HPLC SOP

Door code: 1-34 (as of December, 2008)
Username: 3000Hanover
Password: 3000Hanover

1. Turn on all Hardware (instrument buttons, 6 in total) before the software (computer)
   a. Start at top and work down and right
   b. Hear clicking noises that indicates the start of the pump cycle
   c. To end, make sure Hardware is OFF before the Software is turned OFF
2. Click on the icon for Software Instrument 1 on-line
   a. If software does not load or there is an error, log out and restart the computer
3. Software shows syringe-pump-column-detector, all can be clicked on to change and monitored
4. Sample icon is top left of screen, single or multiple see 1 bottle or numerous bottles
5. Mobile Phase
   a. Individual containers for each solvent
   b. Follow the lines (numbered) from the bottles to the inlet into the column
   c. Mili-Q water only!
   d. MeOH
   e. Make sure containers are full and the software knows how much volume in the containers
   f. If solvent is less than 200 mL, no more injections will be preformed
6. Check for waste flow either
   a. Empties into the waste container, make sure tubing is in the bottle and not the floor
   b. Flows into 2nd detector for further analysis
7. Moisture Check-there is an automatic shut-off if the instrument gets too wet.
   a. Always check for leaks and excess moisture at every fitting
8. Flush the column for 20-30 mins using strong to weak solvent
   a. 100 % H_2O
   b. 100 % MeOH
   c. mixture of H_2O-MeOH
9. Software Pump, click on icon
   a. 1 mL/min flow rate
   b. Increase flow very slowly to not increase the pressure on the column to rapidly, do NOT EXCEED 400 bar maximum pressure
   c. Stoptime = 30 min
   d. Control, click on OK, hear pump turn on
10. Detector, click on icon, depending on matrices and background of sample
    a. Data is stored only to the detector that has the box checked
    b. All data is stored to the end of the run
    c. Stop Time 40 min
    d. Diode Array detector: Range 210-400 nm
       i. Default setting are OK for running
    e. ADC is always ON, not saved, data not needed

        i. Set-up peaks for integration (set-up will prevent integrating every wiggle)
       ii. Width 100
      iii. Reject 1000
      iv. XXX 1000

11. Column Thermostat
    a. $30^{o}C$ is good for a little heat, will feel heat sink warm up that holds the column
    b. $50\text{-}60^{o}C$ is maximum temperature, if needed

12. Injection Volume
    a. Smaller is better for better resolution, separation, and sensitivity
    b. 3-100 μL is range
    c. Start with 10 μL then try 5 μL to 3 μL is concentrated
    d. 25 μL is sample is dilute

13. Always make sure solvent is moving through the lines
    a. Always check for bubbles or air pockets in the sample lines!
    b. If bubble/no solvent flow, OPEN the by-pass valve, black knob, 2 turns to remove all pressure from the system
    c. Increase pump speed to 5 mL/min to flush out the lines
    d. Makes sure to return the pump speed back to 1 mL/min before starting again!
    e. Close by-pass value to re-establish backpressure

14. Samples
    a. Degasses samples are the best, less bubbles
    b. Make sure all samples are filtered before sending through the column, the column is a very expensive filter
    c. Use a syringe and ~25 μm filter

15. Save Method
    a. Save as System File
    b. Load method
        i. File-load-method
    c. Copy spectrum to clipboard, paste into paint, and save a jpg