



Communicating with the Baseline

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What is the baseline telling us?

The baselines observed in liquid chromatography (LC) separations vary widely in appearance. They range from smooth, straight traces to backgrounds that are filled with humps and bumps. However, if these baselines are magnified sufficiently, chromatographers will always be able to see background noise in them. This background noise can manifest itself in a variety of bumps and spikes. Recognizing the different types of background noise can help chromatographers diagnose and correct baseline problems. This month's "LC Troubleshooting" instalment shows how looking at a magnified baseline can help diagnose problems.

I will use several examples to demonstrate the information users can obtain about the source of baseline disturbances.

Look Closely

Many chromatographic methods will produce chromatograms for which the baseline is not completely smooth. This result is especially the situation when workers analyse samples of biological or environmental origin. Baselines in gradient elution methods often contain minor background peaks that can be traced to the water, buffer or other reagents. One goal of the method development process is to adjust the chromatographic conditions so that the peaks of interest are eluted in a region that is free of interfering background peaks.

The gradient separation of Figure 1(a) is an example of this situation. If the users of this method were interested in the peak with a retention time of approximately 4 min and the two peaks eluted just after 10 min, these peaks are sufficiently large

that small irregularities in the baseline would be of no consequence for this application. Although the baseline contains many minor peaks, they can be ignored if they do not interfere. The presence of these minor peaks may come from the matrix or may originate in the reagents and sample preparation process.

Until the baseline is greatly magnified, the chromatogram of Figure 1(a) appears to be normal. However, it looks quite different if the baseline is expanded. Figure 1(b) shows a 1 min section of the baseline expanded approximately 20-fold. Notice that the minor peak in Figure 1(a) marked by an arrow now appears as a chromatographic peak with a typical Gaussian shape. The abnormality is the short-term noise on the baseline. Further expansion (see inset of Figure 1(b)) illustrates the very regular nature of this noise; every data point is discernible.

What could be causing the cyclic baseline seen in Figure 1(b)? Perhaps the first thing to come to mind would be the part of the LC system that is inherently cyclic — the pump. Pump problems, such as bubbles or leaky check valves, may appear as a cycling baseline. These pump problems typically generate pressure pulsation as well. In the present example, the pressure was very steady. Another clue can come from looking at the frequency of the cycling. If the noise were pump-related, users should be able to change the flow-rate and, thus, change the frequency of the cycling. I tried this fix, and the frequency of the cyclic baseline persisted.

By further expanding the baseline of Figure 1, I determined that the frequency was roughly 70–80 cycles/min. This rate is much faster than the cycle of the

pistons in the pump. Furthermore, pump malfunctions don't generally cause such even baseline cycling — they tend to be a bit more random. At this point, I think the evidence is sufficient to abandon the pump as the problem source.

Electronic noise from fluorescent lights and other common sources is often called 60-cycle noise because it coincides with the 60 Hz frequency of the alternating current servicing the laboratory. The noise in Figure 1 is much slower than 60 Hz. In the present situation, I traced the source of the noise to a malfunctioning resistance-capacitance filter in the circuit. When the filter was disconnected, I observed normal random baseline noise. I replaced the filter with a new one, and the baseline appeared as expected.

Normal Background Noise

An amplified chromatographic baseline typically looks more like the one shown in Figure 2 than that in Figure 1(b). In Figure 2, the noise contains more spikes, and the frequency is higher than in Figure 2. The baseline of Figure 2 looks pretty ugly until you look at the scale of the y axis.

For this particular detector — an ultraviolet (UV)-absorbance detector operating at 254 nm — the manufacturer specifies that the noise should not exceed 1×10^{-5} AU with a dry cell. To measure the noise, expand 1 min or more of baseline, as shown in Figure 3. Draw lines roughly parallel to the baseline, so that they barely touch the extremes of most of the noise. Measure vertically between the lines, as shown in Figure 3, and quantify this measurement using the appropriate y-axis scale. In the example of Figure 3, the dotted lines intersect the y axis at

approximately 0.665 and 0.725 mAU for a noise value of roughly 6×10^{-5} AU, so the detector in Figure 3 (not the same one used in Figure 2) exceeds the manufacturer's specifications, but the excessive noise may be a result of the mobile phase and pumping system, not the detector.

