# 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., chloronapthalene, 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)

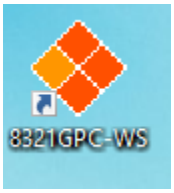
5. After samples are totally finished running and instrument has cooled, clean sample vials, and return vial and cap to [designated storage location]

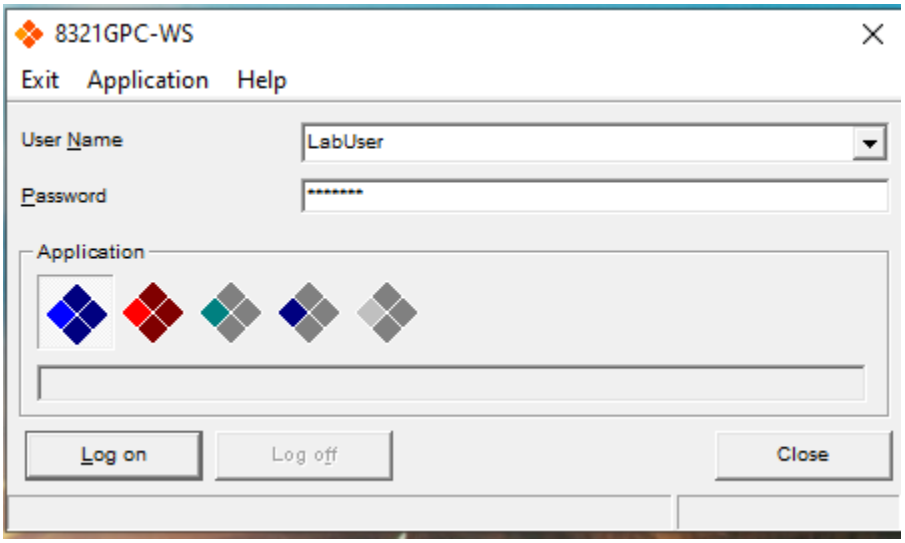
    a. Cleaning procedure for sample vials: clean with hot TCB, then THF or an alcohol to remove TCB

## **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

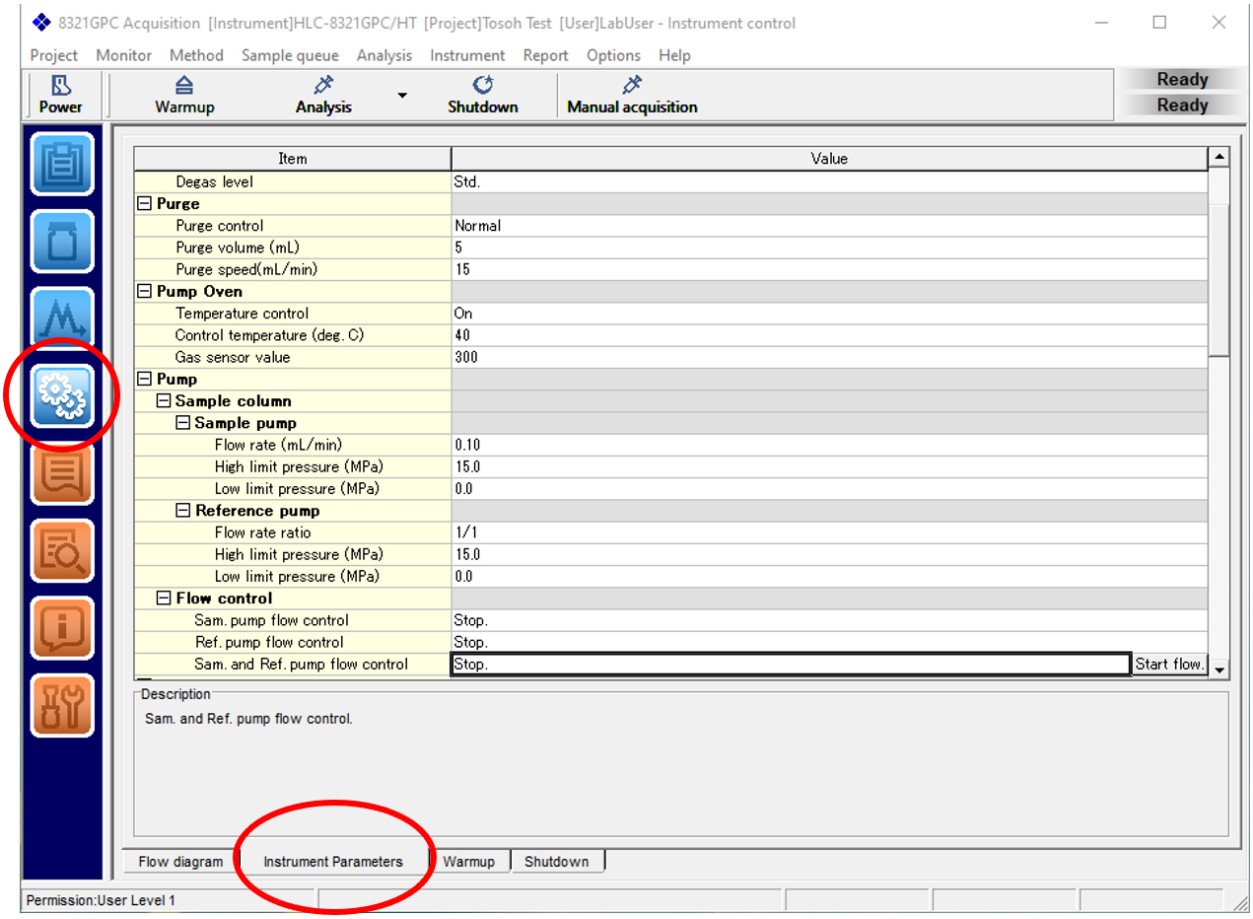
4. Check solvent level and change out solvent/waste bottles if necessary (instructions in next section)

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

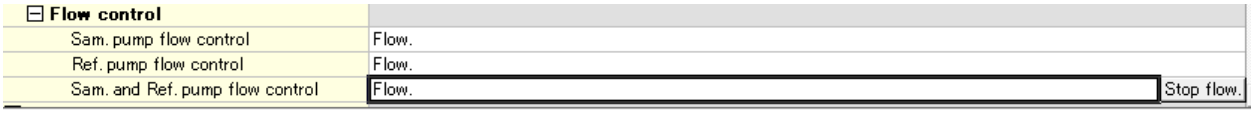
| Solvent Stocker | |
|---|---|
| Temperature control | On |
| Solvent volume (mL) | 0 |
| Waste fluid volume (mL) | 4310 |
| Waste fluid warning volume (mL) | 3000 |

5. Turn on pumps

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



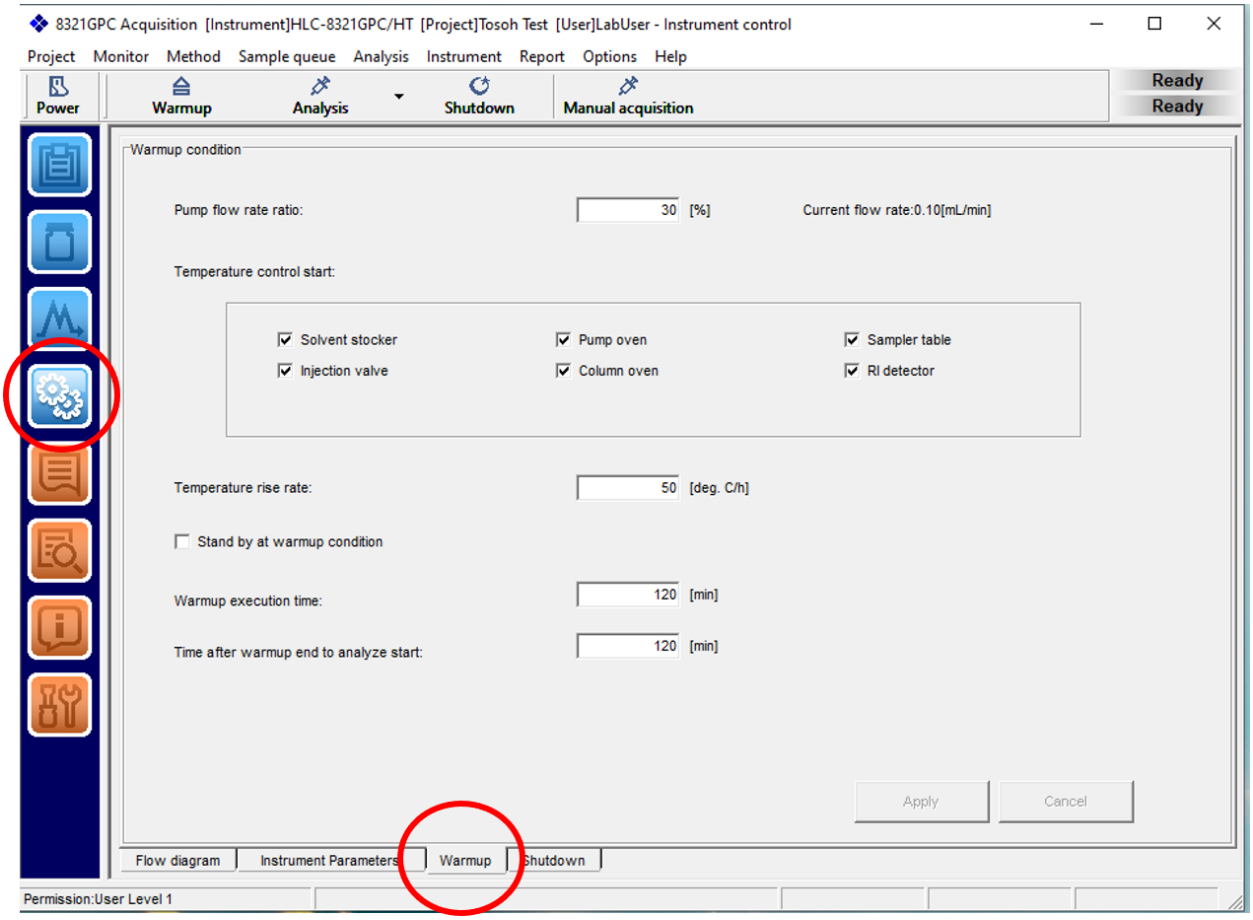

   b. Under “Flow control”, double click “Sam.” and “Ref. pump control”, then “start flow” button will appear.




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)

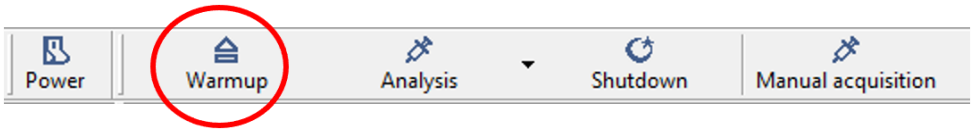
Pump flowrate ratio: 30%
Temperature rise rate: 50 °C per hour
Warm up execution time: 120 min

Time after warmup end: 120 min

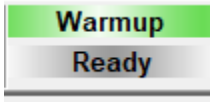
All six temperature control boxes should be checked.

