Liquid Chromatography Problem Solving and Troubleshooting

Question

I am developing a method that uses a sample preconcentration step on a solid-phase extraction (SPE) device to enrich a trace amount of analyte in the sample. After SPE, the concentrated analyte is in 100% methanol. My problem is that, in order to obtain the sensitivity, I have to inject more that 100 µL, my peaks are too broad, and I do not obtain the sensitivity that I need. My mobile phase is a 55% mixture of buffered water in acetonitrile. What can I do?

Answer

First, ask yourself if you can use a different detector. Detectors based upon fluorescence or electrochemistry can achieve orders of magnitude higher in sensitivity than a UV detector. Is the UV detector set at the wavelength maximum of the analyte? Also, consider pre- or postcolumn reactions to derivatize the analyte and, thereby, enhance its ability to be detected using the standard UV detector.

For the remainder of the discussion, I am assuming that you will be using the most sensitive detector you have in your laboratory, and I will focus on what you might do to improve your sensitivity with the chromatography. For instance, you maybe able to modify your sample preparation step. The reason you have too little sensitivity is most likely caused by the fact that the sample is injected into the high-performance liquid chromatograph in too strong of a solvent. The 100% methanol is a stronger solvent than the mobile phase. Therefore, when the sample is injected, the analyte will be carried quickly down the column with the high elution strength of the injection solvent. Consider what happens at low injection solvent strength. The analyte is diluted such that it is enriched (or "sorbed") onto the top of column in a narrow band. This would also be the case at a low volume injection of the sample in methanol. The methanol would be diluted with the mobile phase and the analyte would concentrate on the top of the column and be separated from the methanol. The analyte would move down the column much slower that the methanol moves. At the large injection volume of methanol, the sample solvent overwhelms the column acting, in essence, as a "step" of strong solvent moving the analyte with the methanol until the methanol is diluted with the mobile phase. The result is that the methanol distorts the peak shape at a large volume and not at the smaller one.

So what can you do? There are a couple of possibilities that come to mind. The first is to evaporate the sample to dryness after the SPE step and redissolve it in either a solvent strength equal to the mobile phase or, preferably, in a weaker solvent than the mobile phase, perhaps 90% buffer in acetonitrile. Or, you could try redissolving the sample in a very small amount of methanol. Note: if you change to doing an evaporation step, it would be wise to include an internal standard, but you may already be using one for the sample preparation step.

A second suggestion is to dilute the sample in a large amount of water (or buffer) and then inject a very large volume (1). For instance, if you have a retention time of 4 min with a 10-µL injection and you inject 1.5 mL of sample consisting almost entirely of a water solvent, your retention time would be 5.5 mL. And, the peak shape will be the same width as the peak was with the 10-µL injection size because the analyte has been concentrated onto the column prior to its elution. In this particular case, the sample enriches on the top of the column because the sample is injected in a weak solvent. You are mirroring what is occurring in the SPE activity only on the main column. In such a weak mobile phase, the analyte does not migrate down the column. Once the water-rich injection solvent passes, the sample is swept by the actual mobile phase and migrates down the column following the usual chromatographic process. In this way a very high concentration capability is possible that results in a high sensitivity being attained. In fact, depending upon the sample-analyte matrix, you may be able to do such a large volume injection directly onto the analytical column that the SPE activity might be eliminated. There was not sufficient information for me to make a judgment here as to the appropriateness of this suggestion. For a more complete explanation of this technique (injecting a dilute solution for attaining high sensitivity) refer to the literature (1).

References

1. B.A. Bidlingmeyer. *Practical HPLC Methodology and Applications.* John Wiley & Sons, New York, NY, 1992, pp. 240–41.

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 to: JCS Associate Editor, *Chromatography Problem Solving and Troubleshooting*, 6600 W. Touhy Ave., Niles, IL 60714-4516. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

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