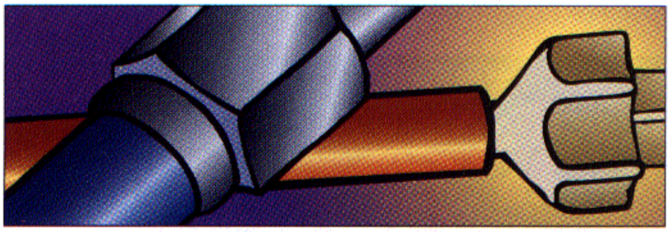
LC Troubleshooting



Noise Problems: A Case Study

John W. Dolan

Electronic noise can be one of the most frustrating chromatographic problems.

Electronic noise in liquid chromatography (LC) can come from a variety of sources, some of which are beyond our ability to control. For example, noise can originate from data system electronics and wiring, detector electronics and lamps, the chromatographic process, and external sources such as water baths and cellular telephones. Fortunately, most problems result from bad detector lamps or the chromatography, so solutions are fairly simple. When problems originate in the system electronics or from external electronic sources, the solutions are beyond most of our skill levels.

This month, I'll look at a problem I recently encountered in my laboratory. The nature of the problem was twofold. Excessive short-term noise was compounded by an elusive, long-term cyclic pattern. The noise was obvious, even to casual observers, as the two chromatograms in Figure 1 illustrate. This figure shows two consecutive runs in a sample sequence. The chromatogram shown in Figure 1a had 5–10 times the noise of the one shown in Figure 1b. It is easy to see that the peaks at approximately 18 min were marginal for quantitation in Figure 1b and impossible to quantitate in Figure 1a. (The scale for all chromatograms in this column is shown in millivolts, where 1 V = 1 AU.)

TRADITIONAL PROBLEM SOURCES

At first, my colleagues and I suspected that the problem was related to the chromatography, so we performed usual procedures such as checking for proper degassing and bubbles in the pump or detector flow cell. No problems were found.

Next, we checked the detector to determine if it was operating properly. The detector specification calls for noise not to exceed 1×10^{-5} AU at 250 nm with a dry flow cell. The run shown in Figure 1b had a noise level of approximately 5×10^{-5} AU at 215 nm, a reasonable level — we expected more noise at low wavelengths and with solvent flowing. A check at 255 nm yielded the run shown in Figure 2, which

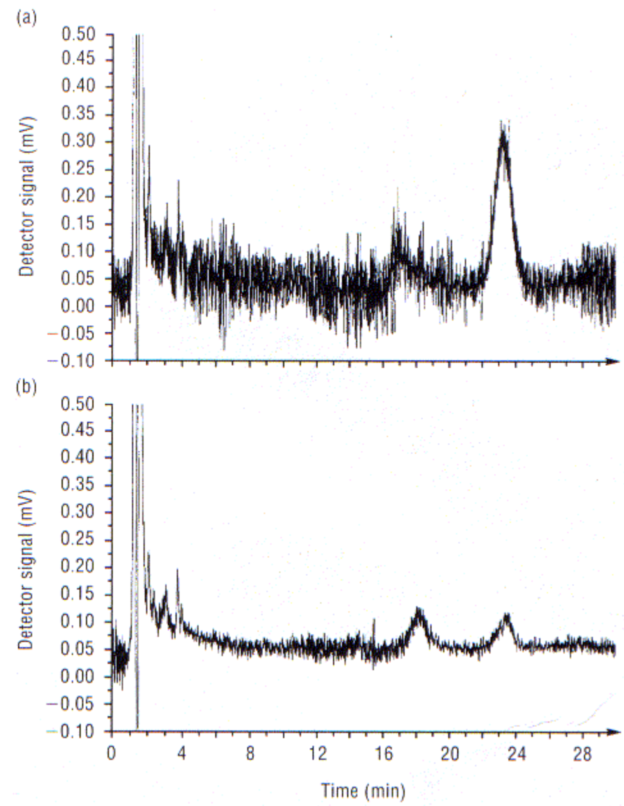


FIGURE 1: Chromatograms from two consecutive runs in a sample sequence. Data-collection rate: 5 Hz; detection: UV absorbance at 215 nm. See text for details.

