Gas Chromatography Problem Solving and Troubleshooting

Question:

I switched from a 30-m x 0.25-mm-i.d. capillary column to a 20-m x 0.18-mm-i.d. one to improve the resolution of some closely eluting peaks and reduce the analysis time. A shorter analysis time was obtained; however, some of the peaks were less resolved than before. Why did the resolution loss occur for the smaller diameter column?

Answer:

Other factors in addition to the column can affect efficiency (i.e., total number of theoretical plates). Any loss of efficiency (decrease in plate number) results in a loss of resolution. When changing column dimensions to improve resolution, other non-column factors may have an influence on the overall resolution. Depending on the dimensional change, resolution may be increased or decreased by these other factors. The total number of theoretical plates is very similar for both columns. The 30-m x 0.25-mm-i.d. column has approximately 142,800 total theoretical plates, whereas the 20-m x 0.18-mm-i.d. column has about 133,300*. This would only account for about a 2% loss in resolution for the 0.18-mm-i.d. column. This resolution difference is not enough to account for the resolution difference between the two chromatograms (Figures 1A and 1B). One or more of the non-column factors are probably responsible for the loss in resolution.

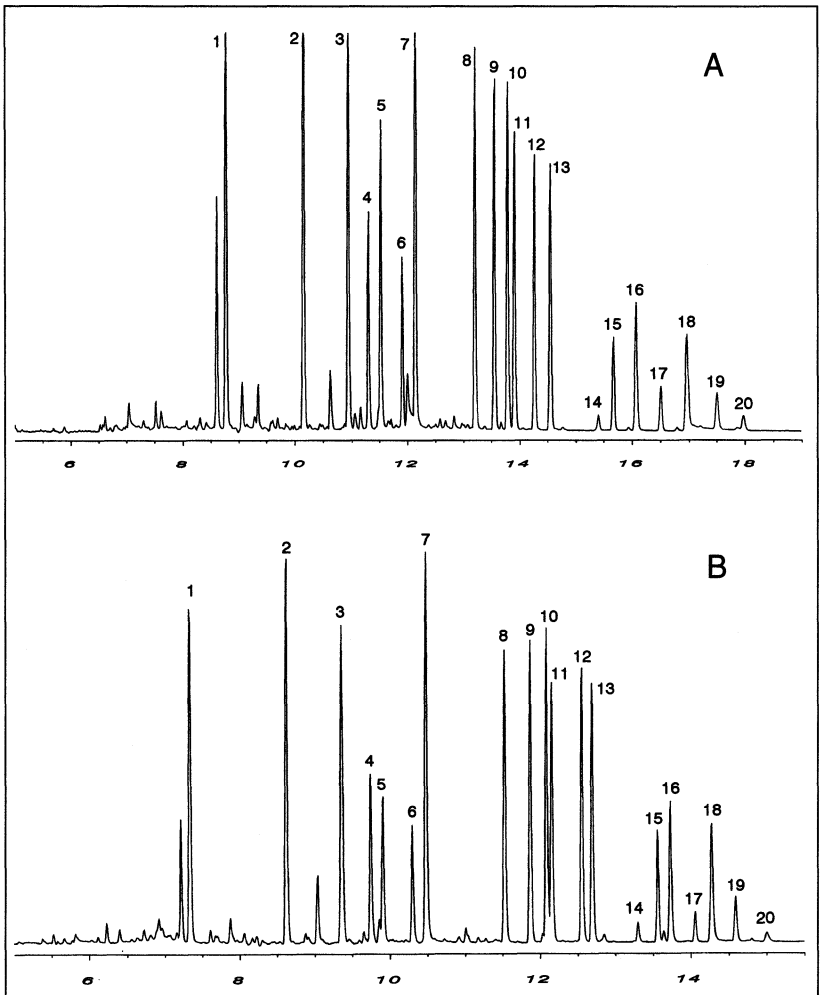

Figure 1. Resolution of chlorinated pesticides using 0.25-mm-i.d. and 0.18-mm.i.d. columns. Column: (A) DB-17ms, 30 m x 0.25-mm i.d. (0.25-µm film thickness); (B) DB-17ms, 20 m x 0.18-mm i.d. (0.18-µm film thickness). Conditions: splitless injector (0.5-min purge activation time); ECD detector, 350°C; nitrogen auxiliary gas at 30 mL/min; hydrogen carrier gas at 45 cm/s; column temperature, 50°C for 0.5 min, 50–150°C at 25°C/min, 150–260°C at 12°C/min, 260–320°C at 15°C/min, and 320°C for 2 min. Peaks: 1, tetrachloro-*m*-xylene; 2, α-BHC; 3, γ-BHC; 4, β-BHC; 5, heptachlor; 6, δ-BHC; 7, aldrin; 8, heptachlor epoxide; 9, γ-chlordane; 10, α-chlordane; 11, endosulfun I; 12, *p,p'*-DDE; 13, dieldrin; 14, endrin; 15, *p,p'*-DDD; 16, endosulfan II; 17, *p,p'*-DDT; 18, endrin aldehyde; 19, endosulfan sulfate; 20, dibutylchlorendate.

One posssible cause of the resolution loss is the injector. Slower transfer of the sample from the injector into the column may negatively affect efficiency, thus resolution. The chromatograms in Figure 1 were generated using a splitless injection. For splitless injectors, the flow rate of carrier gas through the injector is the same as the carrier gas flow rate at the front of the column. At 45 cm/s of hydrogen at 50°C, a carrier gas flow rate of about 1.7 mL/min is obtained for the 0.25-mm-i.d. column and about 1.1 mL/min for the 0.18-mm-i.d. column. For the same volume injector liner, the sample transfer is about 35% slower for the 0.18-mm-i.d. column. Slower sample transfer rates may result in lower overall efficiency depending on the GC conditions, column stationary phase, and sample compounds.

