LC TROUBLESHOOTING

Problem Isolation: Three More Things

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Isolating and correcting LC problems can be straightforward if you take a systematic approach.

Last month's "LC Troubleshooting" column (1) presented three central practices for preventive maintenance of liquid chromatography (LC) systems. Following that theme, this month's column covers three concise rules for problem isolation: the use of reference conditions, systematic isolation, and module substitution. These guidelines will help you to quickly identify and correct problems with your LC system.

REFERENCE CONDITIONS

To know that a problem exists with your method or LC system, you must know how the system works when it is working well. Most of us have a qualitative feeling about what is normal for a particular method. It is also important to have quantitative records of the LC system and of each method in use. I call these records the system reference and the assay reference, respectively. When problems arise, you can rerun these reference conditions to determine if a problem is associated with the system (both system and assay references are bad) or with just the method (only the assay reference is bad).

System reference: The system reference is a test of the entire LC system under conditions that can be readily repeated. The most convenient conditions are the test conditions used for a new column. For a C18 column, these conditions typically include a mobile phase of about 75:25 (v/v) methanol–water with a flow rate of 1.0 mL/min and UV detection at 254 nm. The sample is a column test mixture, often containing uracil, toluene, and a few other aromatic compounds. Record the results of this test for a new column, and you will have a set of reference conditions that can be repeated in the future simply by putting a new column in the system and rerunning the test. Also record the "vital signs" of the system, including pressure, retention time, and capacity factor of each component, and peak height or area. Records should also include all operating conditions and a copy of the chromatogram.

Assay reference: The assay reference is a record of the performance of a particular method when it is working properly. This information is most conveniently recorded each day when the first few standard or control samples are run. Many laboratories perform a system-suitability test each day, which provides a good assay reference. The main purpose of this test is to establish a set of conditions that you can use to determine whether a problem is caused by something related to the analytical method.

Using reference tests: Reference tests help determine whether a problem is associated with the sample, the method, or the LC instrument. When you suspect a problem, rerun the sample to make sure that a problem really exists. Once you confirm that you have a problem, run the assay reference — often this is just an injection of an analytical standard. If the results look OK, the sample is the likely problem source; otherwise, run the system reference test.

If the system reference test is OK but the assay reference is not, the problem is associated with something in the analytical method. If both reference tests fail, you have an instrument-related problem, and the next step is systematic problem isolation.

SYSTEMATIC ISOLATION

Often when a problem occurs, we immediately know the problem source and can correct it. For example, if you see a puddle on the bench and a fitting that is dripping, you know the source of the leak. For many problems, however, the cause of a problem is not obvious, and more work is required to locate the problem source. One such problem is peak tailing in the chromatogram. Probable causes include problems with the mobile phase, column, guard column, or the sample itself. Although it is possible to correct such problems by trying first one fix and then another, a systematic approach will generally solve the problem much more quickly.

Classify the symptoms: Before you can isolate the problem, you must determine the general category of problem you have encountered. One list of categories (2) is

- pressure
- leaks
- quantitation or data quality
- hardware
- chromatogram

It should be clear which category best matches your problem. Each category requires a different problem isolation procedure. Details of problem isolation within each of these categories are beyond the scope of this month's discussion, but more information can be found in references 2 and 3, in past "LC Troubleshooting" columns (4), and in the manuals for your LC system. Following are brief descriptions of approaches to isolating each problem type.

Pressure: Pressure problems fall into three subcategories: high pressure, low pressure, and cycling or erratic pressure. High pressure occurs when the flow is set too high (the correction is obvious) or when the flow path is blocked. To isolate a blockage, systematically loosen the fittings, starting at the detector and working upstream until the pressure drops to normal. The component just downstream of this last fitting is the most likely cause of high pressure.

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Low pressure is caused by flow rate setting problems or leaks. Isolate leaks by examining each fitting until you find the leaking fitting. Start at one end of the flow path and work along it until the leak is located. Tighten or replace the problem fitting.

Cycling or erratic pressure is often the result of solvent degassing problems, partially blocked inlet lines, air leaks, or pump-seal problems. Confirm that the solvent is being properly degassed. Check the solvent inlet lines for proper connections and verify that the inlet line frits are not blocked (remove or replace them to check). Check your maintenance log to see if the pump seals are due for replacement.

Leaks: Leaks are perhaps the easiest problem to isolate. Leaks at fittings usually can be cured by tightening the fittings. If the tightened fitting still leaks, disassemble it and rinse it out or replace it.

Quantitation or data quality: Problems with quantitation or data quality generally fall into one of the following categories: detection problems, injection problems, sample problems, or data-system problems. Be sure to check *LC•GC*'s "Data File" column for related topics. Detection problems usually center on linearity and minimum detectability. Both of these areas likely will have been addressed during method development, so you can refer to your records to be sure your results fall within the tested region.