The run shown in Figure 2 was performed with solvent flowing, so I would expect it to be a bit noisier than a dry-cell run. Even so, the figure shows that the peak-to-peak noise generally falls within the manufacturer's specifications for this detector. Users can perform a dry-cell test by disconnecting the detector from the column and then blowing the

cell dry with nitrogen. Under these conditions, the drift shown in Figure 2 would probably be absent.

The inherent background noise of LC detectors may be no problem, unless chromatographers are performing methods that require detectors to be set on their most sensitive settings. As part of the installation qualification step of validation, analysts should verify that a new detector will meet the noise specification. In addition, it is a good idea to check detector performance when the lamp is replaced. Users can check the baseline noise at any time by running a non-injection blank and then amplifying a section of the baseline until the noise is

measurable. I used this type of in-process check to obtain the chromatograms shown in Figures 2 and 3.

Lamp Failure

The example of Figure 1 showed a high-frequency, regular baseline disturbance. The chromatogram of Figure 4(a) shows a much larger baseline disturbance at a much lower frequency. The large, square peaks do not fall in an exact time cycle, but they do occur roughly every 3 min. Large pseudo-peaks, such as those in Figure 4(a), often make chromatographers suspect air bubbles in their systems. As each bubble passes through the detector flowcell, the optical path is disturbed and a large peak or valley appears. However, air bubbles generally look more like real peaks, in that they will front or tail, rather than exhibit the vertical offset shown in Figure 4(a).

One simple test to confirm the presence of air bubbles is to set the pump flow to 0 mL/min. This setting will allow data collection to continue, but it will stop the flow through the cell. If a bubble is in the cell when the flow stops, it will remain there; if no bubble is present, a new one will not enter the cell. Thus, the baseline tends to be steady when the flow is stopped. Stopping the flow had no effect on the appearance of the chromatogram for the problem shown in Figure 4(a) — the square spikes continued.