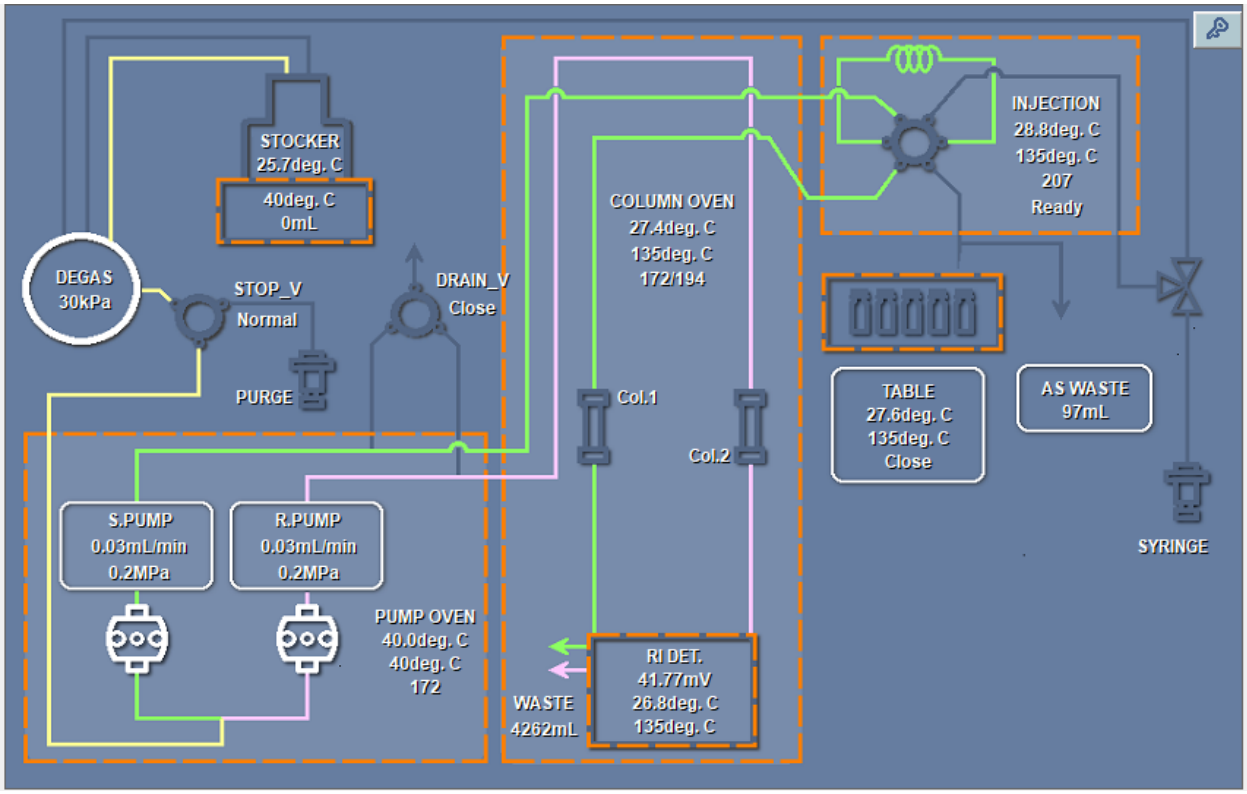
The .

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.

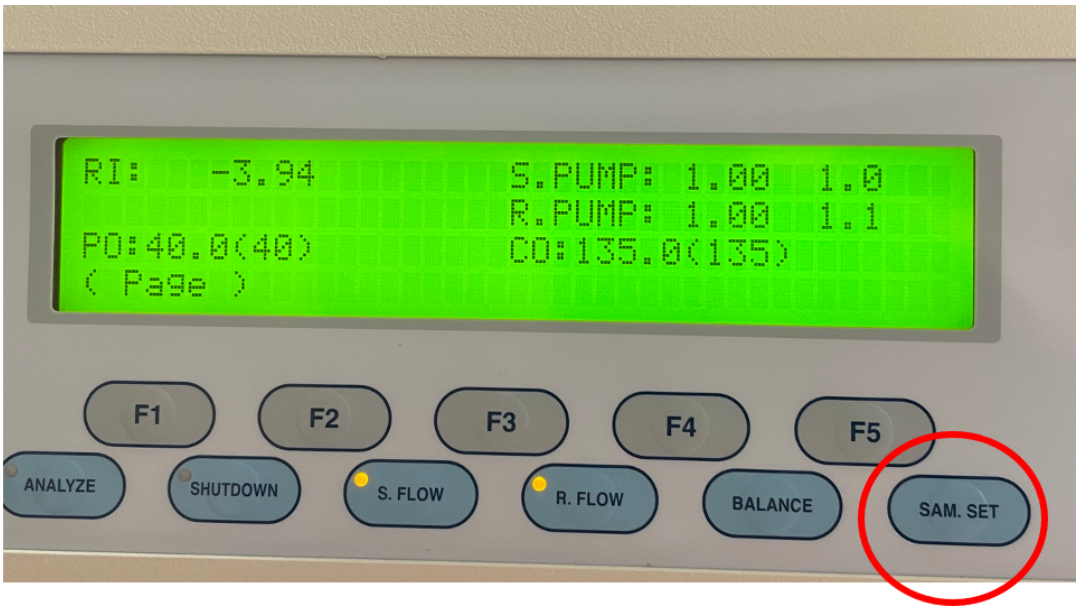


7. Set up sample sequence

    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.



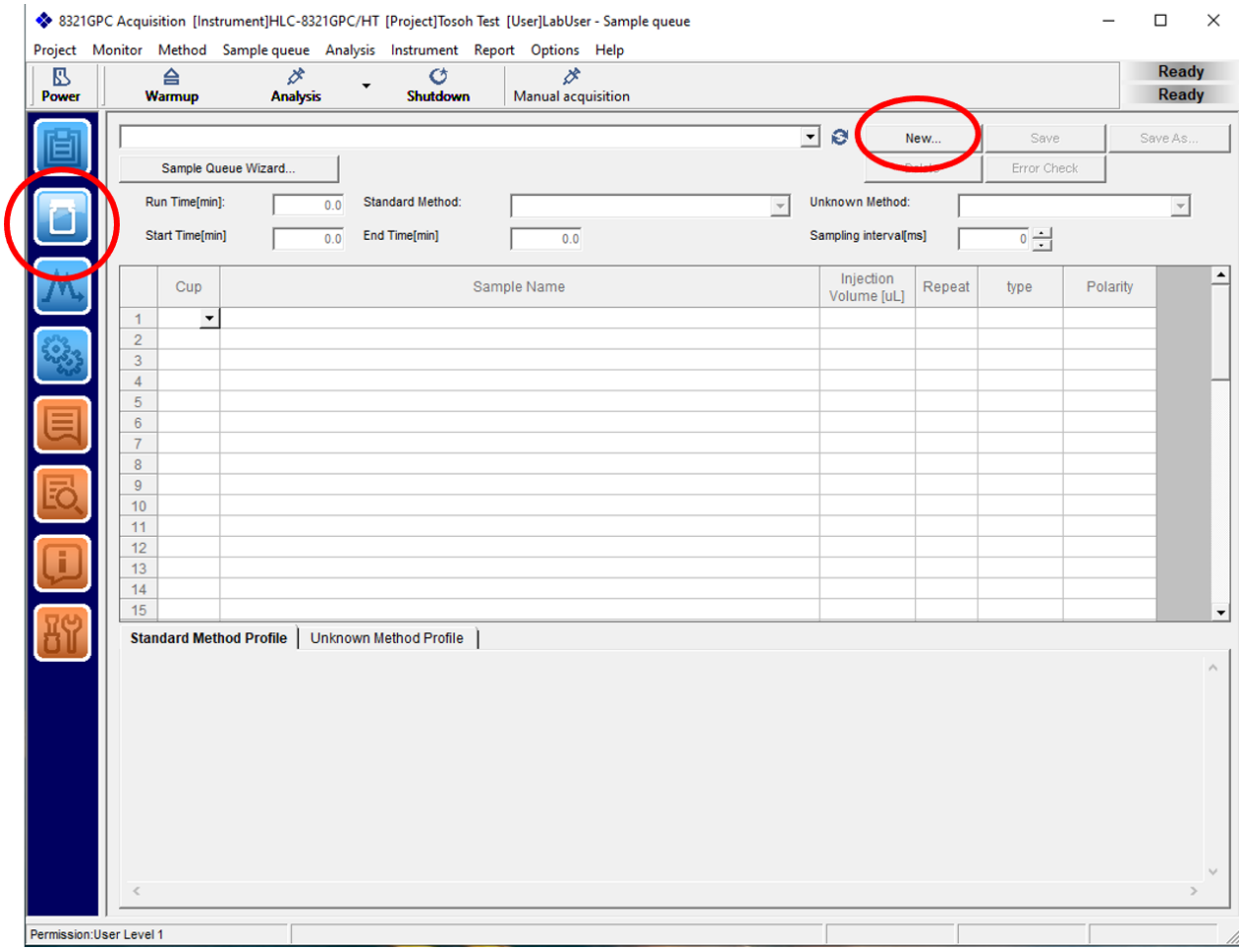

        ii. Open the autosampler door with key (key should already be in the instrument)

        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.



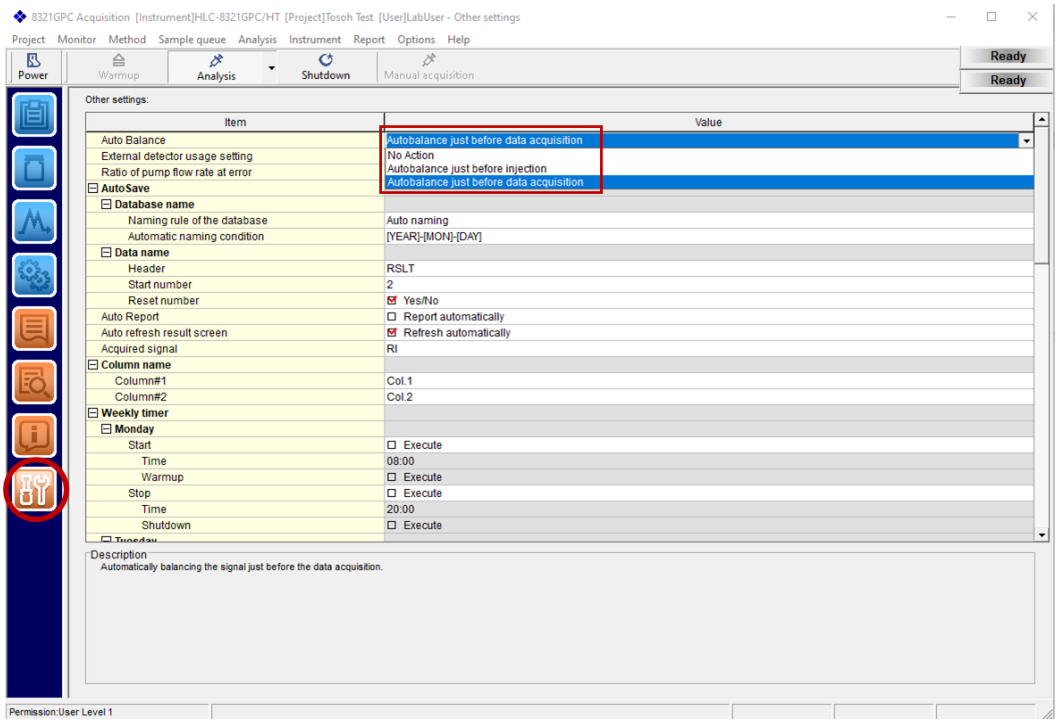

d. Input sample information and run conditions
   i. Click “New” and type in sample name in pop-up (note, this sample will not be saved)
   ii. Input run conditions:

Run time = 35 min
Start time = 0 min
End time = 35 min
Sampling interval = 100 ms
Standard method- default
Unknown method = default

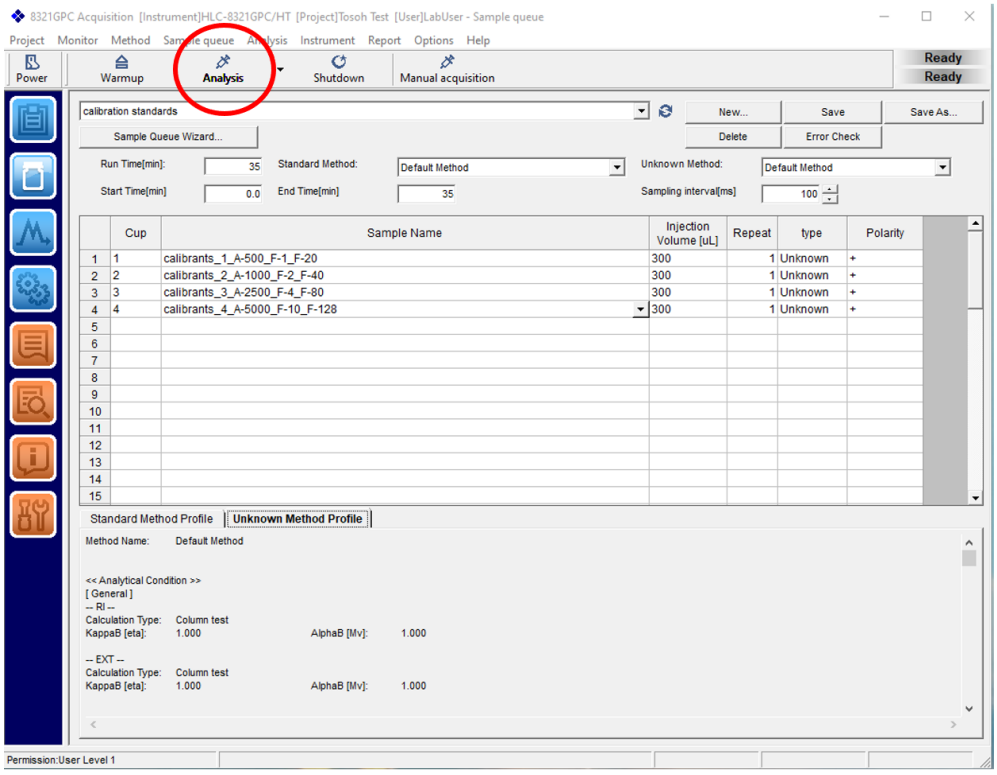
   iii. Type in cup number (where sample was placed) and give the sample a name (this sample name will be saved with the data).

