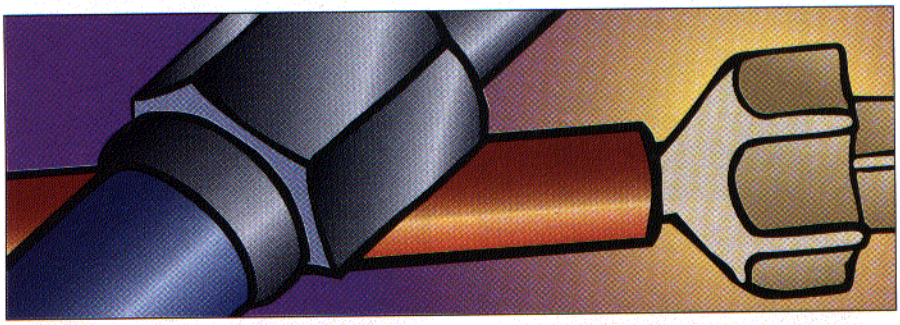
LC Troubleshooting



UV Detector Noise

Michael D. Nelson and John W. Dolan

Baseline noise originating from the detector can be isolated systematically.

Liquid chromatography (LC) detectors can present a mystery when it comes to maintenance and troubleshooting. LC pumps and autosamplers have a significant number of user-serviceable parts. For example, users can repair or replace check valves, pump seals, pistons, valve seals, and needles. In contrast, LC detectors have few parts for users to change. Thus, in many ways, detectors tend to be black boxes that are plugged in and operated until they fail. Users can repair only a few of the parts responsible for detector failure. This month's "LC Troubleshooting" column focuses on one of these items — the detector lamp. Our discussion will center on the widely used deuterium lamps in UV detectors, although some of the information also applies to other lamps and detectors.

QUIET, PLEASE

In our laboratory, much of the LC work involves developing and using stability-indicating assays and bioanalytical methods to support the development of new pharmaceutical products. For stability-indicating assays of drug substances, peaks larger than 0.05% of the main component's peak usually are of interest to our clients. Therefore, the chromatographic baseline must be very steady to enable precise measurements of small peaks. Similarly, baseline stability plays a major role in determining the limits of detection for assays of drugs in plasma or urine.

These requirements mean that a detector usually must operate near its most sensitive region on a routine basis. First, analysts should use the best available reagents so that the background signal is steady. Developing a suitable sample cleanup scheme will reduce the background noise for bioanalytical samples. However, after these parameters are controlled, the performance of the detector itself comes into play.

Analysts should run a blank baseline on a regular basis to ensure that no extraneous peaks are present in the chromatogram due to factors unrelated to samples. The limits of detection and quantitation depend on the signal-to-noise ratio. For example, a signal-to-noise ratio of 5:1 may be selected as the limit of detection for a method. Obviously, if the baseline noise is abnormally high, the detection limits will be compromised, so minimum noise levels are desirable.

The simplest way to check baseline noise is to run the system without making an injection. Display the blank chromatogram with enough magnification so that the baseline width can be measured easily. Figure 1 shows an example. Select a 1-min region of baseline, expand it sufficiently to show the noise, and draw lines that roughly describe the bounds of the noise. The distance between these two lines can be converted to absorbance units (AU). In the present case, the distance between the lines is 0.06 mV and the detector generates 1 V/AU. Thus, the noise is 6×10^{-5} AU. Most detectors have noise specifications of $0.5–1.0 \times 10^{-5}$ AU, so the detector in this figure has excessive baseline noise. The regular nature of the baseline fluctuations suggests that the noise is electronic in nature and may be reduced by adding an electronic filter. More details about the use of these filters can be found in references 1 and 2.

Figure 2 depicts a blank baseline from a typical detector that passes the manufacturer's specifications. Detector specifications usually specify using a dry cell at 255 nm. It is inconvenient to create a dry cell, especially if a noise check is desired under conditions similar to the run conditions. As a general rule, analysts can substitute a steady isocratic baseline for the dry cell technique. If the observed noise under these conditions is within approximately two times the specification, the detector is operating acceptably. The noise at lower wavelengths, such as 215 nm, will be greater than the noise at 255 nm with mobile phase flowing.

NOISE IS A CLUE

The excessive baseline noise shown in Figure 1 can be a clue that a detector problem exists. For the system of Figure 1, the use of a resistance-capacitance filter will reduce the noise to an acceptable level. An earlier "LC Troubleshooting" column provides examples of the use of resistance-capacitance filters to reduce noise (2).

