LC Troubleshooting

Testing Autosampler Performance

John W. Dolan and Travis Culley

Automation doesn't necessarily mean high precision. This month's "LC Troubleshooting" discusses how adding a few steps to a standard operating procedure can increase an autosampler's reliability.

Recently our company purchased two new liquid chromatographs, including autosamplers, for our laboratory. After setting up the equipment, we performed our standard operating procedure (SOP), which we call the Quarterly Check. We use this SOP to test each liquid chromatograph every three months.

In the autosampler check, we test the sample-injection reproducibility in both the filled-loop and partially filled–loop configurations. To test the two new autosamplers, we injected a test mixture seven times under standard conditions and then determined the relative standard deviation (RSD) for the series of injections. For filled-loop testing, we injected 150 µL of sample into a 50-µL loop. This threefold overfill provided maximum precision. For the partially filled–loop sequence, we injected 20 µL of sample in a 50-µL loop. With one autosampler, we obtained a 0.12% RSD — the minimum acceptable RSD is 2% — for the filled-loop test. The partial fill test had a 1.03% RSD — the limit is 3% RSD. The second autosampler produced similarly acceptable test values.

After operating both autosamplers for several weeks, we noticed inconsistencies in their performance. Some samples showed smaller than expected peaks, and others displayed normal peaks. We were injecting 100 µL in a 200-µL loop and using an internal calibration standard that enabled us to quantify the peaks. The variation in peak height, however, indicated that the systems were not performing as they should. We suspected that the needles of one or both autosamplers were partially occluded by pieces of septum, so we removed and cleaned the needles on both systems. This cleaning failed to improve the results, so we concentrated on one of the systems from this point in an attempt to determine the cause of the peak-height variation.

The manufacturer's service representative suggested that we repeat the autosampler-check test. We wanted to eliminate the vial septum as a variable, so we replaced the vial rack with a beaker containing 0.1% acetone in water. We replaced the column with an in-line filter and set the UV detector at 255 nm. A back-pressure regulator placed after the detector provided sufficient pressure for reliable check-valve operation. A mobile phase of 5% acetonitrile and 95% water was delivered at a flow rate of 1 mL/min. We injected 100 µL of the sample into a 200-µL loop. The run time was 3 min.

Figure 1a plots the normalized peak areas vs. run number for 100 injections. (The beaker was uncovered, so selective evaporation of acetone caused some drift. This drift was partially corrected in the plot of Figure 1a; the remaining drift is of no concern for the present discussion.) Injections 12–13 and 36–37 have obvious problems — the peak area is at least 10% smaller than the other peaks. The RSD for the uncorrected data was 19%; after correcting for drift, the RSD was 2.3% for 100 injections. The manufacturer's performance specification under these conditions is less than 0.5% RSD.

The smaller-than-expected peaks have two possible causes: we may have injected a smaller amount of sample or we may have injected air with the sample. Because the autosampler was new, a mechanical error in sample delivery was unlikely, so we suspected that the large deviations in Figure 1a were caused by air in the injection system. Our close examination of the autosampler revealed a small air bubble at the tip of the syringe plunger. We had seen such bubbles before but had ignored them because they are very difficult to purge from the system. It seemed like an air bubble of constant size would not affect system performance. At this point, however, we degassed the autosampler wash solvent (water), thoroughly purged the syringe and associated tubing, and then repeated the precision test. This time we covered the sample beaker with aluminum foil to reduce evaporation. Figure 1b shows the results (corrected for drift). The sample-to-sample noise is reduced and the large deviations are eliminated. The RSD for the uncorrected data was 0.9% and 0.3% for the corrected data.

These results impressed upon us the need to degas the wash solvent and purge the injector before performing a series of runs. Since we have added degas and purge steps to our autosampler routine, we have seen no further reproducibility problems.

ANOTHER BRAND

A few days later we performed a quarterly check on another liquid chromatograph in the laboratory. Two series of seven injections in the partially filled–loop mode yielded RSDs of 2.9% and 4.7%. Although the 2.9% RSD was within our SOP specification of 3%, we routinely see smaller deviations, so we investigated the problem. We broke the needle while attempting to clean it, so we replaced it with a new one. The autosampler with a new needle yielded similar unacceptable results. At this point, we purged the injection syringe thoroughly with degassed water and repeated the test. On retesting, we obtained an RSD of 0.5%.

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LC Troubleshooting Editor

STILL ONE MORE

A colleague reported another autosampler problem. He was injecting 30 µL of sample using a 100-µL sample loop. When the system was started each day, it automatically primed and purged the injection system with a water wash solvent. His first injection of the day produced a chromatogram with sharp, well-shaped peaks. Subsequent injections yielded broader peaks, particularly in the early part of the chromatogram. After a purge cycle, the next injection was OK, but the peaks deteriorated on subsequent injections. The pattern was quite reproducible.

Broad peaks early in a chromatogram are classic symptoms of extracolumn effects. These problems often arise when the sample is too large or the injection solvent is too strong, or with a combination of these two situations.

Several possible causes exist for this problem, but unfortunately we did not hear a final report. In this case, the sample size is unlikely to be the problem source. An injection of 25–30 µL seldom will cause peak broadening unless the peaks are very poorly retained and a strong solvent is used at full strength.