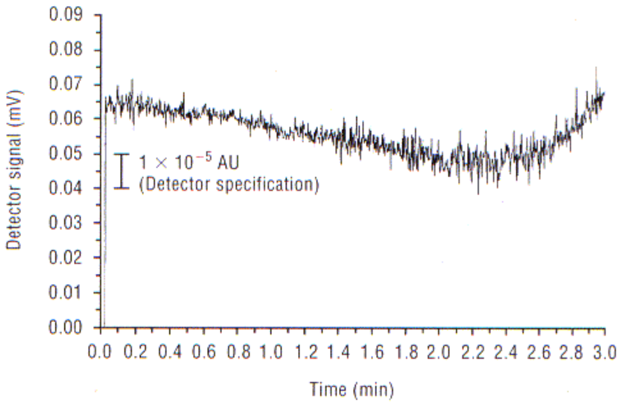


FIGURE 2: Chromatogram showing the detector from Figure 1 performing in accordance with manufacturer's specifications. Noise at 255 nm is approximately 1×10^{-5} AU.

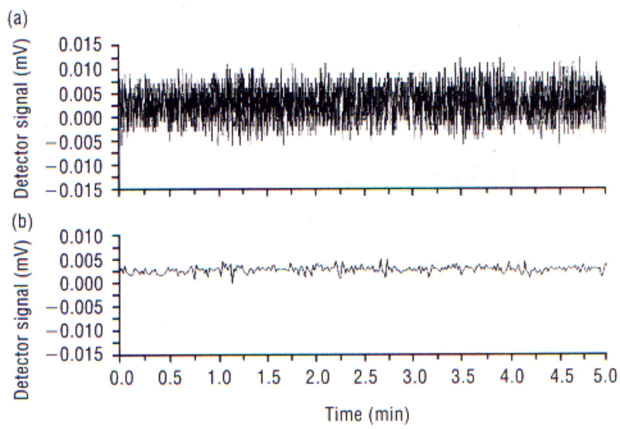


FIGURE 3: Baseline plots generated at data-collection rates of (a) 15 and (b) 1 Hz with data system input leads shorted.

showed a noise level very close to specifications. These data indicated that the detector was operating properly.

As we were making these initial noise investigations, we began to pay careful attention to the occurrence of the problem. As noted above, the problem was not constant but seemed to start and stop randomly. We were running two LC systems into one data station and another three systems into a second station. In our experience, the noise problem was not unique to one instrument or data system; that is, noise might be observed with both data systems on one channel each but not on the other channels. Or a single instrument might show problems. Or all channels on one data system might show problems. As you will see later, these observations may have been misleading because the solution appears to be related to a universal problem.

EXTERNAL SOURCES

External sources of electronic noise can cause data system problems. Often these sources are devices that draw a lot of current or cause a surge on the electric line when they start or stop. The possible sources in our laboratory included a −78 °C freezer, a laboratory oven, a vacuum pump, and an electric furnace. We were unable to correlate the operation of any of these devices with the noise problem. Besides, it seemed unlikely that one of these devices would affect one data channel and not another. Another common source of electronic noise is fluorescent lighting, especially if the ballasts are operating improperly. The data system manufacturer assured us that the system could filter out 60-Hz noise very effectively from such sources. Turning off all the lights had no impact on the noise.

CHECKING THE DATA SYSTEM

To help us track the problem, the data system manufacturer provided a technical note (1) that gave guidelines for reducing noise. Most of these recommendations have general application, so I'll share a few here.

To verify that the analog-to-digital (A/D) converter board was working properly, we shorted the input terminals of the data wires. With the plus, minus, and shield clipped together, the data system had noise amounting to approximately 10 μV (peak to peak), as shown in Figure 3a. This noise level was within specifications for our data system.

Data rate: One way to reduce noise is to reduce the sampling rate. The general rule of thumb is that the data system should gather data for 10–20 points across a peak. When the data rate exceeds this level, the noise level rises without a concurrent improvement in the signal. When the data rate is much less than approximately 10 points across a peak, the noise is reduced, but loss of peak height also may occur.

Our data system averages the signal collected during each sampling period. For example, if the data rate is 1 Hz, the data are collected for 1 s, averaged, and reported. With this design, slower sampling rates average out the high and low noise signals, as you can see by comparing the two traces in Figure 3. In Figure 3a, the data rate was 15 Hz, whereas in Figure 3b, the data were collected at 1 Hz. The signal should improve with the square root of the data rate change; therefore, changing the sampling rate from 15 to 1 Hz should improve the signal roughly fourfold, approximately what occurs in Figure 3.

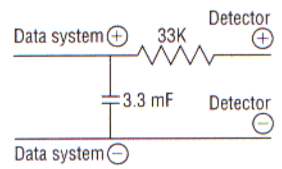


FIGURE 4: Schematic diagram of a 0.1-s R/C filter. See text for details.

