Considerations Prior to Beginning Experiment
- Filter and degas the solvents you will be using with the special suction filtration apparatus and filter. Fill the solvent reservoirs with the solvents. Be sure that all of the reservoirs contain enough solvent to exceed the time necessary for the experiment. (It is wise to double the quantity of solvent you might expect to use to avoid refilling the reservoirs in the middle of the experiment.)

Instrument Start-up Procedure
Start Up Procedures:
1. Open the main valve on the helium tank. Double-check that the pressure is correctly set to ~70 psi.
2. Turn on the components: pump, degasser, autosampler, detector, data collection system (PeakSimple), and computer.
3. Ignite the lamp on the detector by pushing the “LAMP ON/OFF” button and then pushing “ENTER”. The “LAMP” light should turn on. Be sure to give the lamp ~30min to warm up before doing any quantitative work. Detection wavelength can be set by pressing the “λ” button on the detector, entering the appropriate wavelength, and pressing “ENTER”.

Preparing the Instrument for a Measurement
Initiating Solvent Flow:
1. Prior to executing a separation, the solvent lines of the system must be filled. This involves purging the system of air or old solvent.
2. If you have switched solvent systems, or observe air in the solvent lines, you should first manually purge and fill the lines for each solvent you will be using. If the system has been used recently and you have not changed any solvent, skip to step 4.
3. To manually purge a solvent line, disconnect the fitting for that line at the pump inlet (these are finger-tight fittings, no tools are necessary). Attach the tube to the syringe adapter (see photo) and use the syringe to draw solvent through the lines. Continue drawing solvent until no air bubble are visible in the line (this may take several mL). Re-attach the line to the pump and repeat the process for each solvent line that will be used in the separation.
4. Once you have manually filled the solvent lines, use the pump’s purge operation to finish the job. Begin by opening the ball-valve on the pump manifold (see illustration). Opening this valve directs effluent from the pump directly to waste, bypassing the separation column.

5. **DO NOT PURGE THE SYSTEM WITH THE BALL VALVE CLOSED! THIS WILL RESULT IN PRESSURE TOO HIGH FOR THE PUMP TO HANDLE AND CAN RUIN THE COLUMN!!!**

6. Initiate solvent flow by pushing the “PUMP ON/OFF” button on the front of the pump, then press “ENTER”.

7. Push the “PURGE” button on the front of the pump to display the purge menu. Push the “NEXT” or down button to the left of the “PURGE” button until the word “CHANNEL” is displayed.

8. Push the up arrow until the appropriate line (A-D) is selected

9. Push “ENTER” to ensure that the selection is executed.

10. Push the up arrow until you are back to the main “PURGE” screen and press “ENTER” to start purging.

11. Observe the fluid flow lines between the solvent reservoirs and the pump to ensure that all air has been expelled from the lines. Once all air has been expelled (which may take several minutes), push the “PURGE” button again and push “ENTER” to stop purging the system.

12. Repeat the purge operation for all lines that will be in use. You do not need to purge lines for solvents that will not be used during the separation.

**Column Installation and Equilibration:**

1. Prior to beginning a separation, verify that the appropriate column is in place and has been installed correctly. All columns have direction so be sure that the tubing coming from the autosampler is going into the correct side of the column and that the tubing coming from the column goes into the detector. **FAILURE TO DO THIS WILL DAMAGE THE COLUMN!!!**

2. Push the “FLOW” button on the front of the pump

3. Enter the flow rate you would like to use and push “ENTER”. The pump should begin pumping at the desired flow rate.

4. Press the “%” button on the pump to modify the mobile phase composition. Use the arrow keys and number pad to modify the % composition of solvents B, C, and D. The default setting is 100% A. If the total % of solvents B, C, and D is less than 100%, the remaining fraction will be solvent A. For example, to use a mobile phase of 70%A : 20%B : 10%C, enter 20 for %B and 10 for %C and press “ENTER”.

5. Allow the column sufficient time to equilibrate after any changes to flow rate or mobile phase composition.

**Preparing the Hardware for Making a Run:**

As the system is equilibrating, you can prepare the autosampler to make a run. Make sure you have put your sample into a vial appropriate for the autosampler and placed it into the autosampler tray. Prior to starting a run you must program the autosampler with instructions on which vial to sample, how much sample to inject, and a few other things. The default selections are fine for most applications.
1. Push the “METHOD” button found on the front of the autosampler
2. Push the “NEXT” button or down arrow until “MODIFY METHOD” appears, then push “ENTER”. (the words on the screen should become lower case after this happens)
3. Push the “INJ VOLUME” button and enter the volume of solution you want to analyze, then push “ENTER”. (Typically 10-20 µL)
4. Make sure that everything else in that menu is turned off by pressing the “NEXT” button or down arrow until you return to the initial screen.
5. Push the “SPEED” button and check to make sure that the draw speed is set to 200 µL/min and that the inject speed is set to 1000µL/min. These are the default settings and they should not be changed without advanced knowledge.
6. Push the “STOP TIME” button and enter the time the run will end for one analysis, then push “ENTER”. Make sure that all other values in this menu are turned off.
7. Push the “VIAL #” button and enter the first and last tray numbers in the analysis. Push the “NEXT” button or down arrow to enter the number of injections per vial. Push “ENTER” after all information has been entered.
8. Push the “METHOD” button and scroll down with the “NEXT” button or down arrow until “STORE METHOD” appears. Push “ENTER” and all of the information entered for all aspects of the analysis will be saved.
9. Scroll up with the “PREV” button or up arrow until “LOAD METHOD” appears then push “ENTER”.
10. Press the “BALANCE” button on the detector to zero the baseline.

**General Steps for Making a Measurement**

After all of the above has been completed, you should be ready to make a run. To start the sequence push the “START” button, then “ENTER”. The autosampler is connected to the PeakSimple data acquisition system and the software will start collecting data on its own when the injection is made. See the Peak Simple guide for tips on data collection and analysis.

**Data Output and Storage**

See the Peak Simple guide for tips on data collection and analysis.

**Instrument Shut-down Procedure**

1. Adjust the mobile phase composition to run 100% organic phase (MeOH or CH₃CN) through the instrument for 15 minutes.
2. Press the “PUMP ON/OFF” and press “ENTER” to stop fluid flow. Power down each of the instrument components, the data acquisition hardware, and shut down the computer. Finally, close the helium valve on the tank.
3. Dispose of any waste appropriately.

**Troubleshooting Tips**

1. It is important to filter and degas solvents before use. Consult your instructor for procedures.
2. Good detection sensitivity requires appropriate choice of detection wavelength. Collecting a UV-Vis spectrum of your analyte may aid in wavelength selection.