
LC Troubleshooting

# Quiet, Please

**How can you reduce baseline noise?**

Excess baseline noise can be a frustrating aspect of liquid chromatography (LC) separations. Noise can be a limiting factor when chromatographers attempt to use an LC method for trace analysis. Although baseline noise cannot be eliminated, analysts can implement several simple procedures that can help reduce baseline noise to acceptable levels. This month's "LC Troubleshooting" column looks at some chemical, physical, and electronic sources of baseline noise and suggests how to reduce or eliminate them.

## Mobile-Phase Mixing

On-line mixing is a compromise between the completeness of mixing and the time and cost involved. All LC systems mix mobile-phase components by pumping them into a mixing chamber, where mixing occurs with the aid of mechanical stirring or a tortuous path. Low-pressure mixing systems (one-pump systems) meter the mobile-phase components into the mixer in timed pulses at a constant flow rate. High-pressure mixing systems (two-pump systems) control the proportions of the mobile phase by simultaneously pumping the components into the mixer at different flow rates. The volume or time from the point at which the solvents are mixed until they reach the head of the column is called the *dwell volume* or *dwell time*, respectively.

The dwell volume introduces a delay at the beginning of gradient separations that is equivalent to adding an isocratic hold in a gradient program. Dwell volumes generally increase the run time for gradient separations, and they are a primary hurdle in transferring gradient methods from one LC system to another. Much research and development effort has gone into the design and construction of mixing systems that minimize the system dwell volume and maximize the mixing efficiency.

Even so, mixing is never complete with an on-line mixing system. Its inefficiency is obvious if you use a refractive index detector to compare the baselines from analyses using an on-line mixed mobile phase and a hand-mixed mobile phase. The heterogeneity of the mobile phase causes refractive index changes and increased baseline noise. Although UV detectors are less sensitive to refractive index changes, UV detectors at maximum sensitivity settings exhibit similar phenomena.

Reducing baseline noise caused by incomplete mixing is simple for isocratic separations — just hand-mix the mobile phase. Hand-mixing is impossible with gradient methods and might be impractical for some isocratic applications. However, partially premixing the mobile phase generally will improve baselines in both cases. For example, if your method calls for a gradient from 10–85% acetonitrile in water, then hand mix the A solvent to 10% acetonitrile and the B solvent to 85% acetonitrile. In my experience, the initial mixing of organic and aqueous solutions produces most problems, so partial premixing can provide much better overall mixing.

Some LC systems have additional mixing chambers that can be plumbed into the mobile-phase path. For example, some systems in my laboratory have a mixing block with 0.5-, 1.7-, and 2.6-mL mixing chambers that can be used individually or in any combination. Thus, as much as an additional 4.8 mL can be added to the basic mixing capabilities. Chromatographers can purchase aftermarket mixers from many sources. In general, the larger the mixing volume, the better the mixing and the smoother the appearance of the baseline. However, not all mixers are created equal — one manufacturer's 0.5-mL mixer could outperform another's 2-mL mixer.

## System Tune-Up

On-line mixing systems are designed to perform best when all the mechanical components of the system are working at their design specifications; therefore, a leaky check valve or pump seal can cause inconsistent flow rates that translate into substandard mobile-phase proportioning. The result is reduced mobile-phase homogeneity and increased baseline noise. Of course, marginal components also can cause flow-rate and, thus, retention-time changes. To obtain the best system performance, you must ensure that the LC system is operat-

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ing to its specifications. Correct any problems you detect. Some simple tests are listed below.

Simple flow-rate and pressure-decay checks can test system performance. For example, performing a timed collection of mobile phase into a 10-mL volumetric flask will allow you to calculate the true flow rate. A simple pressure-decay test can be performed by plugging the tube connecting the autosampler and the column. Set the pressure limit at 5000 psi (approximately 350 bar) and turn the pump on. When the pressure hits the limit, the pump will shut off. Measure the percentage drop in pressure during a measured time. My laboratory uses a specification of 15% allowable decay in 10 min. You might need to adjust this test to work with your hardware. You can replace suspect check valves, but I generally sonicate a questionable check valve in methanol for a few minutes before replacing it to see if the problem can be corrected less expensively.

You can check mobile-phase proportioning using a step test and a gradient check. Remove the column and replace it with a piece of connecting tubing. Place water in the A-solvent reservoir and put 0.1% acetone in water into the B reservoir. Set the detector to 265 nm. Run a series of 3-min 10% steps; that is, 10, 20, 30% B . . . 100% B with a flow rate of 1 mL/min. By measuring the step height, you can calculate the accuracy of proportioning throughout the range of settings. Similarly, you can run a gradient trace, for example 0–100% in 10 min, and examine gradient linearity and smoothness.

You may need to adjust these tests to work optimally with your LC system. If your system is not performing in accordance to its manufacturer's specifications, you might need to consult your service manual or a service technician.

## Degassing

Most chromatographers consider degassing a technique to prevent bubble problems in pumps or detectors, but degassing can play an important role in minimizing baseline noise. As a general rule, the more dissolved gas removed from the mobile phase, the better the system will perform. Helium sparging is the most effective mobile-phase degassing technique. If you don't already use degassing as a part of your LC procedures, you might find that baselines are less noisy if you incorporate the technique. In-line degassing systems are becoming increasingly popular, and although they seem to perform very well, it would be wise to determine if initial helium sparging further reduces baseline noise with these systems.

