Vasudevan Agilent 1100 Series HPLC w/ DAD & FLD Detector (non-

buffer solvents)

Updated November 14, 2017

Instrument instructions can be found at: http://academic.bowdoin.edu/chemistry/resources/instructions.shtml

If you have any problems with the instrument or would like to get trained, please contact Celeste Morin

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- a. **Read instructions carefully before using instrument**. Reading the bold sentences in each category will tell you what you need to know to run the instrument.
 - i. Bullets are under the bold sentences when more detail is required.
 - ii. At the end of the instructions is a frequently asked questions/troubleshooting section.
- a. Program should be in Method and Run Control view for the following steps.
 - i. Go to View > Method and Run Control.
- b. Turn on computer and login (use your Bowdoin account).
- c. Turn on instrument modules (start with DAD detector).
- d. Operasudevan Lab (Online)".
- e. Add FLD module.
 - i.
 - ii.

detector. It should be the only module that is green on that side.

- iii. Select the module (left click once on the module).
- iv. Click the Add
 - side.
- v. Click OK.
- f. Check solvent and waste bottles. Be sure you have enough solvent for your run and the waste bottle will not be overflowing. If you fill the solvent bottles, adjust the volume levels in the program.
- g. Prepare samples.
- h. Enter sample information into HPLC log sheet.
- i. Turn on detectors.
 - i. Instrument > More DAD > Control.
 - 1. In the Lamps section, select On for Vis and UV.
 - 2. Click OK.
 - ii. Instrument > More FLD > Control
 - 1. In the Lamp section, select On.
 - 2. Click OK.
 - iii. Detector lamps should warm up for twenty minutes before analyzing samples.

- a. Program should be in Method and Run Control view for the following steps.
 - i. Go to View > Method and Run Control.
- b. **Load method** (File > Load > Method).
- c. **Edit entire method** (Method > Edit Entire Method) Check all four boxes.
 - i. FLD Signals
 - 1. Signals
 - a. Enter the Ex and Em wavelength you would like to collect a chromatogram for.
 - 2. Time

a.

- a. Program should be in <u>Data Analysis</u> view for the following steps.
 - i. Go to View > Data Analysis.
- b. Select Integration Task icon
- c. **Integrate spectrum** (Integration > Auto Integrate).
- a. Program should be in <u>Data Analysis</u> view for the following steps.
 - i. Go to View > Data Analysis.
- b. Select Spectral Task icon
- c. Select peak to obtain spectrum.
- d. **Print** (File > Print > All Windows).
- a. Program should be in <u>Data Analysis</u> view for the following steps.
 - i. Go to View > Data Analysis.
- b. **Select report destination and style** (Report > Specify Report).
- c. **Print report** (File > Print > Specify Report and Print)-Edit how you want your
- a. Program should be in Method and Run Control view for the following steps.
 - i. Go to View > Method and Run Control.
- b. **Flush system with appropriate solvent** DO NOT leave water in the system.
- c. **Turn off pumps and detector** (Instrument > System Off). The red button will shut down the lamps and the pump.
- d. Return "Configured Modules" back to original settings.
 - i. Open Configuration window (Instrument > Configure 1100 Access).

ii.

HPLC 1100 Frequently Asked Questions

- a. Remove the standard flow cell and install the standalone cell.
 - i. Unscrew the two front screws.
 - ii. Remove the standard flow cell, leave all tubing connected.
 - iii. Install the standalone cell and tighten the two screws.

iv.

- b. **Turn on the computer and login** (use your Bowdoin account).
- c. Turn on all the modules (starting with the DAD detector).
- d. Open "Vasudevan Lab (Online)".
- e. Enter sample information into HPLC log sheet.
- f. Program should be in Method and Run Control View for the following steps.
 - i. Go to View > Method and Run Control.
- g. **Turn on detector** (Instrument > More FLD > Control).
 - i. Allow twenty minutes for lamp to warm up.
- h. Adjust FLD parameters.
 - i. Instrument > Set Up FLD Signals.
 - ii. Click Full>> button (if it is not already selected).
 - iii. Click Special Setpoints button.
 - iv. enter the excitation and emission scan range.
- i. Load sample into plastic syringe. The needle has a flat edge with a plastic ferrule.
- j. **Insert needle into port that says "In".** The plastic ferrule should be all the way up to cell port.
- k. Slowly inject sample until you see your sample flowing into the waste tubing.
- 1. Keep syringe in the port while running your sample.
- m. **Run sample** (Instrument > More FLD > Take Fluorescence Scan).
- n. Program should be in <u>Data Analysis</u> view for the following steps.
 - i. Go to View > Data Analysis.
- o. **Load signal** (File > Load Signal).
- p. **View isoabsorbance plot** (Spectra > Isoabsorbance Plot).
 - i. Click Exit button when finished.
- q. **View 3D Plot** (Spectra > 3D Plot).
 - i. Click Close button when finished.
- r. Remove syringe and flush out cell with methanol.
- s. Flush air through the cell to remove any solvent.
- t. Remove standalone cell and replace with standard flow cell.
- u. Turn off modules and log off computer.
- a. Program should be in Data Analysis view for the following steps.
 - i. Go to View > Data Analysis.
- b. **Load Signals** (File > Load Signal).

c. In the "Load Signal" window, make sure "Load Using Signal Details" is not checked. This will load all signals collected.