LC Troubleshooting

A Second Shot at Some Troubleshooting Problems

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This month, readers share tips and suggestions for solving problems discussed in past "LC Troubleshooting" columns.

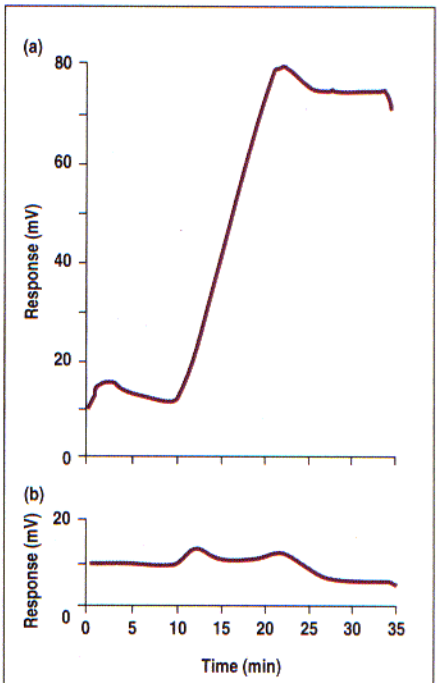

FIGURE 1: Blank gradient baselines for mobile phases containing (a) old hexane and (b) fresh hexane. See text for details.

I get mail from readers who ask a variety of questions about problems or offer helpful suggestions related to topics previously discussed in "LC Troubleshooting." I try to respond directly to my mail so you readers don't have to wait months to find answers to your problems — it may take six months or more before I get around to writing a column about your favorite problem.

Recently, I've been collecting helpful hints and suggestions to solve problems that have puzzled me. This month's column will share a sampling of some of your tips. I'd like to thank all of you for your contributions, whether or not they made it into print.

SOLVENT CHANGEOVER

In an earlier "LC Troubleshooting" column, I discussed conditions for column storage (1). I suggested using the following simple washout and storage procedure: Flush the buffer from the system with unbuffered mobile phase and then switch to 100% strong solvent for a final washout. Store the column in the strong solvent. Before running the next batch of samples, flush and equilibrate the column with mobile phase.

C. Duda of Bioanalytical Systems Inc. (West Lafayette, Indiana) correctly pointed out that switching directly from a strong solvent to a buffered mobile phase could cause buffer precipitation just as switching from a buffered mobile phase to a strong wash solvent does. In both cases, the safest procedure is to wash the column first with unbuffered mobile phase (for example, use 60:40 methanol–water rather than 60:40 methanol–buffer). Approximately five column volumes (10–15 mL for a 25-cm column) will be a sufficient amount for this step. When you use buffered mobile phase to wash strong solvent from columns (or vice versa), the potential for buffer precipitation is high, so this precaution is a wise step.

DIRTY SOLVENT

Last month's "LC Troubleshooting" described some problems related to solvent degradation (2). After press time, T. Michnik and D. Federighi of Cell Therapeutics, Inc. (Seattle, Washington), sent me the pair of blank runs shown in Figure 1. The examples in last month's column focused on reversed-phase solvents; however, this example successfully illustrates potential problems with normal-phase solvents.

The details of the chromatographic conditions are sketchy because the method is proprietary, but the source of the problem is clear. The method involves running a gradient from mobile phase A to mobile phase B, in which A and B contain different mixtures of hexane and isopropanol with a trace of water. The method uses UV detection at 220 nm.

Figure 1a shows a blank gradient for mobile phases containing hexane that was at least one year old. When Michnik and Federighi replaced the hexane with a fresher batch (approximately three months old), they obtained the baseline shown in Figure 1b.

It reminds me of the instructions I once received from a guitar teacher, "If you can't remember when you last changed the strings, they are overdue for replacement." This reinforces a message from last month's column: don't buy more solvents than you expect to use in a few months.

