LC
TROUBLESHOOTING

Pump Shutdown, Sparging Problems, Double Peaks — Answers to Readers' Questions

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The problems this month are of the type that causes us the most consternation — they arise for no apparent reason or result in inconsistent failures.

It is especially aggravating when a method "almost" works. Three of the problems addressed this month fall into this category. A pump that shuts down at inopportune times, a helium sparger that isn't quite balanced, and a method that works only part of the time are the kinds of problems that make us want to transfer to a desk job. These problems can be especially hard to correct because once you fix them, you have to wait and see if the problems are really gone.

PUMP SHUTDOWN

Q: I use my liquid chromatography (LC) system for a routine method that is set up in the late afternoon to analyze ∼40 samples each night. Nearly every night, the pump shuts off sometime during the analysis, and the overpressure warning light is on in the morning. When the autoinjector makes an injection, the pressure rises sharply for just a second or two, then drops to normal. Apparently, the momentary pressure rise during one of the unattended injections exceeds the 6000-psi limit set on the pump and shuts the system down. What can I do to overcome this problem?

JWD: I think you have accurately pinpointed the cause of the problem. It appears that your pump's pressure sensor responds too quickly to the pressure change or that the valve-switching time is too slow, allowing the pressure to remain high for too long. Nearly all injection valves momentarily stop the mobile-phase flow during valve rotation (the Waters U6K injector [Millipore Corp., Milford, Massachusetts] is one exception). You have two choices: either decrease the duration (and thus the magnitude) of the pulse, or reduce the pump's sensitivity to momentary pressure disturbances. Let's look at the options.

First, you may be able to decrease pulse duration by speeding up the rotation of the injection valve. If the valve is pneumatically actuated, this is easily accomplished by increasing the air pressure supplied to the valve to 70–80 psi. In general, abide by the autosampler manufacturer's pressure recommendations, but exceeding these limits by 25–50% should cause no damage to the equipment. If this step doesn't help, operating the injector with helium instead of air or nitrogen will speed valve rotation; the lower viscosity of helium enables the actuator to fill and vent more quickly. If your autosampler uses an electrically operated valve, there is little you can do to speed the rotation (it may be useful to check with the manufacturer).

A valve that has been overtensioned or that contains a worn rotor seal may also have a reduced rotation rate. Try retensioning the valve or rebuilding it. You can check for worn rotor seals by substituting a valve that you know to be good.

If valve adjustments are not possible or not successful, installing a pulse dampener in the system may help. Some LC pump manufacturers and aftermarket suppliers sell Bourdon-tube pulse dampeners or additional mixing coils for use on LC pumps. Another alternative is to use a mechanical (not electronic) pressure gauge designed for LC use. Any of these devices installed between the pump and the injector will provide additional flexibility to the system, "soaking up" the pulses and dampening sharp pressure rises. This modification will effectively reduce the sensitivity of the upper-limit pressure shutoff to momentary changes in pressure.

Finally, the pressure sensor in the pump may be malfunctioning. It is unlikely that you can check for this problem, and generally it is not worth a service call, considering the options mentioned above. The next time the pump service engineer looks at the pump, however, it would be a good idea to verify that this part is functioning properly.

SPARGING PROBLEMS

Q: I seem to have created a problem with the helium sparging apparatus on my LC system. I have a sparging system that uses a cross fitting to split the helium line into three sparging lines, one for each reservoir. Historically, the flow of helium was about the same to the acetonitrile, methanol, and water reservoirs, based on a visual determination of the bubble flow. During a recent preventive maintenance session, the reservoirs were replaced along with the mobile-phase inlet-line frits and the sparging frits. When I used the system again, the reservoirs containing organic solvent were sparged in the normal manner, but the helium flow to the water reservoir was greatly reduced. I checked all the fittings for leaks, I have an adequate supply of helium, and the helium pressure is unchanged at the regulator outlet. What am I missing?

JWD: I've scratched my head over this problem in the past, but the solution is very simple. The problem is likely caused by the use of a sparging frit of the wrong porosity. With a symmetrically constructed system, you would expect the same flow through the three sparging frits. The viscosity of the organic solvents, however, is less than that of water, so an added back pressure is created on the water line. In your case, the added back pressure is enough to shift the split ratio so that the organic solvents are sparged more readily than water. I suspect that you are using frits of the same porosity on all three lines. Try using a larger porosity frit on the water line. For example, use 2-μm or 5-μm porosity frits on the organic lines and a 10-μm frit on the water line. This will help balance the flow. Alternatively, install a needle valve or other restriction device and adjust the relative flow to each reservoir; this step, however, is more expensive and less convenient.

