Waters GPC

Please see Pete for training before your first use.

General

- Check THF reservoir. THF is consumed at 0.35 mL/min when running samples, and there should never be less than 2" of solvent at the bottom of the bottle. See Advanced user topics for refilling THF.

- Check that waste bottle (under sink) can accommodate the expected amount of waste. THF waste in commonly repurposed, so ask Pete before disposal.

- The instrument is controlled using *Breeze* software, which organizes large data sets into *projects*. The active *project* is displayed above the file menu (except when data is being viewed). All samples should be run on the most recent *project*, named Jan + current year. See Advanced user topics to change *projects*.

- The **Navigation** toolbar on the left-hand side of the screen allows you to toggle between functions. *Find Data* and *Sample Queue* buttons are most useful.

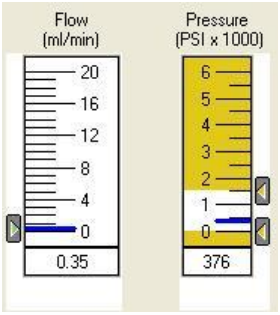
Sample preparation

1. Completely dissolve sample (water and salt-free) at ~ 0.5 mg/mL in **Sample Prep THF** [1:1000 toluene:THF] located next to THF reservoir. Heating may be necessary.
2. Cool to room temp and filter sample through 0.2 µm PTFE filter using a 1 mL syringe into GPC vial and cap. Label vial with page/exp # using permanent marker for future ID. See Advanced user topics for *Low-solubility polymers*.

Run a new Sample Set

Regular users should *Create method template* as described in Advanced user topics

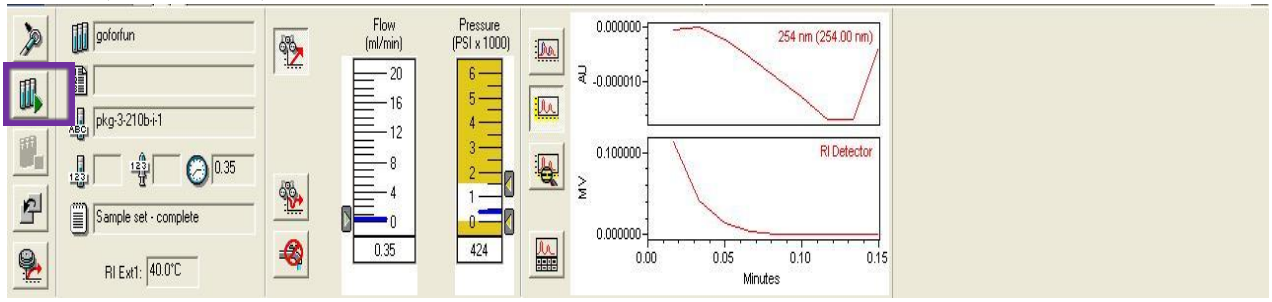
1. Open Breeze and change flow rate from 0.10 mL/min to **0.35 mL/min**. Allow pressure to stabilize at 350 – 600 for > 3 min (20 min is preferable).



2. Open door on Autosampler and wait for the motor noise to subside. Load vial(s) into sample holder, record their position, and close door.
3. Click *Sample Queue* button on Navigation toolbar.
4. Click "File → New Sample Set → New" to clear previous sample set.
    a. Alternatively, previous sample sets can be modified by substituting in your info in *Vial*, *Sample Name,* and *Inj Vol* columns. Ensure that modified sample set follows protocol outline in steps 2-8, then proceed to step 9.
5. Click in the *Function* column in the empty table. A new row will appear.
6. Click on the drop-down menu to change the *Function* to **Condition Column**. Under the *Method* column, select the method with the most recent date (e.g., 06_10_15_NBore_PS_Tol).
7. Set run time to 10 min. If flow rate has been at 0.35 mL/min for 20 minutes or more, the run time can be reduced to as short as 1 min.
8. To add samples, click in the empty part of the table to add a new row. Change *Function* to **Inject Broad Samples**. Change *Vial* and *Sample Name* as desired, and use the same *Method* as your Condition Column. Run time should be 35.00 min.
9. *Inj Vol* can be changed from 5.0-50.0 (µL) depending on sample concentration. Samples that are too concentrated can harm the instrument (injector, columns) and saturate the detector (bad data!). See Advanced user topics for *Low-solubility polymers*.
10. Add additional samples using steps 8 and 9. Adding, inserting, and deleting rows can also be achieved through right-clicking on desired cells.
11. The final row should always be a **Condition Column** using the **Shutdown_NBore** method. Set *Run Time* to 0.15 min.
    a. All sample sets should follow same format, and with 2 samples should appear as:

	Vial	Sample Name	Function	Method	Run Time (Minutes)	Inj Vol (ul)	# of Injs	Next Inj. Delay (Minutes)	Data Start (Minutes)	Label	Label Reference	Processing	
1			Condition Column	03_01_15_NBore_PS_Tol	5.00								
2	1	pkg-3-210b-i-1	Inject Broad Samples	03_01_15_NBore_PS_Tol	35.00	5.0	1	0.00	0.00			Process and Report	Phenc
3	2	pkg-3-212a-i-1	Inject Broad Samples	03_01_15_NBore_PS_Tol	35.00	5.0	1	0.00	0.00			Process and Report	Phenc
4			Condition Column	Shutdown_NBore	0.15								

12. Click on the start button, located on the left side of gray **Acquisition** toolbar (on bottom).

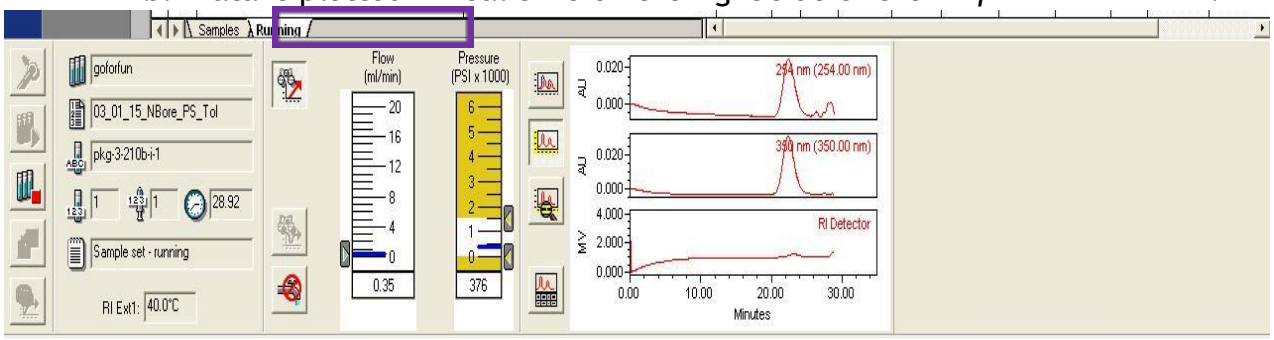


13. A pop-up menu appears that demands that you enter a sample set name. *Settings for this sample set* should be set to *Run Only*. Click run.
    a. The sample set moves to the *Running* tab (bottom left side of the table), and the rows that are running or have run are shown in red.

Active sample set : Pete_is_the_best

	Vial	Sample Name	Function	Method	Run Time (Minutes)	Inj Vol (ul)	# of Injs	Sample Weight	Dilution	Level	Next Inj. Delay (Minutes)	Data Start (Minutes)	Label	Label Reference	
1			Condition Column	03_01_15_NBore_PS_Tol	5.00										
2	1	pkg-3-210b-i-1	Inject Broad Samples	03_01_15_NBore_PS_Tol	35.00	5.0	1	1.00000	1.00000		0.00	0.00			Process an
3	2	pkg-3-212a-i-1	Inject Broad Samples	03_01_15_NBore_PS_Tol	35.00	5.0	1	1.00000	1.00000		0.00	0.00			Process an
4			Condition Column	Shutdown_NBore	0.15										

    b. Data is plotted in real time on the right side of the *Acquisition toolbar*.



Adding samples when GPC is already running

Step 13 from Run a new *Sample Set* can be used to determine whether a sample set is running.