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.”



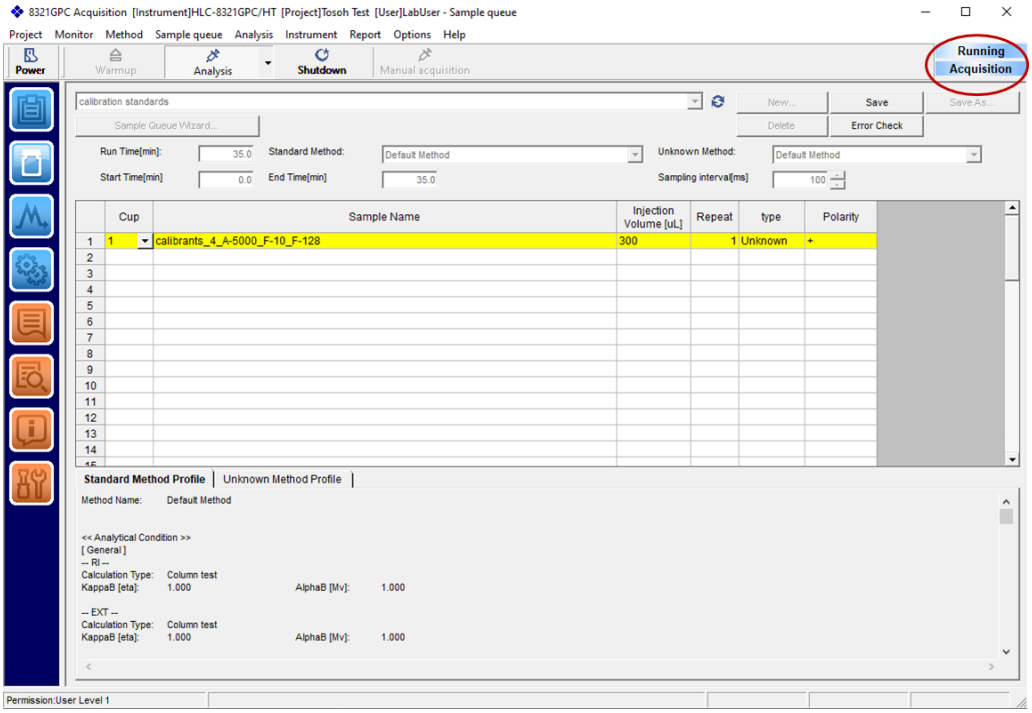

g. Click Analysis button (top tool bar)



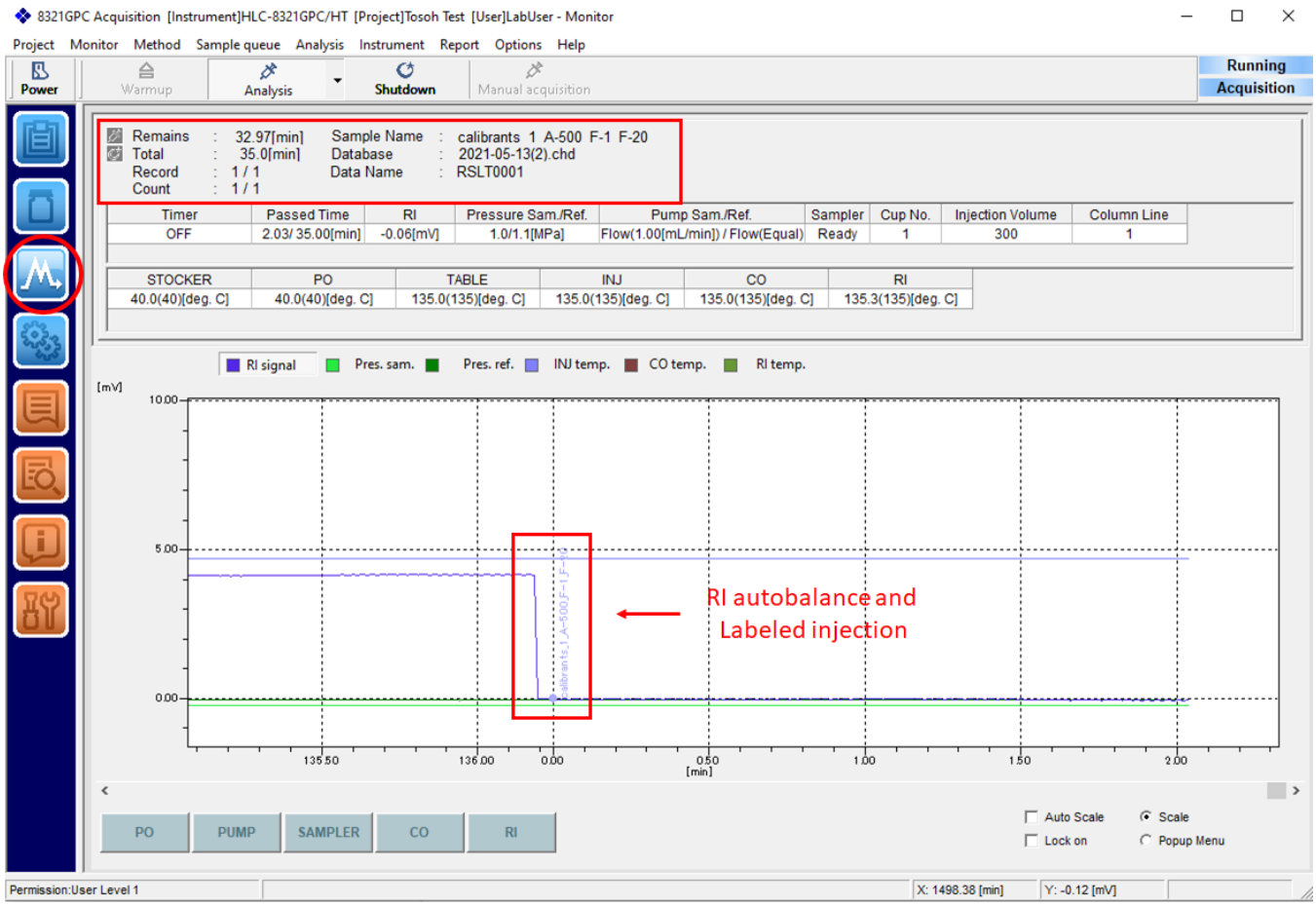
h. The status indicator should turn blue with the text “running” and then “acquisition” once the sample is injected. There is about 7 min between when the sample is marked as “running” and the actual injection.The current sample will be highlighted in yellow ~3 min after the sequence starts.

Before injection:

After injection:

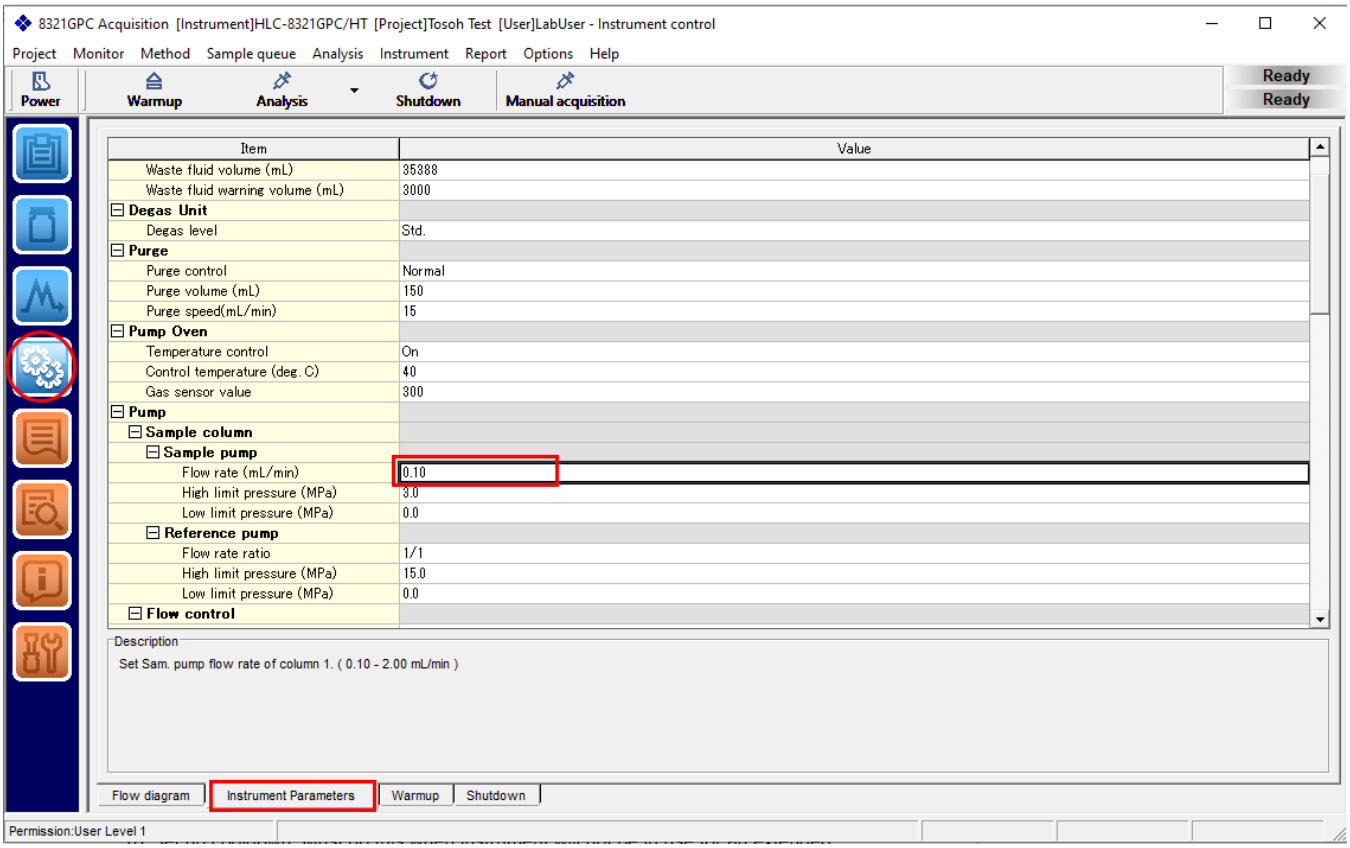



i. On the monitor screen, the current sample and time remaining is displayed in the upper left.



