

# POLYStandard Operating Procedure

## Prepare Sample

1. Prepare samples as fresh as possible. There is a limited time window before samples start to degrade (molecular weight decreases). This time window will vary sample to sample.
  - a. Can test this for new samples by letting the sample sit in the heated sample chamber, and running the same sample every couple hours to monitor degradation.
2. Prepare samples using Tosoh vial and trichlorobenzene solvent. Aim for a **1 mg/mL concentration**, anywhere from **5–10 mL total volume**. **If the solvent level is too low, an error may pop up when running the sample: “A sample cup was not set to the designated cup number.”**

Note: Solvent does not contain a flow marker. If a flow marker is needed, add a small of any solvent that will not boil at elevated temp (e.g., chloronaphthalene, chlorophenol).

3. Seal with foil and special cap.



4. Heat until fully dissolved.

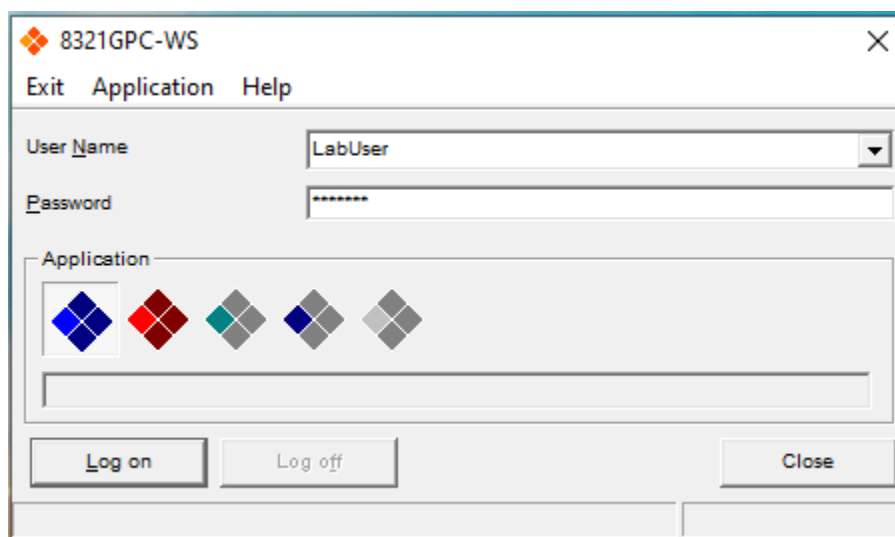
Note: Each sample will require different prep conditions and must be fully dissolved before injecting. As a starting point:

Poly(thiophene): ~145 °C (3-4 hours to fully dissolve)  
Poly(ethylene): ~135–140 °C (2-5 hours to fully dissolve)  
Poly(propylene): ~160–165 °C (4-5 hours to fully dissolve)

5. After samples are totally finished running and instrument has cooled, clean sample vials, and return vial and cap to [designated storage location]
  - a. Cleaning procedure for sample vials: clean with hot TCB, then THF or an alcohol to remove TCB

### Operate Instrument (Warmup, Run/Analyze, Shutdown)

1. Computer login: mcneil-group
2. Open instrument software by click on the orange “flower” icon on desktop, and selecting blue “flower” acquisition application .



3. Log on under “labuser” (in drop down list), password: polymer

4. Check solvent level and change out solvent/waste bottles if necessary (instructions in next section)

Instrument control > Instrument parameters tab > solvent stocker > solvent volume

Solvent Stocker	
Temperature control	On
Solvent volume (mL)	0
Waste fluid volume (mL)	4310
Waste fluid warning volume (mL)	3000

5. Turn on pumps

- a. Click “instrument control” icon on left side toolbar, and select “instrument parameters” tab.

8321GPC Acquisition [Instrument]HLC-8321GPC/HT [Project]Tosoh Test [User]LabUser - Instrument control

Project Monitor Method Sample queue Analysis Instrument Report Options Help

Power Warmup Analysis Shutdown Manual acquisition Ready Ready

Item	Value
Degas level	Std.
<b>Purge</b>	
Purge control	Normal
Purge volume (mL)	5
Purge speed(mL/min)	15
<b>Pump Oven</b>	
Temperature control	On
Control temperature (deg. C)	40
Gas sensor value	300
<b>Pump</b>	
<b>Sample column</b>	
<b>Sample pump</b>	
Flow rate (mL/min)	0.10
High limit pressure (MPa)	15.0
Low limit pressure (MPa)	0.0
<b>Reference pump</b>	
Flow rate ratio	1/1
High limit pressure (MPa)	15.0
Low limit pressure (MPa)	0.0
<b>Flow control</b>	
Sam. pump flow control	Stop.
Ref. pump flow control	Stop.
Sam. and Ref. pump flow control	Stop. Start flow.

Description  
Sam. and Ref. pump flow control.

Flow diagram Instrument Parameters Warmup Shutdown

Permission:User Level 1

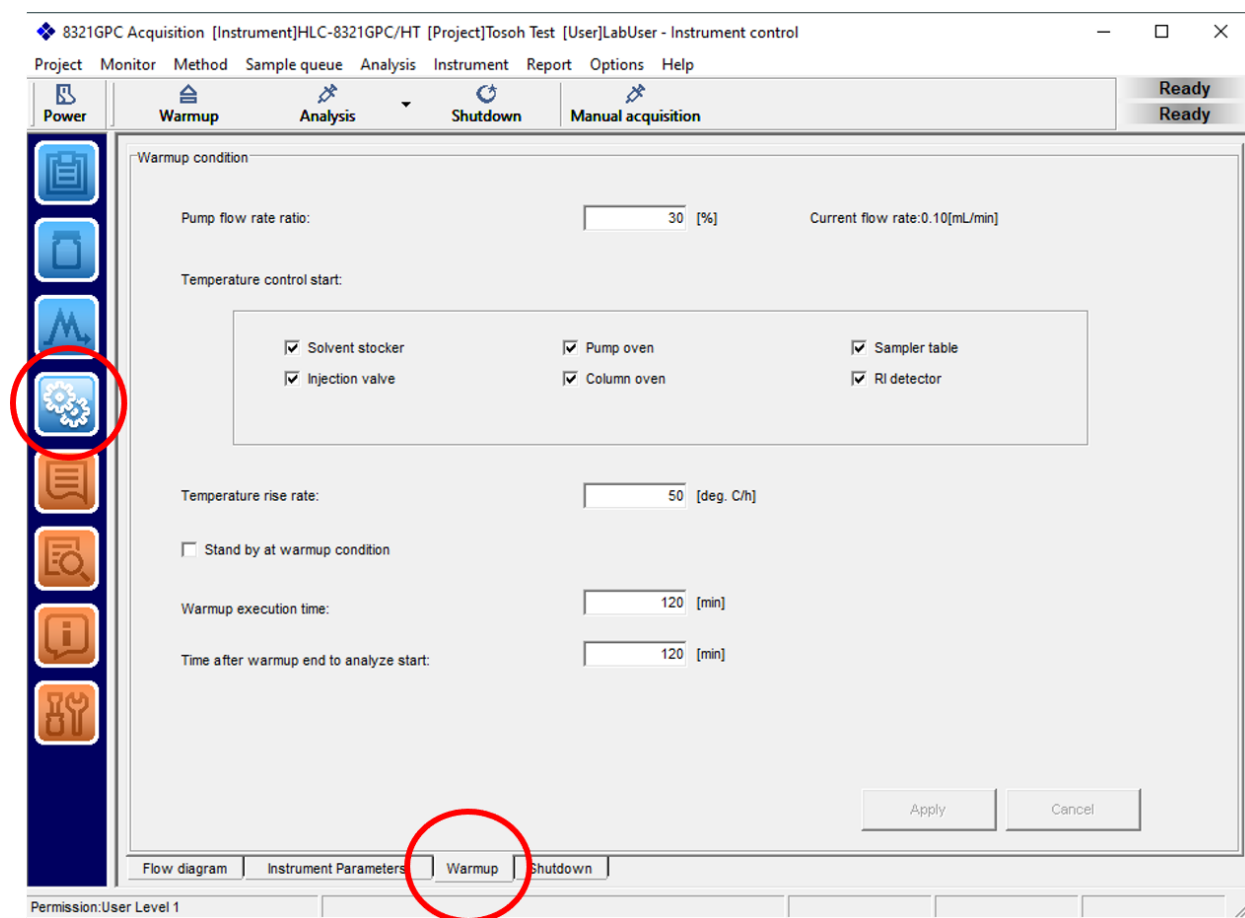
- b. Under “Flow control”, double click “Sam.” and “Ref. pump control”, then “start flow” button will appear.

Flow control	
Sam. pump flow control	Flow.
Ref. pump flow control	Flow.
Sam. and Ref. pump flow control	Flow. <span style="float: right;">Stop flow.</span>

- c. Click “start flow”. Say “yes” to start flow. Should hear the instrument start flowing, see the pressure increase on the instrument display, and see the solvent lines turn green in the flow diagram view of the software.

## 6. Set up warmup condition (4 hours)

- a. Click “instrument control” icon on the left side of the screen, and select “warmup” tab.



- b. Input the following parameters (information is likely already pre-set)

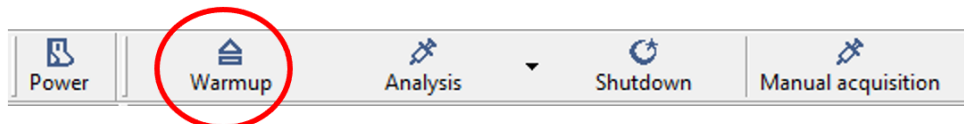
Pump flowrate ratio: 30%  
 Temperature rise rate: 50 °C per hour  
 Warm up execution time: 120 min

Time after warmup end: 120 min

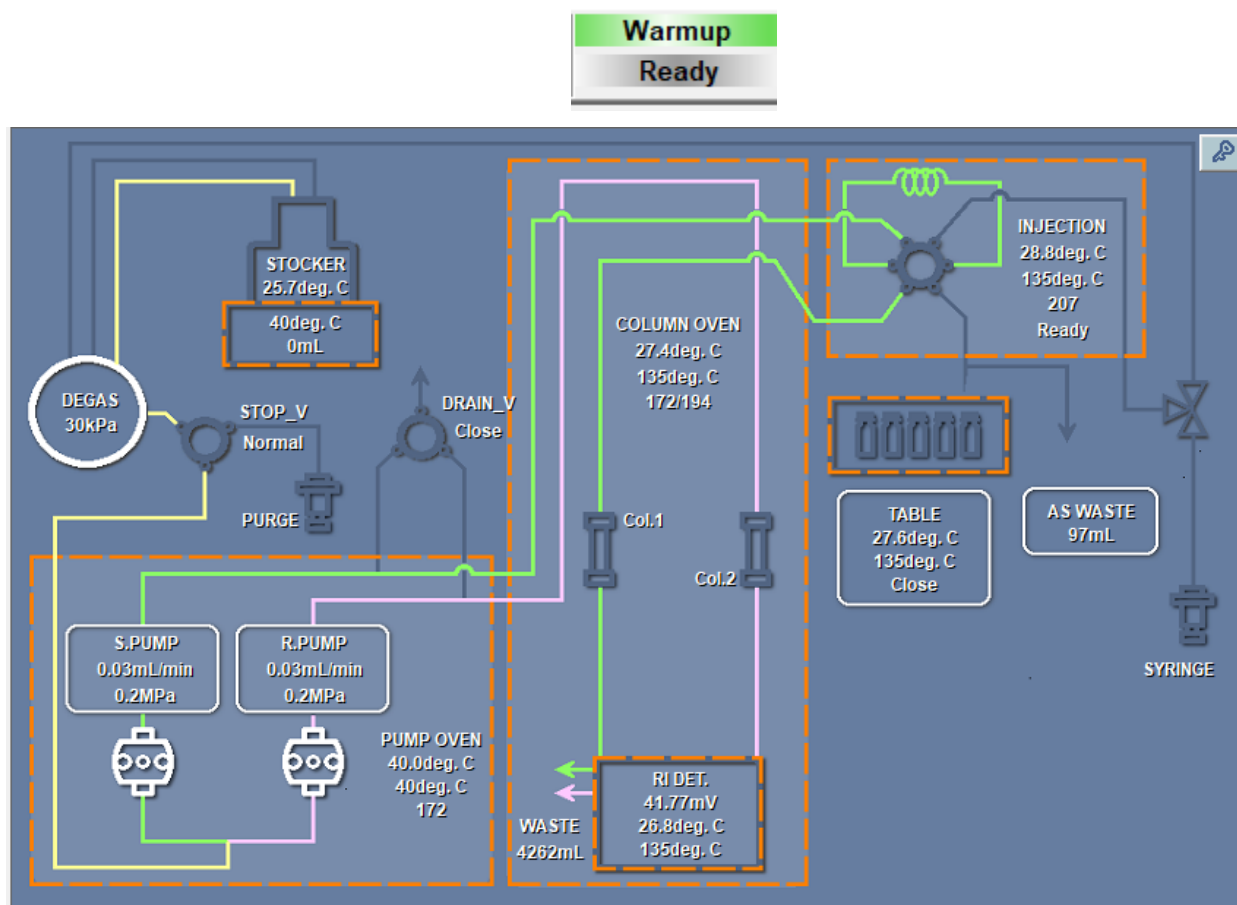
All six temperature control boxes should be checked.