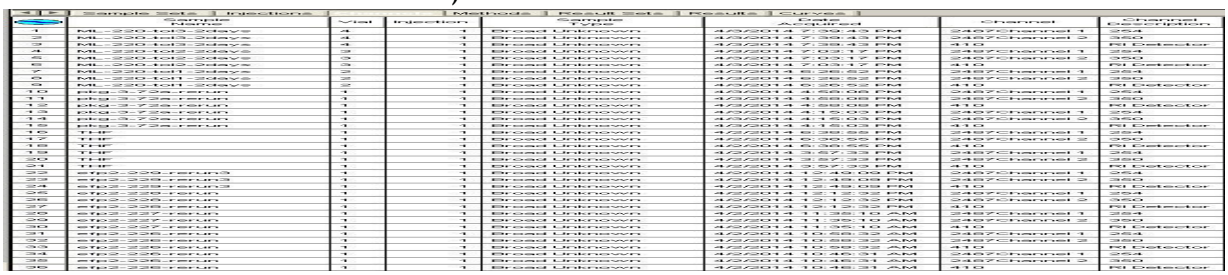
1. "Right click on the sample set table → *Alter Running Samples*". A pop-up warning will appear; click OK. **Note that no further samples will be run until step 3 is completed.**
2. Use steps 2, 3, 8-10 from Run a new *Sample Set* to add samples **before** Shutdown method
3. Follow step 12 from Run a new *Sample Set* to resume acquisition.

Process and view results

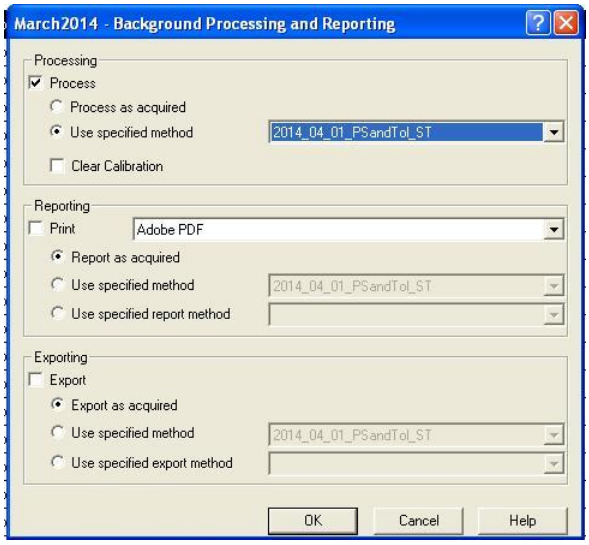
Processing automatically picks peaks, applies baseline correction, and adjusts retention time to the toluene reference peak at ~32.1 min. Without toluene reference, mw data can't be calculated.

1. Click the *Find Data* button from Navigation toolbar to switch over to a list of previously run samples. The settings listed at the top of the page filter what files are observed.

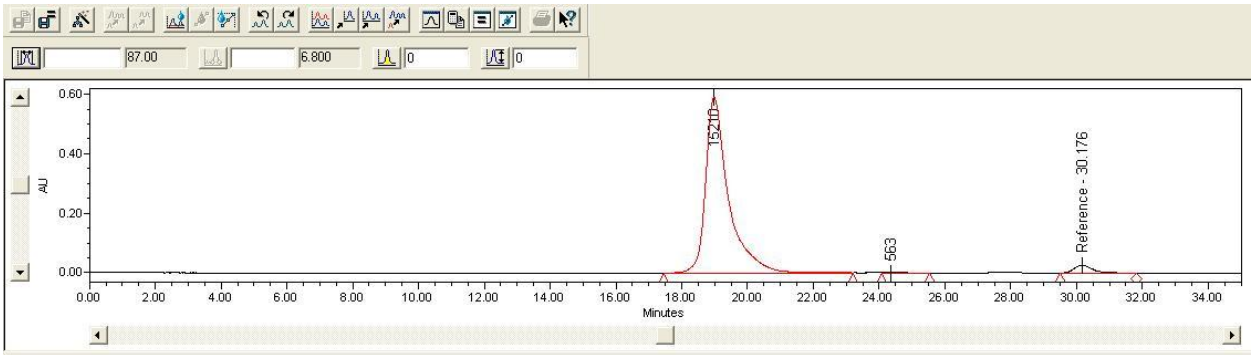
2. The most useful tabs are *Channels* and *Results*. Click on C*hannels*.
3. The *Filter By:* menu allows you to restrict observable files by several time metrics; select the most appropriate one. Click *Update* to apply the filter AND to add recently acquired samples to the table.
    a. Each sample is listed as three separate files according to the method of detection. Only *Channel*s "2487Channel 1" (absorbance at 254 nm) and 410 (refractive index (RI) detector) can be used to calculate molecular weight information (i.e. they calibrated to polystyrene standards and a toluene reference).