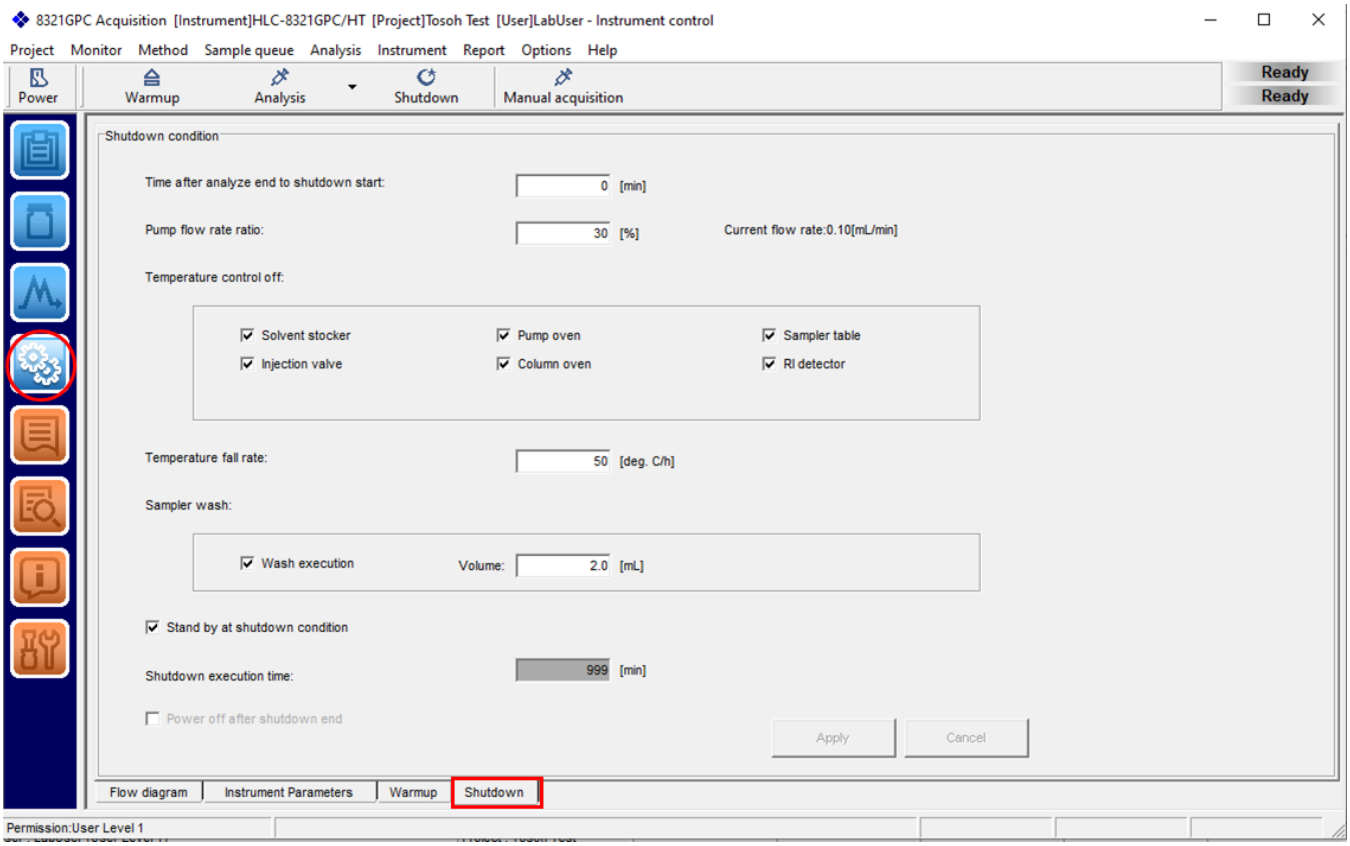

j. When analysis is finished, return “solvent waste” line back to the front solvent bottle, wiping tubing with kimwipe to prevent contamination.

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.




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.

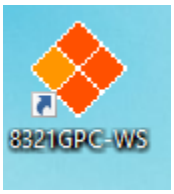
9. If you are ready to shutdown the instrument (weekends, longer periods without use), initiate the shutdown procedure:
    a. Click the “instrument control” icon in from the column on the left and navigate to the “shutdown” tab.




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> sleect 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

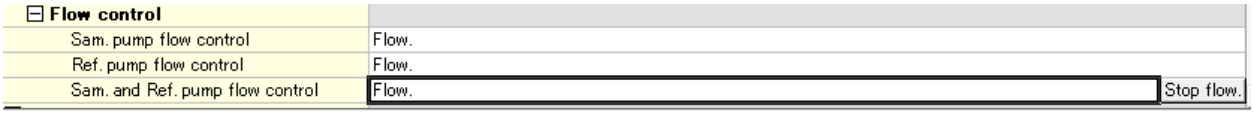
# Upkeep and Maintenance

### 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.

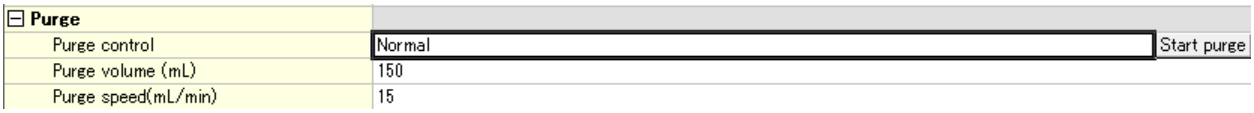



2. Remove cap from current solvent bottle, take tubing off, and then switch out the old bottle for a new one.
3. For waste bottle, must disconnect green wire to remove tubing. Transfer waste into a clear department waste bottle, and return empty amber waste bottle to instrument. Reconnect green wire.

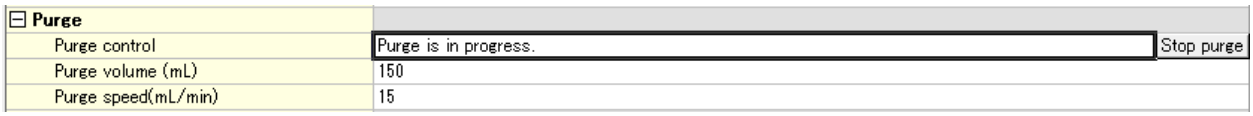
4. Perform purge (system icon), *make sure pumps are off!

   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




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




## 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 ≤ 2 mV over 60 min
  - Noise ≤ 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

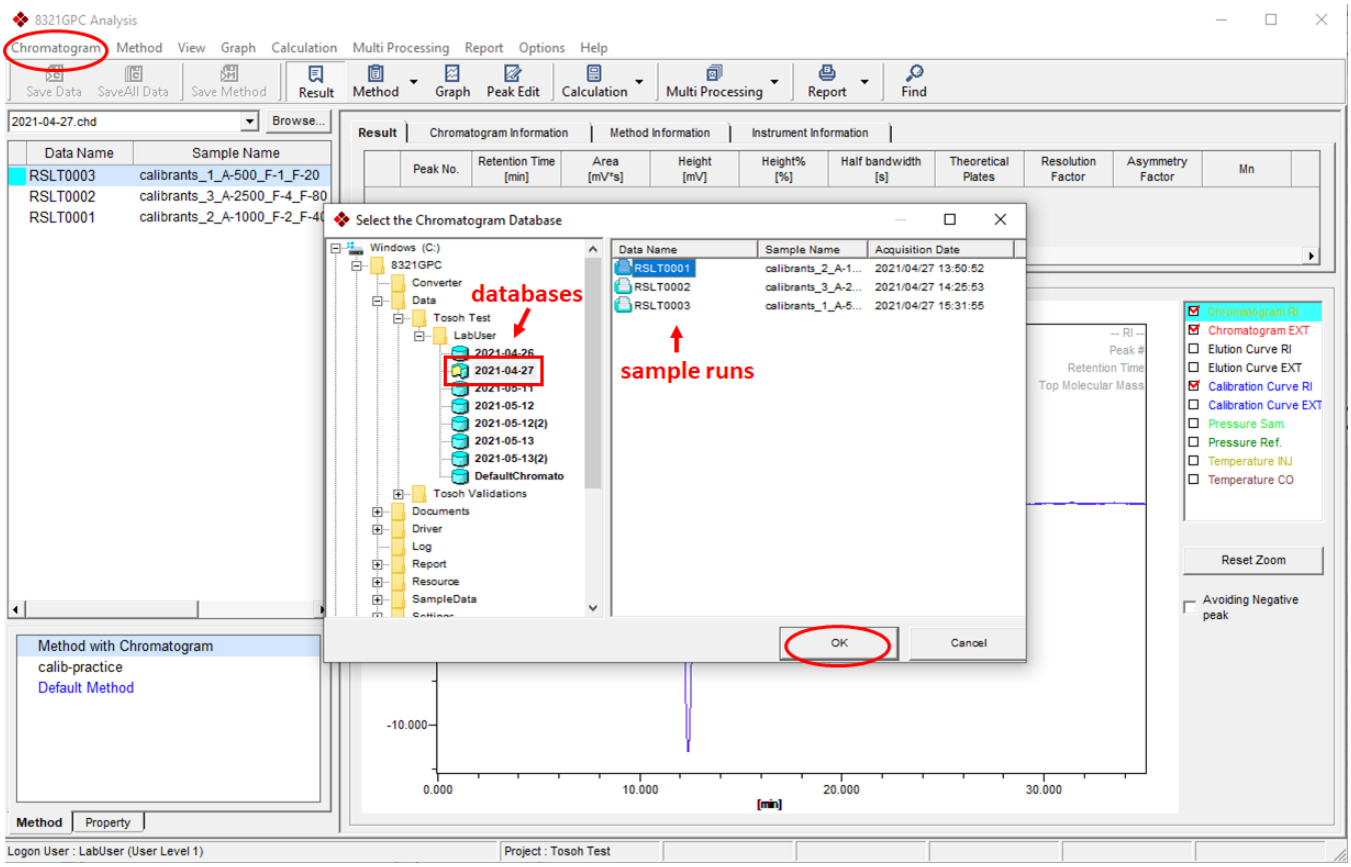
    a. TSKgel standard PS samples are kept in gold box in small refrigerator

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

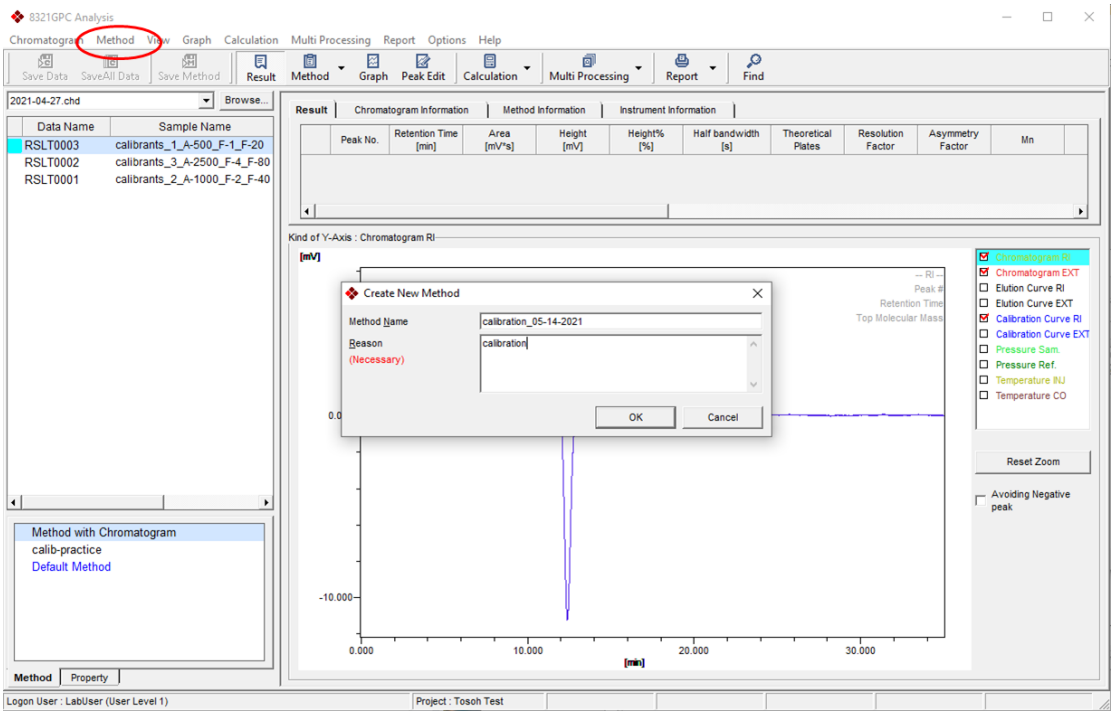
    1: A-500, F-1, F-20
    2: A-1000, F-2, F-40
    3: A-2500, F-4, F-80
    4: A-5000, F-10, F-128