The .

- c. Initiate warmup sequence by clicking the “warmup” button on the top toolbar.



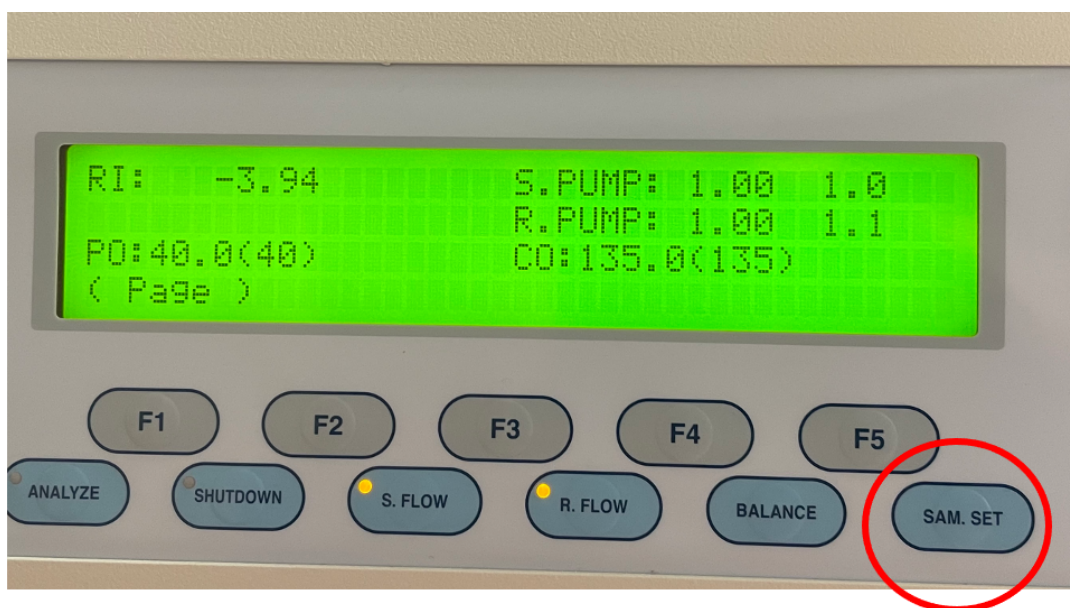
- d. Instrument status (upper right of the top toolbar) should switch from gray “ready” to green “warmup”. Flow diagram should be lit up/colored to indicate that solvent is flowing through pumps and ovens are warming up. Entire warm up sequence takes 4 hours. Can make samples and setup sample sequence during this time.



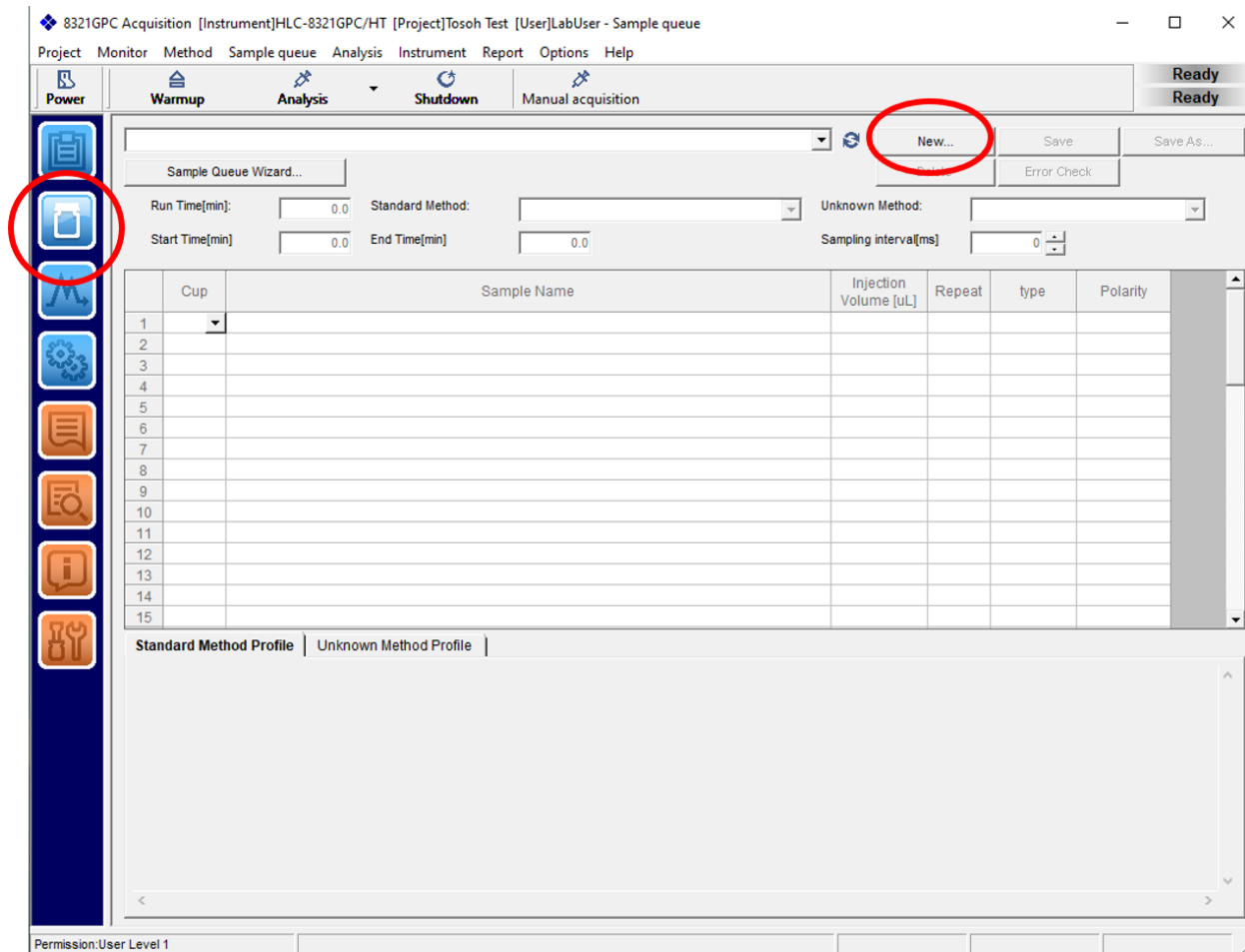
## 7. Set up sample sequence

### a. Place samples in auto sampler

- i. Hit "SAM. SET" button (on front of instrument) to unload auto sampler. The autosampler door must be closed with the key turned for this command to function properly.

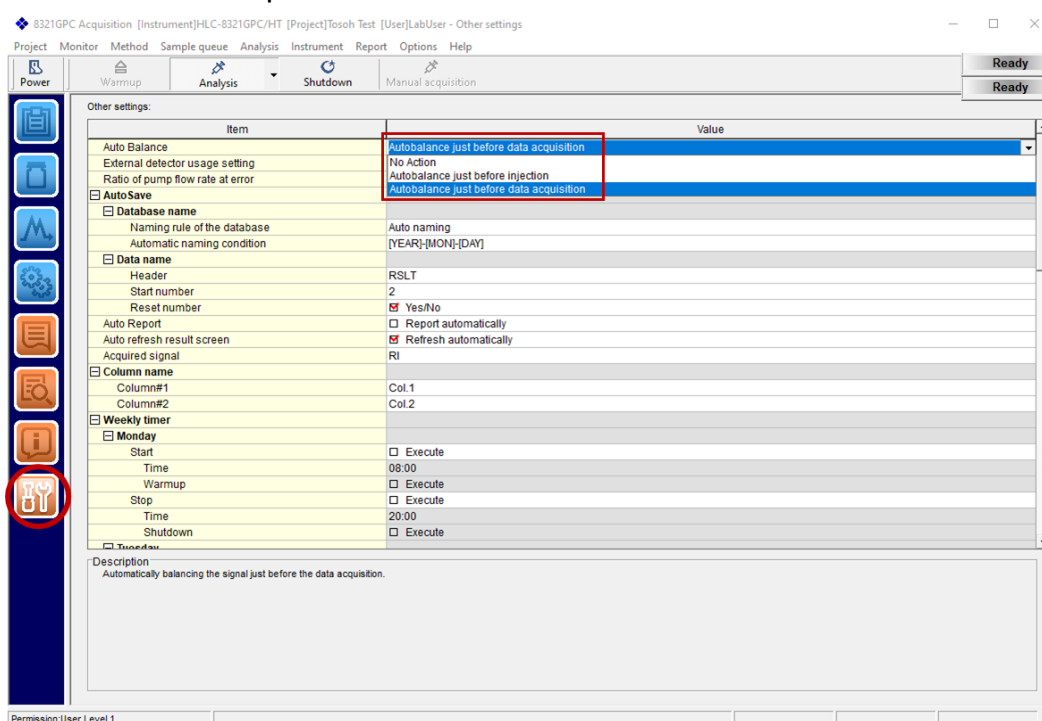


- ii. Open the autosampler door with key (key should already be in the instrument)
  - iii. Place sample vials in numbered tube holders (note they are numbered sequentially spiraling inward) and the close/lock door.
  - iv. Hit "SAM. SET" button again (on front of instrument) to reposition the autosampler.
- b. Move the "solvent waste" line in the waste bottle (plastic tube is covered in red rubber tubing).
  - c. Click "sample queue" button (vial icon) on the left side tool bar.

