Liquid Chromatography Problem Solving and Troubleshooting

Question:

I am attempting to speed up an old, existing method for a basic compound by transferring it over to a 3.5-µ C_{18} short (5 cm) column. My method uses an aqueous/organic mobile phase containing 25mM phosphate buffer at pH 4.5. The existing method uses a 10-µ, 30-cm column and gives satisfactory retention time reproducibility. On the new C_{18} column, I have been experiencing variations (plus and minus) in elution time. Why? What can I do to improve the situation?

Answer:

The reasons why variations in retention times are occurring on the new column and not the old one will be conjecture on my part, but I am happy to offer some thoughts about this. First, I see a problem with using a phosphate buffer at pH 4.5; that is, phosphate does not "buffer" at this pH. Your mobile phase contains a specific-ionic-strength salt solution that modifies the pH, but the mobile phase does not contain a buffer. Phosphate buffers at pH 1.1–3.1, 6.2–8.2, and 11.3–13.3. The issue between buffers and ionic strength is a real difference that I feel is quite important, especially for today's modern high-performance short columns.

For truly rugged performance, chromatographers need to use buffers and not ionic strength modifiers. In fact, if a method is lacking in ruggedness, the cause of the retention time variations can often be attributed to the interactions between the analyte and stationary phase due to secondary equilibria with the silanol population. Using an ionic strength/pH adjustment rather than a buffer does not address the problem of minimizing the interactions with the surface of the silica. It is conceivable that the introduction of the analyte (which has a pKa) modifies the pH in the micro region around the silanols, which in turn influences the secondary interaction with the surface silanols and, therefore, effects the retention reproducibility. This interaction would vary depending on the concentration of the analyte (or analytes) and the variation in the overall pH and ionic strength of the sample.

Using a salt solution (non-buffer) may be alright in some situations where the secondary interactions do not significantly effect retention or the ionic strength solution sufficiently blocks or minimizes the interaction with the underlying silanols. A rule of thumb is that buffers should be used with modern column technologies (short columns with well-packed beds) because small variations in pH are often manifested in viewable differences in retention behavior.

My suggestion is to use the phosphate buffers at pH 3.0 or 6.2 (not 6.0). However, should this pH of 4.5 actually be the best for the separation, a buffer would need to be found at that pH to insure that the best ruggedness would be obtained. Citrate will buffer in this region, and it spans the range of pH from 2.1 to 6.4. However, citrate does have a UV absorption at low wavelength, so detection may be limited below 220 nm depending on the quality of the detector.

Your situation probably reflects that when a method is developed on a 10-µm particle with a long column length, the number of theoretical plates are low and the peaks are relatively broad. Also, these columns contain a relatively large volume, and any perturbation of the secondary equilibrium at the head of the column will be diluted as the sample travels down the column, thus minimizing the effect of perturbing the secondary equilibrium. Because of this behavior, it is probable that the secondary interactions with the silanols may not have dominated the retention time reproducibility on the older column. Modern short columns have much smaller volumes, and any perturbations of the equilibrium responsible for retention will not be diluted and will be more easily seen in the resulting chromatography.

It is indeed an important consideration to update older methods to improve their speed of analysis. However, the behavior of the older columns compared with the newer ones does involve differences in the "rule of thumb" that is used to develop robust methods. Because of this, mobile phases may have to be adjusted to achieve the goal of fast analysis.

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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