Gas Chromatography Problem Solving and Troubleshooting

Question:

Two earlier eluting peaks in my solvent mixture were partially separated using a temperature program that started at 45°C for 5 min. To improve the separation, I changed the initial temperature to 40°C. However, I was surprised to find that the separation became worse. I thought separation improves upon lowering the initial temperature. What happened?

Answer:

In situations of this nature, it is important to determine the peak characteristic that has actually been altered. Peak separation and resolution are often confused and used interchangeably. Separation is the time or distance between two peaks. Peak widths are not factored into this measurement. Peak resolution takes into account the amount of peak separation and the widths of the peaks. Changes in resolution are due to changes in peak separation and/or peak width. Decreasing column temperatures usually increase peak separation but often with a corresponding increase in peak width. If the increase in peak separation is greater than the increase in peak width, improved peak resolution occurs. This is usually the case for earlier eluting peaks (i.e., partition ratios [*k*] of around 5 or less) in the original chromatogram. If the peak separation increase is less than the increase in peak width, peak resolution is degraded. This is usually the case for later eluting peaks (i.e., partition ratios [*k*] of around 10 or more) in the original chromatogram. Lowering column temperature is a common method to improve peak resolution, especially for early eluting peaks. An increase in peak separation is expected; however, in rare cases, this does not occur. This has occurred for the solvent chromatograms in question. The peak widths did not increase, but the peak separation decreased. This caused the loss of resolution.

Figure 1 shows solvent chromatograms using initial temperature values of 45, 40, and 35°C. As the initial temperature was lowered, there was a loss of resolution for peaks 2 and 3, little to no resolution change for peaks 4 and 5, and a resolution increase for peaks 7 and 8. It was peaks 2 and 3 that did not behave in the expected manner. As the initial temperature was lowered, both peaks increased in retention as expected. For the change from 45 to 40°C, the retention time for peak 2 increased by 0.40 min and by 0.37 min for peak 3. This resulted in peak 2 moving closer to peak 3 and a 0.03-min loss in separation. Because the two peaks were only separated by 0.05 min at 45°C, this seemingly small separation loss is significant. Slightly different retention shifts between peaks with a column temperature change is not unusual. However, in most cases, the peaks involved are not close enough to be significantly affected.

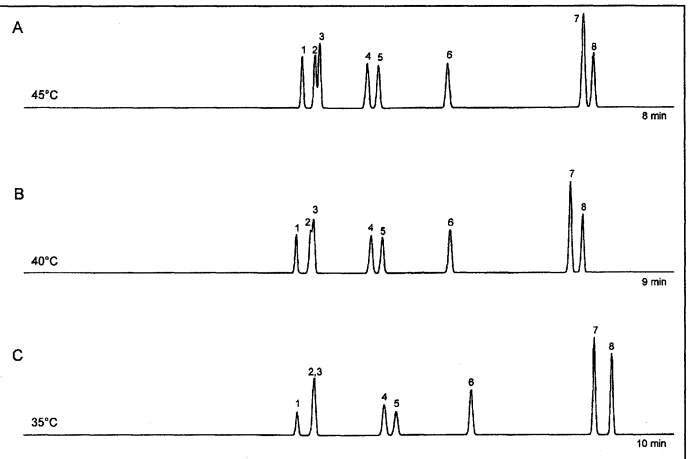

Figure 1. Solvent chromatograms at 45, 40, and 35°C. Column, DB-WAX 30 m × 0.32-mm i.d. (0.5-µm film thickness); split injector, 250°C, 100:1 split ratio; FID detector, 300°C; helium carrier gas, 32 cm/s; column temperatures, (A) 45°C for 5 min, 45–115°C at 10°/min; (B) 40°C for 5 min, 40–110°C at 10°/min; (C) 35°C for 5 min, 35–105°C at 10°/min. Peaks: 1, ethyl acetate; 2, isopropyl acetate; 3, methyl ethyl ketone; 4, isopropanol; 5, ethanol; 6, propyl acetate; 7, toluene; 8, 1-propanol.

If the initial temperature were decreased to below 35°C, it is very likely that peak 3 would have started eluting before peak 2. Eventually a reversal in the elution order would have occurred. This is commonly called a peak inversion. Peak misidentifications and apparent missing or extra peaks are some of the more common errors caused by partial or full peak inversions. Care must be taken when using published chromatograms as a source of GC conditions or peak identifications. An alteration in the temperature conditions may cause a peak inversion leading to a coelution (missing peak) or peak order change (misidentification). If there are closely eluting peaks in the original chromatogram, it may be best to duplicate those conditions as best as possible before making any alterations to the conditions. In this way, the possibility of peak identification errors is reduced.

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