Time constant: Another way to reduce electronic noise is to use an electronic time constant. A resistor–capacitor (R/C) filter can accomplish this task easily. The R/C filter removes short-term noise spikes, thus smoothing the signal. In the past, most LC detectors had built-in detector time constants that filtered the signal fed to a strip-chart recorder. Typically, those systems used a time constant for the 0–1 mV or 0–10 mV outputs, and the 0–1 V data system outputs were unfiltered. For example, users could choose from 0.1-, 0.5-, and 1.0-s time constants. The rule for time constant selection is similar to the data-rate rule — the time constant should not exceed $^1/_{10}$ of the peak width. Thus, a 0.1-s time constant should be adequate as long as the peaks are no narrower than approximately 1 s. With the almost universal use of data systems for LC, most manufacturers no longer build a selection of time constants into their detectors.

The technical support note (1) recommended trying a 0.1-s time constant to remove excessive noise. You can construct a filter from parts that can be purchased at a local Radio Shack or similar retailer. Figure 4 is a diagram of a 0.1-s filter. Our local store did not have the 3.3-mF capacitor, so we had to substitute a 4.7-mF one. This capacitor provided a 0.15-s time constant (time constant in seconds = capacitance × resistance; $0.15 = 33 \times 10^3 \times 4.7 \times 10^{-6}$), which should be adequate for peak widths as narrow as 1.5 s.

We tried the 0.15-s time constant on another detector, and Figure 5 shows the results. The R/C filter was installed, removed after roughly 23 min, and reinstalled after approximately 45 min. The improvement in the baseline is obvious.

ONE PROBLEM REMAINS

Although the R/C filter improved the detector signal as illustrated

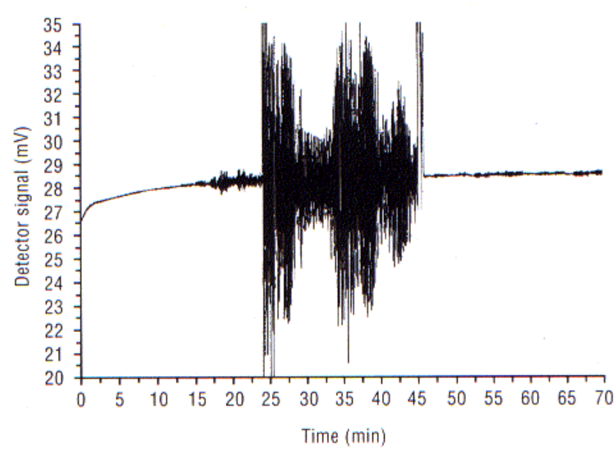


FIGURE 5: Baseline for a second UV-absorbance detector obtained at 255 nm with (0–22 and 46–70 min) and without (22–46 min) the R/C filter of Figure 4 installed. Data rate: 5 Hz. See text for details.

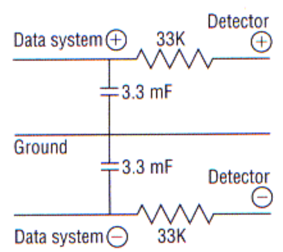


FIGURE 6: Schematic of an R/C filter recommended by a data system manufacturer for removing long-term cyclic noise. See text for details.

in Figure 5, it did not eliminate the noise that occurred at a much lower frequency. Figure 5 illustrates this change in the noise. With the filter installed, the signal was much better in the 0–10 min region than in the 15–23 min region. Without the filter, the same pattern was seen with lower noise in the 28–33 min region than in the adjoining portions of the trace. We observed a cyclic pattern in the remaining noise in the plot of Figure 5 — the noise became better and worse during several minutes with or without the filter installed. Long-term cycles such as these — also called *common mode noise* — can result from a mismatch between the clock cycle of the A/D converter and the frequency of the commercial power supplied to the instrument. The sources of common mode noise are beyond the control of chromatographers, but additional filtering may correct this problem. Figure 6 shows the filter recommended by the data system manufacturer for these situations. After installing this filter, the baseline improved significantly and the periodic noise disappeared.

Figure 7 summarizes the results. The plot of Figure 7a shows the unfiltered signal with 10–20 mV (0.01–0.02 AU) of noise. With the filter of Figure 4 installed, the plot looked like Figure 7b, with approximately 0.4–0.7 mV of noise — a 30-fold improvement. Finally, with the filter of Figure 6 installed, we observed the baseline in Figure 7c. In Figure 7c the noise was approximately 0.05 mV (5×10^{-4} AU), a 10-fold improvement over the simpler filter and roughly a 300-fold improvement over the unfiltered signal. The expanded plots are shown on the same scale in Figure 7d. The final filtered signal was 10-fold more than the detector manufacturer's specification for this detector, but based on our experience with this particular detector, it was as good a baseline as we could hope to see. Tests of the system with real samples showed no loss of sensitivity under our typical operating conditions.