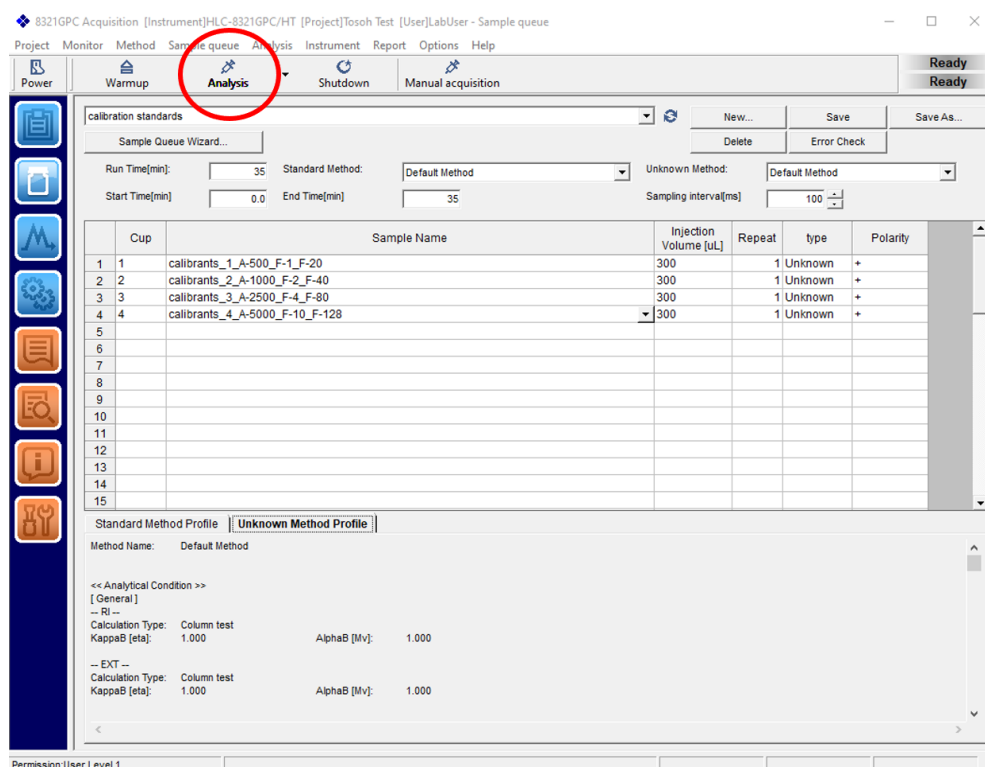


- d. Input sample information and run conditions
  - i. Click “New” and type in sample name in pop-up (note, this sample will not be saved)
  - ii. Input run conditions:
    - Run time = 35 min
    - Start time = 0 min
    - End time = 35 min
    - Sampling interval = 100 ms
    - Standard method- default
    - Unknown method = default
  - iii. Type in cup number (where sample was placed) and give the sample a name (this sample name will be saved with the data).
- e. **Double check** that “solvent waste” line in the waste bottle (plastic tube is covered in red rubber tubing).

- f. Under “other settings” you can select whether to “auto balance” (aka zero) the RI just before acquisition or just before the injection. The default setting is “just before data acquisition.”



- g. Click Analysis button (top tool bar)





- h. The status indicator should turn blue with the text “running” and then “acquisition” once the sample is injected. There is about 7 min between when the sample is marked as “running” and the actual injection. The current sample will be highlighted in yellow ~3 min after the sequence starts.

Before injection:

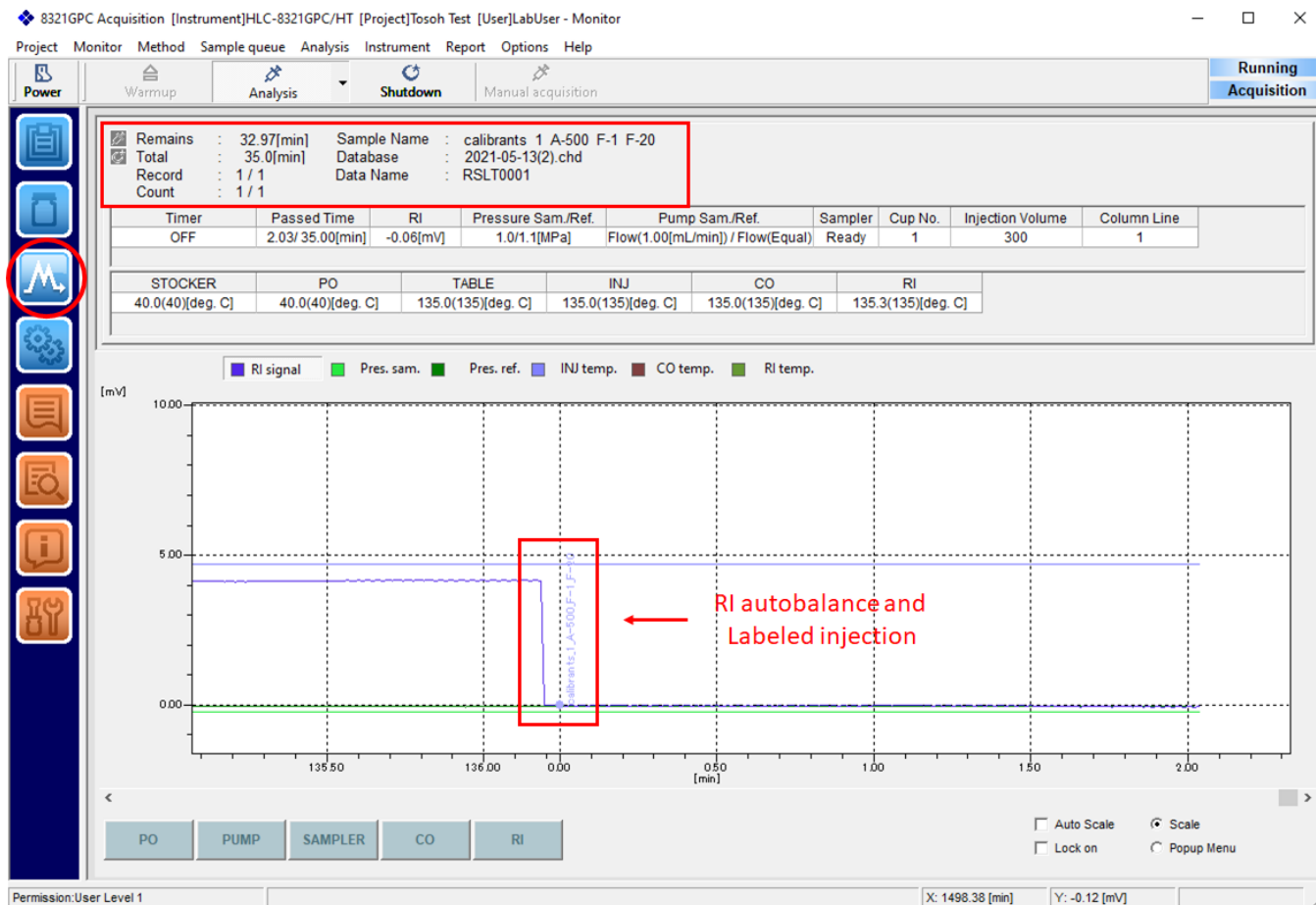
After injection:

The screenshot displays the 8321GPC Acquisition software interface. The title bar indicates the instrument is HLC-8321GPC/HT and the project is Tosoh Test. The status bar at the top right shows 'Running Acquisition' in a blue box, which is circled in red. The main window is divided into several sections:

- Control Panel:** Includes buttons for Power, Warmup, Analysis, Shutdown, and Manual acquisition.
- Parameters:** Run Time (35.0 min), Standard Method (Default Method), Unknown Method (Default Method), Start Time (0.0 min), End Time (35.0 min), and Sampling interval (100 ms).
- Sample Queue Table:** A table with columns for Cup, Sample Name, Injection Volume [μL], Repeat, type, and Polarity. The first row is highlighted in yellow.
- Method Profile:** Shows the Standard Method Profile for 'Default Method' with parameters like KappaB [eta] and AlphaB [Mv].

Cup	Sample Name	Injection Volume [μL]	Repeat	type	Polarity
1	calibrants_4_A-5000_F-10_F-128	300	1	Unknown	+
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					

- i. On the monitor screen, the current sample and time remaining is displayed in the upper left.



- j. When analysis is finished, return “solvent waste” line back to the front solvent bottle, wiping tubing with kimwipe to prevent contamination.
8. If you are done running samples but want to keep the instrument warmed up for future use, reduce the flow rate to 0.1 mL/min.
  - a. Navigate to the “instrument parameters” tab under the “instrument control” screen.

8321GPC Acquisition [Instrument]HLC-8321GPC/HT [Project]Tosoh Test [User]LabUser - Instrument control

Project Monitor Method Sample queue Analysis Instrument Report Options Help

Power Warmup Analysis Shutdown Manual acquisition Ready Ready

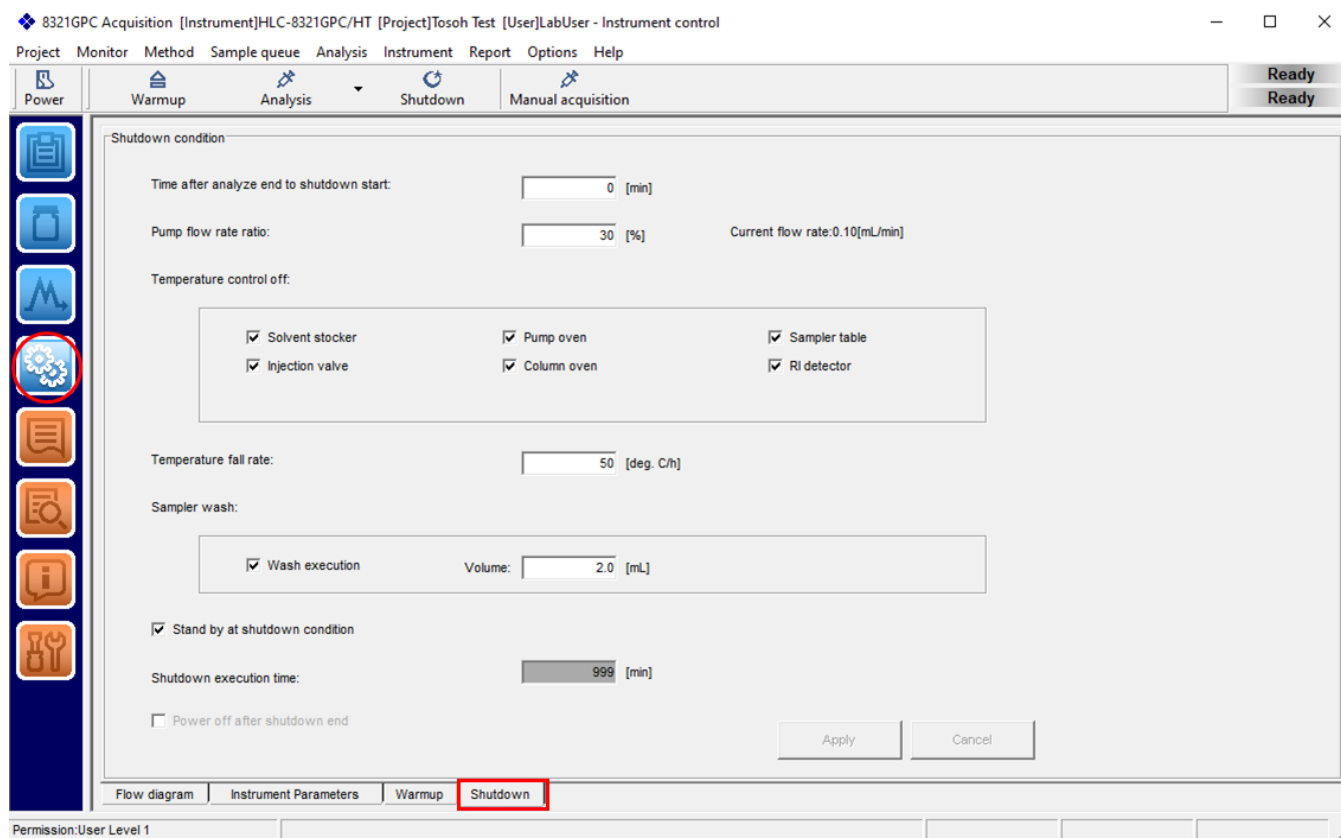
Item	Value
Waste fluid volume (mL)	35388
Waste fluid warning volume (mL)	3000
<b>Degas Unit</b>	
Degas level	Std.
<b>Purge</b>	
Purge control	Normal
Purge volume (mL)	150
Purge speed(mL/min)	15
<b>Pump Oven</b>	
Temperature control	On
Control temperature (deg. C)	40
Gas sensor value	300
<b>Pump</b>	
<b>Sample column</b>	
<b>Sample pump</b>	
Flow rate (mL/min)	0.10
High limit pressure (MPa)	3.0
Low limit pressure (MPa)	0.0
<b>Reference pump</b>	
Flow rate ratio	1/1
High limit pressure (MPa)	15.0
Low limit pressure (MPa)	0.0
<b>Flow control</b>	

Description  
Set Sam. pump flow rate of column 1. ( 0.10 - 2.00 mL/min )

Flow diagram Instrument Parameters Warmup Shutdown

Permission:User Level 1

- b. Double click in the “Flow rate” box to edit the flow rate value by typing the desired value and hitting enter. Drop the flow rate in 0.1 mL/min increments, pausing for ~ 30 s at each increment.
9. If you are ready to shutdown the instrument (weekends, longer periods without use), initiate the shutdown procedure:
  - a. Click the “instrument control” icon in from the column on the left and navigate to the “shutdown” tab.



b. Set the parameters as follows:

- i. Time after analysis: 1 min
- ii. Pump flow rate ratio: 30% (from current flow rate, assumes starting from 1.00 mL/min)
- iii. Temperature control off: make sure all options are checked
- iv. Temperature fall rate: 50 °C
- v. Sample wash: 3 mL
- vi. Shutdown execution time: 120 min
- vii. Make sure "standby" is NOT checked (this will turn the flowrate to 0 mL/min)

1. When "standby" is checked - pumps stay on and column/detector/injection ovens go to 40 C

2. When "standby is not checked - pumps and all ovens turn off

c. Click "shutdown" from the top toolbar.

