

Agilent Technologies 1200 Series HPLC

Standard Operating Procedure

1. Sample preparation prior to analysis
 - I. Powering on and preparing the instrument
 - II. Creating a method
 - III. Creating a sequence
 - IV. Data retrieval and analysis

1. Sample preparation

- Ensure samples are free of particulate matter. A 0.45 μm disposable filter is recommended to filter samples.
- Load samples into labeled HPLC vials (VWR cat # 82030-974 may be used). Each vial holds a maximum of 1.5 mL but do not need to be completely filled; because the autosampler needle collects sample from the bottom of the vial, only ≈ 0.5 mL is necessary. For smaller samples, a glass insert may be used (Figure 1). Glass inserts serve to lower the internal volume of the vial and are simply placed inside the HPLC vial and loaded with sample. The cap is then screwed onto the vial as normal.



Figure 1. An HPLC vial loaded with sample (right) and an optional 400 μL glass insert (left).

- Place sample vials in the autosampler tray, making note of the position of each sample in the tray (you will need this information later).

I. Powering on and preparing the instrument

- Turn on the computer (the password and username can be found underneath the keyboard) and give the old computer ample time to “warm up” (5-10 minutes is recommended).
- Turn on the HPLC by turning on the power strip. The instrument will proceed through its normal startup procedure. You should see an info bubble in the bottom right corner of the computer telling you “1200 is connected.” Wait for the startup procedure to finish; all lights

should be green (or off, in the case of the autosampler module). Orange or red lights indicate a problem.

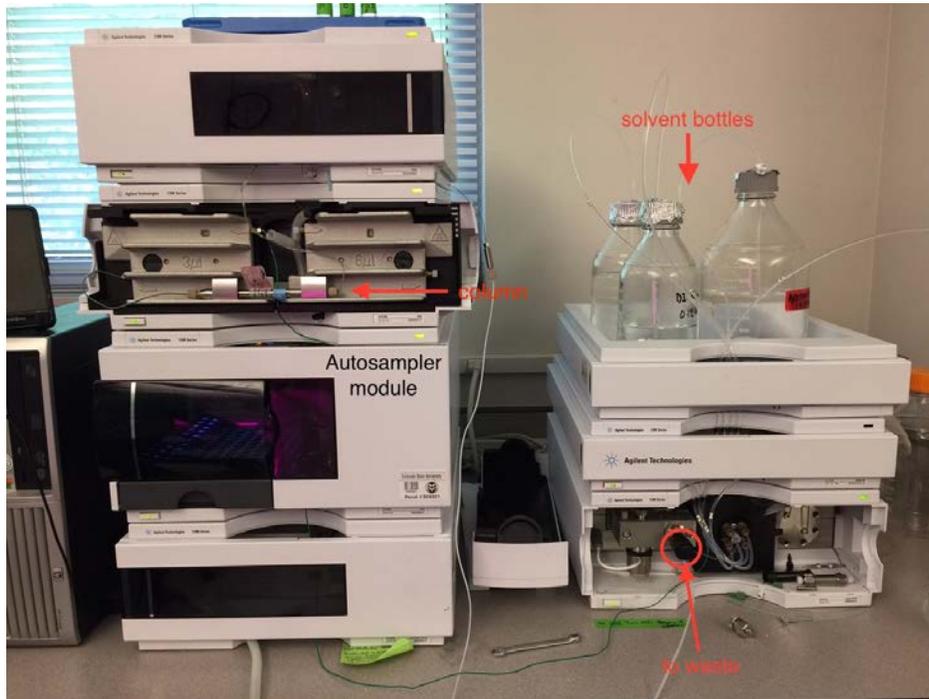


Figure 2. The HPLC as it appears during normal use.

- Open the program “HPLC Online” otherwise known as ChemStation. The program should start up and open after initializing. If the instrument modules are not recognized, repeat previous steps.