CONCLUSION

Noise spikes in chromatograms can come from many sources. Internal noise sources — such as aging detector lamps or bubbles in the flow cell — can be corrected easily. Users can identify and take corrective actions for external noise sources such as ovens, refrigerators, and fluorescent lights. Other noise sources, such as the quality of the power feeding the laboratory, may be beyond chromatographers' control. This month's column used a case study to show how to verify the performance of the detector and data system, use R/C filters, and select the data-collection rate to remove unwanted noise without compromising the signal.

Finally, this case study illustrates the value of instrument manufacturers' technical support staff. Several telephone calls during one week helped solve a problem that was simple for them but difficult for me. Similar advice is available from most instrument manufacturers and is only a phone call away.

ACKNOWLEDGMENTS

I would like to thank Jeff Justice and Tarik Peterson of Justice Innovations (Mountain View, California) for their helpful guidance in solving this problem.

REFERENCE

(1) J. Justice, "Noise Reduction with Chrom Perfect Direct Systems," Justice Innovations (Mountain View, California, 1993).

"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Walnut Creek, California, 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, e-mail 76631.2547@compuserve.com.*

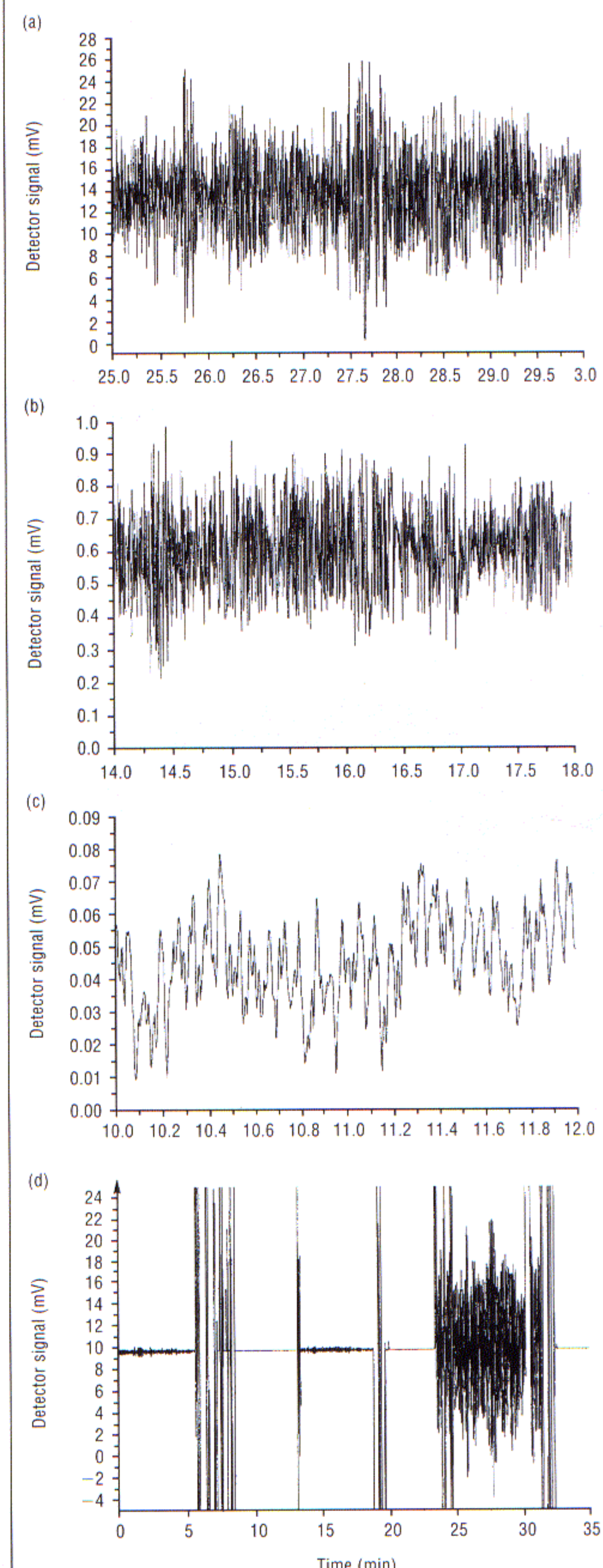


FIGURE 7: Baseline plots generated by the detector in Figure 5 at a 255-nm detection wavelength and a 5-Hz data-collection rate. No filter installed: (a) and 25–30 min section of (d). With filter shown in Figure 4 installed: (b) and 0–5 and 13–18 min sections of (d). With filter of Figure 6 installed: (c) and 8–13, 20–23, and 33–35 min sections of (d).