    b. To view data without processing, follow "right click on desired file → *Review*".
4. Process data (for mw info) by selecting the desired channel (or "ctrl + left-click on multiple channels") then "right-clicking a channel → *Process*".
5. A pop-up menu will be displayed.
    a. Select **Process as acquired** and click OK. Do this 99% of the time.
    b. If your sample was run on the wrong method or the calibration curve was recently updated, you can process your data using a different calibration curve. Select **Use specified method** and choose the desired method from the drop-down menu.



6. Processed samples can be viewed by going to the *Results* tab (adjust Filter and click **Update** until they appear). "Right click your file → *Review*". Alerts may appear asking:
   "…Do you want to replace all your existing data?" – Answer **Yes**
   "…Do you wish to save them before switching?" – Answer **Yes**
   a. M_n and dispersity are displayed for only one peak. Peaks may be added or modified by left-clicking on the plotted line and dragging across the desired time range. Peaks can be removed by "right click → delete" from the table.



	Distribution Name	Elution Volume (ml)	Retention Time (min)	Adjusted RT (min)	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mv (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw	K (dl/g)	alpha	Intrinsic Viscosity (dl/g)	Area (µV*sec)	% Area	Height (µV)	% Height
1		19.058	18.986	19.058	10966	13534	15210		15337	16939	1.234227	1.133168	1.251545				28396690	96.38	590091	95.52
2		24.454	24.361	24.454			563										60079	0.20	942	0.15
3		30.290	30.176	30.290													1006302	3.42	26712	4.32

   b. The plot can be resized by clicking "*View*" from the file menu and selecting one of the following options:
      i. Full View – scales the x-axis to the entire run time.
      ii. Scale to Peak – scales the y-axis to the integrated peak.
      iii. Autoscale – scales axes to max peak height and entire run time.
   c. Manual zoom can also be applied by left-clicking and dragging across a region.
   d. To view groups of samples, "Ctrl + click on desired samples → *Review*"

i. Click the *Overlay* button (above the plot) to view multiple chromatograms

ii. To change which chromatograms are displayed, click the *Channels* tab (below the table) and check/uncheck the *Visible* column.
iii. *Overlay* view remains on for subsequent plots until button is clicked again.

Report (processed data)

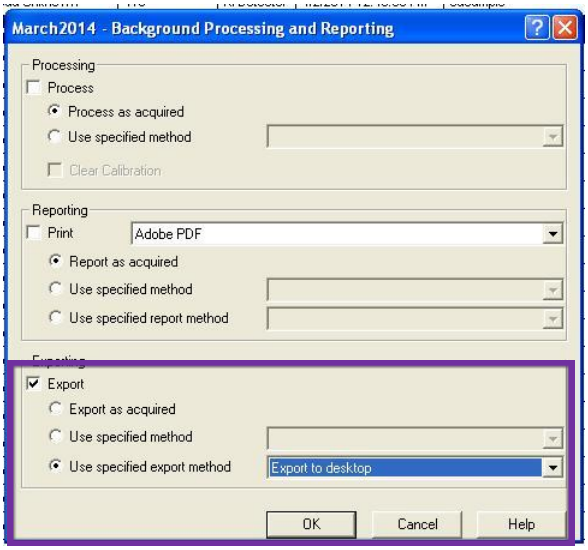
After data has been processed (see Process and review results), chromatogram and associated mw data may be saved or printed as a **Report.**

1. From *Results* tab under *Find Data* button, "right click on desired sample → *Preview*."
2. Select *Use a Report Method…appropriate for the selected data* and click OK.
3. Choose to Save or Print report from the toolbar on top. Note that only the first page(s) contain the most useful information.