- i. When the instrument is shutdown, the flow diagram should be completely grayed out.

d. Alternate option: set automatic scheduled shutdown in "other settings."

## Analyze Data

1. Open instrument software by click on the orange “flower” icon on desktop, and selecting red “flower” analysis application .



2. Login under “lab user”, password: polymer
3. Browse > select user folder > select file > “OK”
4. select file > peak edit >
5. same process: delete all> draw> select baseleine > click before and after peaks > hide front and abck end of calibration
6. · calculation > edited peak (blue check mark)
7. ○ now the molecular weight data should show up on peak
8. ○ save data
9. · lots of options on right hand side
- 10.○ calibration curve RI
- 11.○ right click on screen > can copy data, etc. (will export whatever is checked on the right hand side)
- 12.○ can highlight all injections, right click > overlay graph
- 13.§ can zoom in
- 14.○ results exporter can export data to excel
- 15.○ report > second option (title..) > print preview

## Upkeep and Maintenance

### Changing Solvent Bottle

Note: front bottle is fresh solvent and back bottle is waste.

1. Turn pumps off

- a. Under “Flow control”, double click “Sam. and Ref. pump control”, then “stop flow” button will appear.

☐ Flow control	
Sam. pump flow control	Flow.
Ref. pump flow control	Flow.
Sam. and Ref. pump flow control	Flow. <span style="float: right;">Stop flow.</span>

- Remove cap from current solvent bottle, take tubing off, and then switch out the old bottle for a new one.
- For waste bottle, must disconnect green wire to remove tubing. Transfer waste into a clear department waste bottle, and return empty amber waste bottle to instrument. Reconnect green wire.
- Perform purge (system icon), \*make sure pumps are off!

Purge volume: 150 mL

Speed: 15 mL/min for TCB

Start purge process: purge control > double click to get start button on right hand side > start purge

☐ Purge	
Purge control	Normal <span style="float: right;">Start purge</span>
Purge volume (mL)	150
Purge speed(mL/min)	15

- Manually “stop purge after 20 minutes” and then turn pumps back on.

☐ Purge	
Purge control	Purge is in progress. <span style="float: right;">Stop purge</span>
Purge volume (mL)	150
Purge speed(mL/min)	15

## Things to watch out for

- Normal pressure ranges at room temp: ????
- Normal pressure ranges at high temp (135 C): ????
- The RI should be stable before running samples:
  - Drift  $\leq$  2 mV over 60 min
  - Noise  $\leq$  0.2 mV
- CAN close the software and the instrument will maintain whatever the most recent flow rate/temp settings are.
- Software gets glitchy if it isn't closed and reopened every now and then.

## Yearly Maintenance

- Tosoh sells kit to replace pump hardware (piston, seals, filters etc)
- Can purchase service from Tosoh (tech comes out)
- Bare minimum: replace all the seals on the instrument

## Long-term storage

- Shut down instrument
- Remove solvent reservoirs
- Flush solvent from system
- Cap and store columns

## Creating a Calibration Curve

### 1. Prepare and run calibration samples

- a. TSKgel standard PS samples are kept in gold box in small refrigerator



b. Can combine the following samples into the same sample vial (3 mg polymer each/ 9 mL of solvent total):

1: A-500, F-1, F-20

2: A-1000, F-2, F-40

3: A-2500, F-4, F-80

4: A-5000, F-10, F-128

c. Run each sample and save file



## 2. Open data in GPC Analysis software

- a. Select “chromatogram” from the top toolbar and “Browse chromatogram database” find and open the folder that contains calibration run data. Databases (or sets of sequential sample runs) will save by date acquired
  - i. Chromatogram > browse data> 8321 GPC > sample data > ChromatogramDatabase
- b. Single left clicking on a database then clicking “OK” will open all the sample runs in that database

The screenshot shows the 8321 GPC Analysis software interface. The 'Chromatogram' menu item is circled in red. A 'Select the Chromatogram Database' dialog box is open, displaying a file tree. The folder '2021-04-27' is highlighted with a red box and labeled 'databases'. Inside this folder, a sub-folder 'sample runs' is also highlighted with a red box and labeled 'sample runs'. The dialog also shows a table of data files with columns for Data Name, Sample Name, and Acquisition Date. The 'OK' button is circled in red.

Data Name	Sample Name	Acquisition Date
RSLT0001	calibrants_2_A-1000_F-2_F-4	2021/04/27 13:50:52
RSLT0002	calibrants_3_A-2500_F-4_F-80	2021/04/27 14:25:53
RSLT0003	calibrants_1_A-500_F-1_F-20	2021/04/27 15:31:55

- From the top toolbar, select "Method" -> "New." In the resulting pop-up, name the method whatever you would like and enter a reason. Once you click "OK" the method will show up in the list of available methods at the bottom left.

The screenshot shows the 8321GPC Analysis software interface. The 'Method' menu is highlighted in the top toolbar. A 'Create New Method' dialog box is open, displaying the following information:

- Method Name: calibration\_05-14-2021
- Reason (Necessary): calibration

The background shows a chromatogram plot with a single sharp peak at approximately 12 minutes. The y-axis is labeled 'mV' and ranges from 0.0 to -10.000. The x-axis is labeled 'min' and ranges from 0.000 to 30.000. The 'Result' tab is active, showing a table of peak data:

Peak No.	Retention Time [min]	Area [mV*s]	Height [mV]	Height% [%]	Half bandwidth [s]	Theoretical Plates	Resolution Factor	Asymmetry Factor	Mn

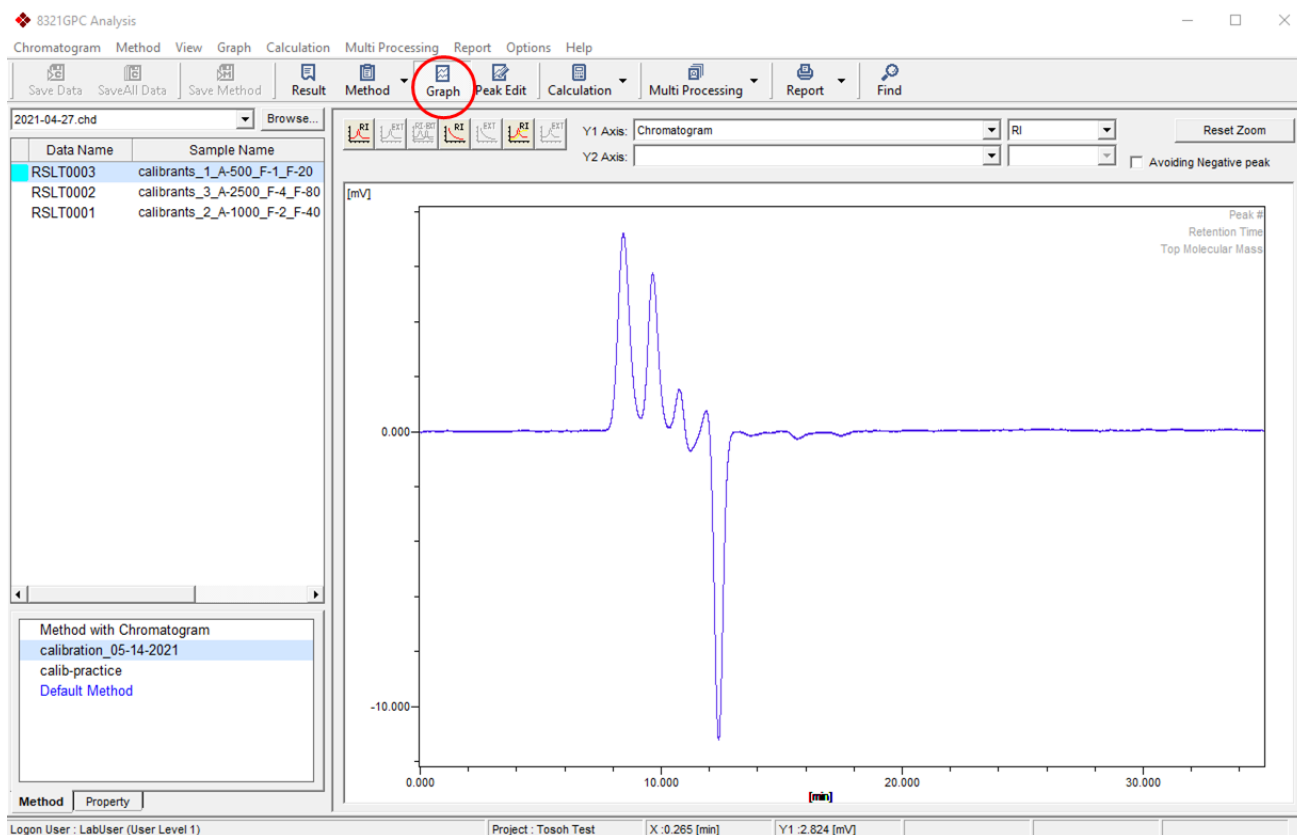
At the bottom left, a list of methods is shown, including 'calib-practice' and 'Default Method'. The status bar at the bottom indicates 'Logon User : LabUser (User Level 1)' and 'Project : Tosoh Test'.

The screenshot shows the 8321GPC Analysis software interface with the 'Analytical Condition 1' configuration window open. The window displays a table of parameters and their values:

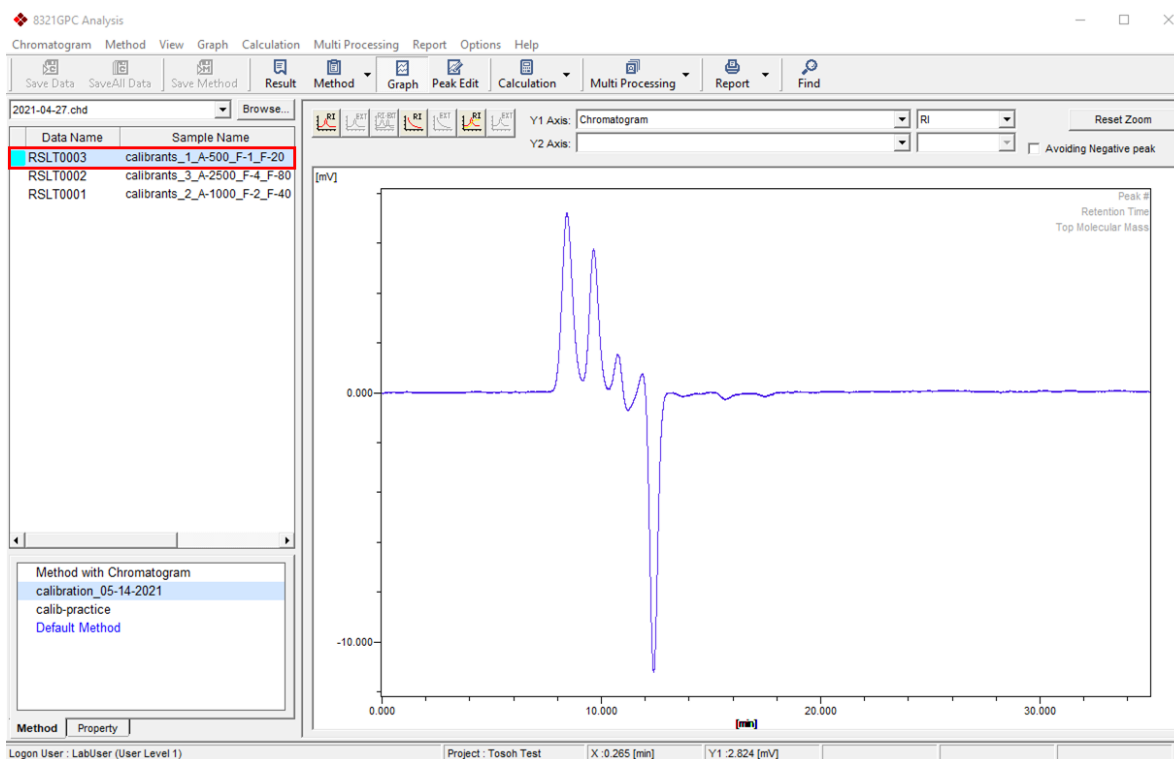
Item	Value
<b>Analytical Condition</b>	
<b>General</b>	
RI	
Calculation Type	Column test
KappaB [eta]	1.0000
AlphaB [Mv]	1.0000
<b>EXT</b>	
Calculation Type	Column test
KappaB [eta]	1.0000
AlphaB [Mv]	1.0000
Output Type	Area
<b>Correction of Calibration Curve</b>	
<b>RI</b>	
Correction by Internal Standard Peak	<input type="checkbox"/> Yes
Retention Time [min]	0.000
Range [min]	0.000
Calculation Internal Standard Peak	Reject
<b>EXT</b>	
Correction by Internal Standard Peak	<input type="checkbox"/> Yes
Retention Time [min]	0.000
Range [min]	0.000
Calculation Internal Standard Peak	Reject
Correction by Lag Time	<input type="checkbox"/> Yes
Lag Time [s]	0.000
<b>Calculation Range</b>	
Description	

