LC Troubleshooting

Autosamplers, Part II — Problems and Solutions

Autosamplers can be great time-savers, but they have their own set of problems.

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Last month's "LC Troubleshooting" column (1) described many of the design and operation features of autosamplers used for liquid chromatography (LC). This second installment about autosamplers covers some common problems associated with these time-saving devices.

Overfilled Vials

Injection reproducibility problems can occur if a sample vial is filled too full and sealed tightly and then a large sample is injected. When sample is withdrawn from a vial, the removed volume must be replaced with something, or a partial vacuum will exist. The partial vacuum generally is no problem if a vial has sufficient headspace; for example, if a vial is filled to less than three-quarters of its capacity. The air in the headspace will expand when the sample is withdrawn. Sample volumes smaller than approximately 50 µL generally present no problem. However, if a vial is completely filled and a large sample — 250 µL or more — is withdrawn, it is possible to create enough of a vacuum that the syringe cannot withdraw the desired volume. In extreme cases, such as with volatile sample diluent, it is possible to cavitate the solvent inside the syringe needle. Cavitation is the formation of bubbles when the pressure is reduced sufficiently that the solvent boils and forms a bubble in the sample needle or tubing. This situation is analogous to a vapor lock in an automobile fuel system.

Some autosamplers overcome the potential for cavitation by using a vent needle that allows air to enter the vial as sample is withdrawn, but this design is uncommon. Using an internal standard will compensate for varying sample size, so this alternative can help. The easiest way to avoid problems related to creating a vacuum in a sample vial is to fill it no more than one-half to three-quarters full.

Needle-Depth Adjustment

If you are accustomed to having plenty of extra sample, you may never have encountered this problem, but needle-depth adjustment can be critical for workers in trace analysis. In the ideal case, you would like the sampling needle to stop just short of the bottom of the vial so that you can make maximum use of the sample in the vial. When sample volume is limited, microvials or vial inserts can be used to increase the depth of sample for a given volume. If the needle-depth setting places the needle too far down, the needle can bend when it hits the bottom of the vial. In some cases, the needle can become blocked when it becomes sealed against the bottom of the vial. At the other extreme, if the needle depth is insufficient, you can try to draw sufficient sample but get only air when the sample depth drops below the needle tip.

Some autosamplers have mechanical needle-depth adjustment features, but most autosamplers control the needle depth electronically. In these cases, users start a teaching program that allows adjustment of the needle height. One trick that I use is making a special vial for teaching use by cutting a large hole in a vial septum and then installing the vial in the sample tray. I adjust the needle to the target depth, then lift the vial by its cap. In the case of my instrument, I expect approximately 1 mm of vertical play if the system is adjusted properly. This play allows a safety margin in case of any variation in vial depth for a given vial model. This depth should be changed when the vial type is changed, for example, from a regular vial to microvial.

Some autosamplers use a needle that has a side port instead of a hole in the end. This configuration helps to avoid needle blockage from septum pieces and eliminates the needle sealing against the bottom of the vial. Some autosamplers use a spring-loaded needle that prevents bending when the needle hits the bottom of the vial. Some microvial inserts have a spring at the bottom, so the needle can be adjusted to touch the bottom of the conical vial tip for maximum sample use.

Needle Position

The needle-depth adjustment discussed above controls the vertical *z*-axis movement of the needle, but the horizontal *x*- and *y*-axis positioning also are important. Whether a needle moves in an *x,y,z* format or a vial moves to a needle as with a rotating tray, it is important that the needle is aligned so that it easily enters the septum on each vial. Misalignment can result in a needle striking the sample vial cap or missing the vial completely. In either case, a bent needle is the likely result. Again, some autosamplers have mechanical adjustments, and others use a teaching mode to control the *x*- and *y*-axis adjustments.

Needle Blockage

The autosampler needle can become blocked with bits of septum if the septum breaks apart during use. Usually, replacing the needle or changing to another type of septum will correct the problem. PTFE-film septa are the least likely to core, but they also provide the least-secure seal for the vial, especially if sample loss due to volatility is of concern. Many workers find that PTFE-faced polymeric septa are the best compromise. As mentioned above, side-port needles generally are free of septum-related problems, but this needle design is not in widespread use.

