

Exploring liquid chromatography with an HPLC simulator

Introduction

The purpose of this activity is to gain some familiarity with the types of parameters and concepts that influence the efficiency of a chromatographic separation. We will be using a website that simulates HPLC which can be found here:

<http://www.multidlc.org/hplcsim/hplcsim.html>

As with other simulator activities, you are encouraged to “play” with the instrument without worry about breaking it. If the simulation gets out of control or nonresponsive, simply reload the web page.

By the end of the activity, you should be able to do the following:

- Manipulate separation math equations to obtain desired parameters such as void time, retention and separation factors, and peak resolution.
- Explain the effect of solvent composition on the efficiency of a separation
- Relate the polarity index of a mobile phase to the selectivity factor
- Identify the optimal flow rate through the construction of a van Deemter diagram
- Develop a gradient method for optimizing the separation of a mixture.

Prior to completing this activity, you will need to know the separation math equations which have been presented in other parts of this course (retention time, void time, retention factor, selectivity factor, resolution, theoretical plate heights and numbers, the van Deemter equation). The various activity parts will ask you to explore phenomena, but not provide step-by-step details of how to perform the tasks.

In the parts that follow, you will find the following commands:

- **[DO]** means you are to follow the directions provided; however, there is nothing you need to respond to.
- **[EXPLORE]** is similar to above; however, you are not provided with strict instructions. Exploring typically will involve looking at the user interface options, moving sliders and in general becoming acquainted with the activity.
- **[REPORT]** is a question or task that requires a response from you. These responses are typically short answer, calculations or table generation.
- **[PLOT]** tasks require you to use Excel (or similar) to create a plot of some sort. In this activity, you may submit a separate Excel workbook with your assignment (no need to embed into this document) and you do not need to format plots properly, save for axes labels
- **[WRITE]** means you are expected to write a paragraph in response to the question or task. It can be included in this document or a separate one.

Part 1: Calculating retention factors, selectivity factors and resolution

Exploring the simulator

The HPLC simulator opens with a set of menus on the left-hand side, a chromatogram and below the chromatogram a table of information.

- **[DO]** Count the number of peaks and compare it to the number of compounds that are displayed.
- **[EXPLORE]** Click on the compound names in the table to highlight each of the compounds. Identify which two compounds are not resolved.
- **[DO]** The void time for the default settings is 31.91 s. Using the retention time in the table for phenol, confirm that the retention factor (k') is being calculated properly.
- **[EXPLORE]** The void time is provided in one of the menus on the left-hand side. Find it.
- **[REPORT]** Calculate the selectivity factors for (a) benzonitrile and p-chlorophenol and (b) p-chlorophenol and acetophenone. Use the retention factors provided in the table.
- **[REPORT]** Calculate resolution of (a) benzonitrile and p-chlorophenol and (b) p-chlorophenol and acetophenone. The width at the base of an ideal chromatographic peak is 4 times the standard deviation (σ) of the peak.

Exploring column temperature effects

Column temperature does not play a critical role in HPLC separations; however, it does have an impact on the separation efficiency. The simulation allows you to change the temperature of the column under the chromatographic properties menu.

- **[EXPLORE]** See how column temperature impacts the shape of the chromatogram.
- **[REPORT]** What temperature do you think results in the best separation of the five components? Describe what has happened to the chromatogram (peak intensities, shapes, locations and elution times).

Part 2: Optimizing a separation using mobile-phase composition

You are now going to revisit the overlapping peaks in the default set of the compounds. In HPLC, mobile phase composition plays a significant role in separation efficiency.

- **[EXPLORE]** Open the mobile phase composition menu and make note of the possible settings. Explore the settings. When done, reset the simulator.
- **[PLOT]** Create a table with the retention factors of benzonitrile, p-chlorophenol, and acetophenone as a function of mobile phase composition. You may change the mobile phase composition in increments of 10 percent. Display these three series on a single plot.
- **[WRITE]** Write a figure description paragraph about the plot you just created. Be sure to include a description of the general trends, any key findings, and unexpected results.
- **[REPORT]** Calculate the selectivity factor between p-chlorophenol and acetophenone for each of the solvent compositions in the previous table and comment on whether or not selectivity factor is a good indicator of the resolution between two peaks.

