GPC User’s Booklet

This handout will give you the minimum information necessary to use the Waters GPC system. If you have further questions or if you experience any problems, please contact Jim Windak at 647-2847 (jwindak@umich.edu).

How to Run the GPC

1) Before running the GPC, you should first check the solvent reservoir to make sure that there is enough THF solvent available for the number of samples that you want to analyze. If the level is less than 1000 ml or if you intend to run a lot of samples, please contact Jim Windak, or the key operator graduate student.

Be sure that there is enough THF solvent in the reservoir

2) Increase the solvent flow to 1.0 ml/minute very slowly. Increase it in steps of 0.1 ml/min, and wait at least 20 seconds between each step. When the solvent flow rate reaches 1.0 ml/min, the solvent pressure should be approximately 300 psi.

Push the Mode button until the display reads ml/min. Then use the up arrow to increase the flow rate in steps of 0.1 ml/min. Wait at least 20 seconds after each step to allow the pressure to stabilize.
3) Turn on the power for the detectors.

The power switch for the detectors is located here.

4) Zero the UV detector using the knob on the front of the detector.

Zero knob

5) Push on the purge button on the front of the Optilab refractive index detector. This allows the reference cell to fill with solvent. Keep it in on for a few minutes until the reading stabilizes. Then push the purge button again to turn it off. Next, push the zero button and wait for several minutes until a reading of “000” comes up on the display.

Purge button  Up
  ↑ Zero button

6) Important! Move the red return line from the solvent reservoir to the waste bottle on the floor.

← Remove the red tubing from reservoir
  → Insert the red tubing into the waste bottle on the floor
7) Log onto Millenium with your username and password.

The Millennium 32 software should startup automatically after you log in. When Millennium is done loading, click on the Login button, and enter your username & password a second time to log into Millennium itself.

Click on the Login button

This box appears. → Enter your username & password

Then this main menu page will then come up.

Then, double-click → Run Samples to bring up the data acquisition page

← First, click on the drop-down menu to select your project
The drop-down menu lets you select a project to use. To run samples, double click the “Run Samples” button. It will ask you to choose a Chromatographic System. Choose the only one, “Waters 440 GPC”, and click on OK. This will take you into the data acquisition page.

The first step is to load the instrument method in the lower right hand box. There is only one default instrument method available. It is called “Waters 440 Default”. To prepare to inject your sample, fill in the sample information. Click on the Function column to get a drop down menu to select the type of sample you want to run, and choose “Inject Broad Samples”. Fill in a Sample Name, set the run time to 35 minutes, and set the injection volume to 75 ul. Also select a Method set. There is currently only one default method set, called “Waters 440 mthd set”. Click on the right-hand injection icon to start the injection process. The status line at the bottom of the box will say that it is now waiting for the sample to be injected.

Be sure Function is set to Inject Broad Samples

After everything is set up, click on this icon to start the run.

Set the instrument method to Waters 440 Default

After you click on the inject icon, the status box at the bottom of the page will say Single inject - waiting
Rinse out the syringe with clean THF. Then draw up 150 ul of the sample solution into the syringe, and remove any air bubbles.

Hold the syringe upside down. Put a Kim Wipe around the needle, and push out some solvent to remove the air bubble.

With the syringe still in the injector, turn the lever to the “Inject” position (up). Then you can remove the syringe, and rinse the syringe with clean THF solvent. Then turn the lever back to “Load” (down), so it will be ready for the next sample.

Sample Preparation Notes:

The sample solutions should be prepared at a concentration of approximately 1 mg/ml in HPLC grade THF. Then add 1 microliter of toluene per milliliter of sample solution, using the syringe labeled
“Toluene Only”. For example, for a 10 ml sample solution, add only 10 ul of toluene. Be careful not to add too much toluene.

The sample solution must then be drawn into a 10 ml disposable syringe (Fisher part # 03-377-29) and filtered through a 0.45 micron PTFE filter (Fisher Part No. SLFH-013-NL), into a clean dust-free container. This is an important step that removes particulate matter from the sample to prevent damage to the system. The solution is then ready for injection and analysis.

How to Process the Data

To review sample data that has been collected, minimize the acquisition window and double click the “Browse Project” icon. Select your project and click on OK. Click on the Channels tab in order to view data channels that have been injected. For each sample, there is one channel for the UV detector output, and one channel for the RI detector output. You must process UV and RI channels separately. You cannot process UV and RI traces together, because they each have their own calibration. Highlight the channels that you wish to use (you can CNTRL-Click to select more than one, or use Shift-Click to highlight a range of them). Then right-click on one of the files to get a menu, and left-click on Review. This will take you into a Review window.
This is a Project window, where you can select data channels to view.