At the bottom left, a list of methods is shown, with 'calibration\_05-14-2021' highlighted in red. The status bar at the bottom indicates 'Logon User : LabUser (User Level 1)' and 'Project : Tosoh Test'.

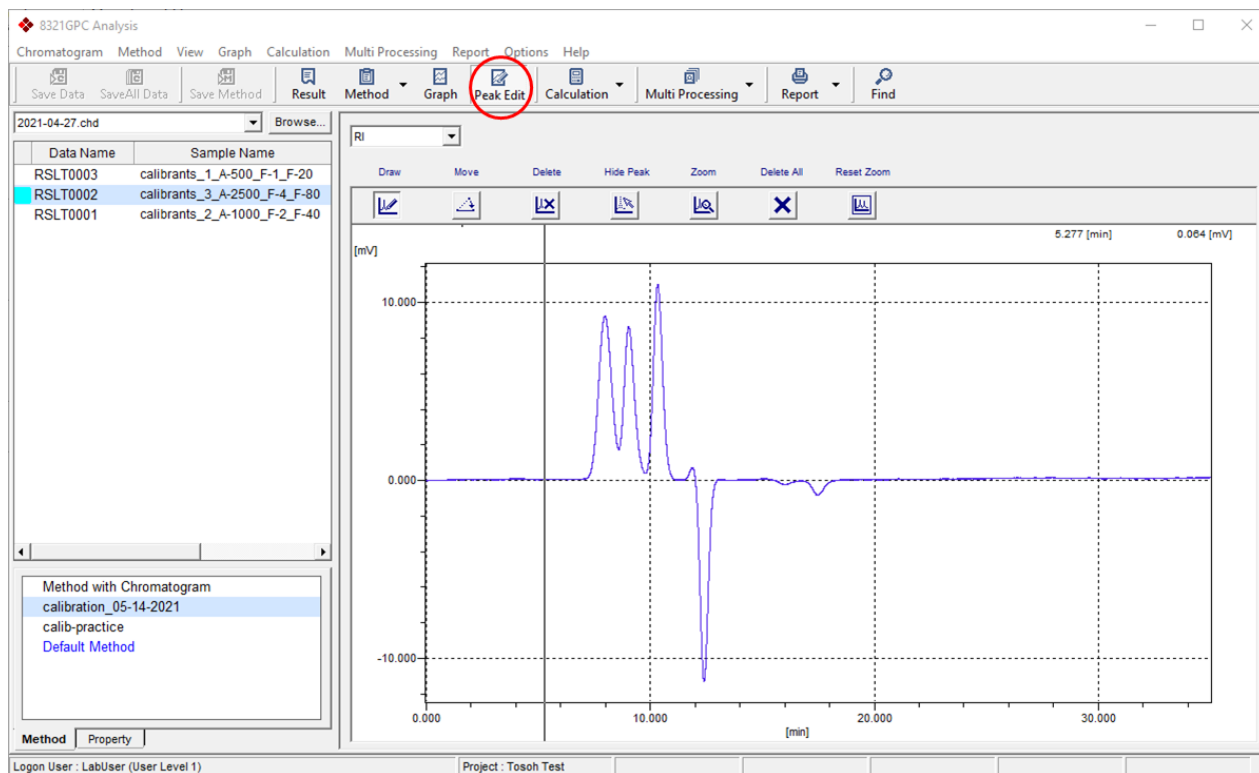
#### 4. To view the chromatograms, select "Graph" from the upper toolbar



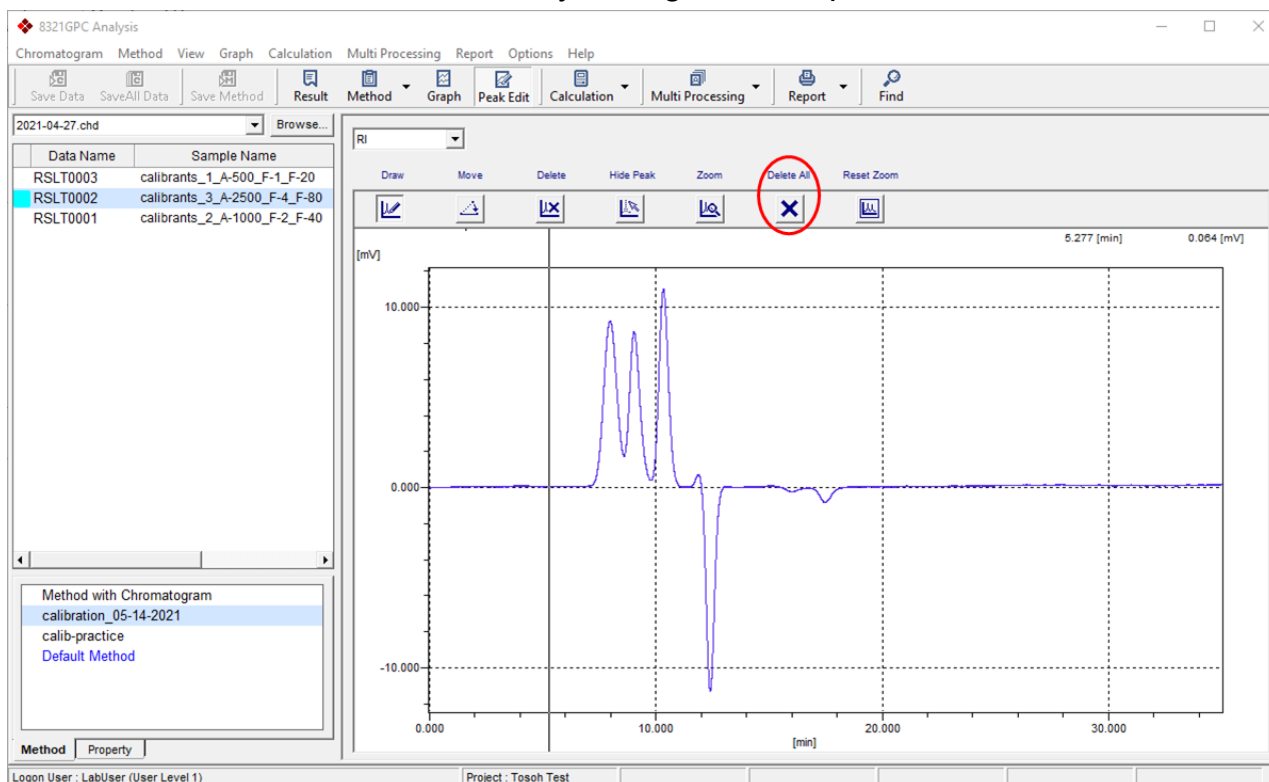
#### 5. Click the sample you wish to analyze from the list at the upper left



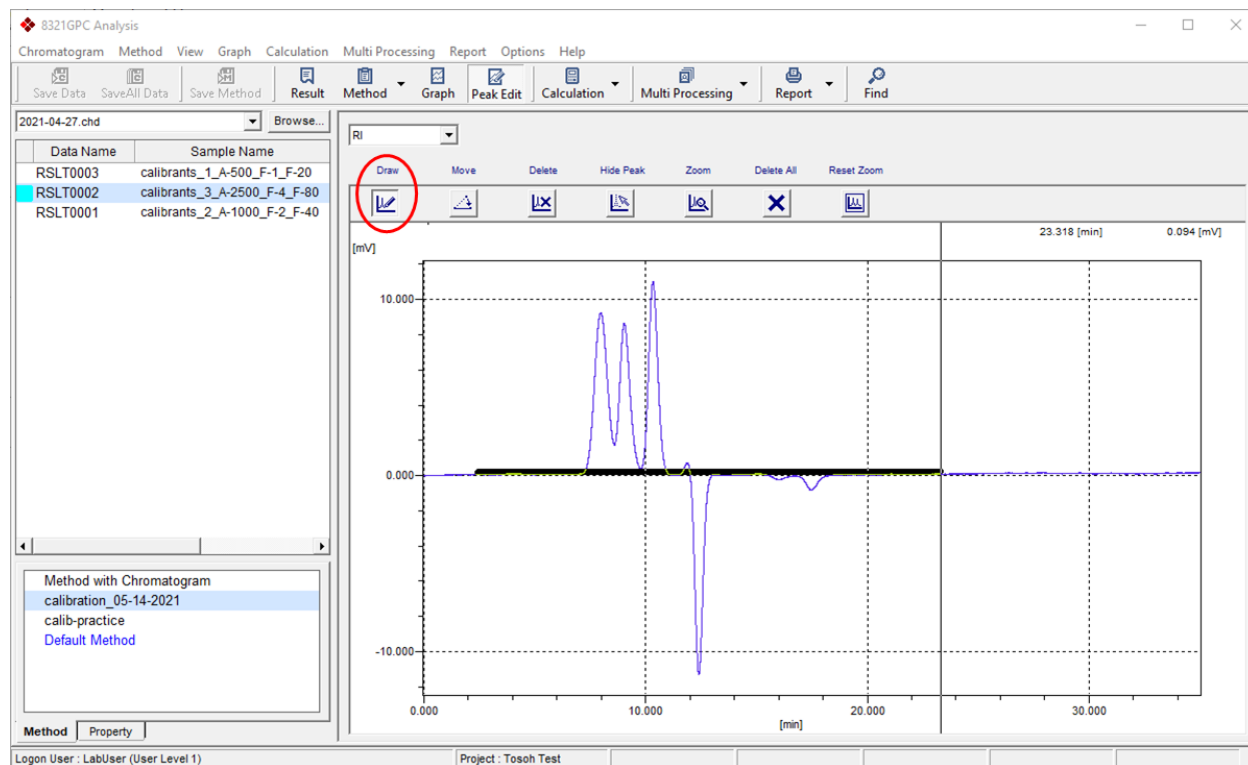
- Click the “peak edit” icon to open the window where you can edit the chromatogram