The type of noise can provide additional clues to its source. The chromatograms of Figure 3 illustrate this situation. The top and bottom traces are for two different detectors under the same conditions. From the scale, the lower trace obviously was generated by a detector with less than 1×10^{-5} AU of noise, whereas the top trace has noise levels of approximately 1×10^{-4} AU, which is a definite problem. When the lower trace is magnified 10-fold, as shown in the middle run, we see clearly that different types of noise are present. The rapid noise in the middle trace could

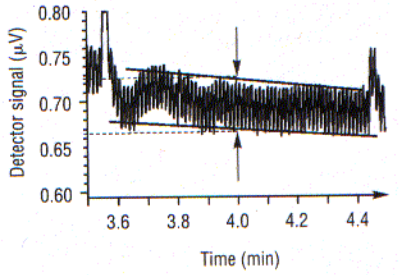


FIGURE 1: Baseline noise determination. See text for details.

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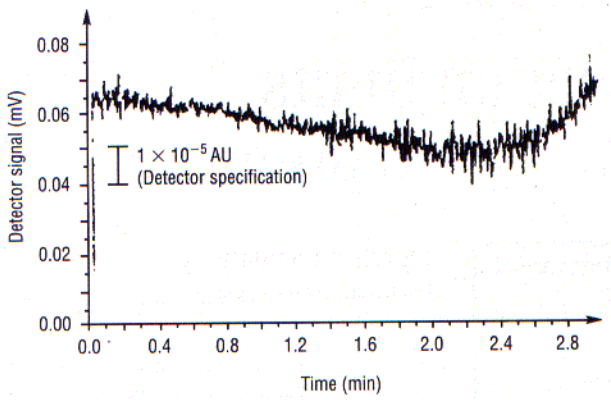

FIGURE 2: Chromatogram generated using a detector performing in accordance with manufacturer's specifications. The noise at 255 nm with the mobile phase flowing is approximately 1×10^{-5} AU.

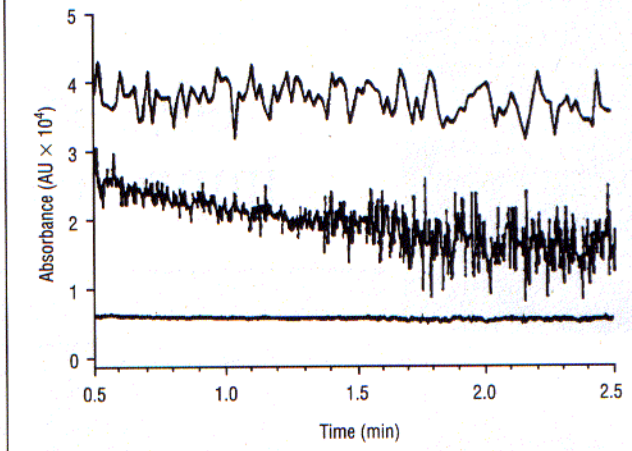

FIGURE 3: Baseline noise for two UV detectors. The top trace shows excessive baseline noise. The bottom trace shows a different detector on the same scale and in compliance with detector specifications. The middle trace is a 10-fold amplification of the bottom trace.

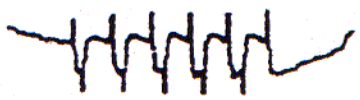

FIGURE 4: Baseline noise following detector lamp failure. Scale and conditions are unknown.

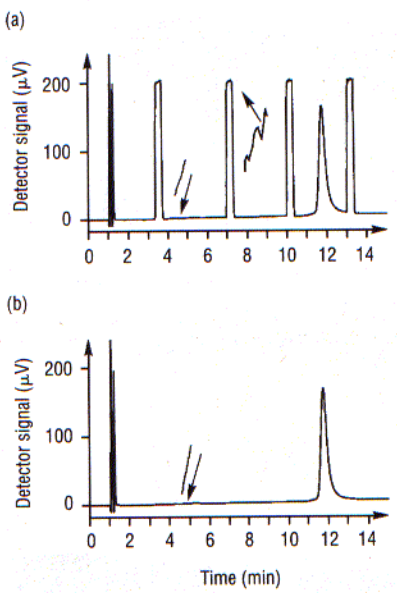

FIGURE 5: Gradient chromatograms generated (a) before and (b) after installing a new lamp. See text for other conditions.

FIGURE 6: Expanded sections from Figures 5a and 5b. The top trace is the 7.1–7.2 min region from Figure 5a expanded 20-fold less than the bottom two traces. The middle and bottom traces are the 4.5–4.6 min regions from Figures 5a and 5b, respectively. See text for discussion.

be reduced further by using a resistance-capacitance filter. The frequency of the noise in the top trace, however, is such that the resistance-capacitance filter is not expected to be beneficial. The next step for this detector would be to replace the lamp.

LAMP FAILURE

Regular cycles in the baseline that are not chromatographic in nature may be clues that the detector lamp has failed or is near the end of its useful life. The baseline section in Figure 4 shows an example one reader observed when the lamp failed.

