenance, troubleshooting, performance tests, and so forth, the data are easy to locate when they are needed. A simple form may prompt for the five W's and the H of a good news story: who, what, when, where, why and how. Just a few words to summarize are all that are needed. Be sure to record part numbers and serial numbers, where appropriate. Armed with this kind of record for each system, it is easy to sort through different troubleshooting episodes and find common causes that may lead to preventive maintenance strategies.

Summary

We have looked at the general problem categories that encompass most LC problems that you will encounter. We have also considered several rules of thumb that can be applied to many different types of problems to help isolate a problem, determine its source and, perhaps, develop a preventive maintenance routine that will help minimize the possibility of future downtime. In future installments of "LC Troubleshooting" we'll look at some of the problem categories in more detail.

John W. Dolan



"LC Troubleshooting" Editor John Dolan has been writing "LC Troubleshooting" for LCGC for more than 25 years. One of the industry's most respected professionals, John is currently the Vice President of and a principal instructor for LC Resources, Walnut Creek, California. He is also a member of LCGC's editorial advisory board. Direct correspondence about this column via e-mail to John.Dolan@LCResources.com.