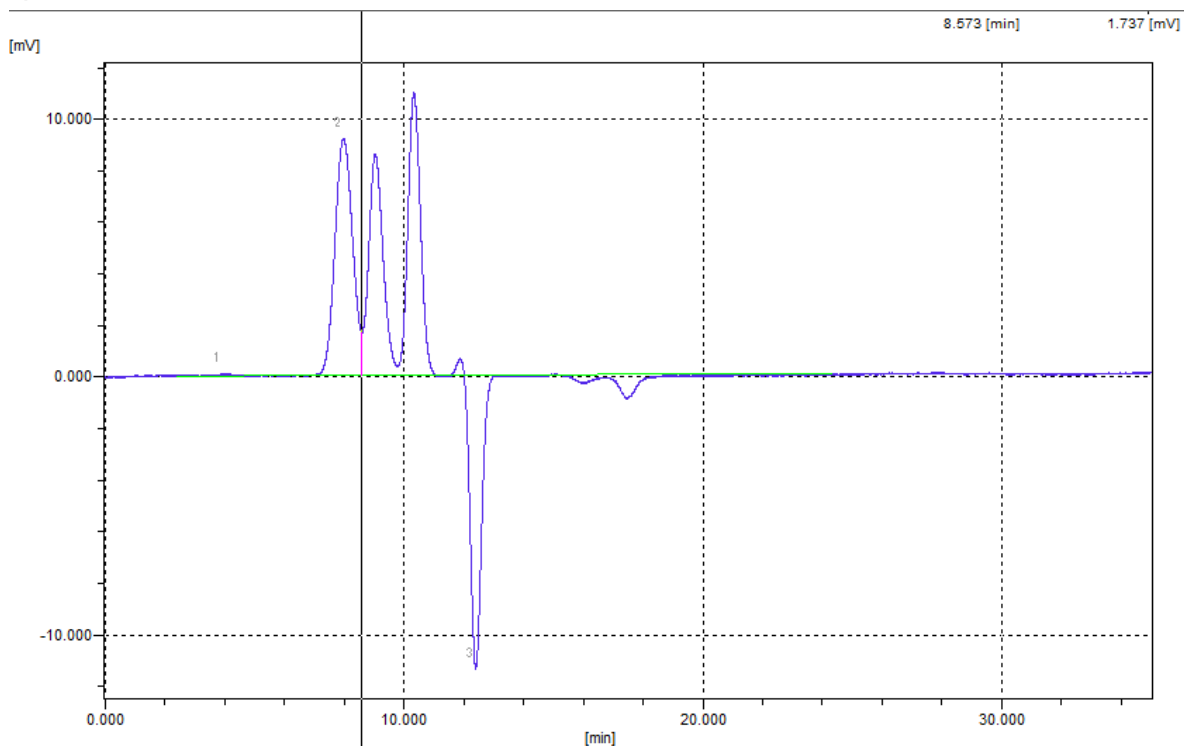
- Click “delete all” to remove any auto-generated peaks/baselines.



8. Select "Draw" then left click and drag to select the baseline. A green line will appear once the baseline is set.

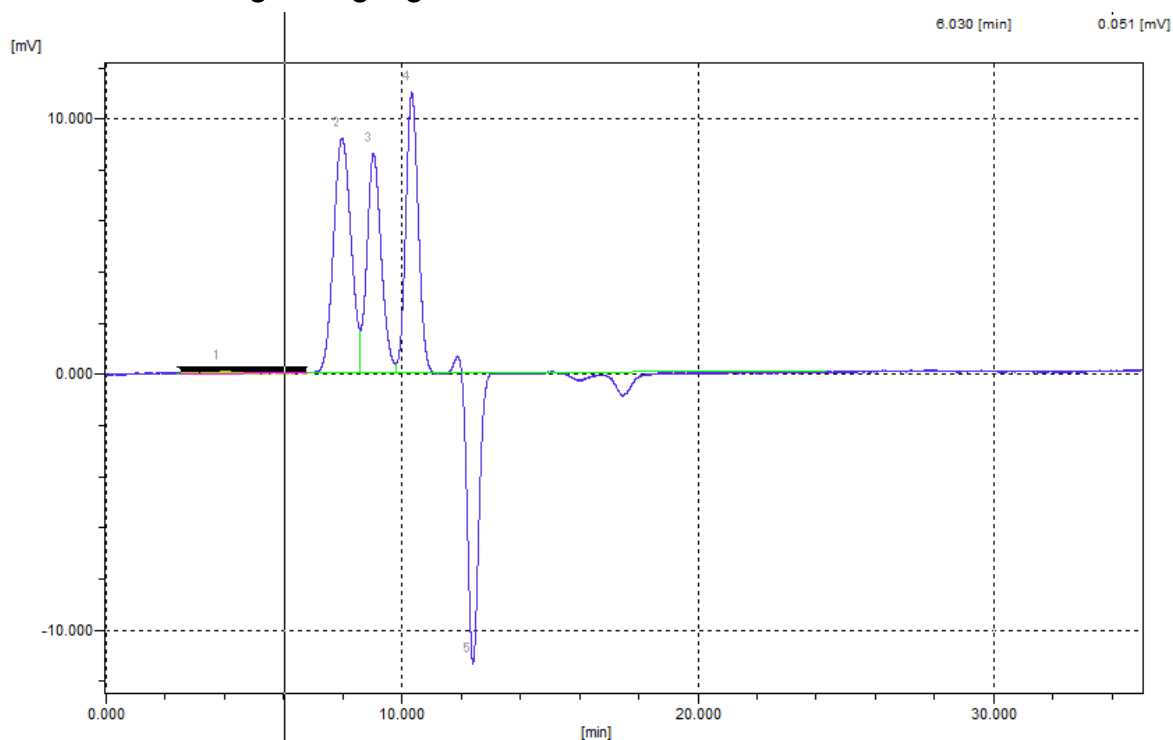


9. Single left click to set the peak limits. Numbers will appear above each selected peak.

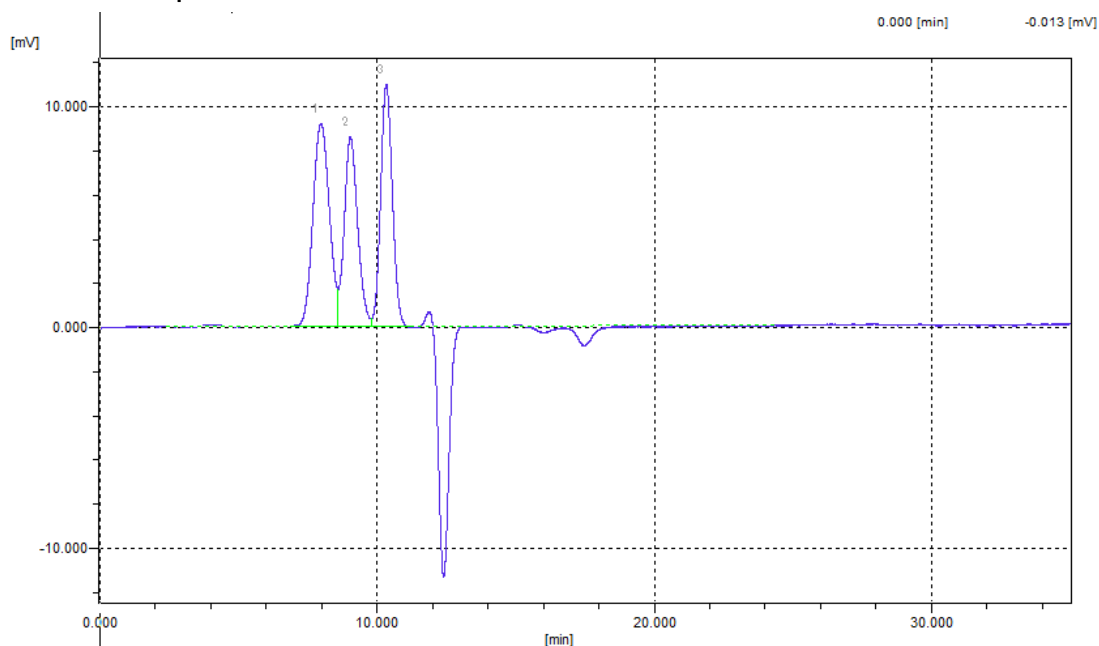


10. Additional peaks will likely appear to the left and right of the true polymer peaks. To remove these peaks from the list of polymer peaks, select “hide peak.” Hovering over different regions will highlight them in black. Single left click on the “peaks” you wish to remove.

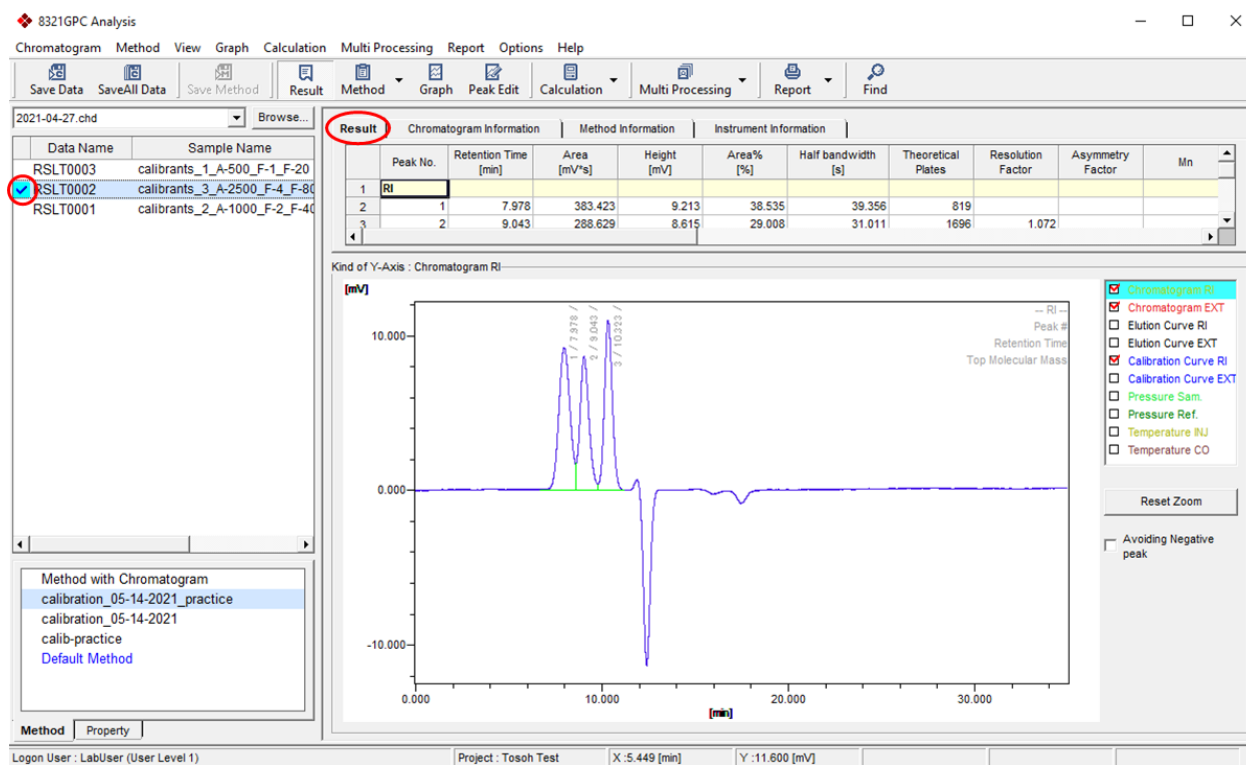
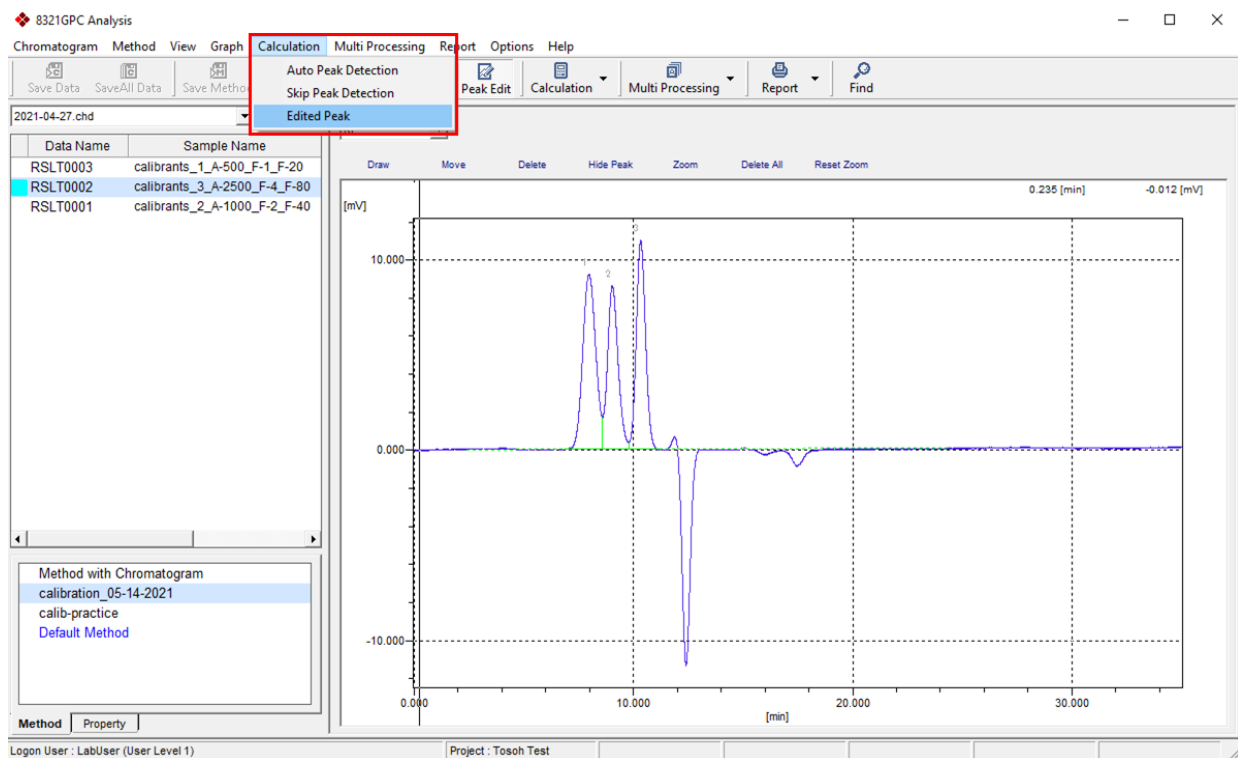
a. Black region highlighted