The mobile-phase inlet-line frits, on the other hand, should all be of the same porosity. I recommend frits with a 10-μm porosity. They will catch occasional bits of lint and other particulate matter that get into the reservoir but will not cause undue resistance to flow. Smaller porosity frits are available, but they are more likely to cause pump-cavitation problems because they create too much restriction in the flow path.

Here's another potential problem: If mobile phase flows back up the helium sparging line when the helium pressure is shut off, it can flow into and contaminate another reservoir. This problem is particularly common with closed sparging systems that maintain a slight head pressure of helium on the reservoir. You can avoid the problem by venting the reservoir (or loosening the cap) before shutting off the helium pressure. Alternatively, install a sparging relief valve in the helium line inside the reservoir; the valve will allow normal sparging to proceed without problems but will open when the pressure drops, so that no mobile phase is forced back up the helium line. Such valves should be available through your favorite LC parts and fittings dealer.

DOUBLE PEAKS

Q: I have been using an LC method to check for product residues after our production equipment is washed down at the end of a production run. Although the sample matrix varies somewhat, methanol is typically my injection solvent. My method uses an 80% acetonitrile–water mobile phase at 1 mL/min with a C18 column. My injection volume is 50 μL. I've used this method for several years with no problems, but now split peaks sometimes appear early in the chromatogram. I've replaced the mobile phase, column, and injector, and I've serviced the pump, all to no avail. Can you give me some help?

JWD: I think your problem is due to injecting too large a volume of too strong a solvent, which washes sample down the column and results in broad bands and sometimes band splitting or tailing, especially for the early bands in the chromatogram. Let's look at some guidelines for injection parameters.

Extracolumn effects result in band broadening, especially for early eluting peaks (1). As long as the injection volume is less than about one-sixth of the peak volume, no problems are likely to arise when injecting mobile phase. For example, you could inject 50 μL of sample as long as the peaks of interest remain less than ~300 μL in volume (0.3-min baseline width at 1 mL/min). This guideline assumes, however, that mobile phase is used as the injection solvent.

> **Injecting too large a volume of too strong a solvent washes sample down the column and results in broad bands, band splitting, or band tailing.**

In your case, your injection solvent has two potential problems. First, it is stronger than your mobile phase (80% acetonitrile ≈ 85% methanol). Instead, try using injection volumes ≤ 25 μL when using injection solvents stronger than the mobile phase. The second problem has to do with the chemical differences between methanol and acetonitrile. Although solvent selectivity differences may or may not cause injection-related problems, I would not hesitate to blame them for the problem you are observing.

Why are you seeing this problem now, but did not see it in the past? There are no definitive answers to this question, but a number of factors such as column temperature or column-to-column differences may be important. I suspect that the method has been working right on the edge of reliability for a long time, but one or more unidentified factors have recently pushed it toward unreliability. The fact that the problem occurs inconsistently suggests that the method is marginally stable.

Assuming that we've identified the problem, what can you do to fix it? Modifying the injection solvent by injecting the sample in acetonitrile–water is probably the surest way to correct the problem. Evaporating the sample to dryness and then reconstituting it in acetonitrile during sample preparation also may help. If you need to continue using methanol, try a smaller sample (for example, 20 μL) or dilute your sample with an equal portion of water and inject twice the volume. When samples are more dilute than the mobile phase, you can generally inject larger volumes because the sample gets trapped at the head of the column until the (stronger) mobile phase begins the elution process.

A CALL FOR INFORMATION

When I teach LC troubleshooting classes, I am often asked questions about system calibration and good laboratory practice (GLP). Of particular interest are routine procedures for testing the calibration of pumps (flow rate and solvent proportioning), injectors (or autosamplers), columns, and detectors. Some laboratories have established procedures, some are trying to implement procedures, and others haven't even thought of it. As far as I know, no "industry standard" exists for instrument testing for GLP.

I would like to devote a future "LC Troubleshooting" column to this topic, based on reader input. If you have some insight or procedures that you would like to share, please write to me care of *LC•GC* or fax me a note at (503) 835-7930. Please include your name, phone number, and affiliation; I will not identify individual readers or divulge proprietary information in the article, but I may want to talk to you to clarify information.

REFERENCE

(1) J.W. Dolan, *LC•GC* **10**(1), 20–25 (1992).

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