Liquid Chromatography Problem Solving and Troubleshooting

Question

I have been trying to separate a group of simple mono- and diprotic organic acids using an ion-pairing reagent in the eluent and have noticed that it seems to have a larger effect on the diprotic acids than it does on the monoprotic acids. Is this what I should expect or am I doing something wrong?

Answer

Although your question does not indicate what you mean by a "larger effect", I assume you are referring to the fact that you have tried a series of ion-pairing (IP) reagents with different alkyl chain lengths in order to adjust the retention. Furthermore, when you did this you found that the relative changes in the retention times of the diprotic solutes were greater than the changes in retention times for the monoprotic solutes as you tried IP compounds with increasing alkyl chain length. If this is what you are referring to in terms of a "larger effect", then the trend that you have observed is what you should expect.

In order to help you understand what is happening, it is useful to briefly review how retention under reversed-phase conditions is influenced by increasing hydrophobicity of the solute. Although this particular concept has been discussed in a previous Troubleshooting note (1) that dealt with methylene additivity and its incremental effect on retention for a series of homologues, it is useful to restate the basic idea that, on a thermodynamic basis, each additional CH_2 unit within a molecule results in a predictable increase in its sorption energy and thus a predictable change in its retention. Because this is a thermodynamic relationship, a plot of the natural logarithm of the retention factor vs. carbon number for homologues is linear, assuming no steric complications. However, if steric considerations are affecting the retention, then the predicted value for k' for the next homologue of increasing size will be greater than the experimentally observed retention factor. Typically, with smaller solutes this is less of a problem than it is for larger, more structurally complex solutes.

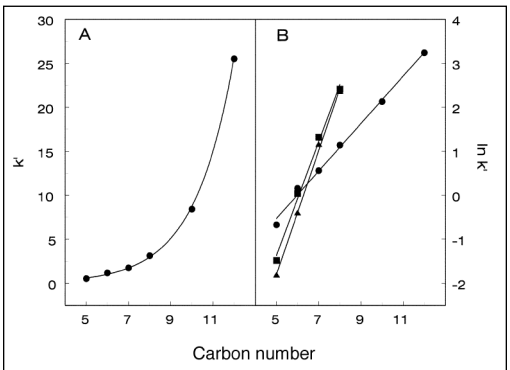

Figure 1. Effect of increasing the alkyl chain length of alkyltriethylammonium IP reagents on the reversed-phase retention of mono- and diprotic acids: (A) benzoic acid and (B) benzoic Acid (filled circles) and 1,2- and 1,3-benzenedicarboxylic acid (filled triangles and squares, respectively). An ODS column was used with 60:40 methanol–water.

Based on what has been stated, if we now consider the influence of the different IP reagents you have tried on retention, the trend you have observed will follow a similar incremental relationship, assuming the different IP compounds are homologues (e.g., a series of $R(R')_3N$ salts, where R' is typically a methyl or ethyl group and R varies between C_4 and C_{12}). Because you are separating organic acids, I assume this is the type of IP you used. As such, under reversed-phase conditions a plot of the retention factor vs. carbon number of the IP reagent homologues will follow an exponential trend that can be linearized via making a plot of ln k' vs. the carbon number.

Shown in Figure 1A is a plot of the retention factor vs. carbon number for benzoic acid chromatographed on an octadecyl column using 60:40 methanol–water and varying alkyltriethylammonium IP reagents ranging from the penyl to dodecyl compounds. Because each additional carbon in the IP reagent contributes incrementally to the solute-pair sorption energy, a plot of ln k' vs. carbon number will be linear. This is illustrated in Figure 1B for benzoic acid, which appears as the data depicted by the filled circles. Also shown in Figure 1B is the retention behavior of two simple diprotic acids (1,2- and 1,3-benzenedicarboxylic acid). If one compares the slopes of these compounds with the slope obtained for benzoic acid, it can be found that they are approximately twice. This is the result of two IP molecules associated with each diprotic molecule compared with one in the case of monoprotic acids.

References

1. R.K. Gilpin. Liquid chromatography problem solving and troubleshooting. *J. Chromatogr. Sci.* **38:** 465 (2000).

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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