

Hewlett Packard 1090 High Pressure Liquid Chromatograph (HPLC)

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.**
- c. **Turn on HPLC** (power button located on front of instrument).
- d. **Open “Instrument 1 Online”.**
 - i. Start > Programs > HP ChemStations > Instrument 1 Online.
 - ii. Click OK if you get error messages about instrument not being configured correctly.
- e. **Check solvent and waste bottles.**
 - i. Check the three solvent bottles in front of the HPLC and make sure there is enough solvent to run your samples.
 1. If you fill the solvent bottles, adjust the volume levels in the program.
 - a. Enter new volumes (Instrument > More Pump > Bottle Filling).
 - b. Click OK.
- f. **Prepare samples.**
 - i. Filter all samples through 0.45 um filter.
- g. **Enter sample information into HPLC log sheet.**
- h. **Turn on lamps** (Instrument > More DAD > Control).
 - i. Wait two hours for lamp to warm up.
- i. **Turn on pumps** (Instrument > More Pump > Control).
 - i. Flush column for fifteen minutes.

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).



- i. Flow, Solvent %, Pressure
 1. Control
 - a. Enter an initial flow rate (if you don't have a pump time table, this will be the flow rate through the entire run).
 - b. StopTime will determine how long each run will take. This will not turn off the pumps.
 2. Solvents
 - a. Use the scrollbars (left of solvent name) to turn the pumps ON and OFF.
 - b. Pumps A-C should have a solvent name next to them, indicating what solvent they are pumping. Verify these are correct.
 3. Create a pump time table (if necessary).
 - a. Enter a time, the % solvent(s), and flow rate.
- ii. DAD Signals
 1. Signals
 - a. Check the boxes to activate one (or more) of the signals (A-E).
 - b. Enter the wavelength at which the absorbance of the sample will be measured.
 - c. Enter a bandwidth (determines the wavelength range over which the absorbance is measured).
 - d. Reference is the wavelength at which a reference absorbance is measured.
 - e. Bw is the bandwidth of the reference wavelength.
 2. Spectrum
 - a. Defines at which point on a signal a spectra will be taken and saved.
 - i. Select "All" - spectra will be taken continuously.
 3. Time
 - a. Time at which the DAD stops an analysis. Set this to "no Limit" so it will stop when the pump stops. This will not turn off the detector.
 4. Required Lamps
 - a. Select both UV and Vis.
 5. Autobalance
 - a. Baseline is reset to zero either before or after a run. Select Prerun.
- iii. Signal Details
 1. The Signals Details dialog box defines which signals will be evaluated during a method run.
 2. Select the signals from the Available Signals pull down menu.
 - a. Click Add to Method button.
 - b. Click OK.
 3. If there are signals you do not want to monitor, delete them.
 - a. Move the cursor to the front of the row - it should turn to a black horizontal arrow.

- b. Select the row.
 - c. Click the Delete Row button.
- iv. Edit Integration Events
 - 1. Default settings are okay. You can optimize this in the Integrate section.
- v. Specify Report
 - 1. Unless you want the report printed automatically, select just Screen in the Destination section.
 - 2. Report Style should be “Short”.
- vi. Instrument Curves
 - 1. Not necessary to check any of these unless you want more curves displayed on your chromatogram.
- vii. Run Time Checklist
 - 1. Select just Data Acquisition and Standard Data Analysis.
- d. **Save method** (Method > Save Method As).



4. Start Run




- a. **Program should be in Method and Run Control view for the following steps.**
 - i. Go to View > Method and Run Control.
- b. **Load sample and inject.**
 - i. Lever should be in the “Inject” position.
 - ii. Turn the lever to “Load”.
 - iii. Insert needle.
 - iv. Inject sample until you see liquid coming out the waste line.
 - v. Turn knob to “Inject”.
- c. **Start Run** (RunControl > Run Method).

5. 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.**
 - i. Auto integrate (Integration > Auto Integrate) – this will integrate everything.
 - ii. Remove unwanted peaks from integration table by selecting icon .
 - 1. Click any labeled peaks in chromatogram you do not want integrated.

6. 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.**
 - i. For a spectrum at the apex click the icon  and click anywhere on the peak in the chromatogram.

- ii. For a spectrum of a different part of the peak (not the apex) click the icon  and click on the point of your chromatogram where you want a spectrum.
- iii. Zoom in using the icon . Zoom out using the icon .
- d. **Print** (File > Print > All Windows).

7. 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).

8. Shutdown Procedure

- a. **Program should be in Method and Run Control for the following steps.**
 - i. Go to View > Method and Run Control.
- b. **Flush out system with appropriate solvent.**
- c. **Turn off pumps and detector** (Instrument > System Off).
- d. **Close ChemStation.**
- e. **Turn computer and instrument off.**