LC Troubleshooting

Mobile-Phase Proportioning Problems and Standard Operating Procedures

Travis Culley and John W. Dolan

Standard performance-checking procedures can speed troubleshooting.

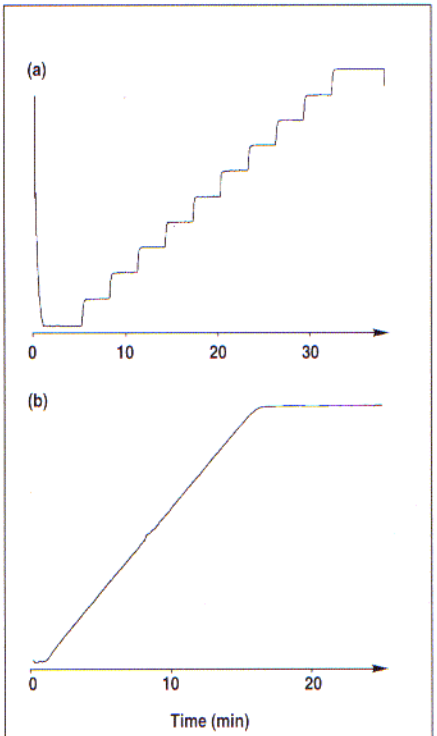

FIGURE 1: Results from acceptable (a) proportioning and (b) gradient-linearity tests. Conditions are described in the text.

Like many laboratories, our facility has a long list of standard operating procedures (SOPs) for verifying instrument performance. These SOPs are used on a predetermined schedule to verify that we are operating within the guidelines of good laboratory practices (GLP). In addition, these tests serve as tools for troubleshooting. This month's "LC Troubleshooting" discusses how we used an SOP to isolate a problem with one of our liquid chromatography (LC) pumps.

THE STANDARD OPERATING PROCEDURE

This SOP comprises two common tests to verify the performance of an LC pump's proportioning accuracy and gradient linearity. The tests require using mobile phases A and B of HPLC-grade water and water with 0.1% acetone, respectively. We remove the column from the system and replace it with a short piece of connecting tubing. We then set the detector at 265 nm and adjust the scale on the output device so that both 100% A and 100% B are on scale. The solvents are helium sparged and pumped at 3 mL/min through the system (this flow rate plus a back-pressure regulator on the detector ensure sufficient pressure for proper check-valve operation).

The proportioning test comprises a 5-min initial hold at 0% B, then steps of 10% B every 3 min until 100% B is reached. Figure 1a shows the data system output for a properly operating system. The steps are consistent with fairly square corners and little or no baseline fluctuation.

The gradient-linearity test is a simple linear gradient of 0–100% B in 15 min followed by a 10 min hold at 100%. The flow rate is 1 mL/min. Figure 1b shows the data system output for an acceptable linear gradient. The plot itself is straight. The ends of the gradient show a slight curvature, which is typical for most LC systems. The slight irregularity in the middle of the gradient is characteristic of this particular pump and occurs when the proportioning valve sequence switches at 50% B. A slight deviation at 50% B is common with low-pressure mixing pumps.

THE PROBLEM

The operator was unable to obtain reproducible retention times on the LC system. After trying some standard fixes, such as changing the mobile phase, he ran the proportioning and gradient-linearity tests. Figure 2a shows the results of the proportioning test, and Figure 2b shows the gradient test results. These results clearly are unacceptable. Neither test was reproducible — each time the test was done, the results were terrible and different from those of the previous run.

