

PerkinElmer Series 200 HPLC: Operating Procedure

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This document outlines the basic operating procedure for the PE series 200 HPLC, UV/Vis and SEDEX Light Scattering Detector. Refer to the complete set of manuals for more detailed information.

- Safety Glasses and gloves must be worn when operating this equipment.
- Before operating this equipment review all safety data specific to the chemicals you will be using. M.S.D.S documents for acetonitrile and methanol are located in the back of this book.
- Check in with lab personnel to review operating procedure before operating this equipment.
- Prepare samples and reagents before powering up the HPLC.
- Make sure you have enough mobile phase.
- For more detailed information see equipment manuals.
- Do not modify this equipment without prior approval from laboratory staff.

The following instructions refer to the block diagrams on the TotalChrom main window, not the toolbar.

- **Create a folder on the C: drive under Student Data to store your data.**

The UV/VIS detector utilizes a tungsten lamp (visible) (Wavelength 360 – 700 nm) and a deuterium lamp (UV) (Wavelength 190 – 360 nm). The detector operates using one lamp at a time. The detector is normally set-up for operation using the UV lamp. The current method sets the wavelength to 238nm. If you wish to use the visible lamp contact lab personnel.

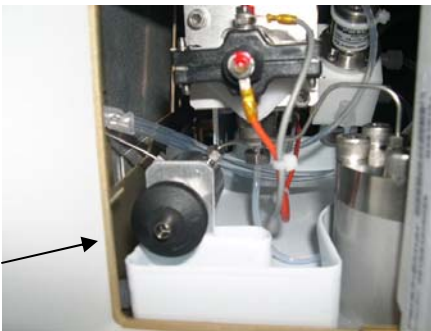
A. Power-up sequence

1. Turn on the PC, log on as user = computer, PW = Letmein!
2. Turn on Dot Link Box and NCI 901.
3. Turn on the pump, switch on right side, UV detector, switch on left rear, Sedex detector, switch on right rear, and column heater, switch on power strip.
4. Turn on Series 200 Vacuum Degasser located next to the mobile phase bottles. Switch located top, rear.
5. Open the Nitrogen cylinder main valve, the pressure should read approximately 40 PSI on the regulator output and 2.9 BAR on the Sedex display.

B. Prime the pump.

This step may not be required if the pump has been operated within the last 72 hours.

1. Open the pump access door on the lower left front panel of the Series 200 pump. This will expose the priming valve as shown below. Rotate black valve knob counter-clockwise approximately 3 turns. See photo below.



Insert syringe / tubing over the valve opening as shown below.



Rotate injection valve clockwise to the inject position so the valve handle is at 10 o'clock.



Using the syringe, pull mobile phase through the injection valve and into the syringe. Observe the tubing connecting the mobile phase bottles to the valve, pull enough liquid through the valve to remove any air bubbles in the tubing.

Rotate the injection valve handle counter-clockwise to the load position (handle at 8 o'clock).

Close black valve, remove the syringe, close pump door, empty contents of syringe into waste container.

C. Starting the Software

1. Double click on the TCNav shortcut.
2. Log in as user = manager, PW = Chrom
3. Select the PE Series 200 LC UV/VIS detector from the instrument window.
4. If the status window displays a com port error, wait 3 minutes for it to clear. If the error does not clear, cycle power on dot link box.

D. Acquiring the hardware – Series 200 UV/VIS

1. From the INSTRUMENT window select PE Series 200 LC
2. Select the RUN box, then select ATTACH.
Wait a minute while the software communicates with the instrument then select TAKE CONTROL from the RUN box.
If ATTACH is grayed out select TAKE CONTROL.
If a seizing error appears on the PE 200, cycle the power on the pump and try again.

It takes a few minutes for these commands to take effect.

E. Load the sequence and method for the UV/VIS detector.

*The mobile phase is set at 50/50 for reagent bottles A&B.
The run time is set to 20 minutes.*

The method defines the operations parameters, the sequence defines the data storage parameters. The sequence references the method. The method and sequence you will use are already built. These files are located at C:\Student Sequences and Methods.

1. From the INSTRUMENT/SETUP window select METHOD then click on the folder to the right of the Method window in the instrument setup menu. Browse to C:Student Sequences and Methods. Open the folder and select 5503 UV VIS Method.
2. Click on the folder to the right of the RAW file path, select the folder you created under STUDENT HPLC DATA to store your data.
3. Repeat step 2. for the RESULTS FILE PATH.
4. In the SETUP INSTRUMENT window enter your unique BASE FILE NAME then click on the icon to the right of the base file name window and select INSTRUMENT NAME, click OK.
5. You have completed loading the method for the UV VIS. Click OK. The status display will read “setting up” then “Ready” This takes a few minutes.