Click on the Channels tab ↓

Highlight the channels you want to review by holding down the Ctrl button on the keyboard, and left-clicking each one you want to select. Then right-click on one of the selected files to get a menu, and left-click on Review.

This is a Review window.

The first step to do in Review is to open a processing method (calibration). Do this by clicking on File – Open – Method. Select the processing method called “UV_Month_yy” for UV channels, or select the method called “RI_Month_yy” for RI channels, and click on Open. Use the most current calibration as indicated by the month & year.

Now you can process the data. Be sure that you are looking at the Main Window under the Window menu. Click and drag across the bottom of a chromatographic peak to get the retention
time on the peak top. This is called integration. Next, click the Process menu and click on Quantitate to assign molecular weights and calculate molecular weight distributions. Click on the Peaks tab near the bottom to view the molecular weight distribution numbers. If you are happy with the results, click on **File – Save – Result.** If you want to look at other channels in the channel table, you can click on the Next channel button on the toolbar.

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**How to Print Reports**

To print a complete report from results that have been saved, go back to the project window. Click on the Results tab to view the result files. Highlight the files you wish to print, and then click on the Tools menu, and click on Preview. Alternatively you can right-click one of the highlighted files and click on Preview. This will launch the report editor. It will first ask if you wish to use the default report method. Click on OK. It will then show you how the report will look. If you want to use this format, just click on the print icon. You can use the Next Report button on the toolbar to view the next report in your list of results.
Click on the Results tab in the project window

Highlight the result files you wish to print. Right-click on the highlighted files, and left-click on Preview, to go into the Report Publisher Preview.

Next & Previous report buttons

Print Button

Preview of Sample Report
How to Shut the System Down

1) Close all of the windows except the main Millennium window.

2) Click on the logout button on the Main Millennium window. Then click on the X to close the Millennium program.

3) Put the red tubing back into the solvent reservoir.

3) Slowly decrease the flow rate down to 0.1 ml/min.
Trouble-shooting Common Problems

1) When I try to integrate and quantitate my sample peak, I cannot get molecular weight distribution numbers. Why not?

There are several possibilities that could cause this problem.

a) Possibility One: Make sure that you have the correct processing method (calibration) open in review. You can check this by looking in the lower right corner of the Review window, where it will tell you what processing method is open.

![This shows what processing method is open.]

If the correct processing method is not open, then do a **File -- Open – Method**, and select the latest calibration processing method.

b) Possibility 2: Check in your Project window to be sure that the channels are called “Broad Unknown” under the heading labeled Sample Type. If not, right click on the channel and left click on Alter Sample. When the Alter Sample window comes up, change the Sample Type to “Broad Unknown”.

Then click on **File – Save** to save the changes to the data.

![Set the Sample Type to Broad Unknown. Then click on File – Save to save the change.]

Next, bring the altered files into a new Review window. Open the correct processing method. Integrate and Quantitate the main peak. You should now see distribution numbers if you click on the Peaks tab near the bottom of the page.
c) Possibility 3: Your sample peak might be out of the range of the calibration. We calibrate with known polystyrene standards from MW 1,000,000 to MW 92. If your sample peak occurs at 10,000,000, for example, it will be outside of the range of the calibration curve. You can extrapolate the calibration curve slightly beyond 1,000,000, but if you try to extrapolate too far, the accuracy will not be good.

The MW 1,000,000 standard has a RT of 15 min. You could extrapolate the calibration curve to as high as 10 minutes, although the accuracy will not be very good. If your sample peak has a RT less than 10 minutes, then you cannot quantify it.

d) Possibility 4: The processing method is looking for the broad unknown peak in the wrong retention time window. To change this, click the Window menu in Review. Click on Processing Method. Click on the Slicing Tab.

Change the retention time and retention time window so that your sample peak will be completely included.

The Retention Time, ± the RT Window value, defines the window that the software uses to search for a broad sample peak. You may need to change these values so that your sample peak falls within this window.

If you change the Retention Time, or RT Window values, you must then click on File—Save—Method. It will ask you whether or not you want to copy the curves with the method. Click on Copy Curves.

Be sure to click on Copy Curves, not the default Clear Curves.
Now, re-open the modified version of your Processing Method in Review. Re-integrate and quantitate your sample peak.

2) The sample peak is very small compared to the toluene peak. Why?

Probably, too much toluene was added to the sample. The height of the toluene peak should be around 200 mV on the UV trace. Add only 1 ul of toluene per milliter of sample solution.