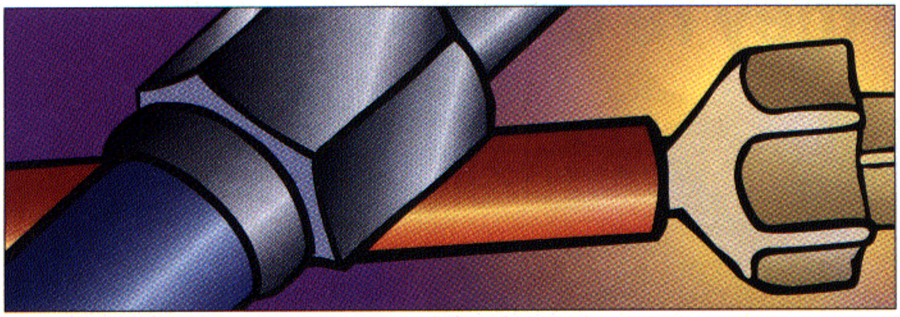
LC Troubleshooting



Baseline Problems — A Case Study

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Sometimes the solution creates new problems.

This month's "LC Troubleshooting" describes a problem we encountered recently in our laboratory. It is an example of the difficulties of isolating a liquid chromatography (LC) problem. We'll also use this example as an opportunity to emphasize the importance of the five *Rules of Thumb* (see sidebar) for troubleshooting and problem isolation.

THE PROBLEM

We first observed the baseline problem illustrated in Figure 1 when we examined the chromatograms from an overnight run series. Figure 1b shows an oscillating baseline with a frequency of approximately 8 cycles/0.1 min. This pattern was consistent throughout the chromatogram and was present in all chromatograms of the series, including standards, controls, samples, and blanks.

The high-pressure mixing LC system included two pumps, an autosampler, and a UV detector set at 230 nm. Mobile-phase A was 0.1% trifluoroacetic acid in water, and mobile-phase B was 0.1% trifluoroacetic acid in acetonitrile. A 15 cm × 4.6 mm, 5-μm d_p phenyl column was operated at 1.5 mL/min at room temperature. The sample was a 200-μL injection of a proprietary compound extracted from plasma. We obtained the separation using a 40–65% B gradient in 9.4 min followed by a 5-min hold at 40% B.

THE SEARCH BEGINS

Almost subconsciously, we should practice the first two rules of thumb: the *Rule of One* (change just one thing at a time) and the *Rule of Two* (make sure the problem is reproducible). The noisy baseline was present in every chromatogram, so we were sure it was reproducible. This fact made it much easier to determine when we solved the problem. By changing one thing at a time, we could isolate the true cause of the problem.

Before we get into the problem-solving procedure, we should acknowledge that more than one way exists to solve LC problems, so your approach may be somewhat different. Each of us tends to approach a problem biased by our experiences with other problems that have similar symptoms.

A cycling baseline can result from a small bubble trapped in the detector flow cell. The bubble can bounce back and forth, causing a regular disturbance in the light passing through the cell. Using degassed solvents and a back-pressure regulator on the cell minimizes bubbles in the detector cell. The back-pressure regulator keeps sufficient pressure on the cell to keep the bubble in solution until after it leaves the cell. We verified that these two practices were used.

We tried two techniques that often remove bubbles from detector cells. The first technique is to increase the mobile phase flow rate, which subsequently increases the system pressure. This step may force the bubble back into solution. Doubling the flow rate did not correct the problem. Another technique that often works is flushing the system with freshly degassed methanol. The pure organic solvent generally has lower viscosity than an aqueous solvent, and the additional degassing increases the capacity of the solvent to dissolve any air trapped in the system. Flushing with degassed methanol did not correct the problem either.

Still convinced that the problem was related to air in the flow cell, we removed the cell from the detector and examined it under magnification. We observed no bubbles in the cell, and the problem persisted when the cell was reinstalled.

Detector lamp failure can generate various baseline problems, although neither of us had seen a correlation of lamp failure with this type of baseline pattern. We checked the internal lamp monitor and found that the lamp had been operated for 995 h. Normally, a detector lamp is useful for 1000–2000 h, so the lamp was entering its caution zone. It was easier to

TROUBLESHOOTING RULES OF THUMB

Rule of One: Change one thing at a time so you are sure of the correlation between the change and correction of the problem.

Rule of Two: Make sure the problem occurs at least twice. It is very difficult to prove you fixed a problem that is not reproducible.

Put it Back: When module substitution is used to help isolate a problem source, put back the good original parts when you are finished. Otherwise, you end up with a lot of used parts that are unlikely to be installed in the future.