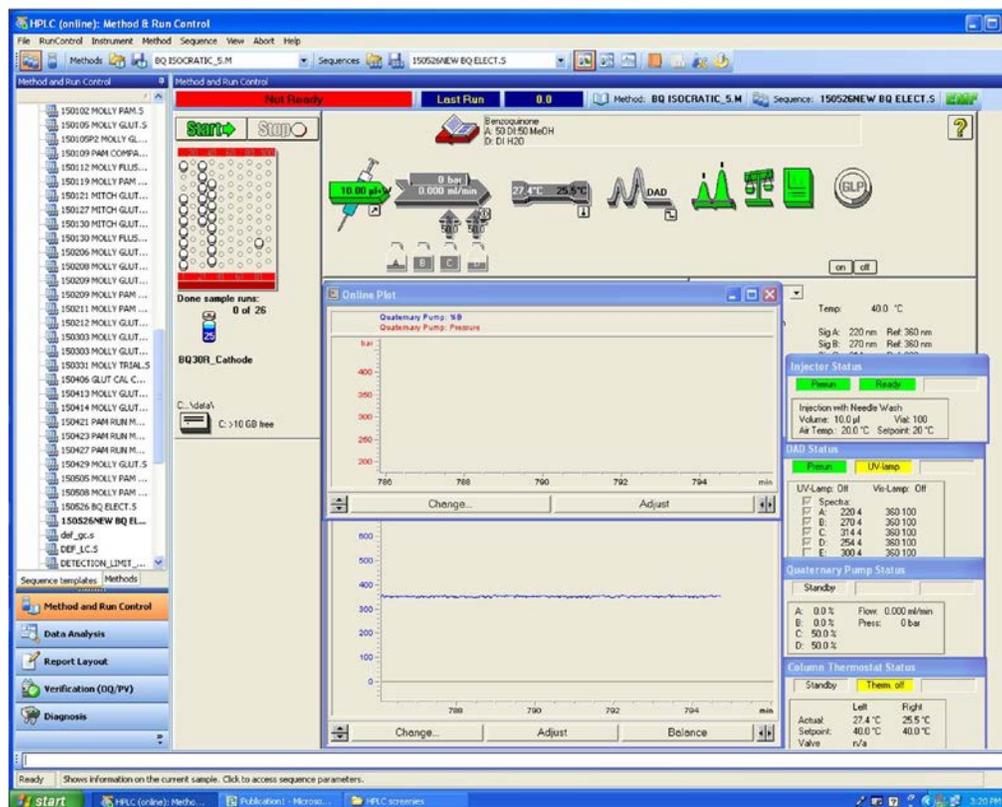


Figure 3. Chem station after it has been opened. Note the entire top panel, including the image of the tray, are all intractable; clicking on images opens a menu with relevant controls.

- To enable instrument control, make sure “Method and Run Control” in the lower left corner of the program is highlighted.
- Ensure solvent bottles are filled with solvent. If running low, fill bottles with HPLC grade solvent. Then adjust the solvent bottle fill level in the program by clicking on the picture of the solvent bottles and selecting “solvent bottles filling,” inputting your new volumes. **NOTE:** the program will automatically shut off if the solvent bottle fill drops to 0, regardless of whether or not solvent is actually in the bottle. If you do not remember to adjust solvent bottle filling, your run will shut off!

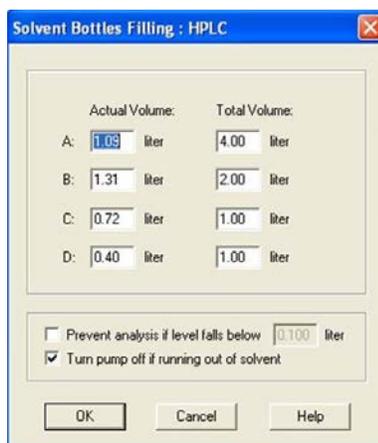


Figure 4. Solvent bottles filling menu.

