

Agilent 1100 Series HPLC w/ DAD & FLD Detector (non-buffer solvents)

Updated May 30, 2008

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

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1. Protocol

- 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.

2. Startup Procedure


- 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).
 - i. Wait until CAG Bootp Server is running before proceeding to next step.
- c. **Turn on instrument modules** (start with DAD detector).
- d. **Open “Instrument 1 Online”.**
- e. **Add FLD module.**
 - i. A “Configuration” window will pop up.
 - ii. On “Available Modules” side, scroll down until you find “FLD G1321A” 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 -> button. Module will now be on the “Configured Modules” side.
 - v. Click OK.
- f. **Check solvent and waste bottles.**
- 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.

3. Create/Edit Method

- 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. Time at which the FLD stops an analysis. Set this to “no Limit” so it will stop when the pump stops. This will not turn off the detector.
 - 3. Multiple Wavelengths and Spectra
 - a. If you would like to collect at different wavelengths, or collect spectra, click on the Full >> button.
 - b. Select Multi Ex or Multi Em.
 - c. Select what signals (B-D) and enter wavelength. Each one of these signals you select will give you a chromatogram.
 - d. Under Acquire Spectra, you can choose to scan a range of wavelengths. Use this if you would like to collect spectra for the peaks in your chromatogram.
 - i. Select “All” and enter a range.
- d. **Save method** (File > Save As > Method).
- e. **Flush system with solvent(s) used in method.**


4. Create/Edit Sequence

- a. **Program should be in Method and Run Control view for the following steps.**
 - i. Go to View > Method and Run Control.
- b. **Select Sequence Task  icon, located in the top left corner.**
- c. **Load sequence** (Sequence > Load Sequence).
- d. **Edit Sequence Table** (Sequence > Sequence Table).
- e. **Edit Sequence Parameters** (Sequence > Sequence Parameters).
- f. **Save sequence** (Sequence > Save Sequence As).


5. Start Sequence

- a. **Program should be in Method and Run Control view for the following steps.**
 - i. Go to View > Method and Run Control.
- b. **Configure monitor analysis window.**
- c. **Verify system is stable.**
- d. **Run sequence** (Run Control > Run Sequence).

6. Integrate

- a. **Program should be in Data Analysis view for the following steps.**
 - i. Go to View > Data Analysis.
- b. **Select Integration Task icon .**
- c. **Integrate spectrum** (Integration > Auto Integrate).

7. Generate Spectra

- a. Program should be in **Data Analysis** 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).

8. Generate Report

- a. Program should be in **Data Analysis** 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 > Report).

9. Shutdown Procedure

- 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).
- d. Return “Configured Modules” back to original settings.
 - i. Open Configuration window (Instrument > Configure 1100 Access).
 - ii. In the “Configured Modules” side, select “FLD G1321A”.
 - iii. Select module.
 - iv. Click Remove button.
 - v. Click OK.
- e. Close ChemStation and CAG Bootp Server.
- f. Turn the computer and instrument modules off.
- g. Remove your samples from autosampler tray.

HPLC 1100 Frequently Asked Questions

1. How do I run the FLD as a standalone unit?

- 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. Connect the waste tubing to the port that says “Out”.
- b. **Turn on the computer and login** (use your Bowdoin account).
 - i. Wait until CAG Bootp Server is running before proceeding to next step.
- c. **Turn on all the modules** (starting with the DAD detector).
- d. **Open “Instrument 1 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. In the “Fluorescence Scan Range” box, 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.**
- l. **Keep syringe in the port while running your sample.**
- m. **Run sample** (Instrument > More FLD > Take Fluorescence Scan).
- n. **Program should be in Data Analysis 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.**

2. How do I print the FLD chromatogram and spectra?

- 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.
- d. **To print FLD chromatogram and spectra, load only the FLD signal(s).**
 - i. Load Signal (File > Load Signal).
 - ii. Select “Load Using Signal Details” box.
 - iii. Click Signal Details button.
 - iv. Add FLD signal(s) to “Signal Description”. Delete all other signals.
 - v. Click OK (Signal Details window).
 - vi. Click OK (Load Signal window).
- e. **Specify report** (Report > Specify Report).
- f. **In report style, select “fld”.**
- g. **Click OK.**
- h. **Print preview** (File > Print Preview > Report).
- i. **If report is okay, print** (File > Print > Report).