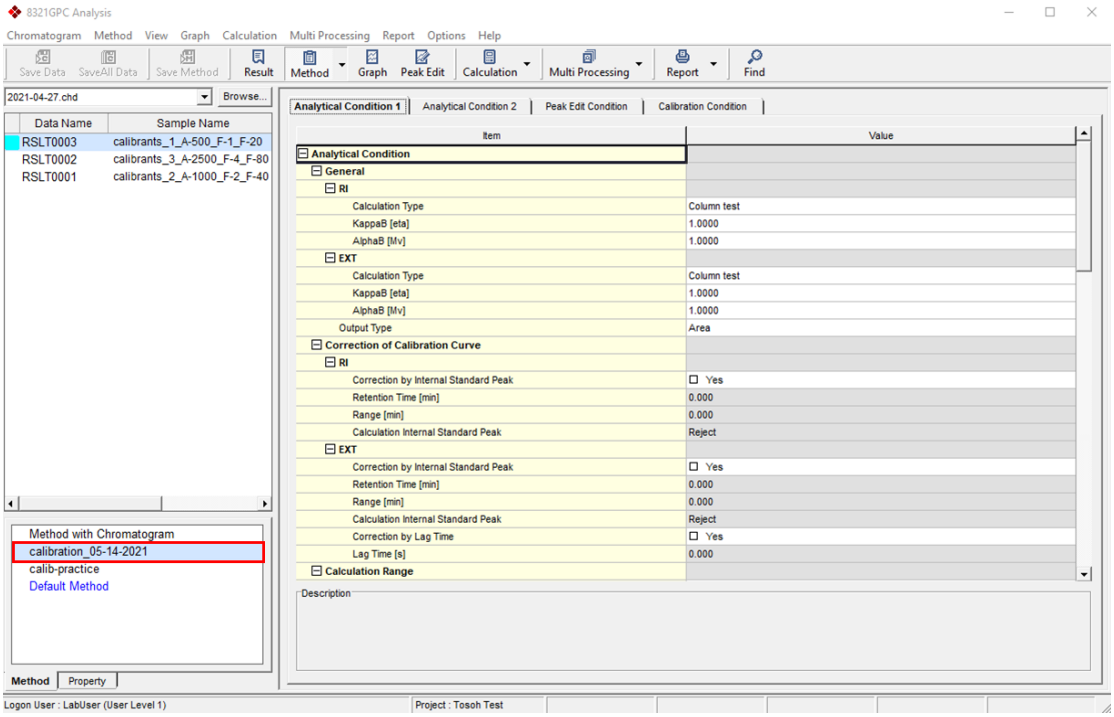
c. Run each sample and save file

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

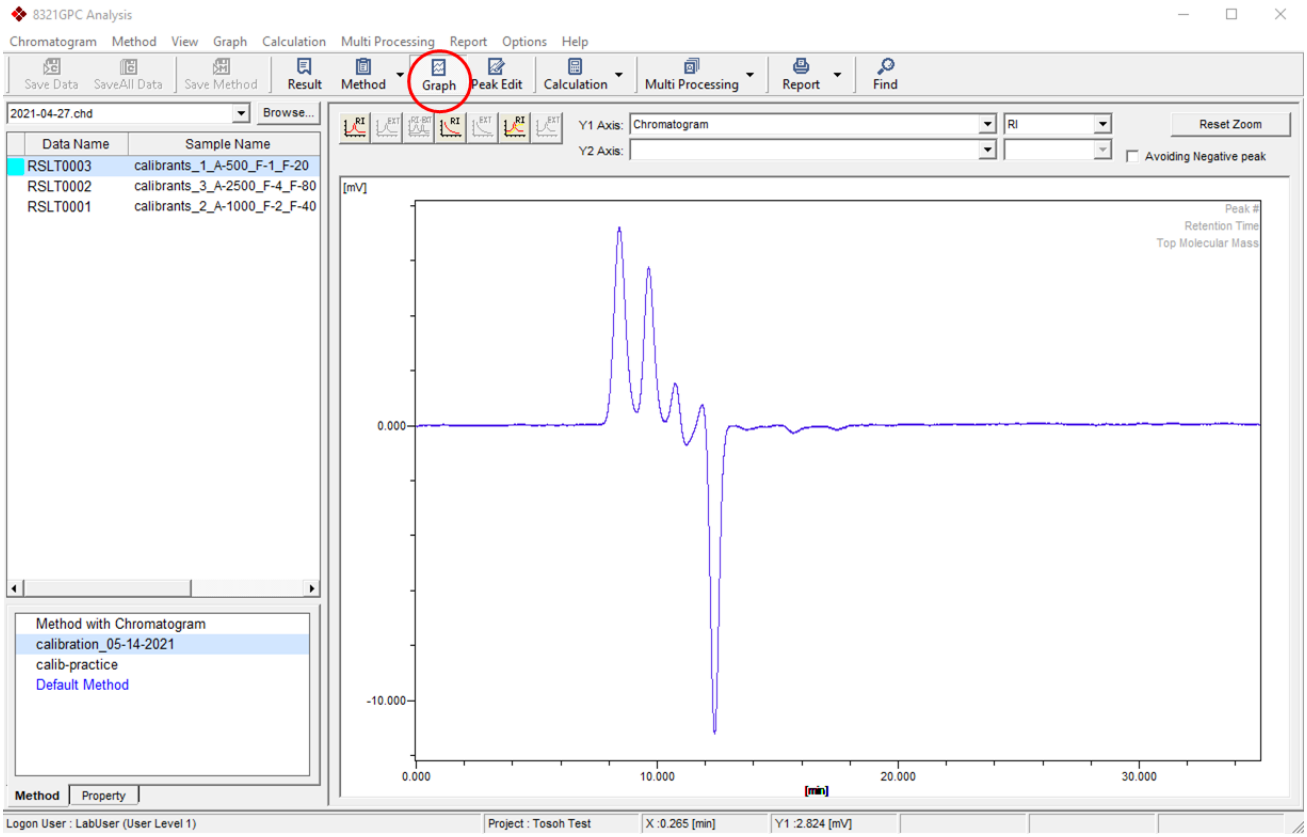


3. From the top toolbar, select “Method” -> “New.” In the resulting pop-up, name the method whatever you would like and enter a reason. Once you click “OK” the method will show up in the list of available methods at the bottom left.

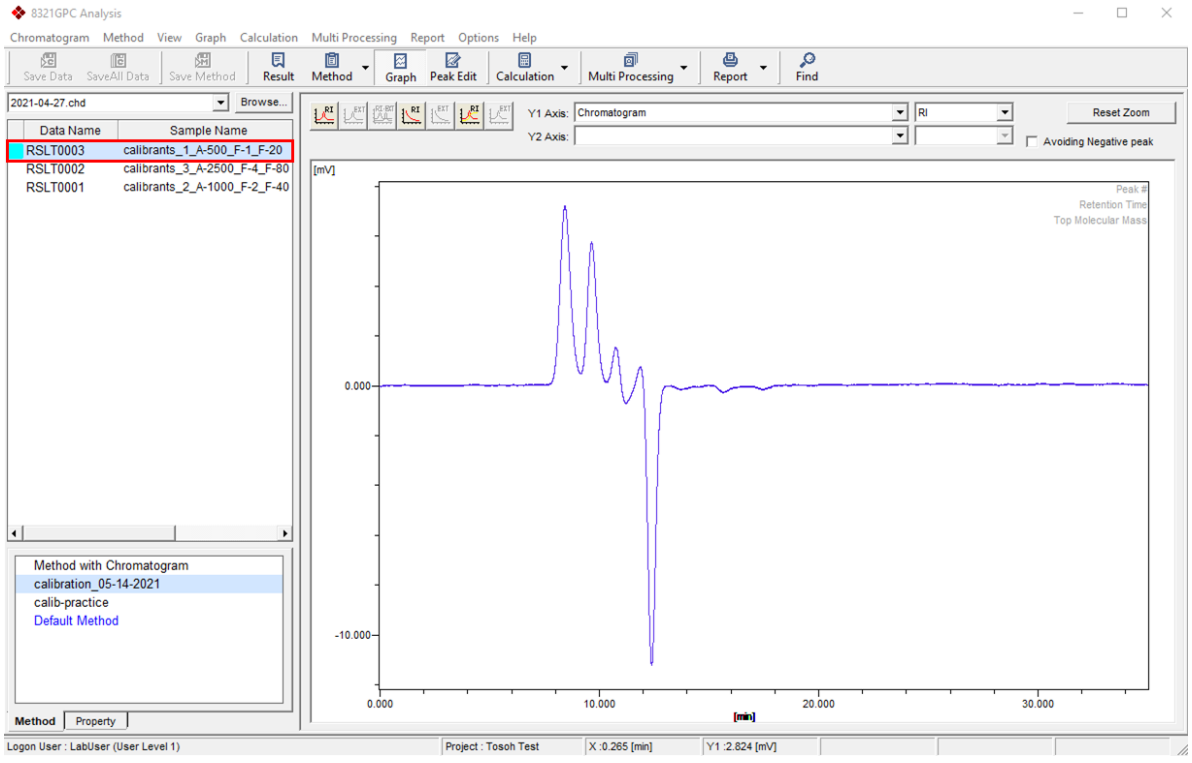


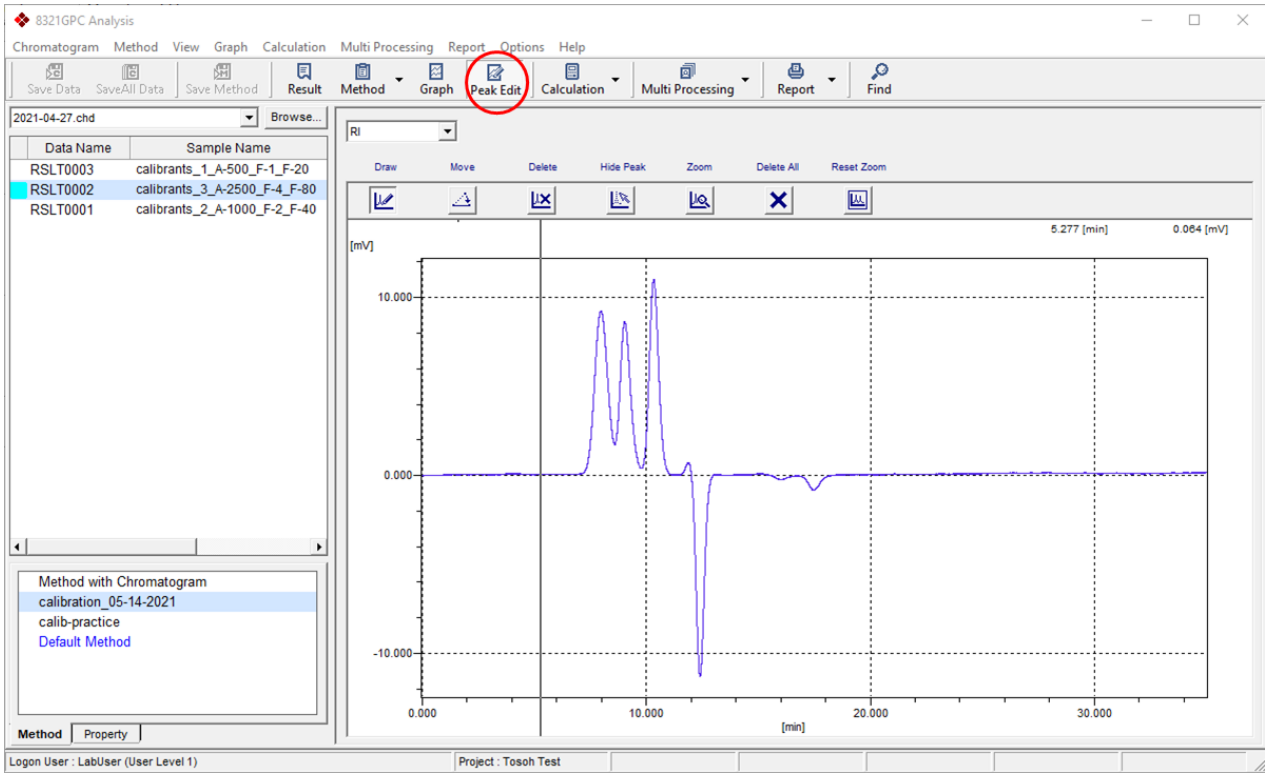
4. To view the chromatograms, select “Graph” from the upper toolbar



