Experiment 2: HPLC Simulation

CH3360: Instrumental Analysis, Plymouth State University


**Introduction**

Due to the variety of stationary and mobile phases available, the ability to mix mobile phases, and the relatively long times required for analysis, the optimization of conditions can be a daunting and time consuming task. In this experiment, you will use a computer simulation of an HPLC system to optimize the separation of a nine-component aromatic mixture. Once an optimized separation has been achieved you will be given a number of unknown samples containing any number of the nine components. You will need to identify the components and report the quantity of each compound.

The HPLC simulator represents the basics of an automated LC system with the following components:

1. A mobile phase supply
2. A pump system with the ability to do binary mixed isocratic or gradient elution
3. An auto sampler with variable injection volumes
4. A UV, diode array detector
5. A computer capable of controlling all the components in the system and analyzing the data.
6. A variety of columns

From the equation for resolution ($R$), you know there are three parameters that can be adjusted to improve the resolution between two peaks: $N$, $k$, and $\alpha$. In this simulation, you will be optimizing the retention factor ($k$), where $k = \frac{t_s}{t_m}$.

To quantify the retention behavior of small solutes in LC, the retention factor, $k$, may be expressed as a function of the mobile phase composition, $\Phi$

$$\log k = \log k_w - S\Phi$$

where $k_w$ is the extrapolated value of $k$ in a poor solvent such as water and $S$ is the rate of change of log $k$ with the solvent composition. Plots of $\log k$ versus $\Phi$ are generally linear, and this relationship may be used to measure the relative retention of a set of solutes and also assess selectivity changes that may be occurring in a given separation. These plots are also suitable for predicting the best solvent compositions to use for a particular separation, rather than adopting a “trial-and-error” approach that could be painstakingly slow in real-life. Points of intersection in the plots of $\log k$ and $\Phi$ represent solvent compositions of co-elution. Therefore, maximizing the differences between $\log k$ and $\Phi$ for a group of analytes contained within a sample results in a value for the most efficient separation conditions, at least with respect to resolution.

To perform this analysis, you will use a pre-developed Excel Spreadsheet. Retention times from the nine-components are entered into the yellow shaded box alongside the solvent compositions at which they were tested. From these retention times, the log retention factor is calculated. Some manual manipulation within the spread sheet is required in order to extract limiting selectivity factors from the data.
**Procedure**

1. Set up the Instrument
   a) Click the “Columns” button. Choose “DuPont C18” and click “OK.”
   b) Click the “Control Panel” button. Turn the Power “On”, set Flow to “1 ml/min”, set Mode to “Isocratic”, set Inj Vol to 10 µL, and set %B to “100”.
   c) Leave the Control Panel open.
   d) Click the “Detector” button. Turn the Power to “On”, set the Wavelength at “254” and the Sensitivity to “.500,” choose “Diode Array”, and click “Done”.
   e) Click the “Computer Data System”. Leave this window open.

2. Set up the Samples
   a) Click the “Samples” button
   b) Inside the Samples window, use the “New Vial” button to create the following:
      - Phenol
      - Standard A
      - Standard B
      - Standard C
      - All 9
   c) Using the “Sample Drawer” to select a sample and the “Add Standards” to set up that sample, make up the samples with 10 mg/mL of each component as follows:

<table>
<thead>
<tr>
<th>Sample: Composition</th>
<th>Phenol</th>
<th>Standard A</th>
<th>Standard B</th>
<th>Standard C</th>
<th>All 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol 2,5-xylenol benzene</td>
<td>Phenol</td>
<td>p-nitrophenol methyl benzoate phenetole</td>
<td>p-cresol anisole toluene</td>
<td>All 9 compounds</td>
<td></td>
</tr>
</tbody>
</table>

   d) To check that your samples are made correctly, open the “Sample Drawer”, choose a sample, and click “View.”
   e) Leave the “Samples” window open

3. Determine the hold up time of the system
   a) Click the “Solvents” button. Choose “Tetrahydrofuran” and click “OK.”
   b) From the Sample Drawer in the Samples window, choose the “Phenol” sample
   c) In the Computer Data System window, inject the sample via Instrument → Inject Sample
   d) Analyze the chromatogram in the Computer Data System window with Data → Integrate Chromatogram → Integration Report

4. Attempt a manual optimization
   a) Click the “Solvents” button. Choose “Methanol” and click “OK.”
   b) Using the “All 9” sample, give yourself 6 runs to attempt to optimize the mobile phase composition (%B).

5. Run the samples
   a) Run each of the Standards at four different phase compositions: 80, 70, 60 and 50% Methanol (%B in the Control Panel). Integrate each chromatogram and record the retention times of the peaks.

6. Find the optimal isocratic composition
   a) Enter the hold-up time and retention times in the Excel Spreadsheet as indicated.
   b) Look at the graph in the “Minimal Selectivities”. The Solvent composition corresponding to the global maximum peak is the optimal %B
   c) Set up and run the “All 9” sample using the optimal conditions
7. Testing Unknowns
   a) In the Sample window, click on the “Unknown” button. Give the sample a reasonable name and enter “3” in the number of components box.
   b) Run the unknown sample using the optimal conditions and integrate the chromatogram.
   c) Determine a way to identify and quantify the peaks in your unknown. Run the necessary chromatograms and analyze your unknown.
   d) Go to the Sample Drawer and View your unknown to see if your analysis was correct.

Analysis and Conclusions
1. Discuss the results of the manual versus the systematic optimizations.
2. Describe the optimal conditions determined in Step 6. Discuss generally why these conditions were optimal, with regard to the resolution of various peaks and length of analysis for other conditions.
3. Describe how you could use the systematic approach to optimize a gradient elution program. (If time permits in lab, you may try this!).
4. What were the results of your unknown testing?