Liquid Chromatography Problem Solving and Troubleshooting

Question:

When I inject a 20-µL sample volume, I obtain a shoulder on the main peak. I am told that the compound is pure and that my column has a void in it. However, other compounds injected on this column have a single peak. Am I observing an impurity? What would you suggest I do? Because of the solubility limitations of the sample, the method uses a stronger solvent than the mobile phase for the injection.

Answer:

It is possible that you are resolving an impurity, but because there is strong sentiment in your organization that the compound is pure, I believe that a more probable cause of the shoulder on the main peak is that the injection of the sample is in a stronger solvent than the mobile phase. An explanation of this phenomenon is that there is limited solubility in the mobile phase. Thus, a small portion quickly and momentarily "precipitates" onto the stationary phase. Then, as more mobile phase flows by, the chromatographic process moves the sample but is restricted because of the solubility limits of the sample. In other words, because there is limited solubility of sample in the mobile phase, the entire amount of the sample is "sorbed" onto the column; then an initial amount of sample easily dissolves into the mobile phase, whereas a remaining portion of sample has to wait a small amount of time to be dissolved from the stationary phase into the mobile phase. The result is that the initial elution occurs in two steps.

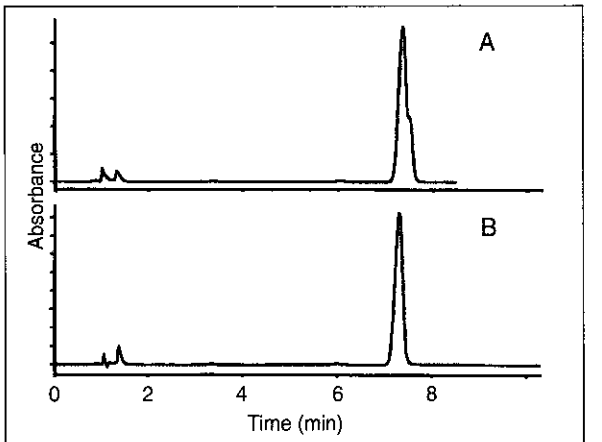

Figure 1. Effect of injection volume of a sample dissolved in a strong solvent on the retention of a sample with limited solubility in the mobile phase. The injection solvent is 44% DMF in water. The mobile phase is acetonitrile–water (12:88, pH=7.0) containing 20mM phosphate buffer. Chromatogram A is a 20-µL injection volume, and chromatogram B is a 5-µL injection.

An example of this phenomenon is shown in Figure 1. The top chromatogram (Figure 1A) is a 20-µL injection of a sample contained in a solvent mixture of 44% dimethylformide (DMF) in water. The DMF is necessary in this method to ensure rapid and complete dissolution of the sample. The bottom chromatogram (Figure 1B) is a 5-µL injection of the same sample. Note that the shoulder in Figure 1A is no longer present in Figure 1B.

Using the approach shown in Figure 1 would be a good test to perform on your sample. Inject a smaller volume and observe the effect on peak shape. If the shoulder disappears, it is due to the injection in the larger volume of strong solvent with a sample of limited solubility in the mobile phase.

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*, 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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