

Standard Operation Procedure for Agilent 1260 HPLC System (LC ChemStation)

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Please schedule training with an experienced lab member before using the Agilent HPLC! Please do not try to work on the system before any training. This SOP might not be self-explanatory without training.

Before Start

- Sign up the “Log Book” with “Column”, “mobile phase”, “sample information”, “column pressure” “Start time”, “No. of samples”, “End time” and “Note”;
- Make sure the HPLC is properly connected and a suitable column is installed.
- **Make sure the solvent bottles are full and waste bottle is empty!**

Start the HPLC system

- Turn on computer. Login info “HPLC_H1136” and password “3000hanover”
- Turn on all four modules for HPLC system by pushing the buttons located on each module’s lower left corner, starting from the top module down. Allow startup procedure to finish; all lights above buttons should be green. Orange or red lights indicate an issue present.
- Double click the “ChemStation Online” icon on the desktop screen to open the ChemStation software.
- ***If** a new mobile phase bottle was used or any mobile phase line seems to be filled with bubbles or the system has not been used for a very long time, it is recommended a purge of the lines be performed before starting an experiment. Open the purge valve (by turning knob counter clockwise) and purge the corresponding solvent line(s) in which mobile phase have been placed by setting the flow rate of the pump as 5.0 ml/min, using “Setup Pump” menu from the panel for few minutes until the solvent line is completely filled with mobile phase. Ensure that no air bubbles are noticed in the solvent line. Close the purge valve when you finished purging.*
- Within the ChemStation program, switch on the instrument (including Injector, Pump, Column and UV lamp) by clicking the “ON” button in the middle right corner of the screen and wait for the light of each module screen to change from red (or yellow) to green. The instrument will

begin to pump solvent. At this point, check your column/system for leaks. **NOTE:** If the instrument detects a leak, it will automatically turn red and shut off.

- Allow your solvent to pump through your column for several minutes before running your samples. Column should be equilibrated until pressure and UV/Vis baseline has stabilized.

Note: Do you plan to use an existing method? If yes, skip “Set Up a Method” section.

Set Up a Method

1. To create a new method click on method file menu, open “Edit Entire Method” displayed and click OK
2. Enter method information if any and then click OK.
3. In “Set up Injector” option enter the injection volume as per requirement. Injection needle wash option highlighted and input solvent wash vial number and click OK.
4. In “Set up Pump” options input flow rate value per/ml of flow required (i.e. isocratic/ gradient) from “gradient type” create time table program for pump as per test requirement. Input stop time for run sample and set maximum pressure to 400psi. And click OK.
5. In “Column Thermostat Method” option, input the required Temperature value and click OK. Then open signal details option click OK. Open Edit Integration Events click OK.
6. In “DAD Signal” option enter the wave length number by numerical key. If required wave length change during analysis create time table program as per requirement, and then click OK.
7. In “Specific Report” option select the report style e.g. ABC, ABC-1, PERFORMANCE etc. and the click OK.
8. In the finish window at the end enter a title to describe the program. Open the ‘file’ menu click “save as” option open “method” and input method name with the help of words key and click OK.

Run a single sample

1. Open the ‘Method’ file and select “Load Method”. Next, select your required method, from the list stored in the file, and click “OK”.

2. Open the "Run Control" file, select the "Sample Information" option, and enter Operator name, Subdirectory name. In data file select manual option and enter data file name. In "Sample Parameter" enter vial location number, sample name, sample amount and comment (if any).
3. Click "Run the Method"

Set Up a Sequence

4. To create a new sequence click on the Sequence file menu, then click "New Sequence". Default sequence will be appear. Alternatively, click "Load Sequence" to use a previous sequence as your template.
5. Open the "Sequence" file menu and click "Sequence Parameter". Make sure to that the "Shut Down" option is checked and added into your sequence. Click "OK".
6. Open the "Sequence" file menu and click "Sequence Table" and begin to build your sequence. Under "Vial", input the number only (DO NOT type "vial") that corresponds to the location of the vial in the sample tray. Sample tray numbered in ascending order (front to back) for each column.
7. Chose a unique name for your sample, then click the "Method Name" box and select your desired method from the drop-down list. At this point, you can continue to fill in information such as injections/vial, 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. Click "OK"
8. Open the "File" menu, click the "Save As" option, and then open "Sequence ". At this point, enter your sequence name to save the sequence, give a title for sequence, and select a location for data source and appropriate directory to save the sequence as applicable. Click "OK".

Run Samples with Sequence

1. Place the 96 well-plate with samples in the plate holder of auto-sampler.
2. Ensure that your samples are in the correct positions in the auto sampler tray according to their entries in the sequence table;

3. Click “Start” to start the sequence run. **NOTE:** Instrument will not run if the auto sampler tray is not secure in the correct position.

After Finish

1. If you run a sequence, make sure to check that you have built “Shut Down” into your sequence. Once all samples have been ran, the instrument will shut down the Injector, Pump, Column and UV lamp. Remember to come back and power off all modules when your sequence is finished later.
2. If you run test samples by single injection, please turn off the system by clicking the “OFF” button on the screen in the ChemStation program. Power down all modules, starting from bottom to top.

Data Analysis

1. Open the “ChemStation offline” icon on desktop
2. Select “Data Analysis” and select the name of your sequence data from the list (or, alternatively, your single run) from the list.
3. Select the individual sample you wish to analyze from the displayed list, by clicking on any file within your sequence; this should bring up the chromatogram.
4. To integrate your peaks, select the “Integration” button to display integration data including Retention Time, Peak Area, Peak Width, etc.