**Instructor Comments.**

Here are some notes about this exercise using the HPLC simulator. The entire assignment can be done as homework, but I found that it works well to do the first part as a class activity. The students are asked to play with the fraction of organic solvent, ø, and to observe the influence that it has on the chromatogram of five compounds. What conditions give the best resolution?

The next task is to come up with a model that will reliably predict the retention of a compound as a function of ø. We assume that if a model works well for two compounds, it will apply to the others also. The students work with one other person to record the retention factor, k’, as a function of ø from 20% to 80%. They can very easily generate a plot of k' vs. ø with a spreadsheet and use the regression feature to find what sort of equation best fits the data. My experience was that some people accepted a poor fit after a hasty guess, however, they were quickly persuaded by other students that an exponential expression (k'=ae-bø) works best. That provided an opportunity to talk about scrutinizing graphical models and minimizing residuals.

I gave them three different models to consider, but I'm not sure that was necessary. However, I do think that it is a lot easier to work with the logarithmic version,

lnk' = a + bø. It’s intuitively friendlier and algebraically easier to find the slope and intercept for a linear equation for each compound using the data from only two chromatograms.

The remaining part of the exercise involves finding the optimum conditions for the best resolution. (It is based on a graphical strategy called “window analysis” developed by Laub and Purnell [*J. Chromatogr.* **1975**, *112*, 71; *Anal. Chem.* **1976**, *48*, 799] for mixed stationary phases in GC and later applied to optimizing mobile phases in LC by Deming [*J. Chromatogr.* **1980**, *199*, 317].) I gave this part as a homework assignment. Once they have the specific equation for each compound, the students can quickly generate a table that calculates lnk' for each compound for an organic fraction from ø = 0.1 to 0.8 in steps of 0.02. In each row (corresponding to a specific value of ø) the two values that are numerically most similar indicate which two compounds are the least well separated under those conditions. The students are asked to create a final column that takes the difference for that one pair. Of course, this is equivalent to the logarithm of the separation factor, a. This is a little bit tedious, since one must visually inspect 40 different rows separately. A graph of this column vs. ø produces triangular windows. The tops of these triangles coincide with conditions where the separation factors are highest for the most difficult-to-separate pair. Going back to the HPLC simulator the students can try the optimum conditions and get the satisfaction of seeing the peaks baseline resolved.

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| **ø** | **cmpd 1** | **cmpd2** | **cmpd3** | **cmpd4** | **cmpd5** | **Min.** |
|  | lnk’ | lnk’ | lnk’ | lnk’ | lnk’ | Lnk’1-lnk’2 |
|  |  |  |  |  |  |  |
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