**HPLC Procedures**

RUN PROCEDURES

1. Turn the **Detector** ON (should be warmed up after about 40 min).
2. Set the **Flow Rate** at 0 mL/min then switch the solvent line from the 75% methanol to the mobile phase of 30% methanol, 68% water, and 2% acetic acid. RINSE the outside of the solvent line with distilled water before putting the solvent line into the new mobile phase to prevent cross contamination of the mobile phases.
3. Set **Flow Rate** to approx. 0.8 mL/min. Make sure pressure is 3200-3800psi.
4. Adjust the UV Detector to zero using “Coarse” and then “Fine” adjustment knobs.
5. NOTE: SAMPLES MUST BE FILTERED PRIOR TO INJECTION. Filtering will extend the life of the column. Filter the sample through the .20 micron mini-filter by drawing the solution into a 3ml plastic syringe, and connecting the filter to the end.
6. Filter the solution into a small vial.
   1. We will use many filters for numerous samples (cost considerations). To prevent contamination between samples you will find that the syringes and filters have been separated into individual bags and marked as to the solution which has been used in them. This will help us reuse some of the filters. If the mini-filter starts to get clogged or you think there might be a problem, use a new mini-filter.
7. Check that the Instrumentation Amplifier is set to +/-1V.
8. Turn on the computer, and open Logger Pro.
9. In Logger Pro, choose the Experiment drop down menu. Select Data Collection. Change duration to 3 minutes. Change sample rate to 120 samples/min. Click “done.”
10. If needed, re-zero the UV Detector using the “Coarse” and “Fine” adjustment knobs.
11. Zero the Instrumentation Amplifier by clicking the “Experiment” tab can choosing zero in the drop down menu.
12. Inject the sample in the “Load” position and immediately switch to the “Inject” position while lab partner clicks “Collect” on the computer.
13. Leave the syringe in while the sample is running.
14. After the sample has completely eluted, move the switch to the “Load” position and remove the syringe.
15. Steps 10-14 can be repeated for all your repeated samples.
16. After the last sample, rinse the syringe with methanol and return to the Styrofoam case.
17. Empty the waste jar.

OVERNIGHT OR WEEKEND PREPARATION

1. Turn the **Detector** OFF.
2. Set the **Flow Rate** at 0 mL/min and switch the solvent line to the 75% methanol solution. (Be sure to rinse the solvent line before you place it in the new solvent).
3. Set the **Flow Rate** to 0.1 mL/min.
4. Place waste jar in back of HPLC to catch waste.

AFTER OVERNIGHT RUN

1. See run procedures

SHUT DOWN

1. Shut OFF the **Recorder** and the **Detector**.

2. Set the **Flow Rate** at 0 mL/min and switch the solvent line to the 75% methanol solution. (Be sure to rinse the solvent line before you place it in the new solvent).

3. Set the **Flow Rate** to 0.8 mL/min and run for 30 minutes.

4. Shut OFF the pump.

5. Empty the waste jar.