The 0.18-mm-i.d. column has a 0.18-µm film thickness, whereas the

* Calculated using the theoretical maximum efficiency for a peak with a *k* value of 5.

0.25-mm-i.d. column has a 0.25-µm film thickness. For splitless injections, thicker films often help to focus the sample into a shorter band at the front of the column. Shorter initial sample bands often result in better efficiency, especially for splitless injections. In addition, the 0.18-mm-i.d. column has less surface area per unit length of tubing than the 0.25-mm-i.d. column. Splitless injections usually rely on the formation of a focused solvent film at the front of the column. If it is assumed that the injected sample occupies the same surface area in both columns, the initial sample band length is longer for the 0.18-mm-i.d. column (a longer length of 0.18-mm-i.d. column is needed to maintain the same surface area as a 0.25-mm-i.d. column). The longer sample band reduces efficiency, thus resulting in a net loss in resolution. Whether a significant resolution loss occurs depends on the GC conditions, column stationary phase, and sample solvent and compounds.

The combination of a slower sample transfer rate, thinner film, and lower surface area for the 0.18-mm-i.d. column may account for its lower resolution of the particular sample. One other factor that should be considered is that the temperature program required to obtain baseline resolution of all peaks may not be the same for columns with different dimensions. The chromatograms in Figure 1 were generated using the same temperature program and carrier gas average linear velocity. A more efficient column may provide lower resolution than a less efficient column when using the same temperature program for both. This usually applies for columns with different lengths and film thicknesses and less for columns with different diameters. The optimal average linear velocity is often different for columns with different dimensions. In this case, the differences in the optimal velocities are relatively small and are thus not responsible for the resolution loss.

When changing to a smaller diameter column (and perhaps a shorter column) to improve resolution and reduce run times, methods developed for the larger column often have to be modified to obtain the desired benefits from the smaller diameter column. In addition, specific pieces of GC hardware such as injectors may not be fully compatible with small-diameter columns. A combination of injector and column factors are probably responsible for a significant portion of the resolution loss. A change in the temperature program is required to fully utilize the ability of the column. A reduction in the middle ramp rate (e.g., from 12 to 8–10°C/min) and/or a reduction in the end point in the initial temperature ramp (e.g., from 150 to 125°C) will probably improve the resolution of the most affected peaks (9 and 10) for the 0.18-mm-i.d. column.

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.

Dean Rood
Associate Editor