Carryover

Carryover is the appearance of a peak in a blank injection after a high concentration of sample. This topic was covered in depth in recent columns (2,3), and I won't discuss it in this installment. The key factors to consider are the selection of the wash solvent, the number of wash cycles, the construction of the sample loop, and the chemical adjustments to the sample solvent to reduce adsorption.

Changing Sample Concentration

Sometimes analysts observe a change in response for repetitive injections that suggests that the sample concentration is changing. Two problem sources are possible: evaporation and sedimentation.

Sample concentration in the sample vial can change when either the sample or the solvent, or both, evaporate. Evaporation of the vial contents is more likely after the septum has been pierced than when the vial is tightly sealed. When using volatile sample solvents such as hexane, methylene chloride, or methyl-*tert*-butyl ether, analysts can observe significant solvent evaporation if vials are not sealed tightly. Aqueous–organic mixtures such as acetonitrile–water are much less likely to display this kind of problem because of their much lower volatility.

Evaporation of the sample solvent will increase the concentration of the remaining sample. This concentration increase is more of a problem when using external standardization than with internal standards, which can compensate for changes in concentration. If the sample itself is quite volatile, selective evaporation of the sample can cause changes in concentration that cannot be compensated for by use of internal standards.

During method development, it is a good practice to measure the stability of the sample on the autosampler tray during a time period that emulates a normal run sequence. An easy way to check for problems is to inject a sample at the beginning of the sequence and then reinject a sample from the same vial at the end of the sequence, for example, 12 h later. If the results are the same, you don't need to worry about evaporation problems.

Sedimentation of the sample in the vial can be a vexing problem. It can occur when samples are frozen and thawed before analysis without mixing. In one example of sedimentation, samples were prepared in a buffer salt solution and frozen (4). Later, the samples were removed from a freezer and placed in an autosampler tray to thaw. The analysis comprised three replicate injections of each sample. The analyst observed that each subsequent injection from a vial followed the same pattern of decreasing peak size. The cause was stratification of the sample. As the samples froze, the water froze first, with the ice floating to the surface. The sample remained in the increasingly salty buffer. Thus, the sample concentration in the bottom of the vial was higher than at the top. When the samples were thawed, the same stratification remained, so the first sample drawn was from the higher concentration of sample at the bottom of the vial. The level of liquid in the vial dropped, so the next injection contained sample at a lower concentration, and so forth. The analysts avoided this problem in future runs by agitating the sample tray briefly to mix the vial contents after the samples thawed.

Flush Solvent

The primary function of a flush solvent is to remove residual sample from portions of the plumbing that are unswept by the

mobile phase. As a result, the wash solvent seldom, if ever, is injected. For this reason, analysts can select flush solvents for their solvating characteristics rather than their compatibility with mobile phases. Of course, a wash solvent should be miscible with the mobile phase, but it doesn't need to match it directly. For example, workers generally can obtain better removal of sample residues by using a solvent stronger than the mobile phase. Thus, if you were using a 50:50 acetonitrile–water mobile phase, you might use 100% acetonitrile for a wash solvent. If the solvent isn't strong enough, then residual sample might not be flushed from the system, and carryover can occur.

Some systems use the flushing syringe to both draw and dispense samples. In these cases, the system will have a hydraulic link between the syringe and the sample needle. This link may be 50 cm or more of 1-mm i.d. tubing. Air bubbles in this line can act as shock absorbers that expand and contract as pressure rises and falls during sample filling and dispensing. This effect can result in variable injection volumes. A simple way to eliminate this problem is to degas the flush solvent and then purge the flush system. For this purpose, most autosamplers have a purge or prime function that flushes approximately 10 mL of solvent through the flushing system. Some autosamplers draw a small bubble before and after a sample to minimize dispersion in the tubing that holds the sample; this bubble should not be confused with unwanted bubbles in the sampling system.

In my laboratory, we routinely replace the wash solvent each day and degas it for maximum reliability. We also avoid the use of buffers in the wash solvents because buffers tend to leave crystalline deposits in the system.

