

Operation procedure for waters HPLC

(SHORT INSTRUCTIONS)

1. Filter and Degas solvents
2. Purge air bubbles from lines with syringe
3. turn on power to pumps
4. Set pumps to remote operation (push menu button until "Mode Local" appears, select edit, push the arrow button)
5. turn on power to detector
6. turn on computer -- run Millenium software -- Choose "Run Sample"
7. Select instrument method on the bottom right part of screen -- edit if you need to change any instrument parameters -- push the "setup" button. This should start the pumps. Remember to push the "run" button on the pumps the first time you start them.
8. Push the monitor button to see the detector output. Let the system equilibrate until the base line detector is stable. To deselect the monitor, click the "abort" button at the top of the screen (The red ball).
9. select "single" to do a single injection.
10. Put in sample name
11. select the method set
12. change run time for your run.
13. Click the inject button -- wait until the system is ready for your injection --

(DETAILED INSTRUCTIONS)

1. **Start up:** Power the system instrumentation according to the following sequence: pumps, detector, and computer.
2. **Filter and degass** solvents. Note: **Top pump is for hexane and the bottom pump is for isopropanol.**
3. **Purging: do not purge the 2 pumps at the same time! Always purge bottom pump (isopropanol) first and then top pump (hexane).**
 - **Turn the purge valve** (located in front of the pump head of the top pump) **to the right.**
 - Press the **menu key on the bottom pump** until the purge function appears.
 - Press **edit/enter** to access the purge function. Press **edit/enter** again to start purging. This will run the pump at 5mL/min for 5 minutes.
 - After the bottom pump complete purging, repeat the process for the top pump.
 - **Turn the purge valve to the left!**
4. **Connect the desired column to the system.** Make sure that the **arrow** on the pump is **the same as the flow direction.**
5. **Set both pumps to remote control:** press **menu** until the mode function appears. Press the **edit/enter**, then use the **up** or **down** key to set the pump to **rem**, press the **edit/enter** again to confirm your selection.
6. Millennium **start up:** double-click the **Millennium** icon on the computer screen. In the **project** pull down menu, choose project **general**. (if you wish to create a new project, please go to Mei's advance classes. Note: You are required to pass a starter's test first!)
7. **Set up run sample conditions:**
 - double-click **run sample** in the millennium login window. The run sample dialog box appears, choose **HPLC**, and then click **OK**.
 - **Maximize** the HPLC in general—quick set window. Find the **instrument method** pull down menu at the lower right corner and choose an existing run condition. For example, 1mLMin 90_10 will provide you a HPLC run condition at 1mL/min in a solvent mixture of 90:10, hexane to isopropanol ratio . If you want to set up a new run condition, see page 3 for instructions about how to set up a new method set.
8. **Start up the system:** Click **set up** button, after about 5 sec, both pumps should start pumping. If not, press the start/stop button on the pumps. Allow the system to stabilize for at least 20 mins before your first sample run. You can click the monitor button to check the baseline. Click abort button (the red- globe shape button in the tool bar) to exit monitor process.
9. **Start a sample run:** click the **single** tab at the bottom of the samples table. Enter the sample **name**, select **inject samples**, select a **method set which matches your run conditions**, change the **injection volume to 20 uL** and enter the desired **run time**. Click

the **injection** button, load your sample into the sample loop and inject. The system will automatically start collecting data once you inject the sample.

10. Click the faucet-like button (located at the bottom of the HPLC in general-quick set window) to stop the system flow after you finish running the last sample.

11. **Data processing:**

- double click **review data** in the millennium login window, choose **injections** and then click OK.
- double click the file (your sample name) you want to process, and maximize the review main window. Click **process-- integrate**. You can highlight a peak by clicking the left mouse key inside the peak of interest. Right click the mouse and choose delete to delete the undesired peak integrations. If you are satisfied with your chromatogram now, from the file pull down menu select save--all results, and then go to step 12.
- **Set threshold:** Use left mouse key to zoom in the noise area, and click **T**(threshold) button in the toolbar to set a new baseline threshold. Right click the mouse and select full view to go back to the full chromatogram.
- **Set Area rejection:** Use left mouse key to zoom the smallest peak of interest, and then click **MA**(minimum area) button in the toolbar.
- **Set Peak width:** use left mouse key to zoom the narrowest peak of interest, and then click **PW**(peak width) button in the toolbar.
- **Save** all the processing results and minimize the review main window.
- Note: you can do data processing while you are running another sample!

12. **Report generation:** double click the **browse project** in the millennium login window, select **general**. In the project window, click the **result** tab. The result view window appears, highlight the file that you want to process, and **right click the mouse**, select **preview** from the context menu. The open report method dialog box appears, select **use the following report method –default**, then click OK. This will provide you a preview of your sample report. Click **print** to get the final report.

Create a new method set

- 1) choose **new method set** in the **edit** pull down menu, say **yes** to question “use the wizard to create this new method set”. And the new method set-select instrument method window will appear, click **create new**.
- 2) In the instrument editor window, select **w2487** in the tree pane:
 - In the **general** tab, select **dual** under the wavelength mode, and **enable** channel 1 and **enable** channel 2.
 - In the **channel 1** tab, set wavelength = 254 nm, and keep all other default parameters.
 - In the **channel 2** tab, set wavelength = 210 nm or any wavelength you prefer, and keep all other default parameters.
- 3) Again in the instrument editor window, select **PCM** in the tree pane:
 - In the **flow** tab, enter **420 as high pressure limit**, and enter **10 as low pressure** limit, choose **gradient** under pump mode, then enter to your desired run condition. Note, **A reservoir is Hexane** and **B reservoir is isopropanol**.
 - In the **events** tab, select **enable events**. Then enter **0.01 for time**, **event 1 for event**, **pulse for function**, and **0.02 for interval**.
- 4) At the **file** pull down menu choose **save as** and name the new run condition. Please name it according to the following format: “?mLMin ?Hex_?lpr”. So other people will know what this condition is.
- 5) Exit and go back to new method set wizard, select the run condition, which you’ve just created as the instrument method. Click next, then next, enter the same name “ mLMin ?Hex_?lpr” as method name, then click finish.