b. Extra peaks removed. Peaks are auto re-numbered.



- Click "calculation" from the top toolbar and select "Edited Peak." A results table will appear and the sample name will be marked with a blue check mark. Repeat steps 6-11 for all samples that you wish to include in the calibration curve.



12. Select “Save All Data” and enter in a reason (whatever you like) in the resulting pop-up window. Click “OK.”

The screenshot shows the 8321GPC Analysis software interface. The 'Save All Data' button in the top toolbar is circled in red. The main window displays a table of peak data and a chromatogram plot.

Peak No.	Retention Time [min]	Area [mV*s]	Height [mV]	Area% [%]	Half bandwidth [s]	Theoretical Plates	Resolution Factor	Asymmetry Factor	Mn
1	8.198	487.725	12.339	55.156	37.257	965		1.326	
2	9.337	214.342	6.656	24.239	29.525	1994	1.207		

The chromatogram plot shows three peaks labeled with their retention times: 1 / 8.198 /, 2 / 9.337 /, and 3 / 10.667 /. The Y-axis is labeled 'mV' and ranges from -10.000 to 10.000. The X-axis is labeled '[min]' and ranges from 0.000 to 30.000.

The 'Save Data' dialog box is shown, asking for an overwrite reason. The text 'practice' is entered in the input field.

Overwrite?  
Changes Reason (Necessary)

practice

OK Cancel



13. Select "Method." Under the "Analytical Condition 1" tab, double click the value for "RI -> Calculation Type." From the resulting dropdown menu, select "Molecular Mass."

The screenshot shows the 'Method' configuration window for 'Analytical Condition 1'. The 'Method' menu is highlighted in red. The 'Calculation Type' dropdown is open, showing 'Molecular Mass' selected. The 'General' section includes 'RI' with 'Calculation Type' set to 'Molecular Mass'. Other parameters like 'KappaB [eta]' and 'AlphaB [Mv]' are also visible.

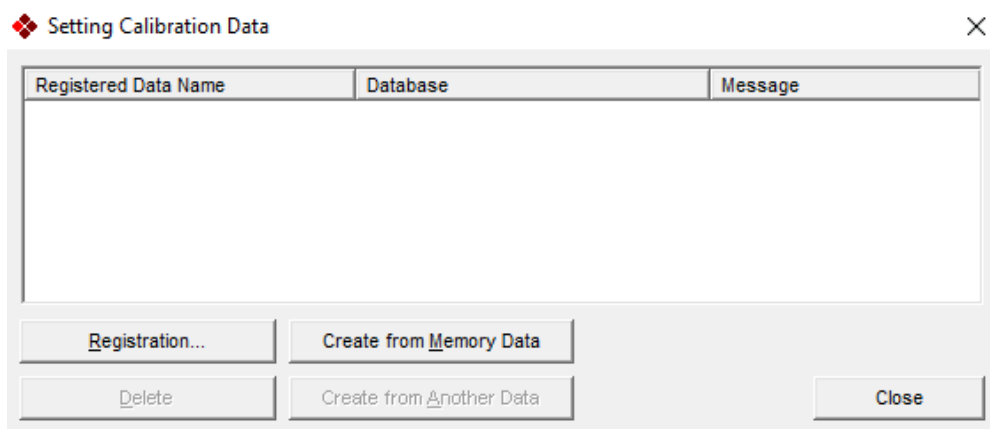
Item	Value
General	
RI	
Calculation Type	Molecular Mass
KappaB [eta]	Column test
AlphaB [Mv]	Molecular Mass
Copolymer	
EXT	
Calculation Type	Column test
KappaB [eta]	1.0000
AlphaB [Mv]	1.0000
Output Type	Area
Correction of Calibration Curve	
RI	
Correction by Internal Standard Peak	<input type="checkbox"/> Yes
Retention Time [min]	0.000
Range [min]	0.000
Calculation Internal Standard Peak	Reject
EXT	
Correction by Internal Standard Peak	<input type="checkbox"/> Yes
Retention Time [min]	0.000
Range [min]	0.000
Calculation Internal Standard Peak	Reject
Correction by Lao Time	<input type="checkbox"/> Yes
Description	The parameter is the calculation type of RI.

14. Under the "Calibration Condition" tab, select "Set the Retention" to add the data from your calibrants.

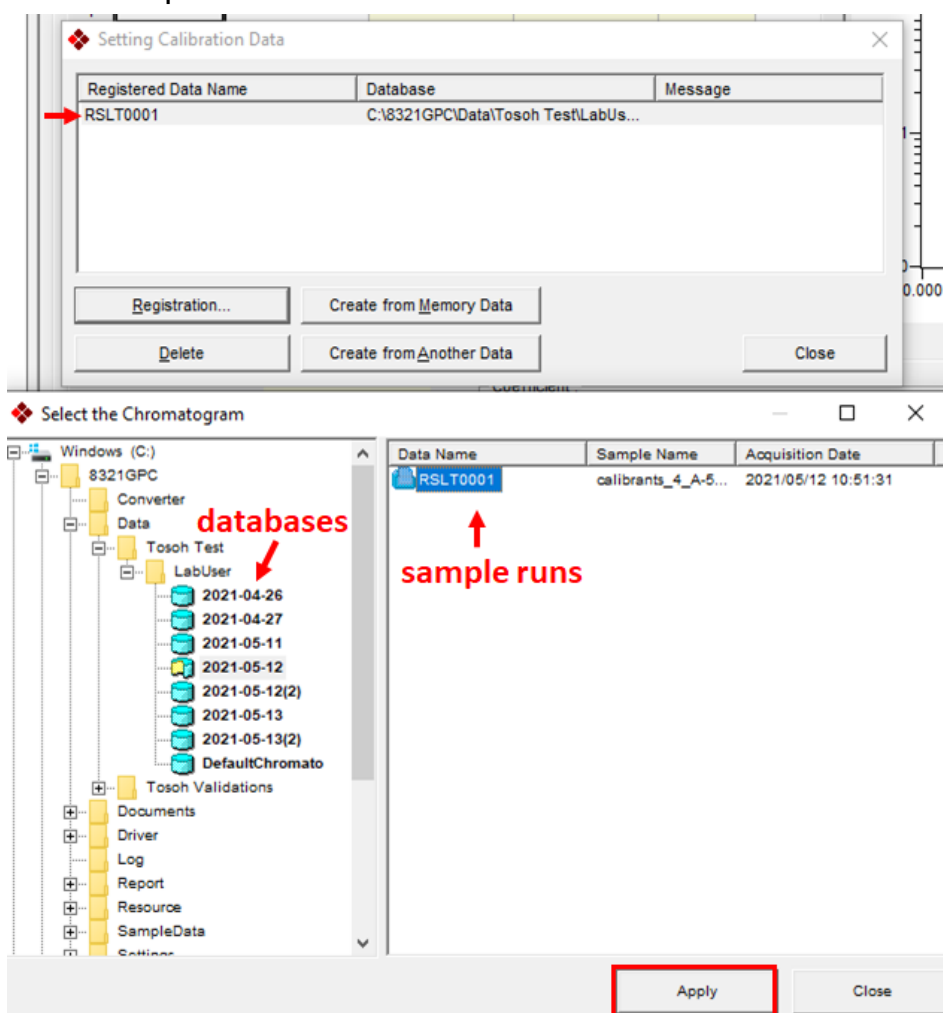
The screenshot shows the 'Calibration Condition' configuration window. The 'Set the Retention...' button is highlighted in red. The 'Calibration Data' table is visible, showing columns for Time [min], Molecular Mass, Correction Value, Approximation, Error, and Weight. The 'Approximation' dropdown is set to 'Linear: At+B'.

Time [min]	Molecular Mass	Correction Value	Approximation	Error	Weight
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					

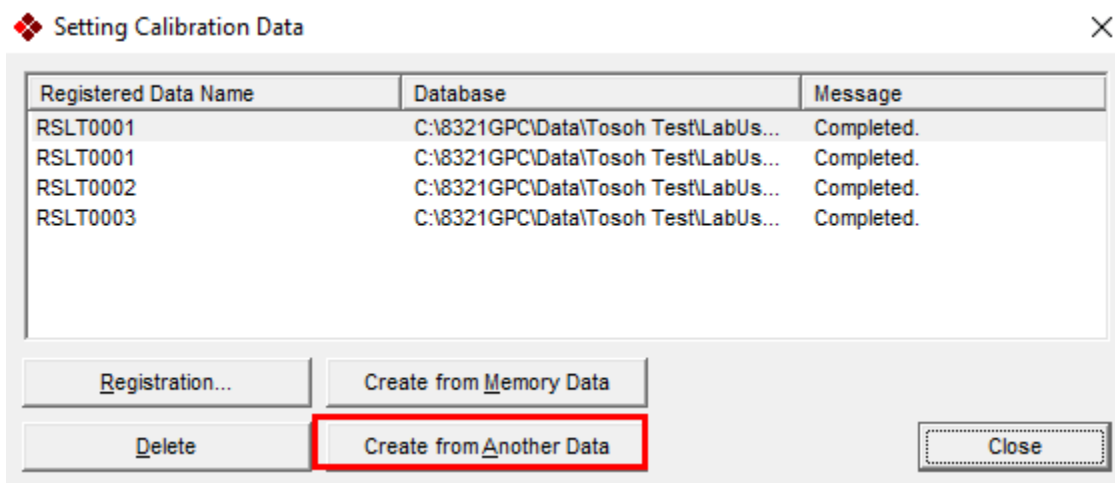
15. In the resulting pop-up, click “Registration” to find calibration data.



