Liquid Chromatography Problem Solving and Troubleshooting

Question

What is meant by the term methylene selectivity and how does it influence what I observe in carrying out reversed-phase separations?

Answer

Methylene selectivity refers to the incremental increase in retention for a series of homologs with the addition of an increasing number of methylene carbons in their structures. Considering this concept in slightly different terms, on a thermodynamic basis, each additional CH_2 unit results in a predictable increase in the sorption energy (ΔH) for the solute and thus a predictable change in its retention.

The effect of additional carbon atoms on the reversed-phase (RP) retention is easily illustrated using a series of closely related neutral solutes such as the parabens (*p*-hydroxybenzoates). The first four homologs in the series are an important group of compounds used as pharmaceutical additives that vary only in their structure by the length of the alkyl chain. Shown in Figure 1A is the RP separation of a mixture of methyl, ethyl, propyl, and butyl parabens (i.e., peaks 2–5, respectively) on an octadecyl surface using methanol–water as the eluent. Under these RP conditions, differences in retention are governed by the increasing hydrophobic character of the alkyl group and its interaction with the solvated bonded octadecyl phase. The effect of increasing hydrophobicity on the chromatographic retention factor (k') is illustrated in Figure 1B.

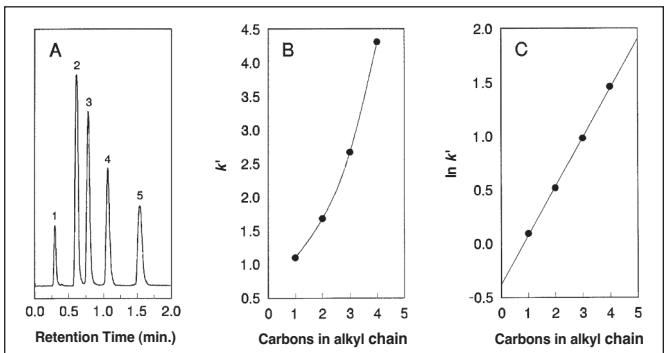

Figure 1. RP separation of paraben homologs.

In general, assuming no steric problems, the relationship between the retention and size (i.e., carbon number) of the aliphatic portion of homologs is logarithmic, and a plot of ln k' versus the carbon number is linear with a slope related to the incremental methylene selectivity (1). This predictable retention relationship is illustrated in Figure 1C. The substitution of a series of other nonpolar groups in place of the methylene units will also result in incremental retention addition (2). Likewise, the addition or removal of a methyl carbon to a molecule will result in similar increases or decreases in RP retention and is often a very useful approach in choosing an internal standard.

It is important to recognize that similar predictable chromatographic behavior is observed with acidic and basic homologs assuming their ionization can be suppressed by the appropriate pH buffering of the eluent. For example, simple fatty acids (having the chemical structure RCOOH and a pK_a of approximately 4.7) follow similar trends if the hydro-organic eluent is buffered to the pH range of approximately 3 to 3.2.

References

1. R.K. Gilpin, M. Jaroniec, and S. Lin. Dependence of methylene selectivity on the composition of hydro-organic eluents for reversed-phase liquid chromatographic systems with alkyl bonded phases. *Chromatographia* **30:** 393–99 (1990).
2. M. Jaroniec, S. Lin, and R.K. Gilpin. Characterization of the surface composition of alkyl bonded phases under reversed-phase liquid chromatographic conditions using homologs of alkanoate and perfluoroalkanonate esters as solute probes. *Anal. Chem.* **62:** 2092–2098 (1990).

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