Liquid Chromatography Problem Solving and Troubleshooting

Question

I would like to improve the signal-to-noise ratio on my UV detector because I need more sensitivity. What tips can you offer me?

Answer

First, let me point out that you have made a common misstatement that I often encounter. I know what you are requesting, but, in the strictest semantic sense, a detector does not have a signal-to-noise ratio. A signal-to-noise ratio is something you set for making decisions about whether a compound is present or not. For instance, you may decide that you require a signal-to-noise ratio of 3 when determining a minimum detectable level, or you may decide that this ratio is 2 or 4. For setting a minimum quantitation level, the signal-to-noise ratio might be 10. The ratio is something you set for a specific compound, not an inherent performance parameter of the detector.

To improve detectability of a compound, either the signal of the detector needs to be increased or the noise level needs to be reduced (or both an improved signal and a decreased noise can be obtained simultaneously). Now, how can we accomplish this improvement in the detection level? The first question to ask is are we using the "best" detector to obtain the highest signal. Given that your molecule absorbs UV light, does it fluoresce? If so, signals for fluorescence are drastically improved over UV. Is there any other detector, perhaps the electrochemical detector, that could be used? If another detector is possible, the signal may be improved enough to improve detectability.

Once you are convinced that you have the maximum signal, things that can be done to reduce the detector noise include having a clean cell window and having a new lamp. The noise of the UV detector is inversely proportional to the amount of light that the photomultiplier or photodiode receives. This is why a dirty cell window and a low intensity light result in increases in noise levels.

Following the same reasoning, noise levels may increase if solvents or mobile phase additives absorb light at the wavelength of interest. For example, at a wavelength of 210 nm, methanol will exhibit some UV absorbance when good acetonitrile is clear at this wavelength. Therefore, you may wish to adjust your solvents in your mobile phase, but this would mean doing some method development to obtain an appropriately good separation in the new solvent/mobile phase. You may also wish to perform a determination of the signal and the noise at a variety of wavelengths. In this way you can determine the best signal with the lowest noise for the sought-after compound. If you have a photodiode-array detector, this experiment is straight-forward, but it can also be done with any variable wavelength detector. Mobile phase additives such as buffers and amines (used to improve peak tailing) can absorb low UV light. If you were operating at 254 nm, buffers such as citrate and acetate (which are good buffers) are very appropriate. However, if you are operating at 210 nm, neither citrate nor acetate would be optimal because they both absorb light at low wavelengths.

Another item to consider is the time constant of the detector. This may be imbedded in the software on some instruments rather than set at the detector. If you increase the time constant, the noise will drop; however, depending upon the peak volume (time), there may also be a decrease in the peak response. This approach may be appropriate, especially if the chromatogram contains well-resolved peaks. The only way to find out if a time-constant change will help is to try it at several values and determine the effect.

Of course, there are other things to try if modifying the chromatography is an option, but I will save these comments for another article.

The purpose of *Chromatography Problem Solving and Troubleshooting* is to have selected experts answer chromatographic questions in any of the various separation fields (GC, GC–MS, HPLC, TLC, SFC, HPTLC, open column, etc.). If you have questions or problems that you would like answered, please forward these to the *Journal* editorial office with all pertinent details: instrument operating conditions, temperatures, pressures, columns, support materials, liquid phases, carrier gas, mobile phases, detectors, example chromatograms, etc. In addition, if you would like to share your expertise or experience in the form of a particular question accompanied by the answer, please forward it to: JCS Associate Editor, *Chromatography Problem Solving and Troubleshooting,* P.O. Box 48312, Niles, IL 60714. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

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