First he guessed that one of the proportioning valves that meters solvent to the mixer was faulty. He tried various valve combinations (A–B, A–C, B–C), but the problem remained, so the valves were not the problem. Then he listed other possible causes: blocked inlet frits,

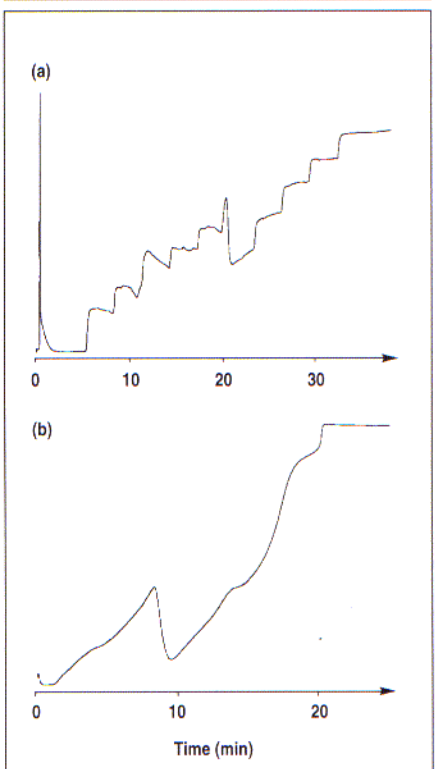

FIGURE 2: Results from unacceptable (a) proportioning and (b) gradient-linearity tests.

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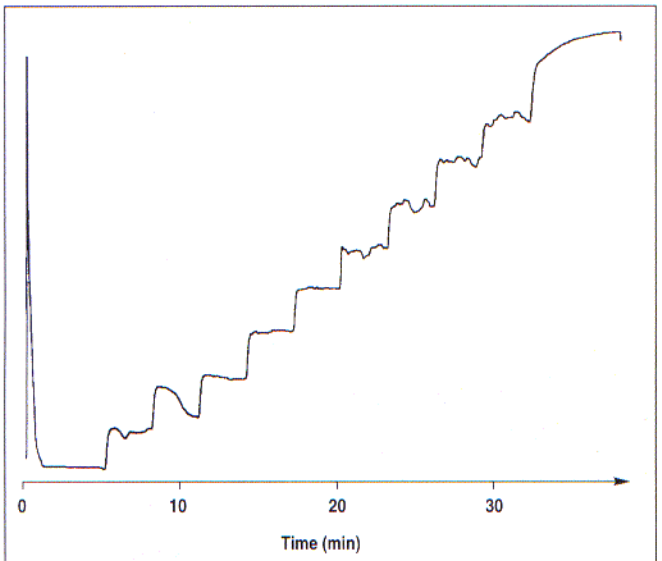

FIGURE 3: Results from proportioning test of Figure 2a after installing larger diameter supply lines.

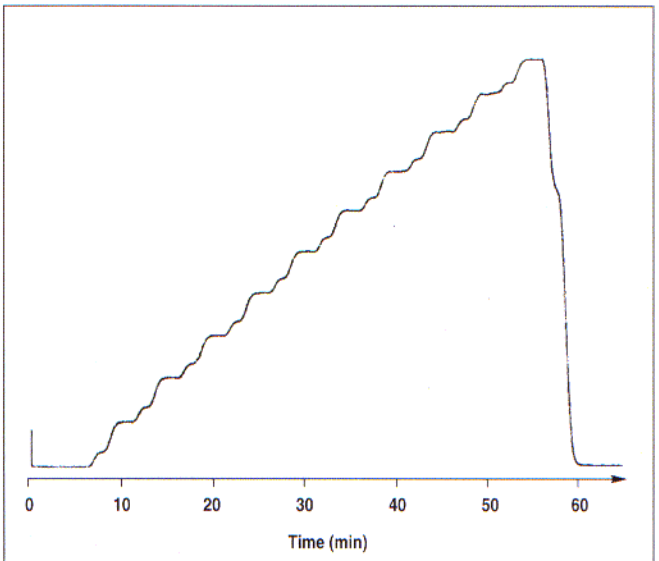

FIGURE 4: Unacceptable step test showing small secondary steps between each major step.

blocked inlet tubing, and a faulty check valve. The reservoirs were helium-pressurized and mounted above the pump, so solvent availability was adequate.

ONE THING AT A TIME

As we have discussed before in this column, it is important to change only one thing at a time when troubleshooting, so that you can pinpoint the true cause of the problem. In this case, our first step was to remove the sinker frits from the inlet lines in the reservoir to see if either was restricted. This change made no difference.