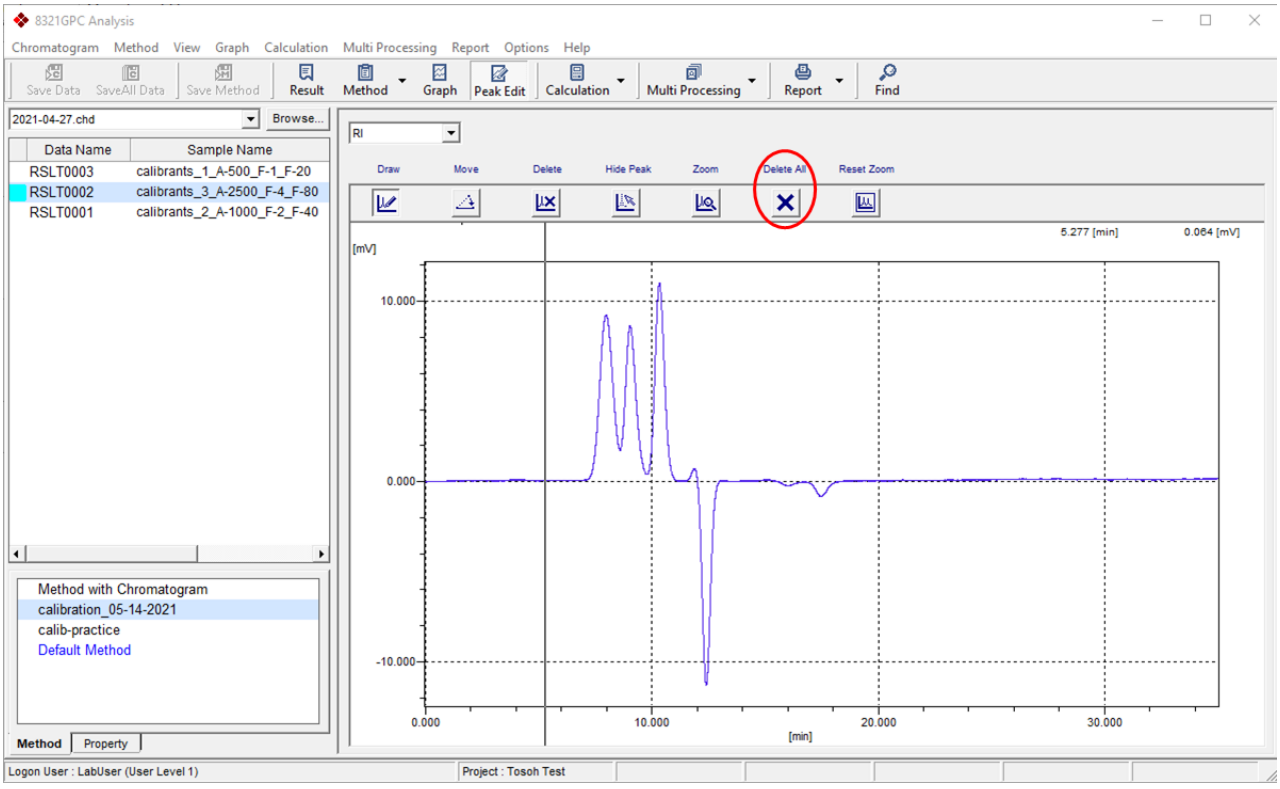

5. Click the sample you wish to analyze from the list at the upper left



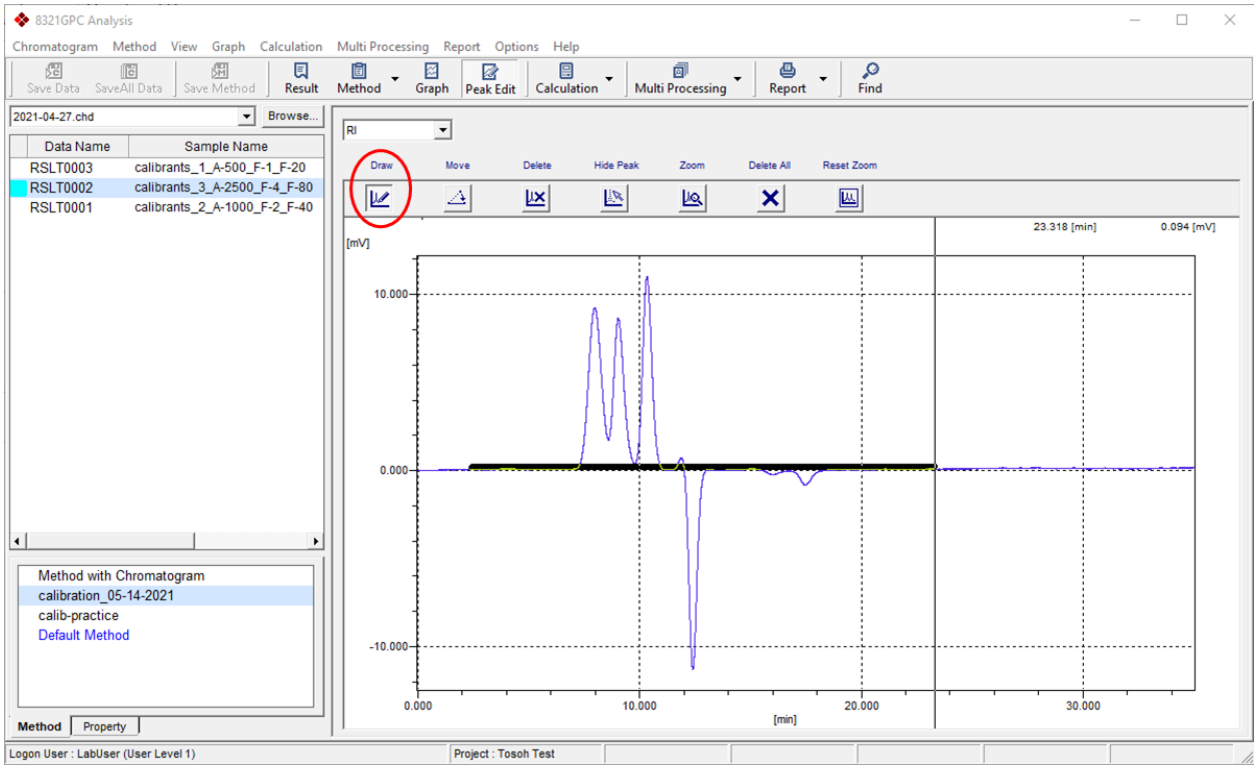
6. Click the “peak edit” icon to open the window where you can edit the chromatogram



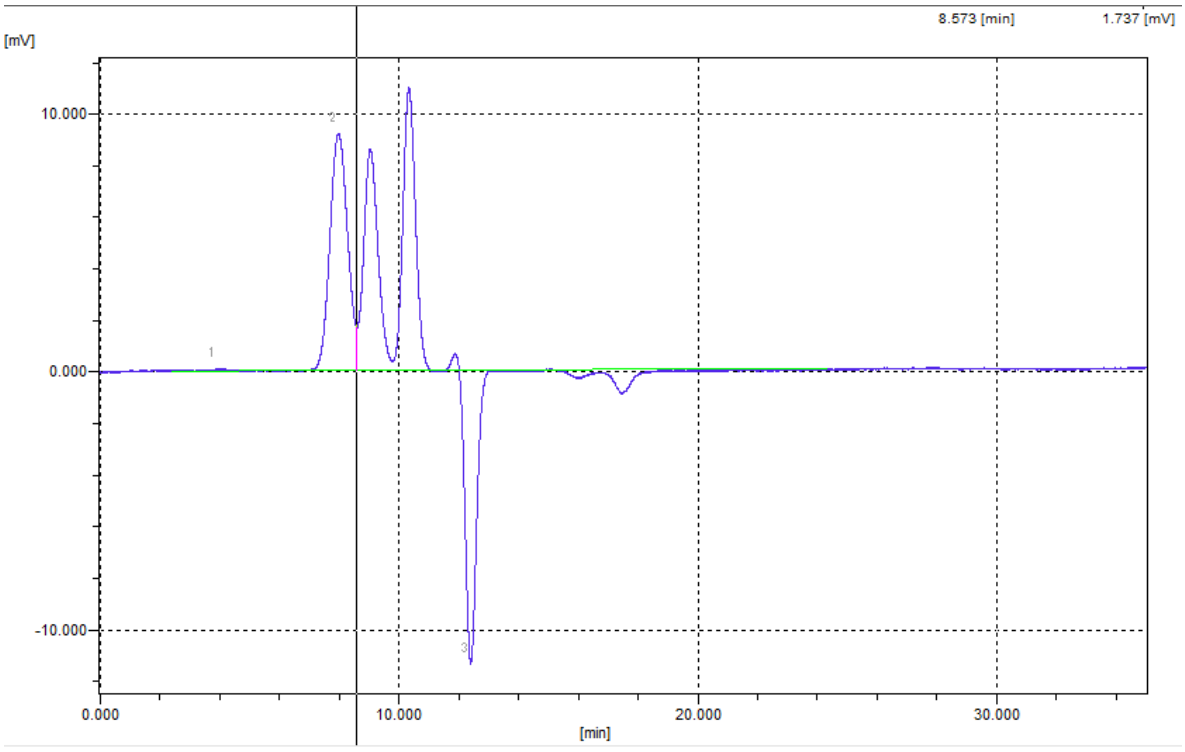

7. Click “delete all” to remove any auto-generated peaks/baselines.



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

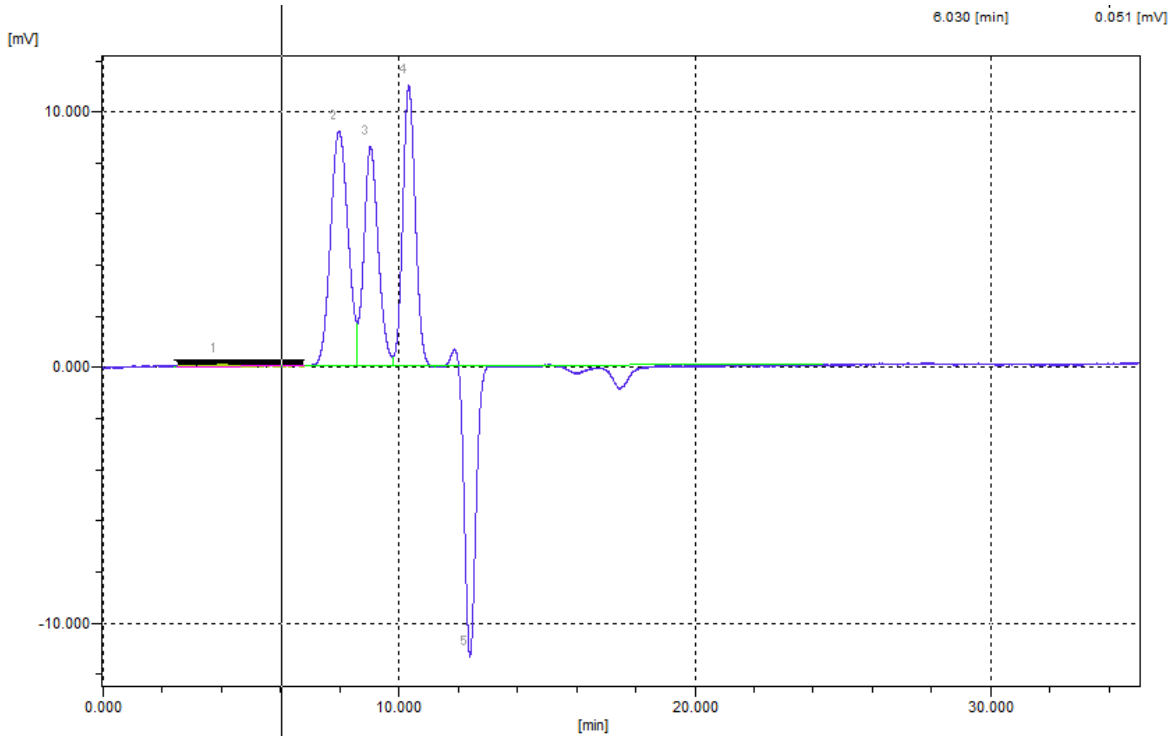



9. Single left click to set the peak limits. Numbers will appear above each selected peak.

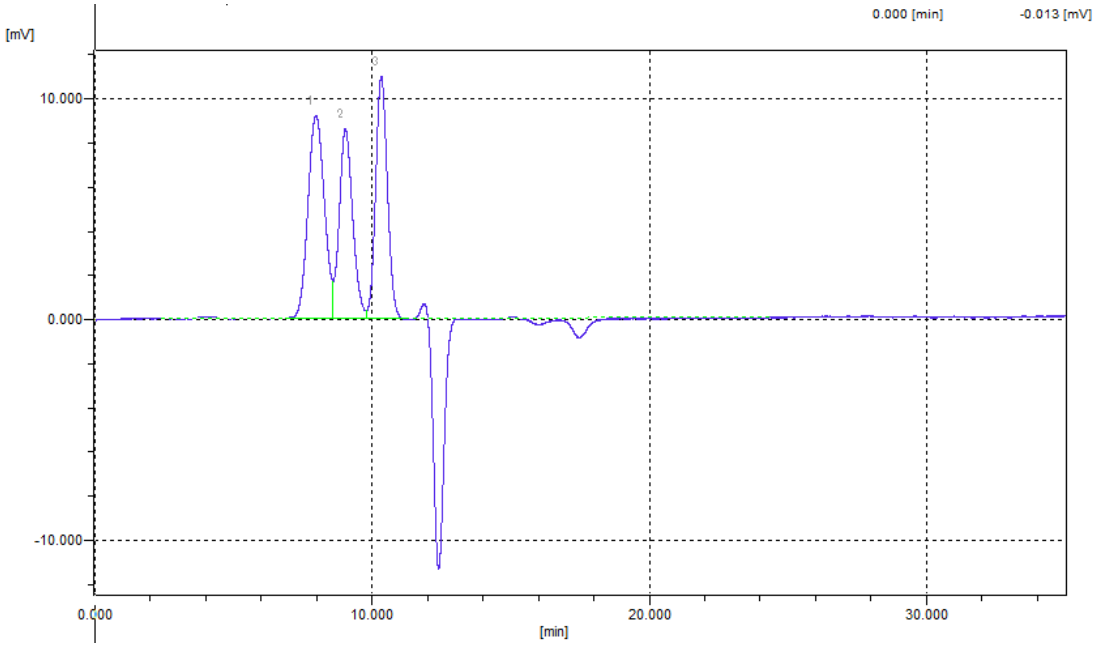


10. Additional peaks will likely appear to the left and right of the true polymer peaks. To remove these peaks from the list of polymer peaks, select “hide peak.” Hovering over different regions will highlight them in black. Single left click on the “peaks” you wish to remove.

