LC Troubleshooting

International Liquid Chromatography Problems

John W. Dolan

LC problems have no national barriers.

I recently completed a three-stop "iron-man" tour of Europe sponsored by *LC•GC International*. On three consecutive days, I taught an LC troubleshooting class in Manchester, Paris, and Dusseldorf. During the trip, attendees asked various questions, some of which I will address in this column.

GRADIENT PROBLEMS

An attendee was using an isocratic method with a C18 column and a methanol–water mobile phase. Unfortunately, the two compounds he quantified had large differences in polarity. Conditions that eluted the first compound — 20% methanol in water — did not elute the second compound. When he selected conditions that eluted the second compound in a reasonable time — 80% methanol in water — the first one was eluted at t_0. Someone suggested that he try a gradient between the conditions that eluted the two compounds. Figure 1 shows the plot produced when he ran a blank gradient of 20–80% methanol with UV absorbance detection at 215 nm. He used a low-pressure mixing system with water as the A solvent and methanol as the B solvent. He vacuum-degassed the solvents using a water aspirator — he didn't have a vacuum pump or helium supply, but he thought degassing with the aspirator was adequate because he obtained a steady pump pressure.

It appears that at least two things were wrong. First, the baseline drift indicates that he was working at too low a wavelength for this mobile phase. Generally, you should avoid using methanol at wavelengths below approximately 220 nm (see reference 1 for further discussion). The drift is caused by a combination of methanol's UV cutoff wavelength of nearly 205 nm and the large absorbance difference between methanol and water at low wavelengths. He was able to use isocratic conditions only because his detector's autozero subtracted the large background absorbance. This large baseline offset narrows the detector's linear range. To alleviate these problems, he either should use a higher wavelength or switch to acetonitrile as the B solvent.

The second problem relates to the noise spikes. I suspect these are caused by very small bubbles passing through the detector. Bubbles often will become momentarily trapped in the detector, causing tailing spikes that are often confused with chromatographic peaks. In this case, however, the spikes were consistent, so I think they were caused by small bubbles passing uninhibited through the detector cell. Each time a bubble passed through, the absorbance appeared to increase because light scattering occured and less light reached the photodiodes in the detector. He must correct this problem before he can continue. Ideally, you should sparge the mobile phase with helium, but he doesn't have the necessary equipment. Three other adjustments may help eliminate the bubble interference.

First, he should premix the A and B solvents to the starting and ending conditions. In his case, solvent A will now be 20% methanol in water and solvent B will be 80% methanol in water. Pure solvents generally hold more dissolved air than solvent mixtures. Premixing the mobile phase may release enough of the dissolved air to reduce the problem. You can further decrease the air solubility by heating the solvent mixtures (I'd begin at approximately 40 °C) while degassing them. Be sure to provide a nucleation site for air bubbles to form. You can combine these tasks by adding a stir bar to the reservoir and degassing the solvents while the reservoir is on a stirrer–hot plate. You can also add a back-pressure regulator after the detector. If you have a limited budget, you can make your own back-pressure regulator by pinching the PTFE detector waste line in several places with a pair of pliers (crimp the tubing when the pump is off so you don't block the flow and damage the detector cell).

Finally, he should check all the low-pressure fittings in the system to be sure they are tight — loose connections might allow air to be drawn into the system. If these changes

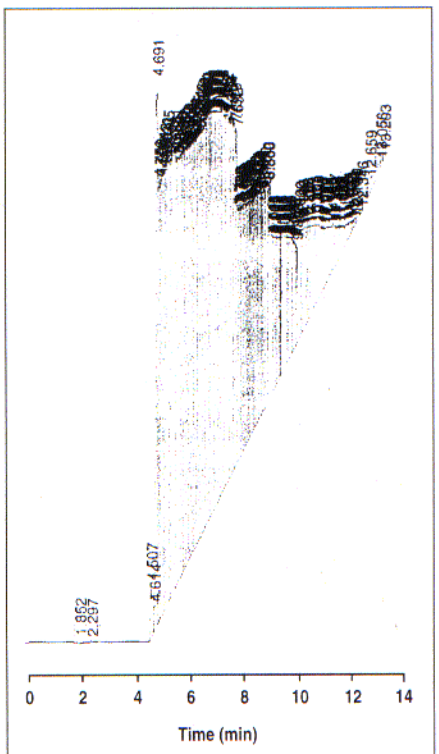

FIGURE 1: Blank gradient of 20–80% methanol–water in 10 min with UV absorbance detection at 215 nm. See text for details.

