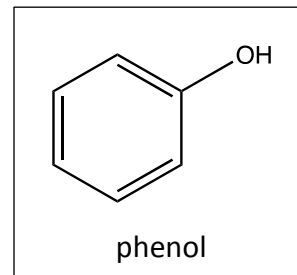
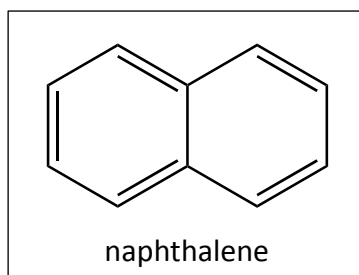
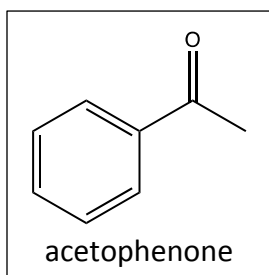

HPLC Dry Lab

In this dry lab you will use an online simulator to examine how different parameters in a high performance liquid chromatography (HPLC) can be used to improve the separation between peaks in the resulting chromatogram. HPLC is used to separate, identify and quantitate multiple components in a mixture. Because of its adaptability and applicability to a wide variety of analytes and sample times, HPLC is one of the most widely used types of measurement used in modern analytical chemistry.

In addition to information provided in the pre-lab lecture and JoVe video (links on OAKS), sections 21-2 and 22-3 in your textbook should be helpful to provide additional information about this type of analysis.

To start the dry lab, go to the “Multi-Dimensional Separations” HPLC Simulator (created by Dr. Dwight Stoll of Gustavus Adolphus College) at <http://www.multidlc.org/hplcsim/hplcsim.html>.

1. Select the following compounds from the “Manage Compounds” list. Make sure all other compounds are deselected.



2. The stationary phase is a C18 column and the mobile phase is a 60%-40% mixture of water and acetonitrile. Would you characterize this as normal phase or reverse phase chromatography?

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The table below the chromatogram lists the compounds in order from first to last eluting compound. The most important headings for this exercise are explained below.

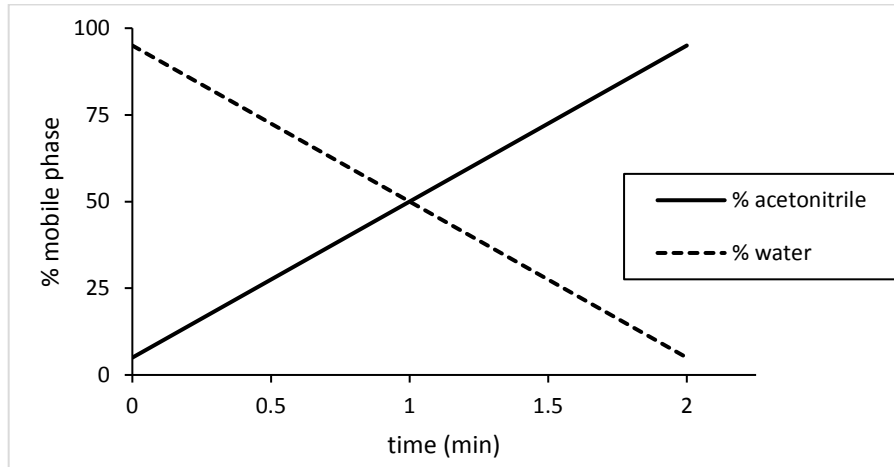
Compound	k'	t _R (min)	σ (s)
phenol	0.9134	0.6105	0.5523
acetophenone	1.9172	0.9308	0.8282
p-nitrotoluene	4.1459	1.6420	1.4483

- **k'** is the retention factor. A larger retention factor denotes a greater affinity for the stationary phase, resulting in a longer retention time.
 - **t_R** is the retention time is the time between the sample's injection and the maximum detector response for the peak.
 - **σ** is the variance, which is related to the width of the peak. If we treat the peak as a perfect Gaussian shape, we can relate variance to the full width half max ($w_{1/2}$) through the following equation: $w_{1/2} = 2.35\sigma$
3. Rank the compounds from most polar to least polar. Use evidence of their elution order to justify your answer.

Mobile Phase Composition

4. The default has the mobile phase composition of 60% water (solvent A) and 40% acetonitrile (solvent B), and is constant throughout the chromatographic separation (isocratic elution mode). Increase and decrease the fraction of acetonitrile to observe its effect on the separation. What is the benefit of increasing acetonitrile (and decreasing the water)? What is the benefit of increasing the water (and decreasing acetonitrile)?

