Lab 8
HPLC Separation of a Mixture of Hydrocarbons

Introduction. One often hears that gas chromatography (GC) performs separations better than liquid chromatography (LC). In a previous lab, a mixture of hydrocarbons (toluene, ethylbenzene and the three xylenes) was separated by GC. In this lab you will separate the same mixture by high-performance liquid chromatography (HPLC) to make a comparison for this sample. An appropriate HPLC stationary phase (a hydrocarbon monolayer) and a mobile phase of a mixed composition of acetonitrile and water will be used. These conditions give separation times comparable to those of the GC experiment.

HPLC Background. There are two physical phenomena that determine whether a mixture can be resolved in chromatography: 1) *efficiency*, *N*, which is expressed as the number of theoretical plates, and 2) *selectivity*, which is expressed as the ratio of capacity factors, *k*'. One common expression for resolution, R_s, shows how these two properties, *N*, and *k*' ratio, combine to describe resolution for two components, **a** and **b**.

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{k'_a}{k'_b}\right)\left(\frac{k'}{1+k'}\right) \qquad 8\text{-}1$$

In the third term, *k*'/(1+*k*'), it can be seen that increased retention increases resolution (larger values for R_s mean better resolution). In GC, the increase in *k*' with decreasing temperature was used to advantage in the recent GC lab to resolve the mixture. In HPLC, the mobile phase composition is typically used to change *k*' for optimizing the separation. The stationary phase is relatively non-polar in this particular LC experiment, so an increasing amount of water in the mobile phase increases *k*' because it drives the equilibrium of the non-polar analyte more toward the non-polar stationary phase and out of the polar mobile phase.. Since it takes time for the LC column to re-equilibrate when the mobile phase is changed, it would not be practical for us to try to change the mobile phase during your lab period. Instead, we provide you here with a chromatogram for the case of 80% acetonitrile/20% water, and you will run the chromatogram for the case of 70% acetonitrile/30% water.

In this lab, you will compare the resolution obtained with GC and LC for the separation of toluene and ethylbenzene. With reference to the three terms of Eq. 8-1, you will determine whether the improved resolution is due to higher *N*, a higher ratio of k'_a/k'_b, or higher *k*'.

GC versus LC. One difference between GC and LC is the column efficiency, which is typically higher in GC. The main reason why GC typically has a higher efficiency than LC is that GC uses a capillary column while LC uses a packed column. There are three contributions to the efficiency, *N*, also known as the number of theoretical plates: quality of the packing (A), longitudinal diffusion (B), and mass transport (C). These are related to the efficiency through the *van Deemter equation*,

$$H = A + B/v + Cv \qquad 8\text{-}2$$

where $H \equiv L/N$, *L* is the column length, *v* is the flow rate of the mobile phase, and *A*, *B* and *C* are constants for a particular column, set of conditions and analyte. *H* is often called the *height equivalent to a theoretical plate*. One can see that the smaller the value of *H*, the higher the

value of N. The A term contributes at all flow rates and is independent of the flow rate v. For a capillary column (as in GC), $A \sim 0$. For a packed column (as in HPLC), A is often the largest term. It is the fact that the column is packed that makes LC typically lower in efficiency. In specific instances, k' ratios or a larger k' can determine resolution, which is why it is only a general statement, rather than a rule, that GC gives better resolution than LC. GC is often faster than LC because high flow rates can be used for gases due to their low viscosities. For resolution at a given separation time, GC is often the method of choice.

So you might be wondering why LC exists if there are so many aspects that make GC potentially better. LC exists because there are many compounds that are not vaporous or that decompose before vaporizing. These include nearly all biological molecules and pharmaceuticals. LC is the mainstay of the pharmaceutical industry.

PRE-LAB ASSIGNMENT

1. *Efficiency calculation.* For the GC lab, you collected a chromatogram at 60 °C. Assume that the peak eluting at $t_r = 3.79$ min can be fit to the Gaussian function below. Calculate the column efficiency using $N = (t_r/\sigma)^2$.

$$\exp\{-(t - 3.79)^2/(2 \times 0.015^2)\}$$

2. *Selectivity calculation.* For the GC collected at 60 °C, calculate values of k' for ethylbenzene and for toluene using $t_0 = 1.6$ min. Calculate the ratio of k' values for these two peaks, using the larger number in the numerator. Note that $k' = (t_r - t_0)/t_0$.
3. At the end of this write-up is a liquid chromatogram for the mixture using a mobile phase composition of 80% acetonitrile/20% water. The peak at 2.79 min. (indicated by arrow) fits well to a Gaussian function below. Calculate the column efficiency for this peak using $N = (t_r/\sigma)^2$. Compare with that for GC.

$$25 \times \exp\{-(t - 2.715)^2/(2 \times 0.022^2)\}$$

4. Do you expect the LC resolution to improve for 70% acetonitrile/30% water compared to 80% acetonitrile/20% water?

EXPERIMENTAL

In this lab, you will be given the same mixture as you were given for the GC lab, the instrument will be set up for the correct separation conditions, and the mobile phase will be an equilibrated 70% acetontitrile/30% water. You will be instructed on how to run the samples. You will see that the three xylenes are not resolved by LC, but that toluene and ethylbenzene are resolved. You will compare the resolution for the 80% acetonitrile/20% water in the chromatogram provided below with that of 70% acetontitrile/30% water. To identify which peak is which, you will run the individual samples of toluene, ethylbenzene and one of the xylenes. Print out the chromatograms in each case and obtain text files on diskette of each chromatogram.

WRITTEN REPORT

1. For the liquid chromatogram of the mixture, label the identity of each peak.
2. Calculate the column efficiency *N* using the ethylbenzene peak.
3. Calculate values of *k*' for ethylbenzene and for toluene. Calculate the ratio of these two peaks, using the larger number in the numerator. Use the value of t_0= 1.2 min.
4. Compare the efficiency *N* and the *k*' ratio with those for GC. Explain which factor contributes more to the better resolution of GC: column efficiency or selectivity.

This lab was created by graduate student Ms. Stuti Christie and Professor Mary J. Wirth in Spring 2003. It was revised by Professor Thomas P. Beebe, Jr. in November 2003.

Figure 8-1. Liquid chromatogram of toluene, ethylbenzene and three xylenes for a mobile phase composition of 80% acetonitrile/20% water. The xylenes elute as one peak for this mobile phase composition.