- Ensure that the waste jug underneath the HPLC is not full, or near full, or it will overflow during the run. Dispose of waste as necessary.
- Install your column, making sure a secure connection is established by tightening screw-plugs to finger-tight. **NOTE:** when putting away columns, ensure they are stored in the box *with endcaps in place*. Columns should not be allowed to dry out.
- Loosen the “to waste” knob (Figure 2) so that flow (and any initial bubbles) will be directed to the waste jug.
- If you already have a method created, load that method (Method→Load Method..., then select your method from the list). If not, navigate to and complete section II: creating a method. Method must be created and loaded to proceed with instrument operation.
- In the instrument control panel in ChemStation, hit the “on” button. The instrument will begin to pump solvent. At this point, check your column/system for leaks. **NOTE:** if the instrument detects a leak via dripping solvent, it will automatically turn red and shut off (and won’t tell you why).
- Depending on your column, it is recommended to flush with organic solvent first until column appears clean (no peaks eluting). Quickly change solvents by clicking on the arrows above the solvent bottles (Figure 3) and selecting “Set up Pump...” Once clean, set the solvent levels to match your initial chromatographic conditions to equilibrate the column. Column should be equilibrated until pressure and UV-vis baseline has stabilized.
- You are now ready to create a sequence to run or, alternatively, perform single runs. Select between these options by clicking the image of three vials (vs. the single vial) in the upper left-hand corner of the chemstation program (Figure 3). A sequence may be started by pushing the “start” button.

II. Creating a method

- Choose a method on which to base your own. Then save it as your own by going to File→Save As→Method... whereupon you will be prompted to select a name for your new method. **NOTE:** it is useful to save your file name ending in a number (ex. Name_1) so that new versions may be saved when changes are made.
- Now edit your method by selecting Method→Edit Entire Method... and select the desired method sections to edit (typically all checkboxes are checked). Select “OK.”
- You will now be able to fill in your method information which should include your analyte, solvents, detection wavelengths, injection volume, flow rate, column type, chromatographic conditions, and total runtime. This information may be edited later. Select “OK.”
- Set up Pump: on this screen you will set your flow rate, stop time, chromatographic conditions, and pressure limits. Initially, ensure your solvents are labeled correctly and the letters A, B, C, and D match the actual solvent bottles. For an isocratic run, only use of the “solvents” section is required. If a gradient run is required, use “timetable;“ create a timetable using the “insert” and “append” buttons to modify your chromatographic run. Make sure to include ample time in your run for your column to equilibrate back to initial conditions. **NOTE:** information in the “timetable” section will always override information in the “solvents” section. Select “OK.”

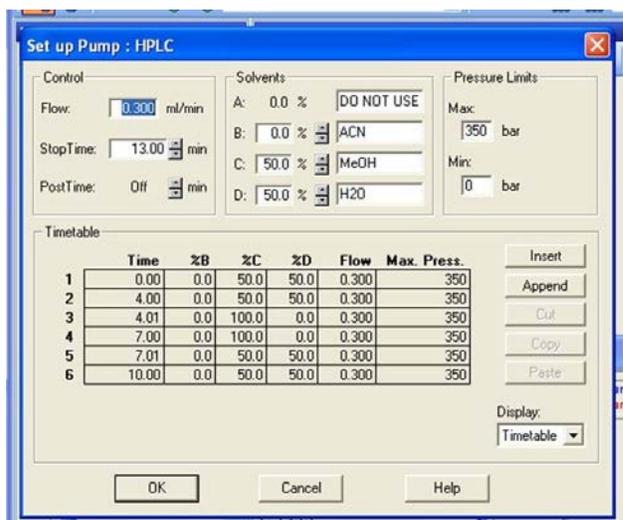


Figure 5. Set up pump menu with a gradient elution scheme.

- Set up Injector: here you will select your injection type and volume. Standard injection of 10 μ L is a recommended starting point. Select “OK.”