## Cleanliness

Baseline noise can come from one of two sources. The techniques discussed thus far address problems originating from incomplete mixing or other disturbances in the mobile-phase composition. Baseline noise also can result from impurities in the reagents or solvents used to formulate the mobile phase. This problem source is more noticeable with gradient elution methods, but isocratic methods also can be influenced by reagent purity. Use high performance liquid chromatography (HPLC)–grade reagents and solvents for the best performance. If you've tried the steps mentioned above and still have more baseline noise than you desire, it could be worthwhile to examine carefully the effect of reagents on the noise.

Reagent purity can be checked two ways. A systematic approach will identify an individual reagent that contributes to the baseline noise. Replace one reagent at a time and examine the baseline for improvements. Alternatively, prepare fresh mobile phase from new reagents. This approach might be faster, but it doesn't identify specific reagent problems. If in doubt, use a fresh container of the reagent or reagent from a different supplier.

Your laboratory equipment or your practices could be another source of contaminants in the system. For example, inserting the pH meter's probe in the bulk buffer can contaminate the entire batch, whereas checking the pH of a buffer aliquot and then discarding it avoids this potential source of contamination. Consider glassware cleaning processes, filtration and degassing equipment, and other items that come into contact with the mobile phase as potential sources of contamination.

The column can cause increased baseline noise as it ages. Many samples contain materials that are strongly retained on a column. Some of these materials have poor UV absorbance or are eluted so late that they don't show up as a traditional peak. However, over time these materials can result in a rolling or irregular baseline. To minimize these problems, flush the column with the strong solvent of the mobile phase (for example, acetonitrile or methanol) after each batch of samples. Dedicating a column to one type of analysis is a technique that many workers use to minimize cross-contamination from other samples. If you suspect the column is contributing to the baseline noise, replace it with a new column. Remember, columns should be considered consumable items, not capital equipment. The cost-per-sample contribution of the column often is a fraction of that of sample preparation, so keep replacement costs in perspective.

Improved sample preparation can decrease baseline noise. When trace analysis is the objective, any change in sample preparation might require validation to enable accurate quantification of the analyte.

## Electronic Filters

Electronic noise in the baseline can originate within the LC system and its associated electronics or from the operating environment. With UV detectors, most electronic noise can be addressed by adjusting the data-acquisition rate or the detector-time constant. These two approaches to reducing electronic noise result in a compromise between improved signal smoothing and loss of signal. The filtering process works by averaging the signal over time, suppressing the highs and lows, and reducing the variation. This process is indiscriminant, so it reduces baseline noise as well. The goal is to provide as much smoothing as possible without suppressing the signal.

A good compromise to obtain a data rate that will give an accurate representation of the detector signal and minimize unwanted signal suppression is to collect 10–20 points across a peak. For example, a 15-cm long, 5-μm particle column might generate approximately 12,000 plates with well-behaved analytes. The column plate number equation:

$$N = 16(t_R/w)^2 \qquad [1]$$

calculates the plate number ($N$) based upon the retention time ($t_R$) and the peak width at the baseline ($w$). Rearranging equation 1 and solving for the peak width allows you to determine that a peak with a retention time of 5 min will have a peak width of approximately 11 s.

Using the general rule above, the data rate should be 0.5–1 Hz. This value probably is close to what the data system will pick by default. Applying the same column to the separation of real samples usually will result in lower plate numbers — 6000–8000 plates per column — so the peaks will be wider. You can measure the peak width manually on the chromatogram or request the peak width as part of the data system report (be sure it is reporting

baseline peak width and not half-height width). To determine if a change in the data rate will improve the baseline noise, you generally can reprocess the same data set with a different data rate. Compare the precision and accuracy of the two data rates to determine if a lower data rate would be better.

The detector-time constant is used less frequently today than it was before the universal use of data systems. However, most detectors have an adjustment for the time constant. This adjustment works in a manner similar to that of the data rate, and generally it is selected so the time constant is approximately one-tenth of the peak width. For the examples above, I would select a time constant of 1 s. Once again, larger time-constant values reduce noise, but they also can reduce the signal strength, so it is a good idea to compare data sets gathered with both settings. Unfortunately, the time-constant changes the data before it is collected, so two data sets must be collected, whereas different data rates can be checked using existing data files. Because the noise-reduction process is different for the time constant and data rate, you might want to check both techniques to determine which will give you the best results or if a combination of the two would be useful.

### Baseline Noise Sources and Noise-Reduction Techniques

**Mobile-phase mixing problems**
- Hand-mix the mobile phase
- Partially premix the mobile phase
- Plumb in additional mixing capacity

**System performance**
- Check or replace the pump seals
- Check, clean, or replace the check valves
- Adjust the system controls

**Degassing**
- Try additional degassing

**Cleanliness**
- Use better-quality reagents
- Flush or replace the column
- Use better laboratory practices
- Improve sample preparation

**Electronic noise filtration**
- Optimize the data rate
- Optimize the detector-time constant


## Summary

Baseline noise can be a limiting factor in the ability to determine trace components in your samples. Baseline noise can originate from a number of sources. This month I've looked at the various sources of noise and simple ways to reduce each. These suggestions are summarized in the accompanying sidebar, "Baseline Noise Sources and Noise-Reduction Techniques."



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