BACK-PRESSURE RESTRICTORS

Usually, I recommend placing back-pressure regulators downstream from detectors to help minimize problems that arise from solvent outgassing in the flow cell. These regulators provide enough pressure to keep dissolved air in solution until it passes through the detector cell. I like to use commercial regulators for

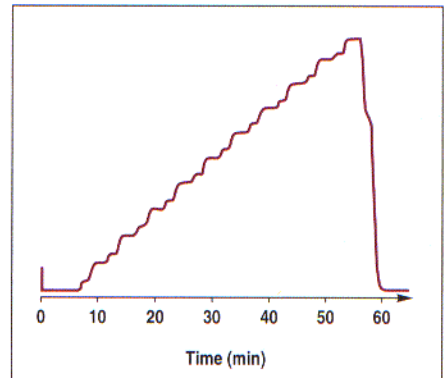

FIGURE 2: Unacceptable gradient step test showing small secondary steps between each major step. See text for details.

two reasons. First, by design, these spring-loaded devices provide consistent back pressure regardless of the flow rate. Second, they act as sinkers so the waste line from the detector stays in the solvent-waste bottle. These regulators are available in stainless steel or PEEK construction with various pressure limits. The 100-psi restrictors are among the most popular because they create enough pressure to keep air bubbles dissolved in the mobile phase but not enough to jeopardize the detector-cell seals.

E.E. Brown of Kalama Chemical, Inc. (Kalama, Washington), reminded me of an inexpensive back-pressure device that I have used successfully in the past — 50–60 cm of 0.01-in. i.d. PTFE tubing added to the detector outlet. This tubing provides enough back pressure for many applications. If you use this technique, be aware that the back pressure is proportional to the flow rate. For example, if you have 100-psi back pressure at 1 mL/min, doubling the flow rate during a column flush cycle may result in a pressure that exceeds the limits of the flow cell. This increase in pressure can lead to problems during column flushing or when changes are made by operators who are unfamiliar with the system's operating restrictions.

PUMP SEAL FLUSHING

In a recent column about pump seal problems, I suggested flushing out salt residues from behind the pump seals to extend seal life (3). When a buffered mobile phase evaporates on a pump piston it leaves an abrasive residue, which can cause premature pump seal wear when the pump is restarted. Flushing the buffer from the pump before shutdown will reduce the problem of buffer accumulation on the piston. Flushing behind the seals with water removes buffer residues and eliminates the pump seal wear from this source.

R.A. Reynolds of The Children's Hospital of Philadelphia (Philadelphia, Pennsylvania) wrote about his method, which uses 0.5 M potassium phosphate as a buffer. Before starting a regimen of seal washing, he was a regular customer of the local liquid chromatograph service technician. He took my recommendation for daily flushing one step further by connecting a gravity-flow system that flushes a continuous stream of high performance liquid chromatography–grade water through the seal-flushing cavity at a rate of approximately 500 mL every 24 h. Since he implemented this system, his pump-related service calls have been reduced to annual preventive maintenance visits.

He cited an additional benefit of his flushing technique — he can monitor the waste with a conductivity meter to determine if buffer is leaking past the pump seal. This method seems like a simple solution for problems of short seal life when analysts must use strong buffers, such as with ion-exchange methods.

MYSTERY SOLVED?

When I discussed mobile-phase proportioning problems in an earlier column, a reader had submitted an odd step-gradient profile (reproduced here as Figure 2) that showed secondary steps between each major step (4). The reader found that he could obtain a normal stair-step profile by replacing two stainless steel filters in his autosampler, but the original reason for the anomalous results was still unidentified. A similar report from a second source confirmed that this problem is not unique (5). In this second case, a partially blocked piece of tubing was the source of the problem.

U.D. Neue of Waters Corp. (Milford, Massachusetts) now has supplied a good explanation for the problem: Some injectors — such as WISP autosamplers and U6K manual injectors (both from Waters) — are equipped with bypass loops through which the flow is directed when the valve is rotated. These bypass loops originally were added to minimize pressure shock when injectors were switched from the load to inject position. In the intermediate position the flow is shut off with a normal valve, but in the bypass configuration all the flow is diverted through the bypass while the valve rotates. Bypass loops are always in line, even when the sample loop is in the fluid stream. Sample dilution is of little concern because the flow through the bypass loop is at least 10-fold less than the flow through the sample loop.