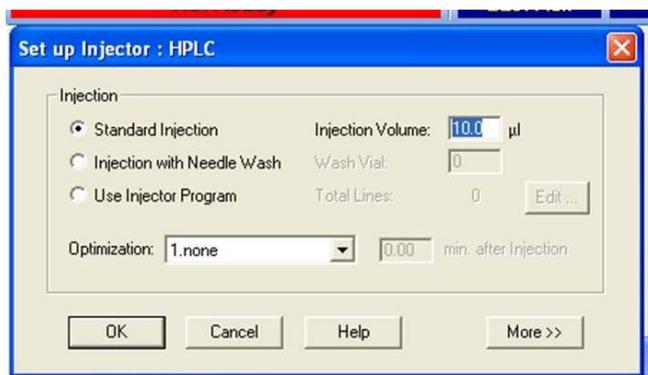


Figure 6. Injector setup.

- DAD Signals: here you will select what wavelengths to monitor throughout your run. Ensure the “store” box is checked so that data is saved! If you require detection in the UV region, make sure the required lamps Vis *and* UV are selected; if the UV region is not needed, unselect this box to preserve the deuterium lamp. Also ensure that your reference wavelength is a clean area in your spectrum- this wavelength should have 0 absorbance. Make sure in the “time” section that “stoptime” is set to “as Pump.” All other conditions may be changed as desired. Select “OK.”

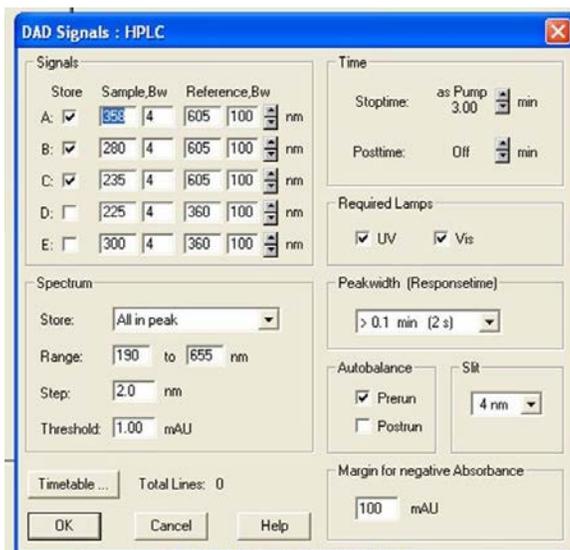


Figure 6. What a functional DAD signals menu looks like.

- Column thermostat method: here you can select the temperature at which the column will be maintained. If temperature is to change throughout a run, push the “more” button and input the timetable desired using the insert and append buttons. Select “OK.”

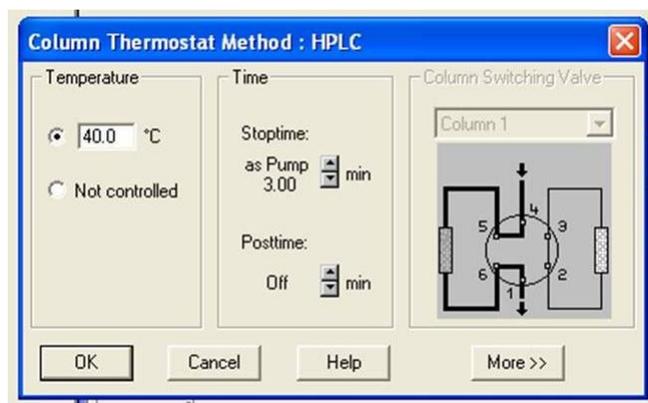


Figure 7. Column thermostat setup.

- Signal Details: Each signal that is to be stored must be added to the method by selecting it from the drop-down menu and then pushing the “add to method” button. In the example shown, three signals have been added and are therefore stored simultaneously. Select “OK.”