Crystal Ball: Try to anticipate what will go wrong next. Use the knowledge of a successful troubleshooting session to modify your preventive maintenance program to minimize the reoccurrence of the present problem.

Write it Down: Good record keeping is key to successful troubleshooting. In the future you can use these records to find failure patterns and strengthen your preventive maintenance program. Regulatory agencies may require that you keep maintenance and repair records.

swap detectors than to install a new lamp, so we replaced the detector with an equivalent detector from another system. (This process, *module substitution,* is one of the most powerful troubleshooting tools available — just replace the questionable module or part with one known to be good.) Unfortunately, the new detector exhibited the same cycling baseline. At least we eliminated the detector as the problem source.

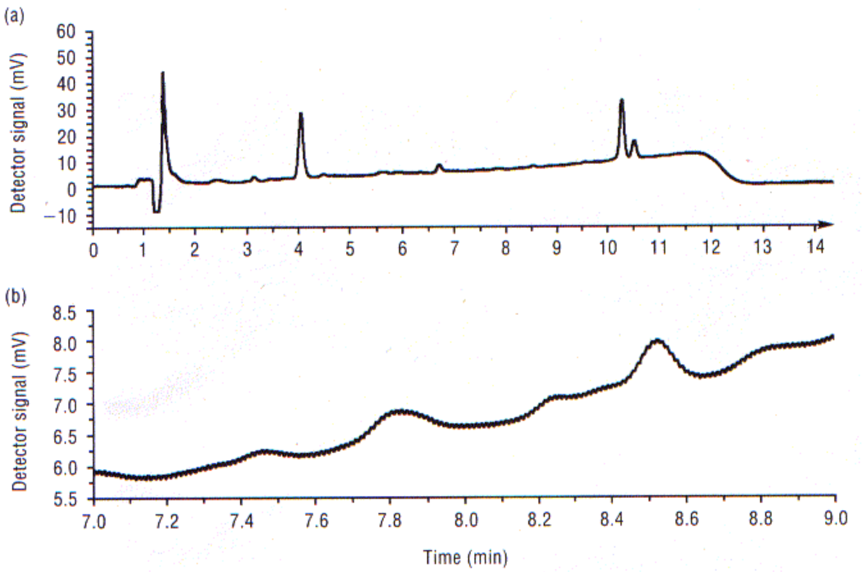

FIGURE 1: Example of the baseline problem's cyclic nature. The chromatogram in (b) is an expansion of a 2-min section of (a).

COULD IT BE THE PUMPS?

With the detector eliminated, we turned our attention to the pumps, which were the next most likely problem area. The pressure reading on the two pumps was almost the same — 89 bar for one and 91 bar for the other. We observed no pressure fluctuation.

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If the problem was related to mobile-phase mixing, we should be able to change the magnitude of the problem by changing the mixture ratio. We switched from 40:60 to 10:90 and then to 90:10 0.1% trifluoroacetic acid–acetonitrile, and we observed no change in the baseline. This observation indicated to us that the mobile-phase mixing process probably was not the source of the problem.

At this point, we were running low on ideas. A malfunctioning check valve or faulty pump seal should generate unstable pressure. The steady pressure we observed made us skeptical that the check valves or seals were at fault. However, it is easy to isolate a pump