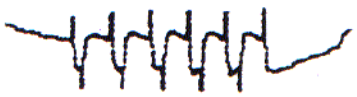

FIGURE 2: Blank baseline showing noise caused by detector-lamp failure. The cycle time is estimated to be approximately 15 s.

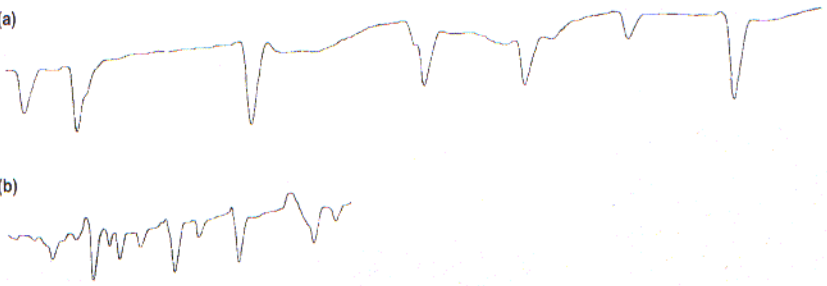

FIGURE 3: Baseline noise caused by inlet-check-valve malfunction on a two-headed pump. Shown are traces generated at flow rates of (a) 1.0 and (b) 2.0 mL/min. The chart speed was not specified.

don't allow him to run the LC system, he will have to find a better way to degas the solvents.

DETECTOR NOISE

An attendee handed me a copy of the plot shown in Figure 2 and asked me to diagnose the problem. I guessed that one of the check valves was malfunctioning. Periodic baseline disturbances often are caused by faulty check valves or by air in pump heads. The attendee then informed me that the cycling did not correspond to the pump delivery — doubling the flow rate had no effect on the baseline. After I made a few more guesses, the attendee told me that replacing the detector lamp corrected the problem.

Now that I know the answer, perhaps I can explain it. Most lamp failures cause sharp spikes in the chromatogram or large squared-off positive or negative deflections. In this case, I would guess that the lamp flickered without ever burning out completely. I'll have to add this to my list of lamp failure symptoms.

CHECK-VALVE MALFUNCTION

After being defeated by the detector-lamp failure, I was hesitant to diagnose the baseline problem shown in Figure 3. The chromatogram was generated by an LC system with a two-headed pump that delivered a manually prepared 75:25 acetonitrile–water mobile phase to a 25 cm × 4.0 mm, 5-µm d_p amino column. A refractive index detector was used. The traces in Figures 3a and 3b were obtained with flow rates of 1.0 and 2.0 mL/min, respectively.

Once again, I guessed that the problem was related to the check valves. Another attendee mentioned that this pattern was characteristic of an inlet-check-valve failure for this particular brand of pump. By carefully examining the plots, you can see the periodic nature of the baseline disturbances, which are characteristic of a pump problem. Reading from left to right in the trace in Figure 3a, the negative dips are spaced at a roughly 1:3:3:2:2 ratio. After doubling the flow, the minimum cycle is reduced