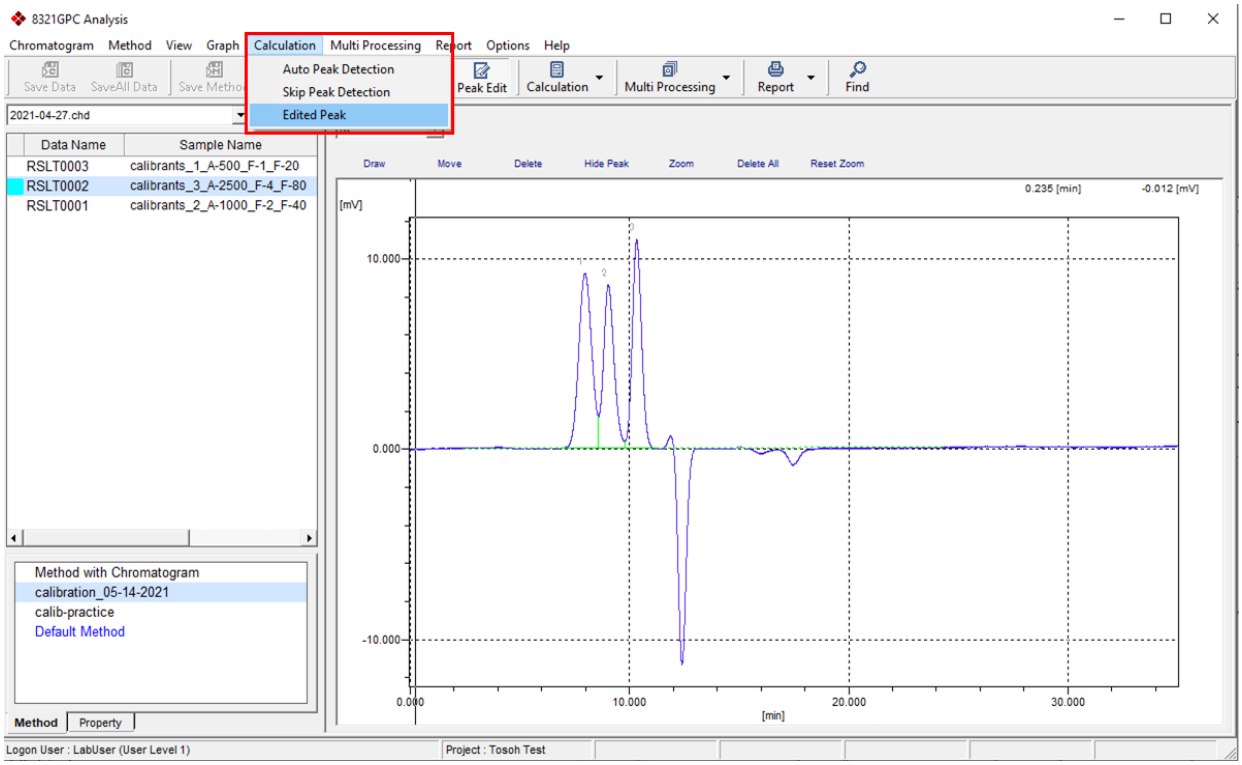
    a. Black region highlighted

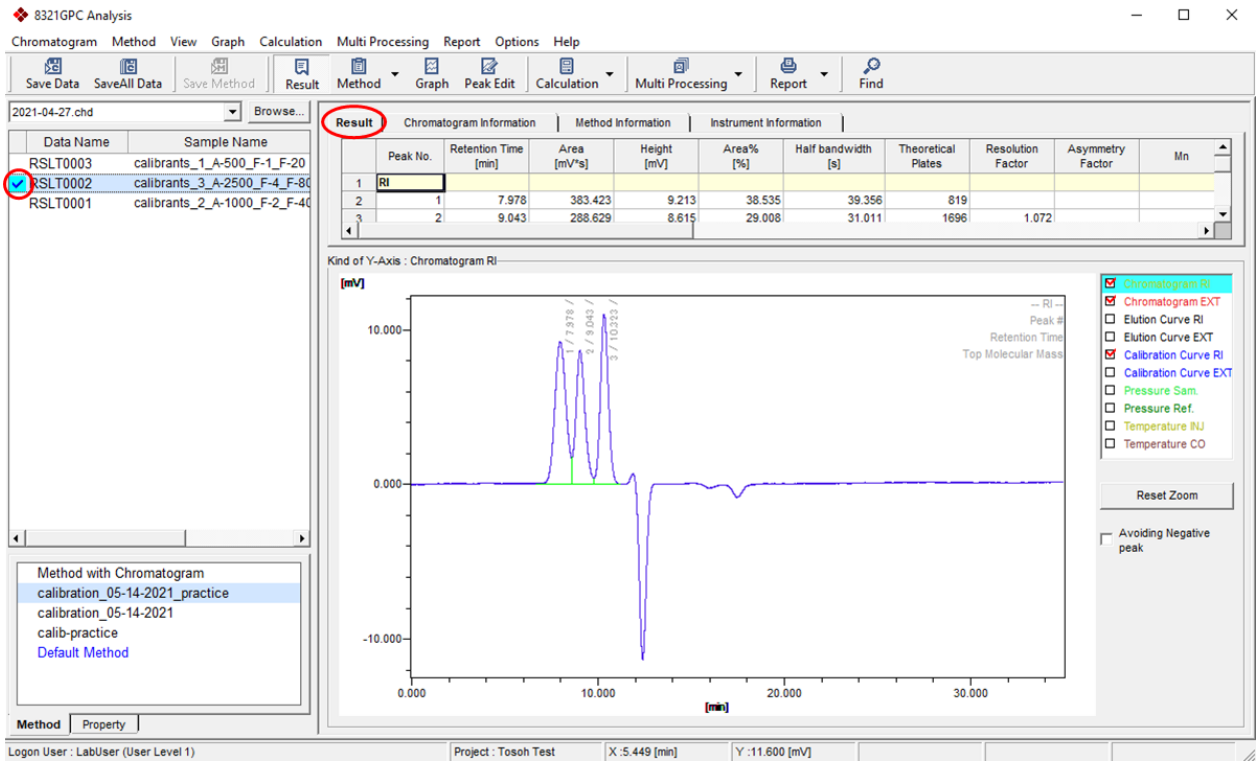



    b. Extra peaks removed. Peaks are auto re-numbered.

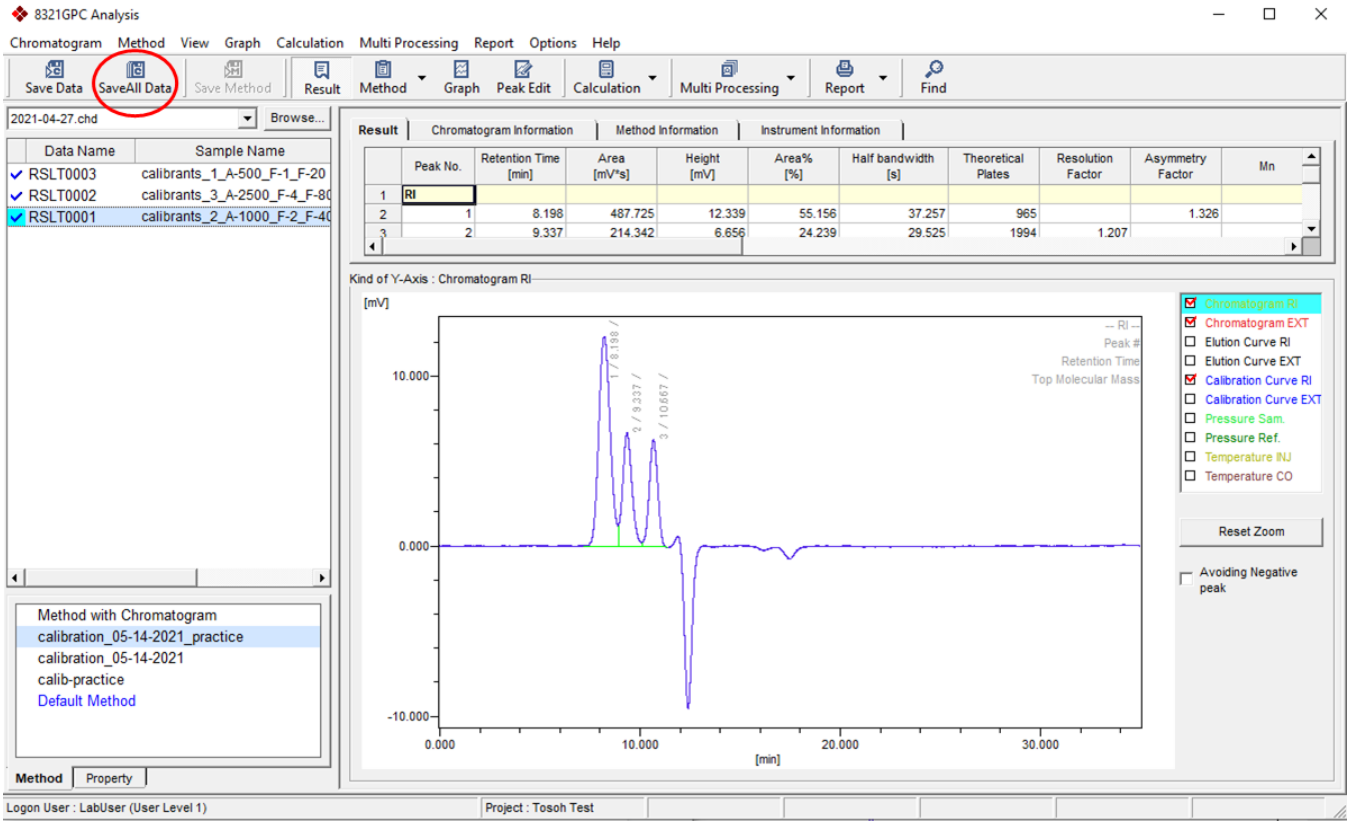


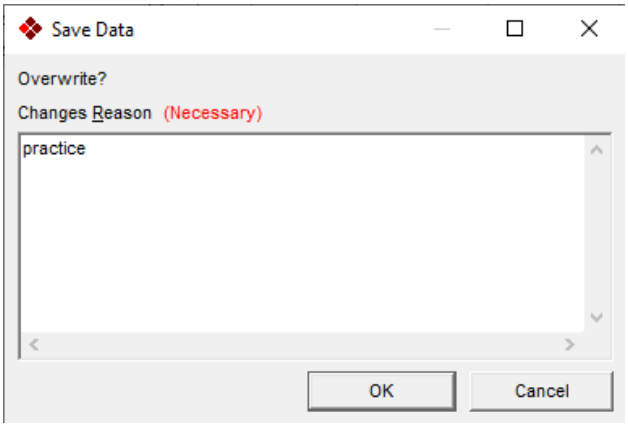
11. Click “calculation” from the top toolbar and select “Edited Peak.” A results table will appear and the sample name will be marked with a blue check mark. Repeat steps 6-11 for all samples that you wish to include in the calibration curve.