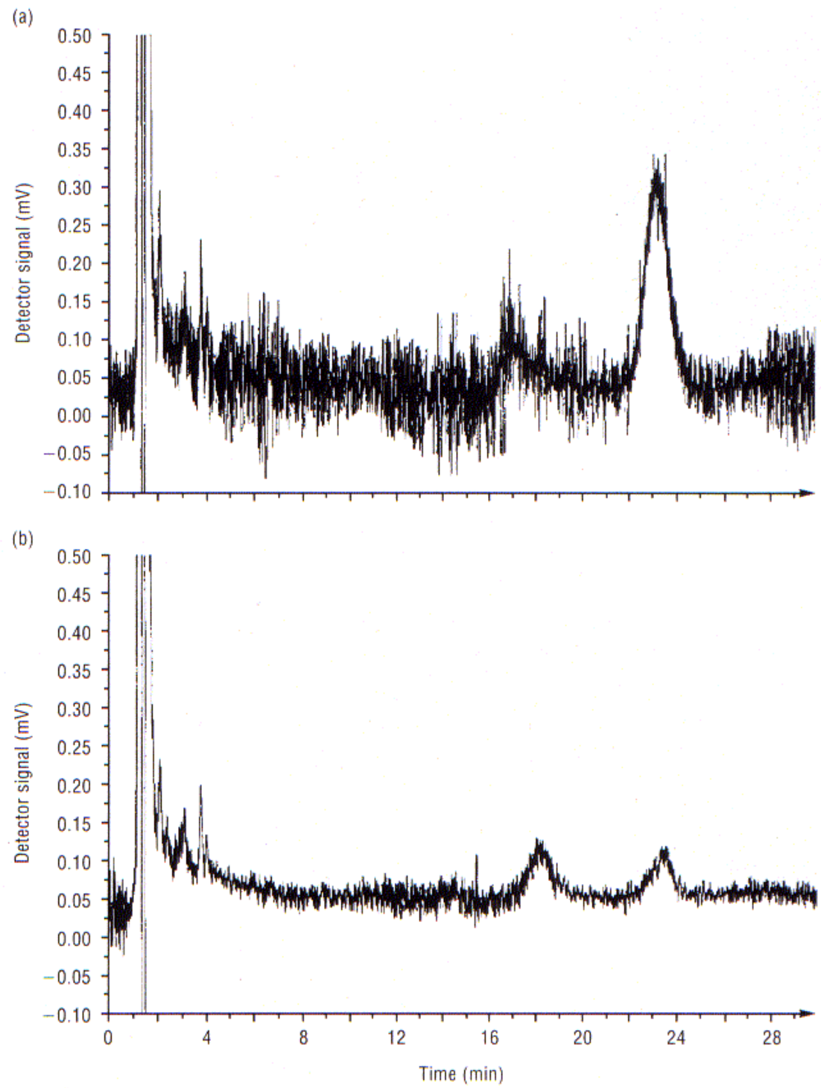

FIGURE 2: Chromatograms from two consecutive runs in a sample sequence. Data-collection rate: 5 Hz; detection: UV absorbance at 215 nm.

and its components. We shut off the flow from one pump at a time. Pumping 100% mobile-phase A or B gave the same cyclic baseline we observed throughout our troubleshooting efforts.

As a final pump isolation technique, we shut off both pumps — surely this step would eliminate the problem. But the problem persisted.

ELECTRONICS?

With the major LC components eliminated, we knew that the mobile phase, pumps, mixing, and detector were not responsible for the problem. We now suspected an electrical interference or electronic failure of some sort.

In an earlier "LC Troubleshooting" column (1), the use of a resistor–capacitor (R/C) filter minimized baseline noise. In that example, we observed high-frequency baseline noise, as illustrated in Figure 2. Figure 3a shows a more severe case of the same type. The noise in this case comprises many sharp spikes superimposed on the normal baseline. This problem was corrected as illustrated in Figure 3b by the installation of the R/C filter shown in Figure 4. Because of the dramatic improvement in the baseline and the recurring nature of this problem in our laboratory, we operate all our LC systems with an R/C filter.

Notice, however, that the noise problems illustrated in Figures 2 and 3 are exhibited as spikes, whereas the noise in Figure 1 is cyclic for the present problem. Our next step was to examine the filter to ensure all the connections were correct. Upon close examination, we observed that the two capacitors in the filter were bent, and the leads were touching. When this unintentional connection was removed, the baseline problem went away. Remaking