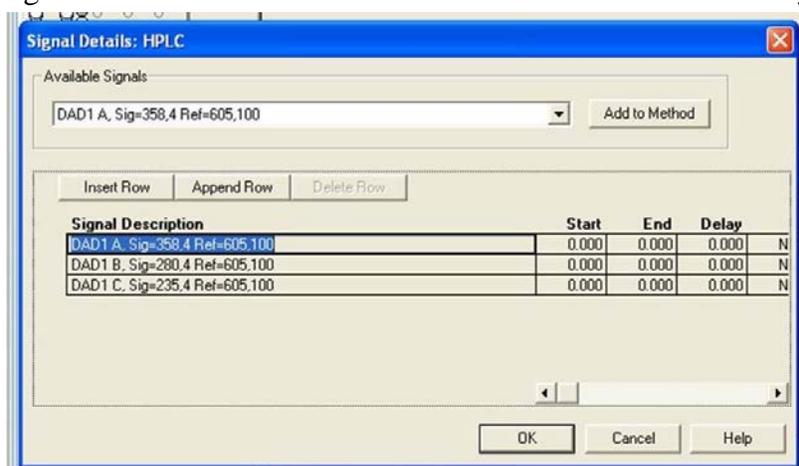
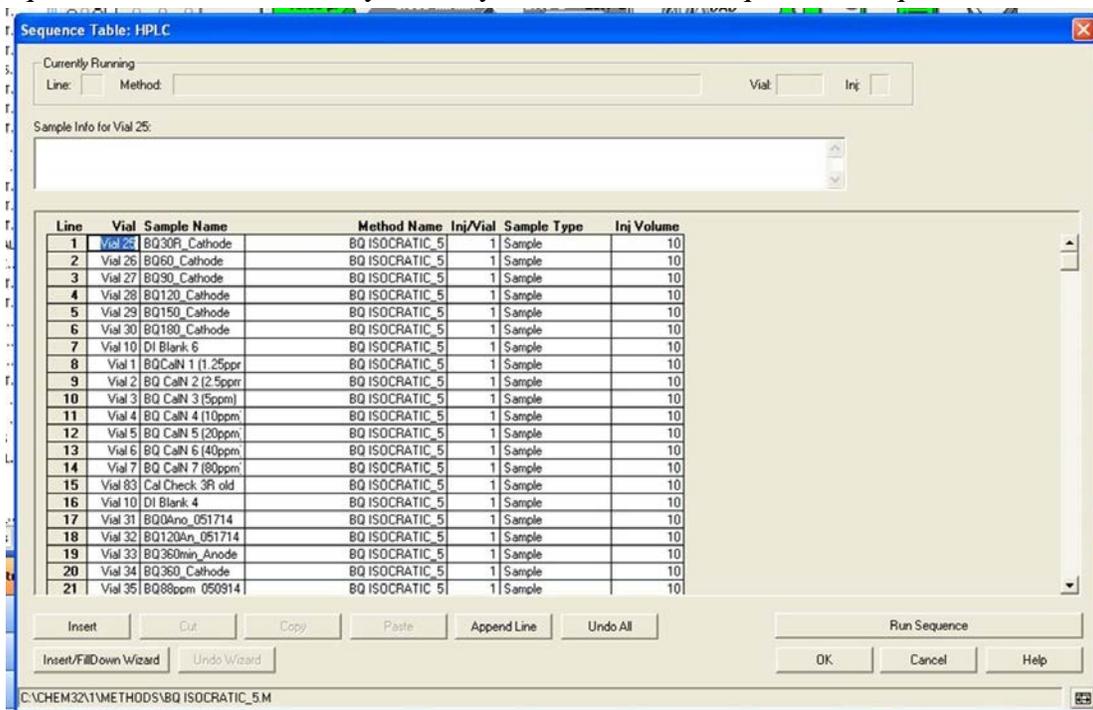


Figure 8. Signal details setup.

- Edit Integration Events: make sure “manual events” box is checked, then select “OK.”
- Specify Report: here you will adjust what displays on the screen, and the default settings are generally acceptable. Select “OK.”
- Instrument Curves: if you wish to store data pertaining to solvent percentage, flow, or temperature, check the appropriate boxes and this data will display in your report.
- Run Time Checklist: the boxes next to data acquisition, standard data analysis, and save method with data should be checked. Select “OK.” Your method is now ready to use.

