Liquid Chromatography Problem Solving and Troubleshooting

Question:

I have recently used a very short 30-mm column of a specific brand and lost more resolution than I had calculated I should lose. I am using a new HPLC and have verified that my system dead volume is appropriate for this short column. My conclusion is that these short columns are not packed as well as the longer 150-mm columns. Do you know if this trend is universal or specific to my particular brand?

Answer:

First let me point out that I am not sure your conclusion is correct, based on the information you have shared with me. As you note, if you measure the resolution obtained on the longer column, the resolution that should be obtained on the shorter column can easily be calculated. This is because resolution is proportional to the square root of the number of theoretical plates, and the number of theoretical plates is directly proportional to column length. Of course, this assumes that both columns were packed equally well, which means the short column would have the same height equivalent to a theoretical plate value as the longer column. If the resolution on the 30-mm column is not as it should be according to the calculation, the short column may not be packed as efficiently as it should be. If you are sure that the shorter column is indeed performing at a much lower level than the longer column, it is appropriate to call the manufacturer and discuss the difference between the performance you obtain and that which the specification says you should obtain.

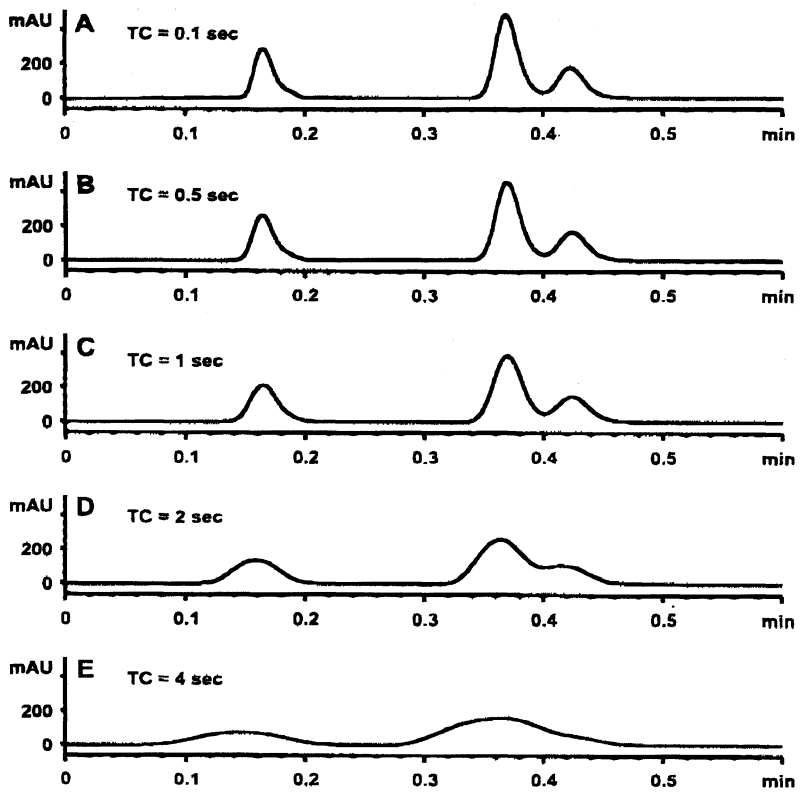

Figure 1. Influence of the detector time constant on resolution in fast HPLC. Column, 4.6 x 30 mm Zorbax SB-C18; flow rate, 1 mL/min; detector, UV at 254 nm; time constant (TC) shown on the chromatograms.

However, before you call the manufacturer, it is a good idea to verify that the lack of performance from the column is not due to something else. You stated that the longer column performed quite well and that you evaluated your system volume; however, another possible cause of the problem is that the detector time constant is not set properly for a fast-eluting peak. New detectors are often shipped with a mid-range time

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.

Brian A. Bidlingmeyer
Associate Editor

constant setting that might not be appropriate for fast HPLC.

Refer to the figure to see an example in which the resolution on a short (30 mm) column was eliminated when the detector time constant was set too high. In this example, two peaks that eluted in under 30 s were resolved appropriately when the time constant was set to 0.1 s and not resolved at all when the time constant was set to 4 s. Because each detector may be different, it is important to read the manual to understand the time constant settings and their influence on data output. A previous article that may be of interest to you examined plate count measurement for a 150-mm column as a function of the detector time constant (1).

In summary, there are good, well-packed, short columns that are appropriate for fast HPLC. However, there may also be poorly packed 30-mm columns. The way to decide between the two is to determine whether your hardware system is performing at the highest level required for the performance desired.

Reference

1. B.A. Bidlingmeyer. Liquid chromatography problem solving and troubleshooting. **34:** 208 (1996).