Nearly a year ago, this LC system was fitted with a commercial helium-degassing system. The degassing system was fitted with 0.040-in. i.d. PEEK pump-supply tubing. The current problem had the appearance of inadequate solvent supply, so we wondered if the PEEK tubing was supplying sufficient solvent for the pump demand. We tested the solvent supply by disconnecting the supply lines at the proportioning manifold and checking to see that they siphoned freely. The PEEK tubing had a siphon rate of approximately 1 mL/min under our test conditions. This siphon rate seemed to be a potential source of pump starvation if we operated the pump at flow rates greater than 1 mL/min. As a reference, we tested a standard $\frac{1}{8}$-in. o.d., $\frac{1}{16}$-in. i.d. solvent-supply tube. A siphon with this tubing delivered nearly 60 mL/min. When we replaced the two pump-supply lines with the standard, larger inner diameter tubing, the pump performance improved (see Figure 3) but was still unsatisfactory. Our next step was to replace the inlet check valve, but this change made no further improvement.

Finally, we carefully removed any air bubbles trapped in the system. First, we forced solvent through the inlet system with a syringe. Next, we set the flow rate at 5 mL/min with the purge valve open. Then we tapped each system component sharply with the handle of a screwdriver to dislodge any stubborn bubbles. This procedure flushed a series of large bubbles from the system. When the tests were rerun, we obtained the results shown in Figure 1.

The internal flow passages of an LC system contain many irregular surfaces and diameters, creating sites that can trap bubbles. In this case, it appears that a trapped bubble interfered with the operation of a proportioning valve, hindering solvent delivery. Often a sharp tap on a system component will dislodge a bubble so that it can be pumped from the system. Use a wooden or plastic screwdriver handle — a metal object, such as a wrench or hammer, could dent or damage the LC system. Remember, you are trying to dislodge bubbles, not vent your frustrations on the hardware.

STRANGER THINGS HAVE HAPPENED

Here's another problem encountered by a reader (1) during a proportioning step test. He ran a test similar to the one above to check proportioning accuracy. Figure 4 shows the test results. Notice the extra small step between each of the major steps in the plot. After the user tried many of the procedures described above, and even replaced the detector, the problem persisted. The retention, resolution, and precision all were acceptable for normal assays, which ruled out a leak or excessive extracolumn volume. To isolate the problem source further, he bypassed the autosampler and connected the pump directly to the detector. The trace then appeared normal, without the secondary steps. This indicated that the problem was associated with the autosampler, not the pumping system.

The user recalled that sample particulates or debris from pump seals can partially block the column inlet frit and result in split peaks. Extending this logic, he wondered if the problem stemmed from a partial blockage in the autosampler. Consequently, he replaced the two stainless steel filters in his autosampler and reconnected the unit — his problem was solved.

It is a mystery why a partially blocked frit in the autosampler caused an irregular step test — perhaps you readers have some suggestions.

REGULAR TESTING

Those two examples illustrate the importance of creating a set of reference conditions that you can use to test your LC system to see if it is proportioning the mobile-phase components properly. When you observe retention problems that persist after you change the mobile phase or try other simple remedies, stop and perform a reference test such as the step test to determine if the LC system is preparing the mobile phase correctly. Many laboratories use SOPs to carefully define the tests and performance criteria. Analysts may perform such tests on an as-needed basis when a problem occurs and on a quarterly basis for quality-assurance purposes. The step and gradient-linearity tests described here work equally well for LC systems with high- or low-pressure mixing. Regular testing will help ensure high-quality results from your LC system.

REFERENCES

(1) C. Dahlheim, personal communication, 1995.

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"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Walnut Creek, California, USA, and a member of the Editorial Advisory Board of LC•GC. *Direct correspondence about this column to "LC Troubleshooting,"* LC•GC, *859 Willamette Street, Eugene, OR 97401, USA.*