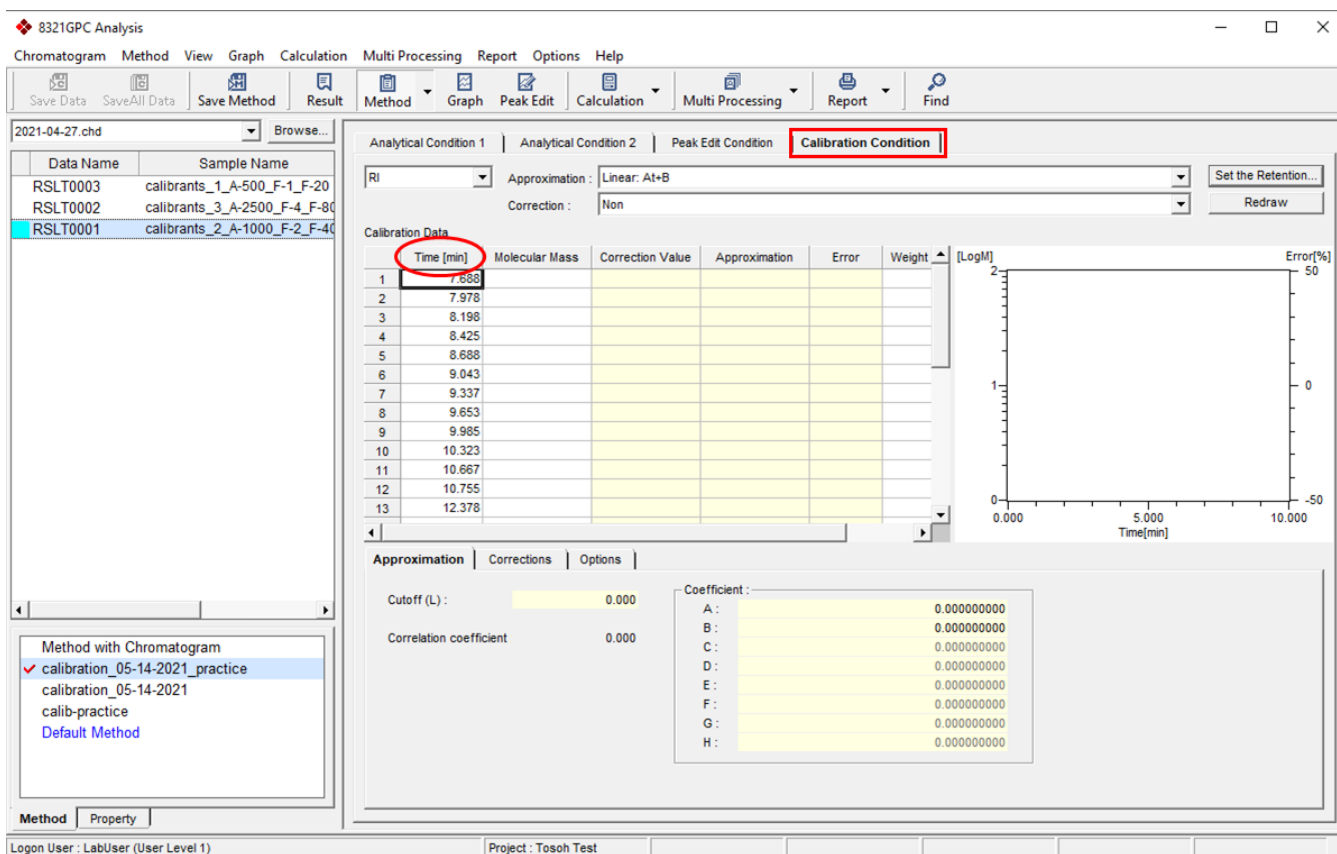
16. Find the calibrant sample runs that you wish to add to the calibration curve. For each sample, click to select it (on the left), then hit apply. Double check that the sample is added to the registration window before adding subsequent samples.



- Once you have added all of the calibration samples you desire, click “close.” In the registration window, click “Create from Another Data.” The word “completed” will auto-populate the right hand column. Click “Close.”



- The retention times from all of your calibrants should appear in the “Time” column under the “Calibration Conditions” tab.



19. From the top toolbar, click “Options” and select “Molar Mass Registration” to open a database of standard data.

The screenshot shows the 8321GPC Analysis software interface. The 'Options' menu is open, and 'Molecular Mass Registration...' is selected. The main window displays a list of data points on the left, a 'Calibration Data' table in the center, and a graph on the right. The 'Options' sub-menu is also visible at the bottom.

Time [min]	Molecular Mass	Correction Value	Approximation	Error	Weight	[LogM]	Error[%]
1	7.688						
2	7.978						
3	8.198						
4	8.425						
5	8.688						
6	9.043						
7	9.337						
8	9.653						
9	9.985						
10	10.323						
11	10.667						
12	10.755						
13	12.378						

20. Select the appropriate group of standards from the list on the left. Our lab uses “TSK Polystyrene Standards.” In the “valid” column near the center, click each standard that was used to generate the calibration curve. Selected standards will have a red check mark. Click “OK.”

The screenshot shows the 'Molecular Mass Registration' dialog box. The 'List' tab is active, showing a list of standards. The 'Detail' tab is also visible, showing a table of standards with a 'Valid' column. A red arrow points to the 'Valid' column header, and another red arrow points to the 'TSK Standard Polystyrene' entry in the list.

Valid	Grade	Lot No.	Molecular Mass	Mw/Mn
<input type="checkbox"/>	F-288		2890000	1.09
<input checked="" type="checkbox"/>	F-128		1090000	1.08
<input checked="" type="checkbox"/>	F-80		706000	1.05
<input checked="" type="checkbox"/>	F-40		427000	1.02
<input checked="" type="checkbox"/>	F-20		190000	1.04
<input checked="" type="checkbox"/>	F-10		96400	1.01
<input checked="" type="checkbox"/>	F-4		37900	1.01
<input checked="" type="checkbox"/>	F-2		18100	1.01
<input checked="" type="checkbox"/>	F-1		10200	1.02
<input checked="" type="checkbox"/>	A-5000		5970	1.02
<input checked="" type="checkbox"/>	A-2500		2630	1.05
<input checked="" type="checkbox"/>	A-1000		1050	1.13
<input checked="" type="checkbox"/>	A-500		495	1.14

21. Double click on the first box in the “Molecular Mass” column and select “Setting Values” from the resulting dropdown menu.

The screenshot displays the 8321GPC Analysis software interface. The main window is titled '2021-04-27.chd'. The interface includes a menu bar (Chromatogram, Method, View, Graph, Calculation, Multi Processing, Report, Options, Help) and a toolbar with icons for Save Data, Save All Data, Save Method, Result, Method, Graph, Peak Edit, Calculation, Multi Processing, Report, and Find.

On the left, there is a 'Data Name' and 'Sample Name' table:

Data Name	Sample Name
RSLT0003	calibrants_1_A-500_F-1_F-20
RSLT0002	calibrants_3_A-2500_F-4_F-80
RSLT0001	calibrants_2_A-1000_F-2_F-40

The main area shows 'Analytical Condition 1' | 'Analytical Condition 2' | 'Peak Edit Condition' | 'Calibration Condition'. The 'Calibration Data' table is visible:

	Time [min]	Molecular Mass	Correction Value	Approximation	Error	Weight
1	7.688					
2	7.978					
3	8.190					
4	8.425					
5	8.688					
6	9.043					
7	9.337					
8	9.653					
9	9.985					
10	10.323					
11	10.667					
12	10.755					
13	12.378					

A dropdown menu is open over the 'Molecular Mass' column of the first row, showing options: 'Immediate Number' and 'Setting Values'. The 'Setting Values' option is highlighted in blue.

Below the table, there are sections for 'Approximation', 'Corrections', and 'Options'. The 'Approximation' section shows 'Cutoff (L): 0.000' and 'Correlation coefficient: 0.000'. The 'Options' section shows a list of coefficients (A through H) all set to 0.000000000.

At the bottom left, a 'Method with Chromatogram' list is visible, with 'calibration\_05-14-2021\_practice' selected. The status bar at the bottom shows 'Logon User : LabUser (User Level 1)' and 'Project : Tosoh Test'.

22. The software should auto-populate the rest of the table with known data from the standards that you just selected and should auto-calculate the calibration curve. The error values for a good calibration curve should be less than 10. A red checkmark will now be next to the calibration method name at the lower left.

The screenshot shows the 8321GPC Analysis software interface. The main window displays the Calibration Data table and a calibration curve plot. The table has the following data:

Time [min]	Molecular Mass	Correction Value	Approximation	Error	Weight
1	7.688	1090000	1090000.00	1205799.35	-10.62
2	7.978	706000	706000.00	597432.96	15.38
3	8.198	427000	427000.00	350687.74	17.87
4	8.425	190000	190000.00	202390.57	-6.52
5	8.688	96400	96400.00	107053.20	-11.05
6	9.043	37900	37900.00	45316.34	-19.57
7	9.337	18100	18100.00	22236.29	-22.85
8	9.653	10200	10200.00	10345.06	-1.42
9	9.985	5970	5970.00	4629.95	22.45
10	10.323	2630	2630.00	2042.26	22.35
11	10.667	1050	1050.00	887.84	15.44
12	10.755	495	495.00	717.44	-44.94
13	12.378				

The calibration curve plot shows [LogM] on the y-axis (ranging from 3 to 6) and Time [min] on the x-axis (ranging from 8.000 to 11.000). The plot displays a series of blue data points and a red linear fit line. The error values for each point are shown as red bars extending from the fit line.

In the left sidebar, the method list includes:

- Method with Chromatogram
- Calibration\_05-14-2021\_practice
- calibration\_05-14-2021
- calib-practice
- Default Method

23. Click "Save Method" and "OK." The method is now ready to use!

The screenshot shows the 8321GPC Analysis software interface, identical to the previous one, but with the 'Save Method' button in the top toolbar circled in red. The 'Save Method' button is located in the top toolbar, between the 'Save All Data' and 'Result' buttons.

## Other Information

### Some key differences between the high-temp system and a typical SEC system:

- The instrument is designed to be turned on and off without ruining the columns
  - Less frequent on/off the better (i.e., turn on for M-F, off for weekends rather than on/off every day)
- Instrument needs ~2h warm up/cool down to power on/off
- Might see higher pressure on the instrument readout because the solvent is more viscous at lower temperatures (at operating temp of 135 C, the pressure should be lower)
- Instrument has several ovens to heat the different parts - surfaces can be hot while it is turned on!
- Solvent (1,3,5-TCB) can degrade with heat (gels and releases HCl), tubing will start turning red
  - Flush the instrument with new solvent every 1 or 2 months
- Should turn on the instrument ~1/wk to check that things are running smoothly

### Manual control of systems parts

#### “Monitor” screen

- When you open the software, should see “monitor” screen by default
- Look for grey “ready” (as opposed to “disconnect”)
- Real time RI signal is in blue, pressure is green, reference pressure is dark green
  - Monitor -> monitor settings to see other signals
  - Left click and drag to translate, right click to zoom
- Check the pressure - should be **2 mL/min** bc running 1 mL/min thru both columns

#### “Flow diagram” screen

- Shows flowchart of the whole instrument, can control individual parts from here by clicking
  - 6 heating regions (orange)
  - Live solvent flow (green)
- Solvent reservoirs should be kept at 40 C at all times
- The RI detector and the columns should always be kept at the same temperature
- The sample holder can be kept at a lower temp if desired (prevent sample decomposition for long autosampler runs)
- There’s a gas sensor on this screen 300 (?? not sure what this means) to detect leaks
  - Tighten the lower pressure sensor limit as you use the instrument
  - Record the pressure at 0.5 mL/min (which is the warm-up flow rate)
  - 1 or 2 mPa lower than the above pressure is the lower limit
  - The high pressure limit should be listed on the column spec sheet

General instrument things:

- Two solvent bottles - fresh (front), waste (back)
  - Solvent is degassed before entering the system
  - When replacing solvent, perform 150 mL pump purge (to take care of any air exposure)
- Two feeds for solvent into the instrument (column 1 = sample, column 2 = reference)
- Three feeds for solvent out of the system (columns + autosampler/injection waste)
  - Autosampler/injection waste reservoir is 300 mL, keep an eye on it
- Uses dual pump system for pressure stability
- Uses two columns, a sample column and reference column (for the continual flow RI reference cell)
- Two sets of keys are available to lock the instrument doors

## Software

*The analysis software can be downloaded onto personal computers!*

Username: LabUser

Password: polymer

1. Blue icon = live instrument software
2. Red icon = offline software (data workup)
3. Teal icon = database manager