Gorske Agilent 1260 Series HPLC with DAD Detector (non-buffer solutions)

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
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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. **If not on already, turn on computer and login** (use your own Bowdoin account).
   i. First time users only.
      1. Create folders to store your data, method, and sequence.
         a. Right click on Windows Icon, lower left of the screen, and click Open Windows Explorer .
         b. Go to Local Disk (C:) > Chem32 > 1.
         c. Create a data, methods, and sequence folder.
            i. Click once on the folder to highlight it.
            ii. Go to New .
            iii. Type in your name or initials to name that folder.
            iv. Repeat for the methods and sequence folder.
      2. Configure a network printer and set it as default. Carbon is currently the default printer on this computer. It is located in the photocopier room near the stockroom.
         a. Make sure you are connected to the Bowdoin network.
         b. Go to Start > Run.
         c. Type in the location of the printer.
            i. Ground floor - \\bradbury\
            ii. First floor \\bradbury\werner
            iii. Second floor \\bradbury\
         d. Click OK.
         e. Set printer as default.
            i. Start > Printers and Faxes.
            ii. Right click on printer you just added.
            iii.

b. **If not already on, turn on the instrument modules. Start with DAD, then work your way up to the top.**

c. **After a few minutes, the instrument will be done initializing, and then Open Agilent 1260 (Online) by clicking the icon on the desktop.**

d. **Check solvent and waste bottles.**

   i. Check the four solvent bottles on top of 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. The instrument will shut down if the solvent bottles get below a set point.

         a. nter new volumes.

         b. Click OK.

         c. Be sure there are no bubbles on the filter. If there are, open the purge valve (black knob located on front of the pump module turn it left to loosen but do not turn it all the way) and pump the mobile phase at 5ml/min. until the bubbles have been removed. If necessary, shake the line to dislodge the air bubbles, being careful not to break the solvent filter by hitting the glass sides too hard. Stop the pump and close the purge valve. Do this for all lines that have bubbles in the solvent filter.

   ii. Check the waste bottle in the cabinet. If full, change bottle and bring the full

a. Use the scrollbars (left of solvent name) to turn the pumps ON and OFF.
b. Pumps A-D should have a solvent name next to them, indicating what solvent they are pumping. Verify these are correct by tracing the lines from the solvent bottles to where they enter the pumps.

3. Create a pump time table (if necessary).
a. Enter a time, the % solvent(s), and a flow rate.
b. To see a graphical representation of the timetable, select the Display combination box and select Flow/Pres or Solvents.

ii. Set up Injector.
1. Typically a Standard Injection is used. Injection volume will vary.
2. Draw speed and eject speed can range from 10ul/min to 1000ul/min. **Typically 500ul/min is used.** Default is 100ul/min. More viscous samples should be injected more slowly.

iii. 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. - spectra will be taken continuously.
3. Stoptime
a. As Pump/Injector
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.

iv. 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./F1 12 Tf1 0 0 1 201.65 209.21 Tm0 g0 G[Autob

a. Move the cursor to the front of the row

c. Click the little scrollbars next to each box to increase or decrease value. The first box controls lower range, the second box controls the upper range.

7. Generate Spectra

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.**

   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).

8. Generate Report

a. **Program should be in <u>Data Analysis</u> view for the following steps.**
   i. Go to View > Data Analysis.

b. **To print report** (File > Print > Specify Report and Printer) [illegible]

9. Shutdown Procedure

a.

HPLC 1100 Frequently Asked Questions

1.

i. **Another box will come up ask you to enter the ending value**.
j. **Once done, the results will be printed out on the default printer**. The results will be:

5.

i. To highlight, move the cursor to the start of the row. The cursor will turn to a black horizontal arrow. When it does, click the left mouse button. When the row is highlighted, delete it.

l. **Click OK when finished.**

m. **When you view your report now, only the signal you selected will be displayed.**

8.