If the loop (or an associated frit) becomes partially blocked, the resistance of the sample loop becomes comparable to the resistance of the bypass loop. In this situation, the flow ratio through the loops is changed. However, the sample loop usually has a much larger volume than the bypass loop, as when an autosampler is fitted with a 1-mL loop and used in a partial-fill mode. If a step change in solvent composition enters this system, a two-step change may occur at the detector. The first step appears when the new solvent going through the bypass reaches the detector. In the present case, if the resistance of the two loops

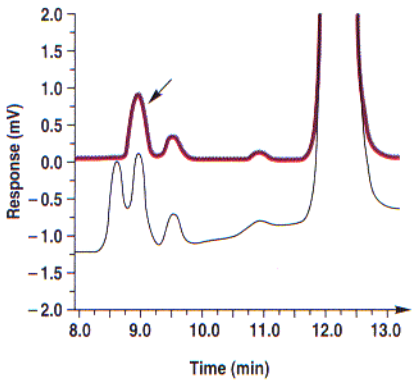

FIGURE 3: Degradation products of a major peak at 254 nm (upper chromatogram) and 215 nm (lower chromatogram). See text for discussion.

is equal, then this stream will be diluted 1:1 with the previous solvent exiting the sample loop. When the sample loop is flushed fully, the two streams are again the same, so no dilution takes place. Therefore, the step will double in size. The resulting plot of a step test would show a substep approximately half the size of each major step. This explanation neatly fits the symptoms of the problem of Figure 2 in which a small step was observed and then the normal major step appeared.

As a footnote to the explanation, the reader suggested that the size of the substep can be used to calculate the relative resistance of the sample and bypass loops. Once you know this value, you can use the flow rate to determine the volume of the sample loop. As one of my professors used to say, "Proof is left to the student . . ."

DUAL-WAVELENGTH DETECTION

J.R. Kern of LC Resources Inc. (Walnut Creek, California) submitted a good reminder about the importance of using more than one wavelength if peak purity is a concern. His laboratory was trying to isolate and identify degradants of a new, synthetic pharmaceutical product. The laboratory personnel had developed a reversed-phase method that separated at least three impurities eluted before the main peak when the eluate was monitored at 254 nm (upper chromatogram in Figure 3). They used an LC system with a diode-array detector to calculate a peak-purity index for each peak.

The peak-purity index can be calculated by various methods, but all are based on measuring absorbance at more than one wavelength and at more than one point on each peak. Some LC units use two wavelengths and ratio the measured absorbance at a defined point on the up and down slopes. Other systems use more complex algorithms. This particular system reported a peak-purity index of 1.54 for the first small peak (denoted by the arrow in Figure 3), indicating that the peak was partially overlapped with another peak (a purity index of 1.0 indicates a pure peak). Visual inspection of the chromatogram shows only a slight suggestion of a peak on the leading edge of the peak of interest. When the eluate was monitored at 215 nm, however, the overlapping peak is obvious (lower chromatogram in Figure 3).

This example illustrates the importance of using more than one wavelength when trying to detect and identify minor chromatographic peaks. If the eluate had been monitored only at 254 nm, the reader would have missed the presence of a potentially important degradant. Even if a diode-array detector is unavailable or your detector doesn't calculate peak purity, you can perform a similar operation by making two runs at different wavelengths for a comparison. For example, monitor one run at the normal detection wavelength, then monitor a second run at a wavelength of less than 220 nm.

CONCLUSION

I think these comments reinforce the adage that "two heads are better than one." For the problems covered in previous columns, I usually could assign a feasible solution, but as we've seen this month, this was not necessarily the only solution. We all should keep this in mind when addressing our problems — don't be afraid to ask for a second opinion.

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(3) J.W. Dolan, *LC•GC* **13**(7), 530–532 (1995).
(4) T. Culley and J.W. Dolan, *LC•GC* **13**(6), 456–458 (1995).
(5) T. Eidenberger, personal communication, 11 July 1995.

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ERRATUM

The equation for the aldol condensation reaction provided in the November installment of "LC Troubleshooting" (*LC•GC* **13**[11], 862 [1995]) was incorrect. The correct equation is shown below.

$$CH_3–COH=CH_2 + CH_3COCH_3 \rightarrow (CH_3)_2COH–CH_2COCH_3$$