A electronic circuit used to start and maintain lamp operation can be complex, with different voltages used for different functions. The electronics also determine if the lamp is on or not. If the lamp is not lit and the switch is on, the system attempts to start the lamp. The chromatogram of Figure 5a dramatically illustrates this situation. The chromatogram shows the expected sample peak at 12 min, but the four square-topped peaks are abnormal. A gradient method is used in this example, and a baseline noise value is difficult to obtain with a gradient because when the baseline is magnified sufficiently to measure the noise, the drift is nearly vertical. The expanded 4.5–4.6 min baseline segment of Figure 5a demonstrates this phenomenon. This baseline section is expanded 1000-fold, and still it is insufficiently magnified to determine baseline noise. Examination of the expanded chromatogram at the top of one of the square peaks is instructive, however. The expansion of the 7.1–7.2 min section of Figure 5a is only a 50-fold increase over the full run, yet the choppy nature of the noise is reminiscent of the trace of Figure 4. Furthermore, the regular spiking nature of the square peaks suggests that the lamp ignition circuitry is trying repeatedly to light the lamp. So it appears that the trace shows a repetitive cycle of the lamp lighting, going out, and lighting again. In this example we were lucky that the peak came out in a section of the chromatogram in which the lamp was on. Replacement of the lamp eliminated the unwanted square-topped peaks (Figure 5b).

Figure 6 compares the expanded baseline segments of Figures 5a and 5b on a different scale. The middle and bottom traces are the 4.5–4.6 min baseline segments in Figures 5a and 5b, respectively. On the scale shown, the baselines for the old and new lamps are nearly identical. The top trace (20-fold less magnification) clearly shows the difference in the nature of the noise when the square-topped peaks were displayed. It isn't clear whether the lamp was off at this time, in the ignition cycle, or experiencing some other lamp phenomenon, but something definitely was wrong.

OTHER CLUES

Detectors today have additional tools to help diagnose lamp problems. Some or all of these features are present in most detectors currently manufactured. When a lamp is first started, an internal calibration cycle may occur. The detector checks the wavelength accuracy using a holmium oxide filter or a characteristic of the deuterium spectrum, and a lamp energy meter may display a value that analysts can interpret to determine if the lamp is performing properly. Many detectors now track the number of hours the lamp is ignited. A lifetime of 1000–2000 h is typical for a modern deuterium lamp, but lamps can operate even longer. We recently replaced a suspect lamp that had 2500 h on the meter, and the new lamp made no difference, so we reinstalled the old one. Even though lamp life may vary, the meter reading can be a helpful guide for troubleshooting. For example, a meter reading of 350 h suggests that the lamp might not be the source of a baseline problem, but a meter reading of 1500 h indicates that the lamp could be faulty.

Don't confuse an air bubble in the detector with a lamp problem. Air bubbles may flow through the detector and cause a sharp spike or a disturbance that looks like a chromatographic peak. In other cases, the bubble may stop in the flow cell and cause a dramatic baseline shift, usually off-scale. Analysts can identify bubble problems by shutting off the mobile-phase flow. If the problem is a bubble, the baseline will remain steady, on- or off-scale. An electronic or lamp problem, however, will persist when the flow is stopped. Two simple practices will reduce the frequency of air bubbles in the detector. First, degas the mobile phase so that air bubbles are less likely to form. Second, use a back-pressure regulator after the detector to keep sufficient pressure on the detector cell so that bubbles don't come out of solution. Such regulators contain a spring-loaded valve that maintains the pressure at 80–100 psi. They can be purchased from most LC supplies vendors. Be sure to select a regulator that has a specification less than the detector cell's upper pressure limit.

CONCLUSIONS

It is important to know how the LC system works when it is working well so that you can tell if something is wrong. Even if the baseline noise is not checked on a regular basis, the practice of making blank runs on a daily basis provides a source of reference data that can be consulted at a later date. Modern UV lamps provide extended life, and in most laboratories, the detector is on only part of the time. Therefore, detector lamps may last a year or two before failure. When a component fails so rarely, setting up a preventive maintenance schedule for lamp replacement doesn't make much sense. Be observant of the symptoms, and you should be able to correct a lamp failure problem quickly. The problem shown in Figure 5 resulted in less than an hour of down time from the first appearance of the problem until the detector was back in use with a new lamp.

REFERENCES

(1) K.L. Christianson and J.W. Dolan, *LC•GC* **15**(10), 928–924 (1997)
(2) J.W. Dolan, *LC•GC* **14**(5), 378–382 (1996).

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"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Walnut Creek, California, and a member of LC·GC*'s editorial advisory board. Direct correspondence about this column to "LC Troubleshooting,"* LC·GC*, 859 Willamette Street, Eugene, OR 97401, e-mail John.Dolan@LCResources.com.*

ERRATUM

Last month's "LC Troubleshooting" column included discussion about isocratic retention and separations (*LC•GC* **16**[12], 1080 [1998]). In a description of Figure 1, the text should have stated that isocratic retention decreases as temperature *increases* (rather than as it *decreases*).