III. Creating a sequence

- Access the sequence table by clicking on the image of the sample tray and selecting “sequence table.” Alternatively this may be accessed via Sequence→Sequence Table...



Sequence Table: HPLC

Currently Running

Line: Method: Vial: Inj:

Sample Info for Vial 25:

Line	Vial	Sample Name	Method Name	Inj/Vial	Sample Type	Inj Volume
1	Vial 25	BQ30R_Cathode	BQ ISOCRATIC_5	1	Sample	10
2	Vial 26	BQ60_Cathode	BQ ISOCRATIC_5	1	Sample	10
3	Vial 27	BQ90_Cathode	BQ ISOCRATIC_5	1	Sample	10
4	Vial 28	BQ120_Cathode	BQ ISOCRATIC_5	1	Sample	10
5	Vial 29	BQ150_Cathode	BQ ISOCRATIC_5	1	Sample	10
6	Vial 30	BQ180_Cathode	BQ ISOCRATIC_5	1	Sample	10
7	Vial 10	DI Blank 6	BQ ISOCRATIC_5	1	Sample	10
8	Vial 1	BQCaN 1 (1.25ppr)	BQ ISOCRATIC_5	1	Sample	10
9	Vial 2	BQ CaN 2 (2.5ppr)	BQ ISOCRATIC_5	1	Sample	10
10	Vial 3	BQ CaN 3 (5ppm)	BQ ISOCRATIC_5	1	Sample	10
11	Vial 4	BQ CaN 4 (10ppm)	BQ ISOCRATIC_5	1	Sample	10
12	Vial 5	BQ CaN 5 (20ppm)	BQ ISOCRATIC_5	1	Sample	10
13	Vial 6	BQ CaN 6 (40ppm)	BQ ISOCRATIC_5	1	Sample	10
14	Vial 7	BQ CaN 7 (80ppm)	BQ ISOCRATIC_5	1	Sample	10
15	Vial 83	Cal Check 3R old	BQ ISOCRATIC_5	1	Sample	10
16	Vial 10	DI Blank 4	BQ ISOCRATIC_5	1	Sample	10
17	Vial 31	BQ0Ano_051714	BQ ISOCRATIC_5	1	Sample	10
18	Vial 32	BQ120An_051714	BQ ISOCRATIC_5	1	Sample	10
19	Vial 33	BQ360min_Anode	BQ ISOCRATIC_5	1	Sample	10
20	Vial 34	BQ360_Cathode	BQ ISOCRATIC_5	1	Sample	10
21	Vial 35	BQ88ppm_050914	BQ ISOCRATIC_5	1	Sample	10

Insert Cut Copy Paste Append Line Undo All Run Sequence

Insert/FillDown Wizard Undo Wizard OK Cancel Help

C:\CHEM32\1\METHODS\BQ ISOCRATIC_5.M

Figure 8. An example of what a sequence table should look like.

- Here you will program your sequence. Under “vial,” input the number *only* (do not type “vial”) that corresponds to the location of the vial in the sample tray. Chose a unique name for your sample, then click the “method name” box and select your desired method from the drop-down list. Then continue to fill in injections/vial (typically 1), sample type (typically Sample), and injection volume (which should ideally match your method). Use the “insert” and “append line” buttons to add samples to the sequence list.
- When sequence is created as desired, select “OK.”
- **SAVE YOUR SEQUENCE!** Select Sequence→Save Sequence As... or File→Save As→Sequence... You will then be prompted to enter a name for your sequence. **NOTE:** this name is how you will locate your data later so it is important to remember the name you select.