Bypass Problems

When an injection valve is rotated between the load and inject positions, a momentary stop in flow from the pump to the column occurs. This scenario is illustrated in Figure 1a, in which the valve rotor is in the intermediate position. (For a review of how the valve operates, see last month's "LC Troubleshooting" column [1].) During this transition period, the pump pressure rises, and the column pressure drops, so a pressure surge occurs when the rotation is completed. In the early days of LC column development, chromatographers observed

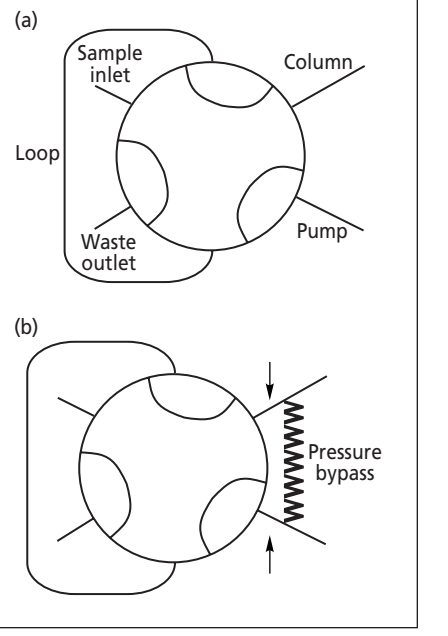

Figure 1: Schematics of (a) a conventional injection valve with the rotor positioned midway between the load and inject positions and (b) the same valve with the addition of pressure bypass plumbing. Arrows show the locations of potential blockages. See the text for details.

that these pressure surges could quickly destroy poorly packed columns.

One clever way to avoid pressure surges was by using a pressure bypass. The pressure bypass simply is a length of capillary tubing that connects the pump and column, as shown in Figure 1b. The resistance to flow across this capillary and the back pressure through the normal flowpath determine the split ratio of flow. For example, the split ratio may be 20:1 under normal conditions (load or inject position), so 5% of the flow goes through the bypass and 95% goes through the valve. The valve is blocked during the transition between load and inject positions, so 100% of the flow goes through the bypass. Thus, the bypass prevents interruption of the flow to the column and greatly reduces, if not eliminates, pressure surges. The advent of better column-packing techniques and more stable column packings reduced the need for pressure bypasses; however, some autosampler manufacturers still include pressure bypass functions.

Although a pressure bypass minimizes pressure surges, it is not without potential problems. Problems occur if the flow between the bypass and the valve becomes restricted; for example, at the arrows in Figure 1b. If restriction occurs, the split ratio is changed, so the 20:1 split ratio normally observed may change to 1:1. Thus, half the flow goes through the valve, and half goes through the bypass. The practical effect of this blockage is equivalent to diluting the sample with mobile phase. So a 100-µL injection with a 1:1 split ratio will become a 200-µL injection. This change can have a significant effect on the widths and shapes of all peaks in a chromatogram. The symptom of a poorly operating bypass is when all peaks are broadened. The fix is to disassemble the plumbing and flush all the valve passages and tubing or to replace the parts.

Because of the insidious nature of bypass blockage, some workers prefer to avoid them. An alternative to protect against pressure surges is using a Rheodyne Make Before Break (MBB) valve. This valve has a clever design that connects a bypass passage only during the transition from load to inject, so analysts gain the advantages of the bypass but avoid the problems. The valve substitution is straightforward for autosamplers that normally use Rheodyne valves.

Summary

Although autosamplers can be great time-saving devices, they introduce their own unique set of problems. I discussed some of the more common autosampler problems in this column. Each autosampler design has its own most common problems. An instrument's owner's manual is another source of information about problems related to that specific autosampler.

References

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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.com.

You Asked for It

I often am asked when I'm going to compile a selection of "LC Troubleshooting" columns. During the past 17 years, *LCGC* has published more than 180 installments of the column, and few readers have access to all the past columns. So I have compiled the columns, from the first issue in 1983 to the last issue of 2000, scanned them into PDF files, and placed them on a CD-ROM disk for ready access. The CD-ROM disk also includes a database file that is searchable by keyword and hot-linked to the articles. I've titled this CD-ROM disk "The LC Troubleshooting Bible." You can purchase a copy through LC Resources or from the LCResources.com web site. So if you want specific information about electrochemical detector problems, stainless steel passivation, or some other topic, you can search the database library and quickly find the information you need.