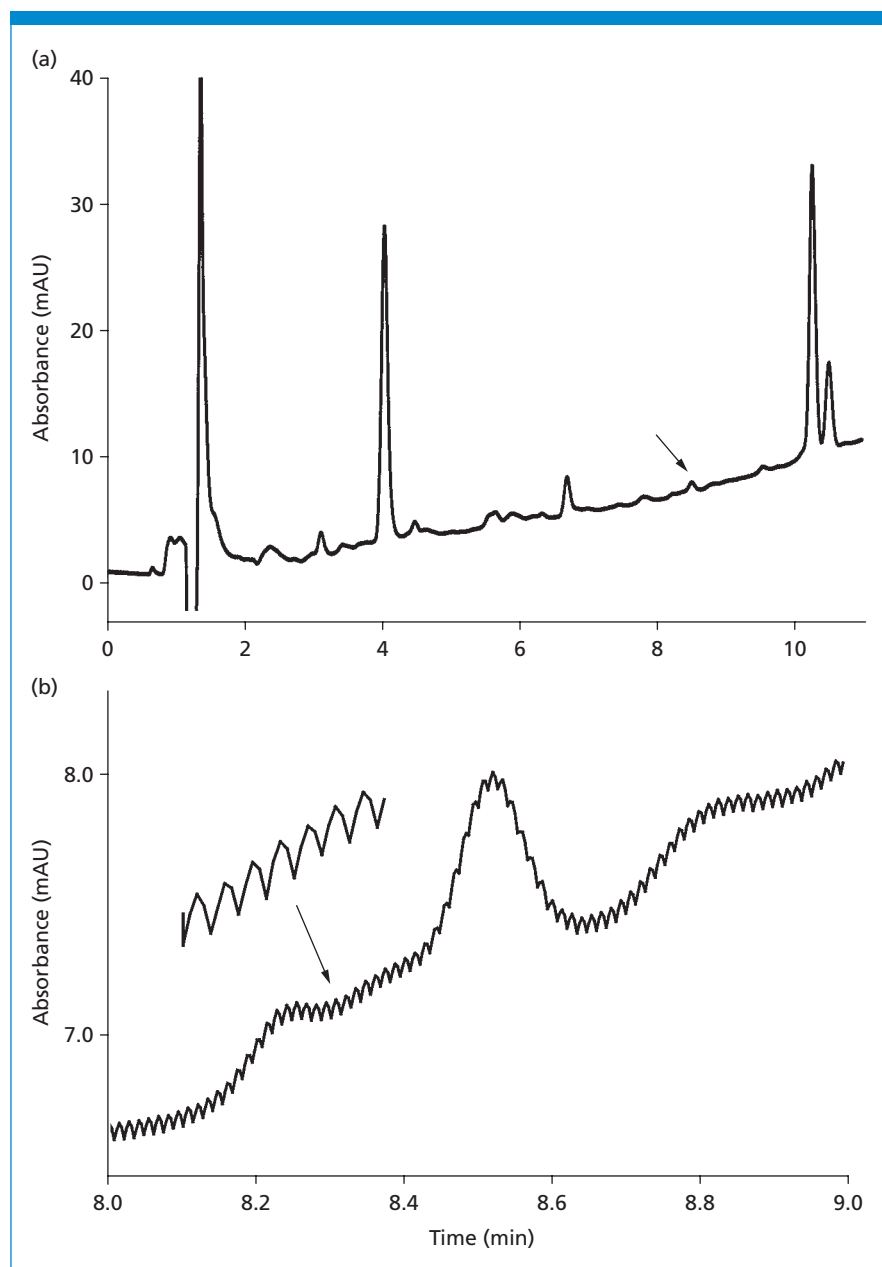


Figure 1: Baseline noise resulting from a malfunctioning resistance-capacitance filter. Shown is the baseline (a) under normal amplification and (b) greatly amplified.

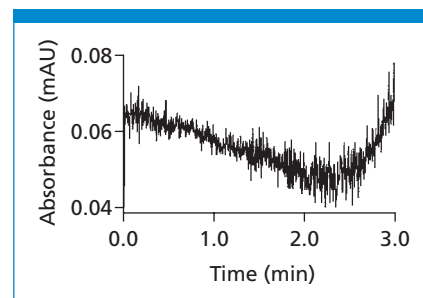


Figure 2: Baseline noise for a UV detector. See text for details.

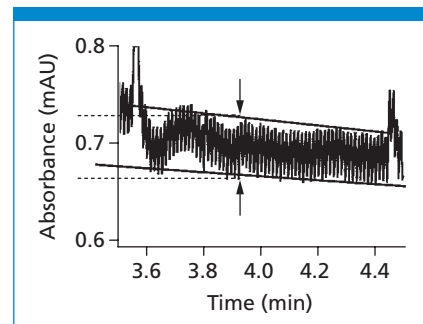


Figure 3: How to measure baseline noise. See text for details.

Another source of large baseline offsets can be external electrical equipment that draws a large current, such as a water bath or a motor during its start-up cycle. For example, the heater on a water bath may cycle on and off at approximately 20 s intervals, and a disturbance in the electrical circuit can occur each time the heater turns on. If the LC system electronics are insufficiently protected against these surges, the chromatographic baseline can reflect the surge as a spike. In the instance of Figure 4(a), I could identify no such power-hungry devices.

Eventually, the problem was tracked to the failure of the detector lamp. Detector lamp failure in UV detectors can cause a variety of symptoms. The most common observation by far is an increase of background noise beyond what the method can tolerate. If you suspect this mode of failure, run a quick background noise check and compare it with the detector specifications. Remember that if mobile phase is flowing, you may find that the background noise is 5–10-fold greater than its specifications under normal conditions.

