

Agilent HPLC Operating Instructions
Version 1.0
10/2/09

- 1) Turn the instrument on by pushing the power buttons for each desired module. If the fluorescence detector is not needed/desired, it can be bypassed in the flow path and also removed from the computer configuration. Seek assistance from the Lab Manager to perform these actions, if desired.
- 2) Double-click the "Instrument 1 online" icon to launch the software if it is not already running. The LC software is called ChemStation. If only data analysis and/or re-processing are desired, the "Instrument 1 offline" icon can be clicked instead. The software opens to the Method and Run Control section of ChemStation. In this section, the components of the LC are displayed graphically in the upper portion of the screen. Click the *on* button in the lower right corner of this upper section to turn on all of the instrument components. To edit any component, place the mouse over its respective icon, single click, and a drop-down menu will appear. Edits to the various components can be made in this way or alternatively through editing the entire method, which is explained below.
- 3) Methods and sequences are also created in this section of ChemStation. The method and sequence that were used last are displayed above the blue bar that says "Method and Run Control".
- 4) To open a different method, click *Method* → *Load Method*, and select the desired method from the list. You may have to open the correct folder on the right side of the window first.
- 5) To create a new method, click *Method* → *New Method*, which opens the "Def_LC" method. Next click *Method* → *Edit Entire Method...* Alternatively, just click *Method* → *Edit Entire Method*, which will edit the currently loaded method. Go through the various pop-up windows and make changes to the method as desired. When changes have been completed, go to "Method - Save Method As..." and save the method to a new file name. Window 1: Method Sections to Edit: click all 4 boxes. Window 2: enter any method comments. Window 3: enter the pump parameters, such as flow, stop time, and post time. Select the mobile phase solvents and their percentages. The pressure limits can be modified, if desired. If a gradient is desired, insert lines in the timetable and enter the parameters accordingly. A graphical display of the gradient can be viewed by selecting "Solvents" in the display drop-down menu. Window 4: enter desired injection volume. Window 5: enter desired column compartment temperature. Window 6: enter desired diode array signals. Make sure the reference bandwidth is at least 100 nm above the sample bandwidth. Select which portions of the spectrum to store. If the wavelengths will be switched during the analysis, enter these parameters in the timetable. Window 7: enter the excitation and emission wavelengths for the fluorescence detector. If the wavelengths will be switched during the analysis, enter these parameters in the timetable. Window 8: enter the signals that will be processed by the data analysis section

of ChemStation. Window 9: enter any desired integration events. Window 10: specify the report parameters for the data. Window 11: select instrument data curves to overlay. Window 12: edit desired calibration settings. Window 13: click OK to calibration table. Window 14: select the box "Save Method with Data" on the Run Time Checklist.

- 6) To open a different sequence, click *Sequence* → *Load Sequence Template*, and select the desired sequence from the list. You may have to open the correct folder on the right side of the window first.
- 7) To create a new sequence, click *Sequence* → *New Sequence Template*, which opens the "Def_LC" sequence. Next click *Sequence* → *Sequence Parameters...*. Alternatively, just click *Sequence* → *Sequence Parameters*, which will edit the currently loaded sequence. Fill in operator name. Fill in the proper subdirectory (this is the folder the data files will be stored in). If this is a new folder, the software will inform you that the subdirectory doesn't exist and asks if you want to create it. Click Yes. If you want the data files to be named automatically by the software, click the "Auto" radio button, or if you want to supply a prefix and a counter, click the "Prefix/Counter" radio button and fill in the desired prefix and counter in the white boxes. If a shutdown is desired at the end of the sequence, click the "Shutdown" check box, and select "macro "Shutdown.mac",go" from the drop-down menu. Enter a sequence comment if desired, then click OK. Click *Sequence* → *Sequence Table*. Enter a line for each sample to be analyzed. In the "vial" column, enter the position of the vial in the autosampler rack. In the "sample name" column, enter the sample name. In the "method name" column, select the proper method for each sample from the drop-down menu. In the "inj/vial" column, select the number of injections for the selected vial. In the "sample type" column, select whether the sample is a calibration sample ("calibration") or an unknown sample ("sample"). In the "cal level" column, enter the proper calibration level for each calibration standard. The calibration standards should be analyzed from lowest to highest concentration, and numbered from 1 on. In the "Update RF" column, select whether you want to replace, average, or have no update to the response factor for that standard. In the "Update RT" column, select whether you want to replace, average, or have no update to the retention time for that standard. Enter sample info for each sample in the large white box at the top of the window if desired. If lines of the table are not desired, click on the line number to highlight the entire line in black, then click "Cut". For additional sample table lines, either use the "Insert" or "Append line" commands. Click OK when finished setting up the table. Go to *Sequence* → *Save Sequence Template As...* and save the sequence to a new file name.
- 8) Place the standards and samples in the autosampler rack in the positions indicated by the sequence. Double-check that there is enough mobile phase for the run, as well as enough capacity in the waste bottle to collect the waste solvents. Fill the solvent bottles or empty the waste bottle as necessary. Double-check that the proper method and sequence are loaded.
- 9) To start the analysis, click the "Start" button above the graphical display of the autosampler rack. Alternatively, *Run Control* → *Run Sequence* will also start the analysis.
- 10) Once the samples have been analyzed, the data can be viewed and processed in the Data

Analysis section of ChemStation. Click the "Data Analysis" icon to go to this section. A list of data folders will be displayed on the left side of the screen. Open the proper folder, and double-click the desired sequence file to open it. To see the calibration table/curve, click the "Calibration" icon in the upper middle portion of the screen. If the calibration was already part of the method, nothing needs to be done. If this is a new calibration table, enter the amounts (in ng/ul) of the calibration standards into the calibration table. The sample table is displayed at the top of the screen. Double-click the desired sample to open it in the graphical window(s) below.

- 11) Samples can be re-processed with a modified method or with a different method. Select the desired method by using the drop-down menu in the top-left corner of the sequence window. To re-process, click the green arrow icon in the top bar of the sequence window.
- 12) To generate a report, click *Report* → *Specify Report...*. Select the desired report options and click OK. Select *Report* → *Print Report*.