Injection problems can be checked by changing to a sample that is known to perform well (for example, an analytical standard). If the problem persists, compare manual and automatic injection. Comparing the results of the analytical standard or control sample with your samples will help you isolate sample-related problems. Look for sample degradation in the autosampler, sample interaction with other sample components or LC system components, matrix effects, and interferences.

Some data-system problems will be apparent from a data-system self-test. Others may be complicated by detector linearity or other problems. The simplest way to check data-system performance is to substitute a known good system for the questionable one (see the discussion on module substitution below).

Chromatogram problems: Chromatogram problems are the most difficult to isolate. Start by rerunning the assay reference test. If this test produces normal results, you know that the problem is somehow related to the samples and how they interact with the system. The first step most of us take is to replace the guard column and analytical column to ensure that the system is working at its best. If this fails to correct the problem, try to correlate the problem with some other event. Does the failure occur late in a series of runs rather than at the beginning? (Such a pattern might point to sample degradation, mobile-phase degradation, or column fouling.) Does the failure occur for some samples but not others? (This pattern might suggest matrix effects, interferences, or storage problems.) Does the failure have a sudden onset? (Sudden failures might result from a hardware problem or the breakthrough of a contaminant from the guard column or analytical column.) The key to isolating these problems is to devise tests that clearly eliminate possible problem sources. You will soon identify the cause of the problem.

Hardware: If you suspect a hardware problem, and the solution is not obvious, rerun the system reference test. If this test fails, you know the problem is not related to the sample or the analytical method. Some hardware problems are routine and show up as other changes in the system. For example, check-

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valve and pump-seal problems often result in pressure problems. Detector lamp problems will result in excess noise in the chromatogram. Some mechanical problems may cause module failure, such as electronic failures in control circuits.

Module substitution can help you isolate which system component or subassembly has failed. The repair and maintenance sections of the users' manuals for each module are the best source of information about isolation and correction of specific hardware problems.

MODULE SUBSTITUTION

Module substitution is the replacement of a questionable system component with one that is known to be working properly. Most of us do this when we suspect there is a problem with the column — we just put a new column in the system to see if the problem goes away. This technique is useful for isolating problems that are difficult to locate. If you can't determine whether a problem is caused by the autosampler or the data system, for example, you might replace the autosampler to see if

the system returns to normal. You can exchange subassemblies, such as circuit boards, to isolate a problem within a specific module.

Because module substitution is such a powerful way to quickly isolate LC problems, it presents a strong argument for standardizing your LC equipment with one manufacturer. Standardization is especially important when the system is under the direction of a central system controller because most controllers talk to only one brand of equipment. For isocratic systems used in routine work, you can often substitute one brand of pump for another during troubleshooting, but gradient systems and integrated LC systems may require that all modules come from the same manufacturer.

AND FINALLY . . .

There's nothing magic about the guidelines discussed above — for the most part, they are just common sense. Keep in mind the "Five Rules of Thumb" for troubleshooting (5).

First, change just one thing at a time. It takes a little longer to isolate a problem in a stepwise, plodding manner, but the knowledge you gain about the problem and its cure will speed up the process the next time the problem occurs.

Second, a problem isn't a problem until it occurs at least twice. Reference tests are useful in determining the problem source. Confirm that the problem is reproducible. Don't waste time chasing down a nonexistent problem.

Third, put known good parts back into service. Module substitution is a quick and easy way to solve a problem, but it can be wasteful if you throw away parts that are perfectly good. I think we all have replaced questionable columns with new ones only to find that the problem lay elsewhere. Yet the new column remained in the system and the old one was relegated to the "column drawer" where it remains, unused, because no one wants to risk putting a used column into a system. And don't forget to throw away all failed parts so they will not get confused with new ones. If you choose to save good used parts, be sure to label them clearly as to their quality and history.

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Fourth, try to anticipate what will fail next. By keeping track of pressure trends, lamp usage, and column history, you will be able to correct many problems before they become significant.

Fifth, keep good records of your maintenance and troubleshooting efforts. These records can be invaluable in tracing failure patterns or helping to identify problems that rarely occur.

Finally, make good use of the resources available for troubleshooting. Your knowledge and that of co-workers is your first line of defense. Users' manuals contain invaluable information about specific hardware modules. If the information you seek is not in the manuals, don't hesitate to call the manufacturer to ask for help (ask for technical support). You can get free troubleshooting pamphlets from several manufacturers, and books (2) and computer programs (3) are commercially available. If you can't solve the problem with one of the tools listed above, it may be time to request a service call to get your LC system back in operation.

REFERENCES

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