Deuterium lamps in most modern UV detectors will last for more than 1000 h in normal use. Most of these detectors have a feature that allows users to determine how many hours of use the lamp has experienced. Check your detector's operator's manual to determine how to find this record. Also, remember to reset the lamp hour counter when you install a new lamp.

Although most lamps fail because of excessive noise, dramatic failures can also occur. The chromatogram of Figure 4(a) shows an odd-looking baseline. A square peak is present at approximately 3 min intervals. In this situation, the lamp would not stay lit, and when it went out, the detector lamp's start circuitry engaged. When this happened, the elevated baseline occurred. After the lamp started, the baseline returned to normal until the lamp went out again after a few minutes. After replacing the lamp, I obtained the baseline of Figure 4(b). At the same amplification as used in Figure 4(a), the baseline is flat (lower trace of Figure 4(b)). A 100-fold amplification of the baseline (upper trace) shows background peaks but no signs of the spiking observed before lamp replacement.

What about LC-MS spectrometry?

When using UV detectors, chromatographers are used to a continuous response function and smooth peaks, such as those depicted in Figure 1. When using a mass spectrometer as a detector, the baseline is somewhat different. If the mass spectrometer is set in the single-ion monitoring mode, the detector responds only when an ion with the desired mass is present.

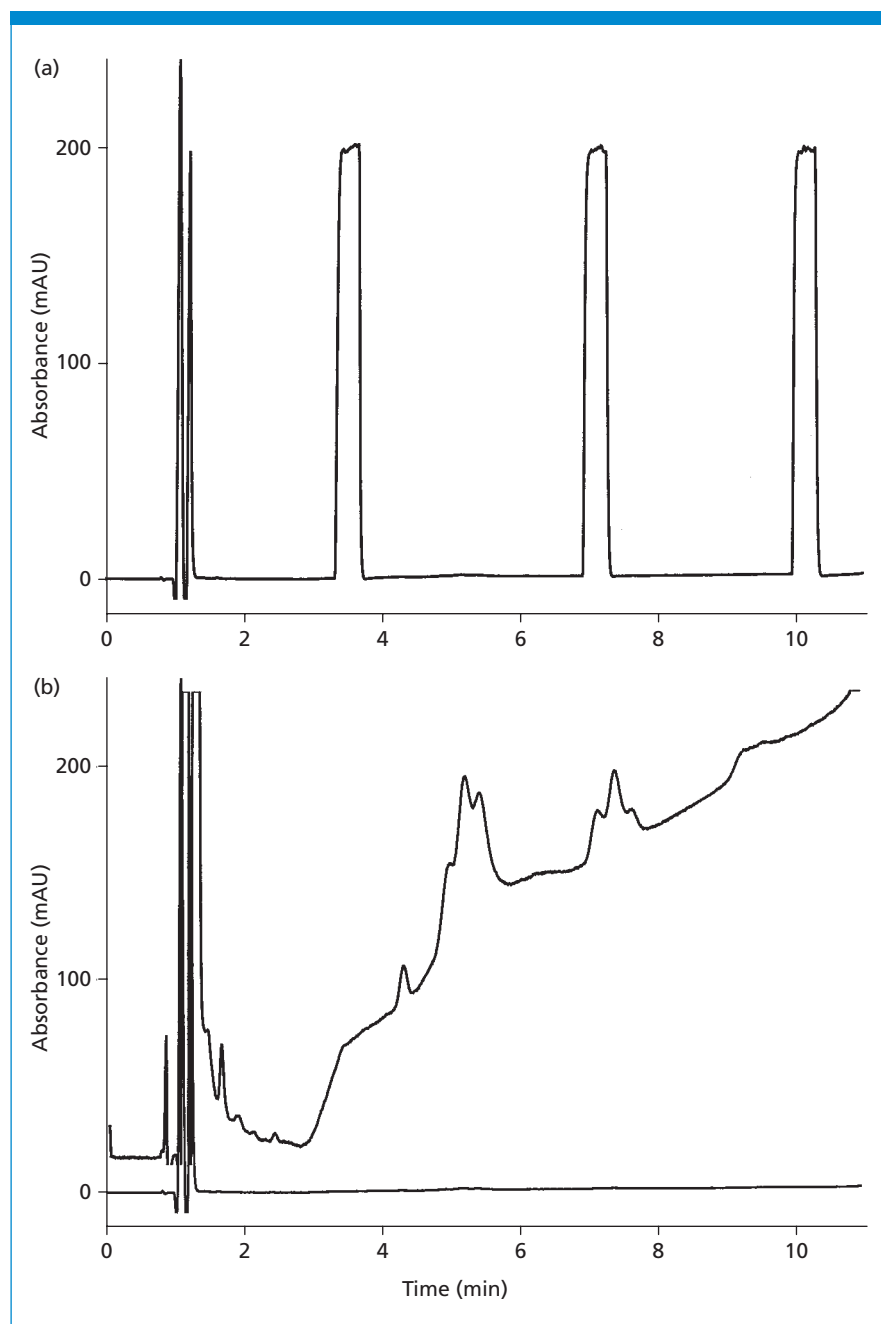


Figure 4: UV detector lamp failure. Shown are (a) square peaks caused by the lamp-start circuitry and (b) baselines obtained with a new lamp at the same settings as (a) (lower trace) and with 100-fold amplification (upper trace).

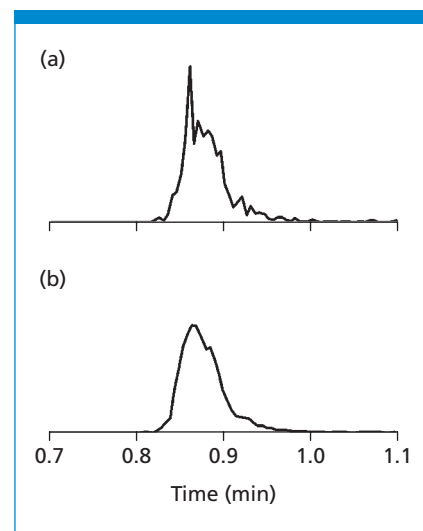


Figure 5: LC-MS peak obtained (a) without and (b) with signal smoothing.

"A close examination of the chromatographic baseline can help chromatographers understand how well an instrument is operating."

Multiple-reaction monitoring in an LC–MS–MS set-up creates a situation in which no signal — other than shot noise from the photomultiplier — is present, so the output is, at least theoretically, zero when no target ions are present.