Export (raw data)

Export time and absorbance data (from each channel) to plot chromatograms in Sigma Plot or Excel (see *Process exported data* in Advanced user topics). Regular users should make your own export method following procedures under *Create export method* in Advanced user topics

1. From *Channels* or *Results* tab, "right click → **Export**". Opens menu
2. Ensure that *Export* box is checked, and **Export to desktop** is the *specified export method*.
3. File will appear on the desktop as "*Export to Desktop*" + a random series of numbers. Sample name and several acquisition parameters are listed within the spreadsheet.
4. Extract the data that you need (or copy the file to a flash drive) then delete the file from the desktop. Files like these clutter the desktop and may be deleted periodically.



Advanced user topics

Acquisition

THF refill

If only a small amount of additional THF is needed, it may be added to the current reservoir. Otherwise, THF should be replaced with a completely new bottle of stabilized THF using the following instructions.

1. Adjust flow rate to 0.00 mL/min through Breeze software.
2. After flow stops, replace empty bottle with full one. Make sure that metal filters are still attached to the green and thick white lines AND that the lines are completely submerged.
3. Adjust flow rate to 0.10 mL/min through Breeze software.

Create a method template

1. From *Sample Queue* in Navigation toolbar, go "Edit → Open Sample Set Method Template"
2. Open template named *Template*. Click through the wizard, making sure to check *Inject Samples* and set parameters for a typical run. Near the end, you can define which parameters will change from run to run.
3. Save template under a different name.

Low- solubility polymers

Polymers with a low solubility in THF should be prepared as dilute solution with a high injection volume (~ 50 µL). If aggregation or precipitation of the polymer occurs at these concentrations, the sample can be prepared immediately prior to injection. In this case, the polymer can be passed through the 0.20 µm PTFE filter while the THF is still hot, and injected before it cools.

View Acquisition toolbar

The *Acquisition* toolbar (on the bottom of the screen) displays the run controls, flow-rate, pressure, and data from the detectors. There is a check-box (located below the Navigation toolbar) to view the *Acquisition* toolbar. Always make sure that the *Acquisition* toolbar is visible when you leave the computer.

Process exported data

Normalize to compare chromatograms as overlaid or stacked plots.

1. Open *Normalize* or *Normalize with graph* excel file from *Documents* folder. Do not save any modifications to these files.
2. Copy all absorbance values from exported data, and paste into columns B-G under *Enter data here!Normalized to 1* tab.
3. Data is normalized to 1 a.u. and is simultaneously formatted in three different ways:
    a. The *Enter data here!Normalized to 1* tab directly overlays chromatograms.
    b. The *Normalized short stacked A top* tab formats data with 0.5 a.u. spacing between curves, which is useful for plotting multiple similar chromatograms.
    c. The *Normalized stacked A on top* tab formats data with 1 a.u. spacing.
4. Paste normalized values into Sigma Plot or use plots generated in *Normalize with graph*.

Create an export template

1. From *Find Data* in Navigation toolbar, select a file then "*Database* → *Export Data*" from the file toolbar.
2. Select Template as export method and click Edit Method to open Export Method Editor.
    a. Editor opens under the *Fields* tab, which exports experimental parameters and processed values as a text file. If this information is desired, change file name/ location and report parameters
    b. *Raw Data* should always be included in your export method. Change file name/location and modify desired parameters.
3. "File → Save As" to alter the name.

Change Projects (find old data)

Breeze software organizes large data sets into *Projects*. The active *project* is displayed above the file menu (except when data is being viewed). All samples should be run on the most recent *project*, named Jan + current year. Only the files (data, methods, calibration curves) created within the active *project* can be accessed. To find old files from a different *project*:

1. Click on *Manage Breeze* on the Navigation toolbar → **Projects**.
2. The *Comments* column describes the time range over which data was collected. "Right click on the desired project → *Switch To…*" and select desired *project*.

This can be performed while samples are still running in the most recent project.

3. After the old data is viewed/extracted, switch back to the most recent project.

Alter sample information

Sample Name and *Sample Type* can be edited post-run, so data can be retrieved even if you used the wrong name or ran your samples as *narrow standards*.

1. Click on *Find Data* in the Navigation Toolbar.
2. Select the *Injections* tab and find your sample(s).
3. "Right click → Alter Sample", then change *Sample Name* and *Sample Type* as appropriate. All other items are related to the run conditions, and cannot be modified.