- **[REPORT]** Calculate the resolution between p-chlorophenol and acetophenone for each of the solvent compositions in the previous table and comment on what value results in a poorly resolved set of peaks.
- **[EXPLORE]** Find the mobile phase composition that gives baseline separation of the five compounds **and** is completed as quickly as possible (we are all impatient).
- **[REPORT]** It is possible to get an even shorter run time if we sacrifice peak resolution. Find a mobile phase composition that resolves all of the peaks *at least half-way* (so the peaks are separated at $\frac{1}{2}$ the maximum height at least). Report the solvent composition, the run time and the worst peak resolution. (What are the two compounds and what is their resolution.)

Part 3: Exploring mobile-phase further

In the previous activity, we explored the impact of solvent composition but did not know why it was happening. Separation efficiency is based upon the polarity of the mobile phase, stationary phase and analytes. We can explore this concept with a new term, the polarity index. Look at page 794 of your textbook for calculating the polarity index. You will explore two questions in this part of the activity: do two mixtures with the same polarity index yield the same separation? How does polarity index affect retention factor?

- **[DO]** For this part you will change the compounds to phenol and bromobenzene. *Note: I sometimes found that the simulator wanted me to put more compounds in the list, go ahead and do so if you need to and just ignore the others.*
- **[DO]** Find the retention factor for each solute at solvent compositions varying from 100% water to 100% acetonitrile (in increments of 20%). Place the % composition in column A of an Excel sheet and the retention factors in columns C and D (we'll use B in the next step).
- **[DO]** Refer to page 794 of your textbook and calculate the *polarity index* for each one of the solvent compositions.
- **[PLOT]** Create a plot of $\text{LOG}(k)$ as a function of P for each of the solutes. Place the data on the same graph.
- **[REPORT]** Theory predicts that the plot you created will be linear with a slope of 0.5. Comment on how accurately theory predicted your experimental results.
- **[DO]** For each of the polarity indices calculated above, find the corresponding composition of water and *methanol* that gives the same value. For example, a 40% water/acetonitrile mixture has a polarity index of 8.44. Using the information from page 794 in your textbook, you can find that a 34.5% mixture of water/methanol will yield the same P value.
- **[DO]** Switch the solvent composition to methanol and create a new table of retention factors at the solvent compositions you just calculated. Add these two new datasets to your plot.
- **[REPORT]** If the solvent does not interact with the solute, the same P -value should yield the same retention factors. Using the slopes and intercepts of the four lines you have plotted, comment on how (or if) the nature of the solvent is impacting the separation process.
- **[WRITE]** Create a figure description paragraph of the $\text{Log}(k)$ vs. P plot. You may (should) use the findings that you “reported out” to construct this paragraph.

Part 4: Optimizing flow rate

In this part of the activity you will explore how to optimize the mobile phase velocity using the van Deemter equation. You will also obtain the parameters for the van Deemter equation under a different set of conditions and report on the effects.

- **[DO]** Reset the simulator. Open up the Chromatographic Properties menu to see the flow rate and the height of theoretical plates.
- **[PLOT]** Create a table exploring how flow rate influences the height of theoretical plates. Change the flow rate in increments of 0.2 up to 2.4 mL/min and then by increments of 0.5 up to 7 mL/min.
- **[DO]** Use Excel's solver routine to find the van Deemter parameters. A separate video will be created to show you how to do this.
- **[DO]** Change one parameter. You can either change the solvent composition (double the percent acetonitrile), change the solvent type (switch to water/methanol) or change the column temperature (double the temperature). Create another van Deemter plot using the new condition. (Put this plot on the same graph as the previous one.) Find the new van Deemter parameters.
- **[WRITE]** Write a figure description paragraph describing the change. Discuss the difference in optimal mobile-phase velocities and use the calculated parameters to identify which one (or ones) had the most effect on the change.

Part 5: Gradient mobile phase for complex mixture

Lastly, you will explore how a solvent gradient can dramatically improve the separation efficiency when a wide range of solutes are in the mixture.

- **[DO]** Reset the simulator and add valerophenone and hexanophenone to the compound list.
- **[EXPLORE]** Find (if possible) conditions that will separate all seven species within a reasonable (10 min) amount of time.
- **[DO]** Change from isocratic mode to gradient mode. In gradient mode, the solvent composition is gradually changed from one ratio to another over a fixed period of time. Typically, one traverses 95% water to 5% water (note that the simulator allows you to change B, so the numbers are backwards). You will keep these fixed and only change how steep the gradient is by changing the ramp time.
- **[REPORT]** What are the conditions for an ideal separation of this mixture? (solvent composition, gradient time, base-line separated peaks and short run time)