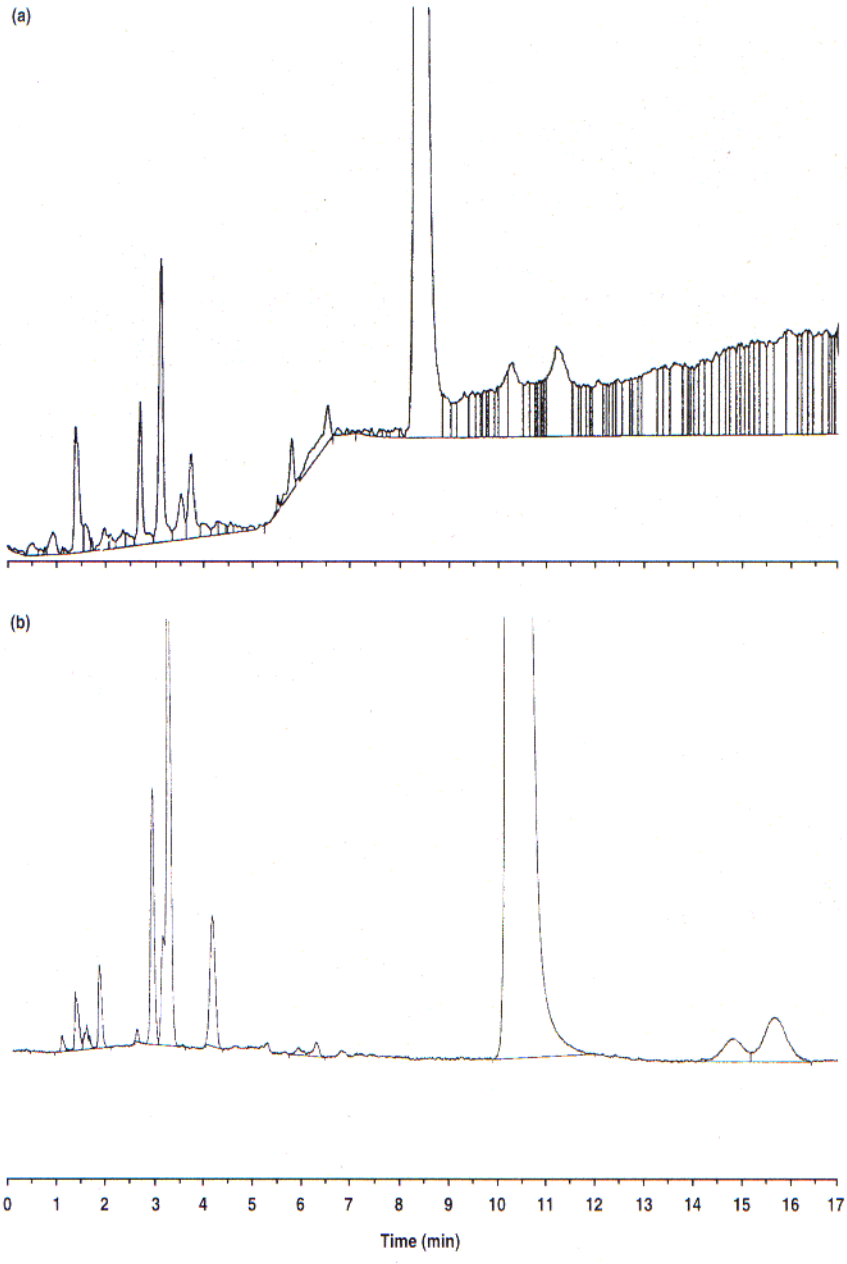

FIGURE 4: Chromatograms generated (a) before and (b) after replacing a faulty solvent proportioning valve. See text for details.

to nearly half. At the same time, we see that the failure is more pronounced at the higher flow rate, which is common with a faulty check valve because it has increased demands when it cycles more quickly.

If you suspect a check-valve problem, systematically isolate the faulty valve by replacing first one valve, then the next, and so on. Repeat the baseline test between each replacement. When you identify the faulty valve, discard it and leave the replacement installed. Reinstall any properly functioning check valves.

LEAKY PROPORTIONING VALVE

Figure 4a illustrates problems with an existing method. After a long track record of reliable operation, this separation began to exhibit erratic baseline shifts accompanied by shorter retention times (compare the major peak's retention time of 8.5 min in Figure 4a with its 10.5-min retention time in Figure 4b). The isocratic method was run on a low-pressure mixing system with an acetonitrile (solvent B)–buffer (solvent A) mobile phase. The C reservoir was not used and contained methanol.

After numerous false starts, the analyst realized that the retention shift was caused by changes in mobile-phase composition — not in flow rate. Manually prepared mobile phases yielded slightly better results but still caused baseline and retention shifts. The analyst suspected a problem with the proportioning system, so he bypassed the low-pressure mixer, allowing the pump to draw mobile phase directly from a single reservoir containing manually mixed mobile phase. With this change, the separation returned to normal. The analyst reinstalled the proportioning valve and mixer and exchanged the reservoirs. As long as the C reservoir contained one of the mobile-phase solvents, the baseline and retention remained stable. However, if the C reservoir contained acetonitrile or methanol, retention times were shorter than when it contained the buffer. All the results suggested that the proportioning valve for the C reservoir was leaking. Figure 4b shows that when the analyst replaced this valve, the system again performed satisfactorily.

The results can be explained as follows: The C reservoir contained acetonitrile, and its leakage yielded a mobile phase enriched in organic solvent, thus generating shorter retention times. Conversely, when the unused reservoir C leaked water into the mobile phase, the mobile phase was weakened and longer retention times resulted. This leakage occurred even when the A and B components were premixed but drawn through the proportioning manifold. The baseline stabilized only when the analyst used premixed solvents and bypassed reservoir C. The baseline shifts appear to be the result of a change in the absorbance of the mobile phase as the mobile-phase composition changed. The abundance of integrator marks on the baseline of Figure 4a indicate that the integration parameters were set improperly.

REFERENCE

(1) C. Seaver and P. Sadek, *LC•GC* **12**(10), 742–746 (1994).

"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.*