IV.. Data Retrieval and Analysis

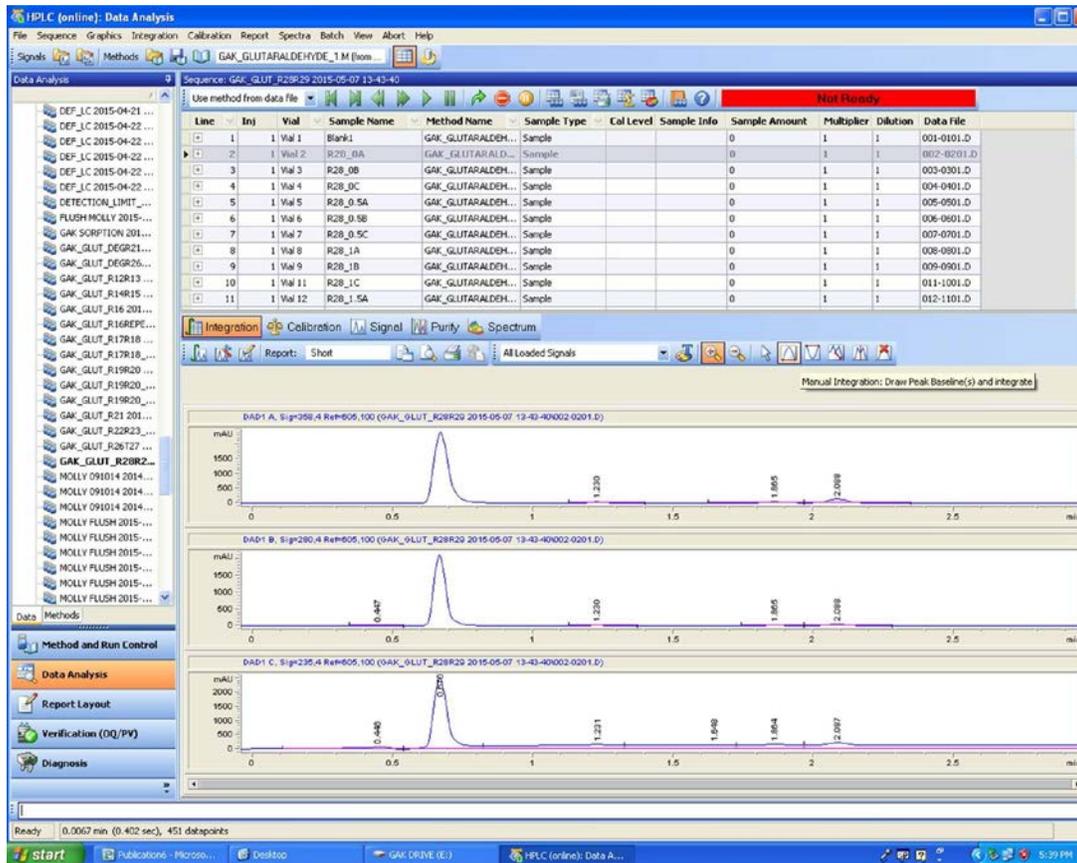


Figure 9. The data analysis screen in chemstation.

- In the lower left-hand panel of chemstation select “Data Analysis”
- Select the name of your sequence (or, alternatively, your single run) from the list to the left
- Now you may select the individual sample you wish to analyze from the list displayed at the top of the screen. To view all spectra collected for this sample, show “all loaded signals” as in the example shown. Alternatively, if only one signal is desired, select it instead; this is recommended for integration.
- The “integration” and “spectrum” buttons below your sample list will be most frequently used. To view a complete UV-vis spectrum of any of your chromatographic peaks (as long as you opted to save this data in your method) click the “spectrum” button and click the appropriate “select spectrum...” button that appears in the toolbar below.

- To integrate your peaks, select the “integration” button (shown in Figure 9). Select the desired signal you wish to monitor from the drop-down menu as described before. Automatic integration results may now be read or, if desired, manual integration may be performed by clicking the “Manual Integration” button (highlighted in Figure 9) and then drawing your own baseline under the peak(s) of interest.
- Record your integration results. This is your data! Congratulations.
- When finished with HPLC, shut down the system to conserve energy. This is done by turning off the power strip behind the instrument (**note: do not touch the buttons on the instrument**) and turning off the computer.