At the end of each run (or at the beginning of the next if no flush cycle is used), the sample loop will contain mobile phase. Normally, this mobile phase is displaced by the sample, and the loop is backflushed onto the column so that the sample arrives first and is followed by the remaining mobile phase in the loop. If the injector was misplumbed and prevented the sample loop from being backflushed, as we suspect in this instance, the residual mobile phase can dilute the sample before it reaches the column, which can increase band broadening. When the prime–purge cycle flushes the loop with water, the 70 µL of water included in the injection will help on-column concentration of the sample, and thus yield narrower bands. You should compare the actual sample-valve plumbing with the plumbing diagram in the operator's manual to see if the plumbing is correct.

If you cannot determine the cause of a problem like this, you could add an autosampler purge cycle between each injection. A purge cycle would not add significant time or effort to most methods because the purge could occur during data processing at the end of the run.

A more common but related problem occurs when an autosampler wash solvent is stronger than the mobile phase. For example, a method might require that you use 100% methanol as a wash solvent and 60% methanol in the mobile phase. A pulse of strong solvent injected with the sample can have the opposite effect of on-column concentration — broader bands occur when the loop is purged. The solution is simple — use a wash solvent that is no stronger than the mobile phase.

CONCLUSION

Our experiences with three autosampler problems have led us to change our laboratory's SOP for LC system operation to include degassing the autosampler wash solvent and purging the autosampler before running a sample series in which injection volume is important. Autosamplers are available in many different designs, and bubble problems may occur more frequently with some brands and models than in others. We see similar performance problems with LC pumps — some will pass air bubbles with no problems, whereas others require thoroughly degassed mobile phase to run at all. Degassed mobile phases will improve the performance of all LC pumps, even if this procedure is not mandatory. Similarly, the current results suggest that degassing the wash solvent and purging the injection system will improve the reliability of autosamplers.

How should autosamplers be tested? Our quarterly check determines overall performance of the LC system under conditions similar to those of everyday use. We feel that this is the best way to test all components of the system. We can test individual components of LC systems to assure their performance, but these tests do not guarantee that the systems will work as a unit. The autosampler procedure using acetone described above tests the autosamplers as independent units. This test is especially convenient for making a large number of injections because the run time is short. We can also use this test to isolate a problem if it is not obvious.

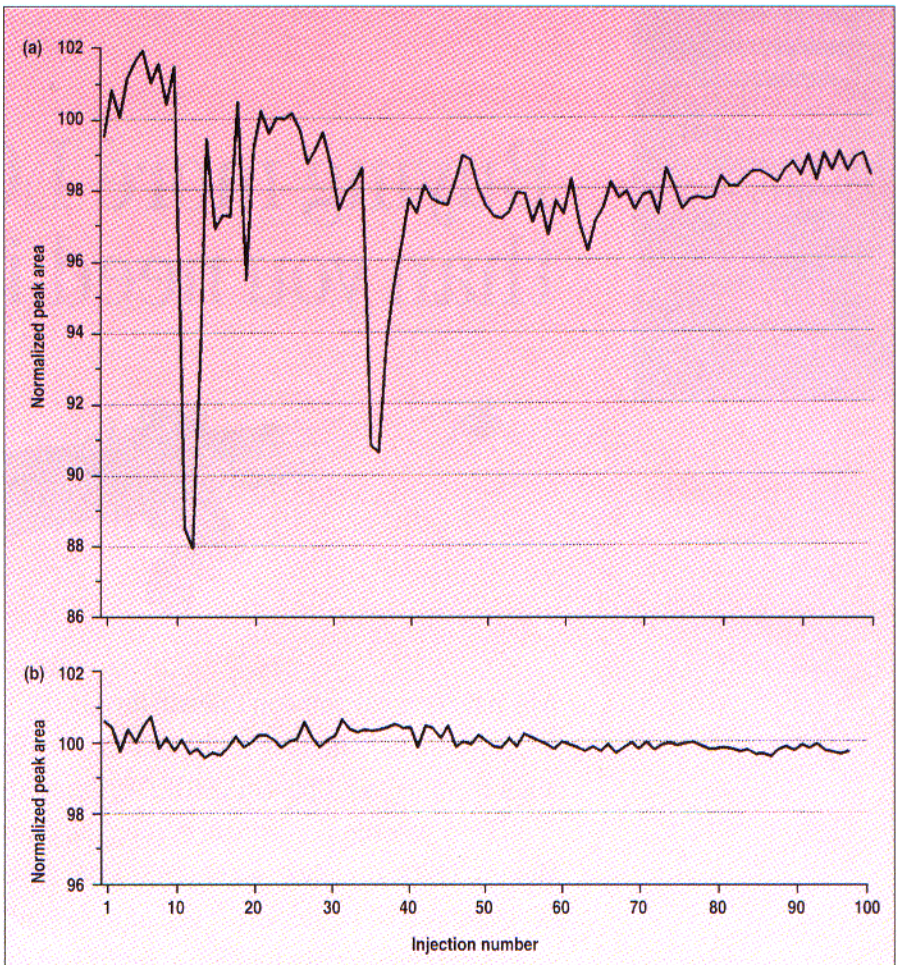

FIGURE 1: Plots of normalized peak area vs injection number for 100 consecutive injections (a) before and (b) after degassing the autosampler wash solvent and purging. The plots have been corrected for drift. See text for details.

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