Liquid Chromatography Problem Solving and Troubleshooting

Question:

Why is solute retention often more erratic for normal-phase separations than reversed-phase separations? What can I do to minimize these problems?

Answer:

When carrying out normal-phase separations, two of the more commonly encountered problems are day-to-day fluctuations in retention and slowly drifting retention, which occurs when the eluent is first introduced into the column or a change in eluent composition is made. These problems result respectively from variations in laboratory temperature and slow column equilibration rates.

In the first case, erratic changes in a solute's k' value can be minimized through the appropriate temperature control of the column and eluent. In general, for most separations carried out under reversed-phase conditions, a ± 1°C change in the laboratory temperature (i.e., associated change in the column temperature) will result in a relatively small (2–4%) variation in k'. However, for normal-phase conditions in which heats of adsorption are higher, it is not uncommon to obtain 2–3 times this change in retention. Also, under certain conditions, even greater variations may be observed as the result of changes in the sorbed layer of the solvent present on the surface (1). This latter problem arises because a completely dry silica surface is significantly more retentive toward polar solutes than one that contains a layer of physically sorbed water or other polar modifier such as methanol or iso-propanol.

Temperature control is especially important when normal-phase separations are carried out on highly polar surfaces like silica or alumina using nonpolar carrier solvents such as *n*-hexane that contain small amounts of a highly polar modifier such as an alcohol or traces of dissolve water. These same types of conditions also lead to slowly drifting retention when the column is initially exposed to the eluent, because several hundreds of column volumes of the eluent may need to be passed through in order to reach equilibrium in terms of the sorbed surface layer of the polar modifier.

For example, assuming a very simple single-site titration-type adsorption model for the formation of the surface layer, it would take several hours for a standard 4.6- × 250-mm column containing approximately 4 g of 60 A porous silica to reach equilibrium using *n*-hexane containing 0.5% 2-propanol as the eluent. An even worse condition exists in terms of slow equilibration when *n*-hexane or another nonpolar hydrocarbon solvent is used by itself, because dissolved water is present in only very small amounts. Often these problems can be minimized if an adequate separation can be developed using an eluent that contains larger quantities of a less-polar modifier and by more careful control of the temperature and quantities of trace water dissolved in the nonpolar carrier solvent (2). In the latter instance, one approach has been to use water-saturated hydrocarbons.

References

1. R.K. Gilpin and W.R. Sisco. Effect of temperature on precision of retention measurements in liquid chromatography. *J. Chromatogr.* **194:** 285 (1980).
2. W.R. Sisco and R.K. Gilpin. Role of temperature in normal-phase chromatography using water as the modifier. *J. Chromatogr. Sci.* **18(1):** 41–45 (1980).

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 or answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions or answers.

Roger Gilpin
Associate Editor