Quantitative Analysis Assignment – Contributed by Professor Anthony Borgerding, University of St. Thomas

Chem 300
HPLC Lab on Theory Plus a Measurement of PFCs by HPLC-MS Using an Isotopically-Labelled Internal Standard

Part II. Impact of column and mobile phase properties on chromatographic separation. This project will be performed using the wonderful HPLC Simulator developed by Dwight Stoll and colleagues. **The report for Part II will be simply to answer the highlighted questions posed as you go through the procedure using the simulator**.

1. Log on to http://www.multidlc.org/hplcsim/hplcsim.html. This program uses equations from the Golay equation, along with physical properties of various analytes, to predict the retention time, peak width, and other properties of analytes in an isocratic or gradient separation. All of the simulations assume a packed column and a nonpolar stationary phase, which is by far the most common column type for HPLC analysis.

2. You should see a default chromatogram containing peaks for phenol, benzonitrile, p-chlorophenol, acetophenone, and nitrobenzene. You will only see 4 peaks because benzonitrile and p-chlorophenol co-elute.

3. You should also see a stack of 5 pull-down menus: "Manage Compounds", Mobile Phase Composition", "Chromatographic Properties", "General Properties", and "Column Properties".

4. Click on "Manage Compounds". From the list of compounds, click the box next to ethyl benzene, p-xylene, propiophenone and Loratadine. You should see four additional peaks in your chromatogram and 9 compounds listed on the table of data below it.

5. Click the "Manage Compounds" bar again to minimize the pull-down menu.

6. Studying the impact of mobile phase on retention and peak width. Click on the "Mobile Phase Composition" bar to open the pull-down menu. Note that there are two liquids that can make up the mobile phase: water and acetonitrile. The ratio of these two liquids determines how polar the mobile phase will be (lower % acetonitrile means more polar). Note also that you can choose between "Isocratic Elution", which maintains a constant ratio of mobile phases (similar in behavior to isothermal in GC) and "Gradient Elution" in which the ratio of water and acetonitrile changes as the separation progresses.

    a. Using isocratic conditions, click and drag the circle/bar graphic that allows you to rapidly change the %B (% acetonitrile), and observe the changes in retention time of the analytes. **Q1**: *Are the analytes retained more at a*

higher % acetonitrile or lower? Does this correspond to a more or less polar mobile phase?

b. Adjust and set the %B to the highest value that allows for baseline separation of benzonitrile and p-chlorophenol (peaks 2 and 3 when resolved). **Q2**: *What is the highest %B where that happens?* Note that, under these conditions, the peaks for ethylbenzene and p-xylene are unresolved and very wide. In fact, the resolution between these two very non-polar compounds gets worse as their retention increases (verify this for yourself).

c. Switch to "Gradient Elution" Set the %B at time zero to the value you calculated in the previous step. Note that the peaks are not well resolved. This is because the ramp begins immediately at time = 0 minutes and because the polarity decreases so fast (to 95% acetonitrile in only 5 minutes). Decrease the %B at time zero to 25 and increase the time in the second row to 12 minutes. Note that all of the peaks except for ethyl benzene and p-xylene are resolved. **Q3**: *How does the overall time of analysis compare to the step above (6.b)? What caused that change?*

7. Studying the Impact of Flow Rate (Part 1).
   a. Click the "Chromatographic Properties" bar to open its pull-down menu. Change for "Flow Rate" from 2 to 1 (units are mL/min). Observe the resolution, retention times, and value of HETP (calculated in the table below). Record the value of HETP and the open tube Flow Velocity (for comparison later). **Q4**: *Explain these observations in terms of plate theory. Specifically, is HETP more impacted by longitudinal diffusion or by resistance to mass transfer under these conditions?*
   b. Record the backpressure calculated at the bottom of this pull down menu (for comparison later), then click on the "Chromatographic Properties" bar again to close the pull-down menu.

8. Studying the Impact of Column Properties. Click on the "Column Properties" bar to open its pull-down menu.
   a. Change the "Particle Size" from 3.0 to 1.5 um, which is the state of the art for the most expensive columns today. Observe what happens to the resolution between ethylbenzene and p-xylene. Also go back and click on Chromatographic Properties to see what happened to the value of HETP compared to the one you recorded in step 7a. **Q5**: *Explain these observations in terms of plate theory. Which term is most impacted by particle size?*
   b. On the Chromatographic Properties pull down menu, record the backpressure and compare it to what you recorded in step 7b. **Q6:** *Explain your observation.*
   c. Decrease the Inner Diameter from 4.6 mm to 2.0 mm. Observe the changes in retention time and resolution. On the Chromatographic Properties pull down menu, record the open tube flow rate and compare it to what you recorded in step 7a. **Q7**: *Explain these observations in terms*

of plate theory. Specifically, given that mass transfer in the mobile is minimal for packed columns, is longitudinal diffusion or resistance to mass transfer more important under these conditions?

9. Studying the Impact of Flow Rate (part 2): Click the "Chromatographic Properties" bar to open its pull-down menu. Change for "Flow Rate" from 2 to 0.38 mL/min. Note that the open tube Flow Velocity is approximately equal to the 4.6 mm diameter column at 2 mL/min. Observe the changes in resolution, retention times, and value of HETP. **Q8**: *Explain these observations in terms of plate theory. Specifically, is longitudinal diffusion or resistance to mass transfer more important under these conditions?*

Report for part II. Type your answers for each of the 8 questions posed during the simulations you were asked to do.