Liquid Chromatography Problem Solving and Troubleshooting

Question

A colleague of mine asked if I could explain what "HILIC" meant. This is a new term for me. What is it and how does it work?

Answer

HILIC is an abbreviation that stands for "hydrophilic interaction chromatography." As you may be aware, the language of chromatography is filled with jargon terms, and this is one of the most recent to be used. The first use of the term HILIC appeared in biochemical separation literature to describe the use of a polar stationary phase (polyhydroxylethyl aspartamine) to separate proteins, peptides, and other polar compounds in an aqueous-organic mobile phase (1). Also, because the manufacturer of this column wrote this paper, the term was used to highlight the differentiation of this column from other phases used for separation in this area. Furthermore, the term HILIC more specifically presented this column as unique for a new separation technique for biological molecular separations.

Only recently has this term been applied to small molecules and thus is now being used as a general term. In the most general sense, HILIC is nothing more than using a polar stationary phase in traditional reversed-phase eluents. Such polar stationary phases include bare, unbonded silica, propyl alkyl amine, and ion-exchange-type columns, to name just a few. Although the technique is not new (2), the use of the term HILIC for these small-molecule separations is. One of the earliest reports (2) that describes the chemical interactions involved when using polar columns with reversed-phase eluents used silica gel with methanol–water (pH adjusted) mobile phases. That report demonstrated that retention was a result of two main forces, a polar interaction (e.g., electrostatic) and a weak hydrophobic interaction. We do not often think of silica gel as being weakly hydrophobic, but it can be with respect to a particular sample's polarity. What is relevant for people doing chromatography is that for separations of very basic compounds, a polar column can be used to attain good peak shape (no tailing) and reasonable retention compared with that obtained when using a traditional reversed-phase column in the same type of mobile phases. Thus, this paper demonstrates that it is often possible to overcome problems of tailing and excessive retention of polar compounds on a C18 stationary phase by simply using a polar (e.g., silica) stationary phase in the reversed-phase mode. An example of this is shown in Figure 1. It should be pointed out that the method development for HILIC follows the usual strategy that is used when using a nonpolar bonded phase, except that one uses a polar functionality on the surface of the packing (i.e., alumina, silica, NH_2, and diamine).

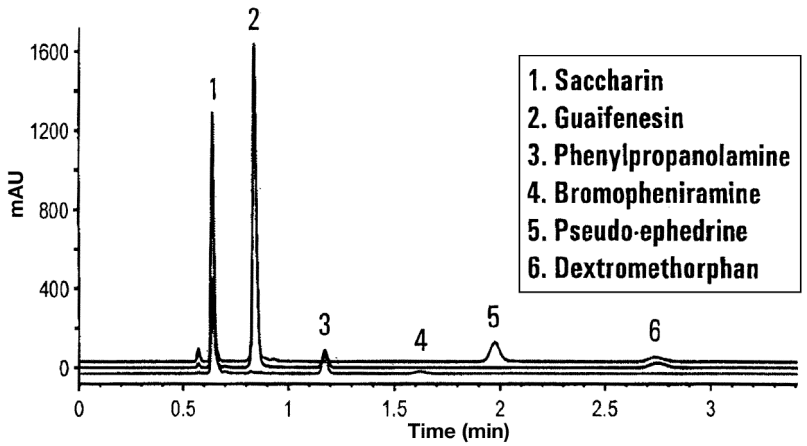

Figure 1. Example separation on a polar column that uses a reversed-phase eluent (the overlay of three different cough syrup formulas). The mobile phase used was MeOH–water containing 20mM dipotassium phosphate (69:31, pH 7). The column used was a Zorbax Rx-sil (silica) (4.6 × 150 mm).

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 it 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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Unfortunately, chromatography has too many jargon terms. Also, there has been a propensity to add more terms as the technology grows into new application areas. The result is increased confusion in how to use various chromatographic techniques. Instead of adding jargon terms, one may wonder why we do not focus on unifying the underlying separation mechanisms in order to attempt to classify mechanistic similarities in the various existing modes. The conclusion I come to is that since HPLC has had an experiential development in a number of scientific disciplines, there has been the tendency to name "new" approaches—at least new to the developer. Also, I guess that if it was easy to unify things, someone would have attempted to do it already.

References

1. A.J. Alpert. *J. Chromatogr.* **499:** 177 (1990).
2. B.A. Bidlingmeyer, J. K. DelRios, and J. Korpi. Separation of organic amine compounds on silica gel in the reversed-phase mode. *Anal. Chem.* **54:** 442–47 (1982).