F. Load the sequence for the UV/VIS detector.

1. From the INSTRUMENT/SETUP box select SEQUENCE, select the sequence folder – C: Student Sequences and Methods.
2. Open the folder and select 5503 Student Sequence.
3. Select your data file for the RAW FILE PATH and the RESULTS FILE PATH.

The sequence is set up for 20 samples. If you need to take more samples increase the ENDING ROW number as needed, then click OK.

The status window will display “setting up” then change to “ready”.

Once the display changes to ready you can proceed.

G. Configuring the SEDEX detector.

1. From the INSTRUMENTS window on the left, select SEDEX. Select the RUN box, then select ATTACH. Wait until the status window displays “SEDEX No Method”
2. From the instrument / setup box select METHOD, then select the method folder on the right, go to C: Student Sequences and Methods, select 5503 SEDEX Method.
3. Select your data files for the RAW FILE PATH and the RESULTS FILE PATH.
4. On the instrument setup window enter your unique base file name, the same one you used for the UV VIS method. From the drop –down menu located on the right side of the base file window append the instrument name to your base file name then click OK.

The status window should read SEDEX SETTING UP, after a few seconds this will change to SEDEX READY.

H. Load the sequence for the SEDEX detector.

1. Under INSTRUMENT / SETUP, select SEQUENCE then select the sequence folder, C: Student Sequences and Methods, 5503 Student Sequence.
2. Select your data file for the RAW FILE PATH and the RESULTS FILE PATH.
3. Set the starting and ending row values to match the method values. The default is 1, 20 respectively. This is the number of injections you can have within a sequence.
4. Click OK. After a minute the status window should read SEDEX ready.

After you have loaded the correct method and sequence for each detector you need only load the sequences for each detector as they reference the methods.

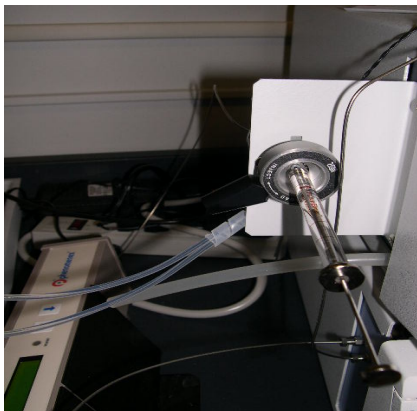
I. Start the Pump.

From the instrument window select the PE Series 200LC. Click on the HANDS ON window. Click START PUMP then close the window.

Note: maximum operating pressure for the Phenomenex and Inertsil columns is 3500 PSI. The pump will automatically shut off if this pressure is exceeded.

J. Injecting the sample.

If the injection valve is not already rotated to the left, rotate the injection valve lever counter-clockwise about $\frac{1}{4}$ turn until it stops. Using the proper syringe, one without a beveled edge, insert the syringe into the injection port as shown and inject the sample.



This loads the sample into the sample loop. Remove the syringe. Now rotate the injection valve lever clockwise until it stops, **this injects the sample into the column and starts the software.** Once the sample is injected into the column wait 30 seconds, then rotate the sample valve lever counter-clockwise back to the load position. Rinse the syringe with D.I water.

- K. Viewing the Chromatograph.
1. Select Real-Time Plot to view the data.
 2. Select instrument from the tool bar to select the plot for each detector.
 3. Select options to rescale.
- L. To stop the run you must stop the run for both detectors by selecting the PE Series 200 from the instruments window then select run, then stop pump. Repeat the procedure selecting the SEDEX.
Canceling the run will not save the data.
- M. To stop the pump select the PE Series 200 LC from the instruments window then select Instrument / Hands on, stop pump.
- N. To change the run time stop the pump and the run for both detectors, select MODIFY, Downloaded method, from the top tool bar select instrument, control options, pump program. Under step 1 of the pump program adjust the run time then select the detector tab and adjust the detector time to match the run time. Click on the wavelength box to complete the change then click OK. Then select file, save.
- O. Printing and saving the data.

At the end of each run the data file report and the chromatogram will automatically print from the printer. The data from your run is automatically saved in the file you set up earlier. The location of your data is printed on the data printout. The peak integration data is printed on page one of the printout. Page two shows the chromatogram with retention times.

P. Viewing previously saved chromatograms.

Click on the chromatogram icon. Select your data file.
View and print as needed.

Q. Shutting down.

1. Turn off all equipment – degasser, column heater, pump and detectors.
2. Clean / rinse all syringes with D.I water.
3. Label all your chemicals containers.
4. Clean up the work area.
5. Log off the computer.
6. Check out with lab personnel.