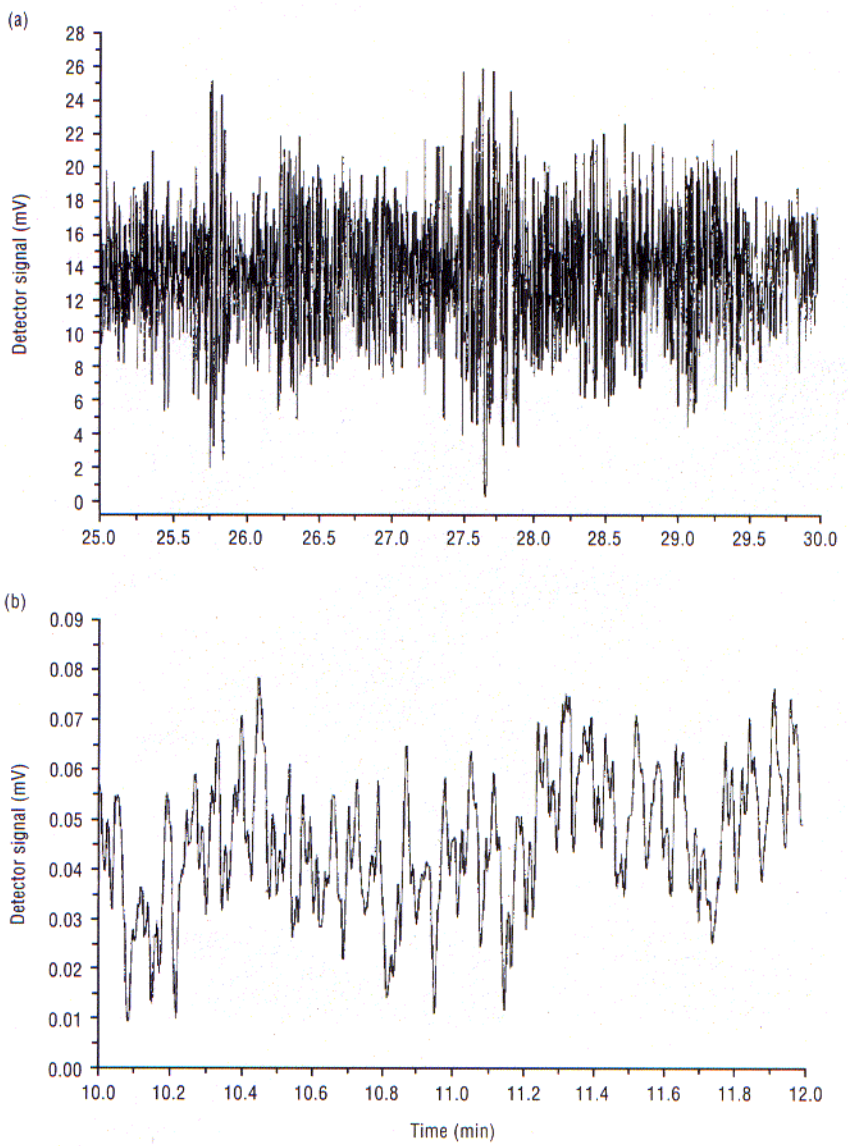

FIGURE 3: Baseline plots generated (a) before and (b) after installation of the R/C filter shown in Figure 4. Note that the *y*-axes are different in (a) and (b).

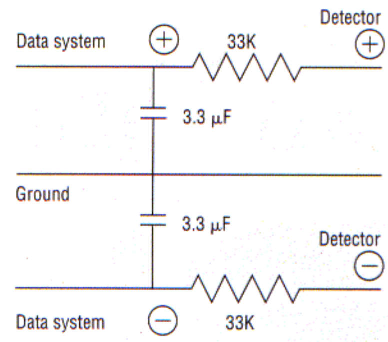

FIGURE 4: Schematic of an R/C filter for the removal of noise spikes.

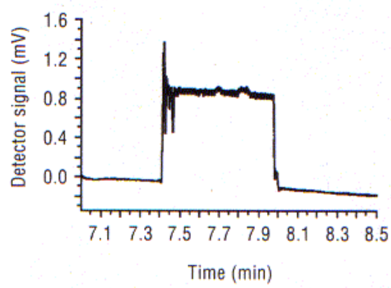

FIGURE 5: The effect of shorted capacitor leads on the cyclic baseline problem. The leads were shorted from 7.4 to 8.0 min.

this unintentional connection recreated the problem. The baseline of Figure 5 shows this situation.

CLEANING UP

Now that we had solved the problem, we needed to review the remaining rules of thumb to ensure that we covered all the bases. The *Put it Back* rule tells us to reinstall any good original parts that we replaced during module substitution. In the present case, the substituted detector was still in place, so we reinstalled the original detector and replaced the borrowed one.

The *Write it Down* rule reminds us to update our system records to record the troubleshooting incident, its symptoms, and the solution. We maintain a system logbook for each LC system, so we filled in the appropriate form for our records.

Finally, we must consult the *Crystal Ball* to anticipate how we can use what we learned from this problem to prevent similar problems in the future. We learned that the way we constructed our R/C filters made them susceptible to similar problems when analysts brushed against the filters during servicing. A slight reconfiguration of the system should eliminate the possibility of this problem's reoccurrence.

CONCLUSIONS

So what did we learn from this case study? Our first, often unstated, assumption was that the problem resulted from a single source. This assumption was valid in the present case and is likely to be true in most cases. This approach makes troubleshooting much easier. By using the five *Rules of Thumb* either consciously or subconsciously, we take advantage of a framework for problem solving that ensures we don't overlook any important areas. Finally, by approaching the problem in a logical, step-by-step manner, the solution came quickly — the entire troubleshooting session took roughly 30 min. We now can add this problem to our "I'll never do that again" list.

REFERENCE

(1) J.W. Dolan, *LC•GC* **14**(5), 378–382 (1996).

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