5. Gradient elution is used when the mobile phase composition is changed during a separation. For example, set % B to 5% at 0 min and % B = 95% at 2 min. This means the mobile phase composition changes as follows:



Compare the resulting chromatogram obtained with the gradient elution conditions described above to the chromatogram obtained with isocratic elution set at the initial (5% acetonitrile) mobile phase composition. Compare the gradient elution chromatogram with one obtained with the final conditions (95% acetonitrile). Based on your observations, what is the benefit of using gradient elution in HPLC?

Column Properties

Return the mobile phase conditions to 40% acetonitrile in isocratic elution mode. Next, examine the conditions under the heading “Column Properties.”

6. Examine the role of column length by doubling it to 200 mm and then halving it to 50 mm. Suggest a benefit to a long column in chromatography. What about a short column?

- After returning the column length to 100 mm, investigate how particle size influences the separation. Adjust the particle size between 1 – 10 μm . Based on your observations, are small particles or large particles preferred in liquid chromatography?
- Expand the tab under “Chromatographic Properties.” What happens to the back pressure when the particles size is changed? What could be a potential problem with small particles for liquid chromatography?

Resolution and Theoretical Plate Height

Return the column properties to the default settings of 100 mm length and 3.0 μm particle size.

- Complete the table below for the three peaks in the chromatogram in the order they appear. Note you will have to calculate $w_{1/2}$ in minutes from variance (given in seconds) with $w_{1/2} = 2.35\sigma$.

Compound	Retention Time (min)	Variance (σ)	$W_{1/2}$ (min)
1.			
2.			
3.			

- Calculate the resolution between the critical pair, the two most closely eluting peaks.

$$Resolution = \frac{0.589 \Delta t_r}{w_{\frac{1}{2}avg}}$$

Where Δt_r is the difference in retention time of the two peaks and $w_{1/2avg}$ is the average full width, half max. In analytical chemistry a resolution greater than 2 is desirable because it means there is negligible overlap between two neighboring peaks.

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11. In chromatography “theoretical plates” are used as a measure of the efficiency of a separation. It is desirable to have a large number of theoretical plates (N) and a small theoretical plate height (H), which are calculated for each compound with the following equations. (L in the second equation is for column length.)

$$N = \frac{5.55t_R^2}{w_{1/2}^2}$$

$$H = \frac{L}{N}$$

Find the number of theoretical plates and plate height for the three compounds in the chromatogram.

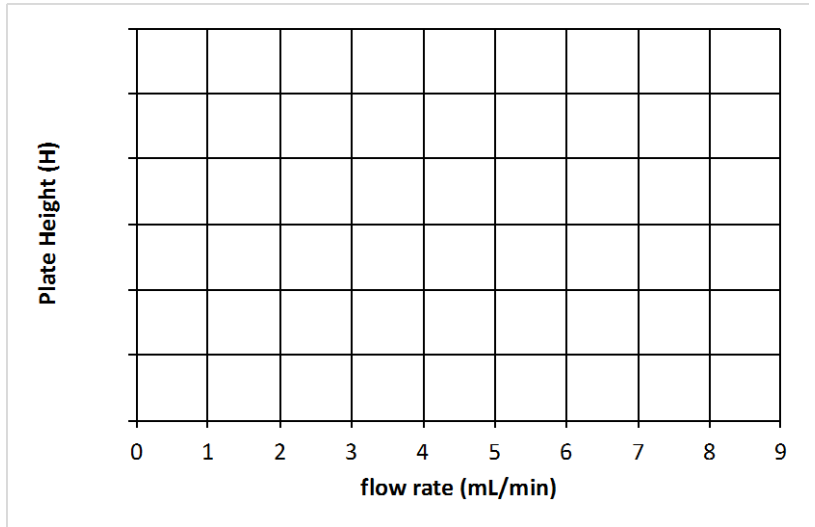
Compound	Number of theoretical plates (N)	Theoretical plate height (mm)
1.		
2.		
3.		

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Flow Rate

12. Under “Chromatographic Properties,” change the flow incrementally from the lowest allowed value (0.05 mL/min) to the highest allowed value (8.0 mL/min). Make a rough sketch of how the theoretical plate height for **naphthalene** changes with flow rate. (Use your Excel skills to make this one easier!)

Flow rate (mL/min)	H (mm)
0.05	
0.1	
1.0	
2.0	
3.0	
4.0	
5.0	
6.0	
7.0	
8.0	



13. Based on your data above, what flow rate do you recommend for the best separation of naphthalene? Justify your answer.