Analysts can think of multiple-reaction monitoring detection as the counting of ions that have a particular mass. Because the photomultiplier responds to discrete events, the output signal is much more step-like than that of the traditional smooth Gaussian chromatographic peak. This situation is illustrated in Figure 5(a) for an unsmoothed signal. Signal averaging or smoothing will reduce the noise significantly, as Figure 5(b) shows.

Any time that signal averaging is used, whether it is with an LC–MS system or a time-constant or data-rate filter with a UV detector, chromatographers lose signal. Analysts face a trade-off between obtaining a more meaningful smoothed peak and having the data output truly representative of the peak area. In the instance of Figure 5, I lost less than 1% of the peak area through the smoothing process — an insignificant amount for this particular assay. Even with smoothing, however, the typical low-level LC–MS peak, as shown in Figure 5(b), is very poorly shaped when compared with traditional LC peaks. Don't be led astray by this type of peak-shape observation — it is the nature of the detector and has little correlation to the usefulness of the collected data.

Conclusions

A close examination of the chromatographic baseline can help chromatographers understand how well an instrument is operating. All detectors have an inherent level of background noise. It is wise to quantify this noise when a detector is new and known to be operating properly. In this manner, users have references for future comparison when they suspect detector malfunctions. It is also important to remember that different detectors have different noise characteristics, so the appearance of a normal chromatogram from one detector may be unacceptable if the data were collected from another detector.

"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 Europe. Direct correspondence about this column to "LC Troubleshooting," LC•GC Europe, Advanstar House, Park West, Sealand Road, Chester CH1 4RN, UK, e-mail: dhills@advanstar.com

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