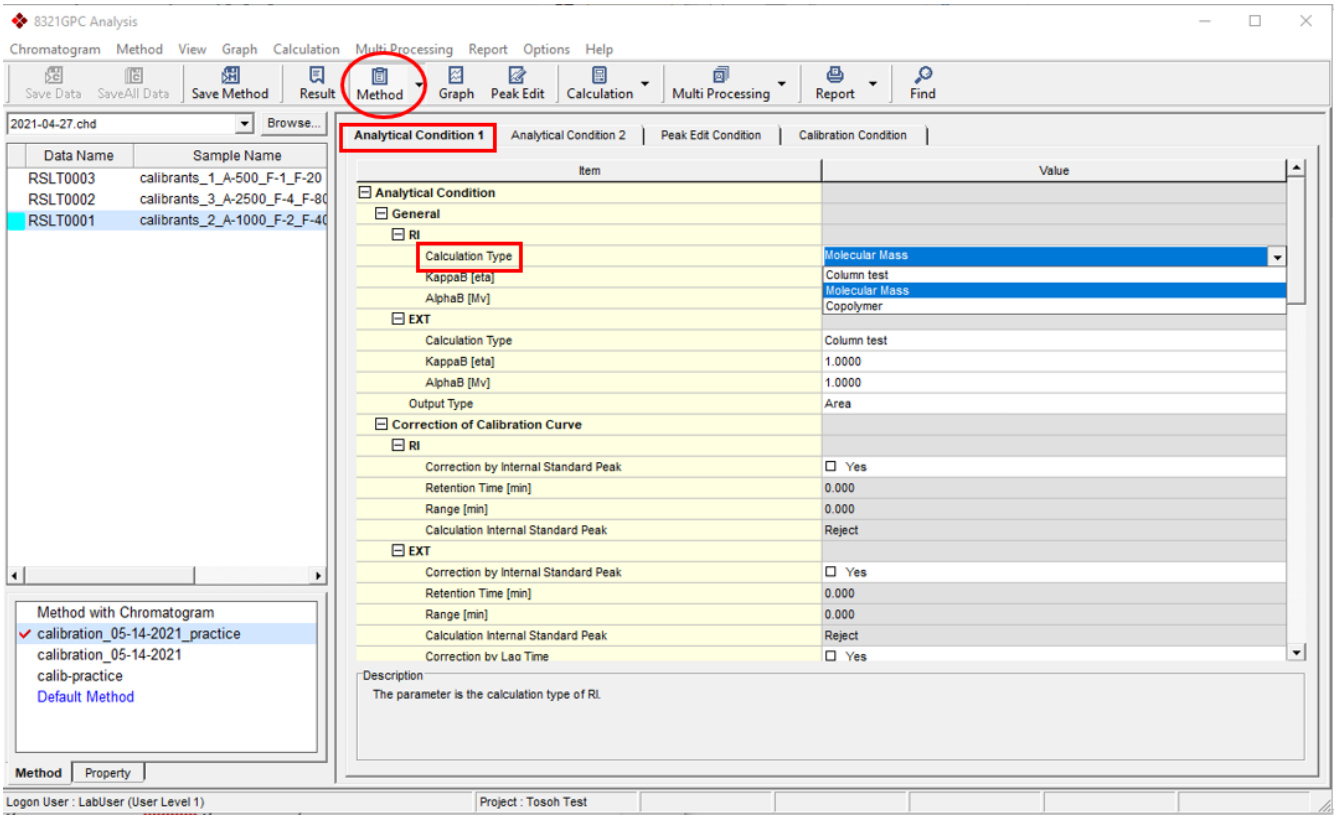



12. Select “Save All Data” and enter in a reason (whatever you like) in the resulting pop-up window. Click “OK.”

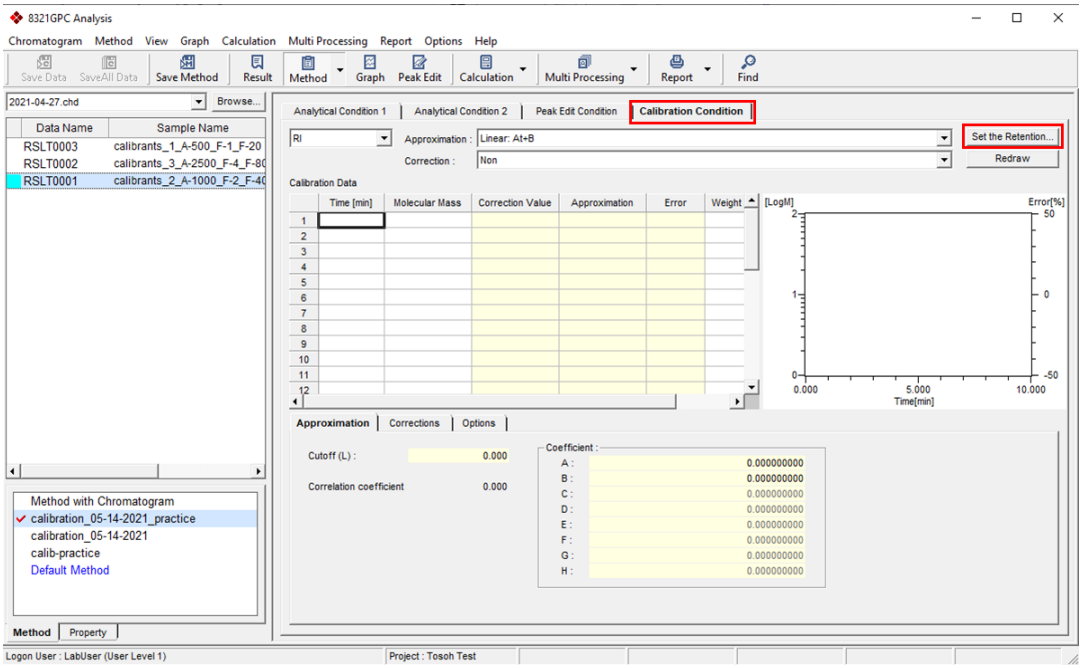


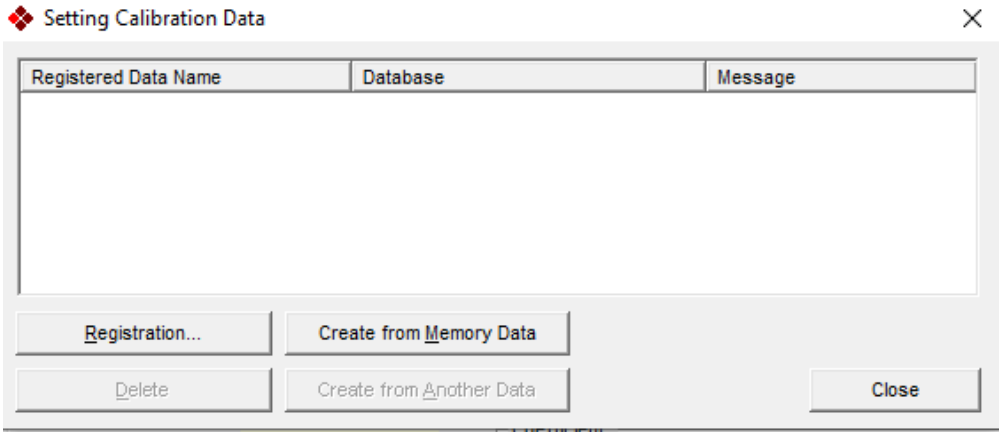
13. Select “Method.” Under the “Analytical Condition 1” tab, double click the value for “RI -> Calculation Type.” From the resulting dropdown menu, select “Molecular Mass.”



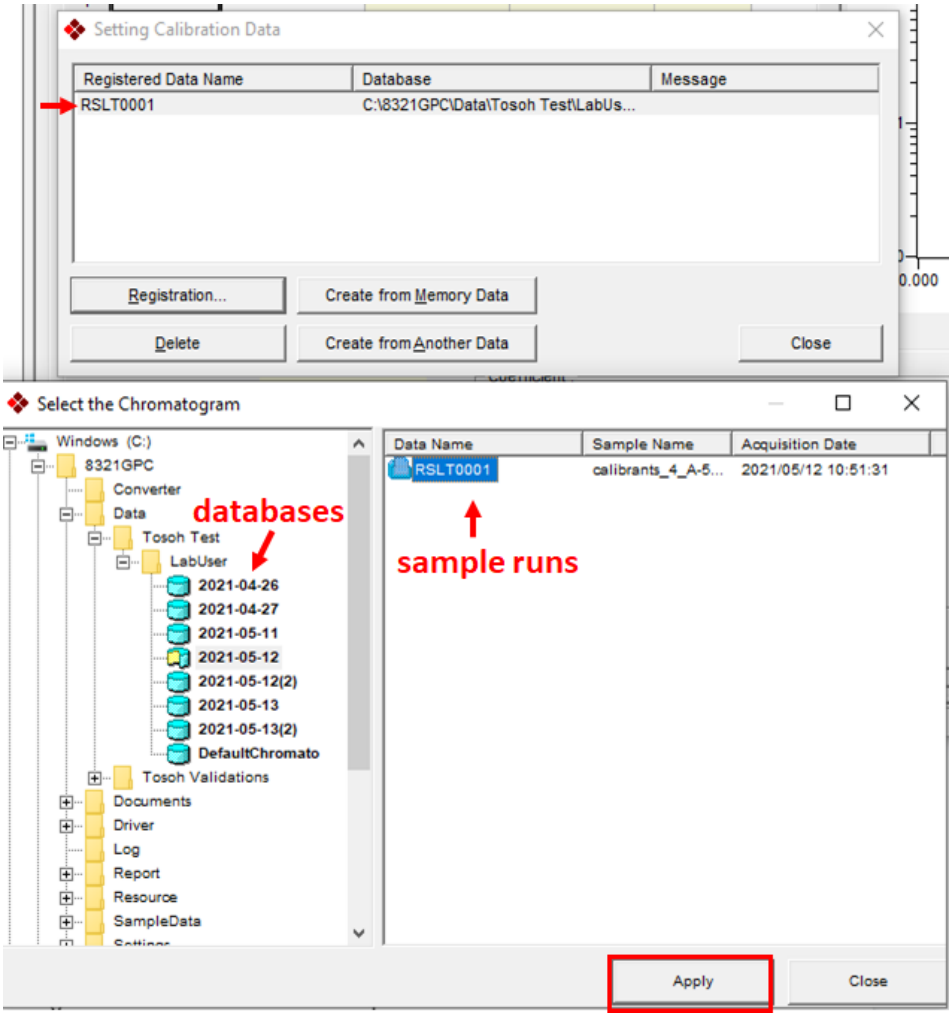

14. Under the “Calibration Condition” tab, select “Set the Retention” to add the data from your calibrants.



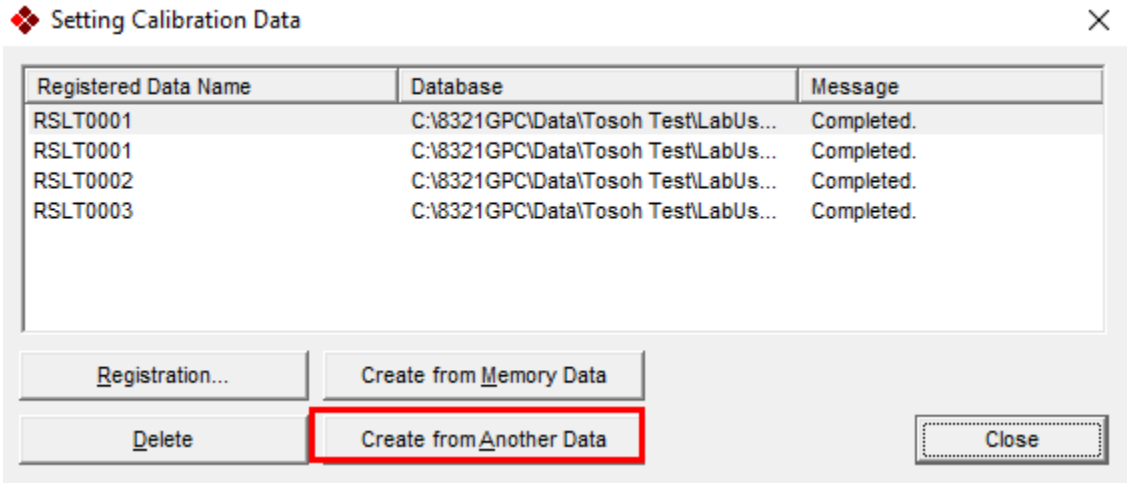
15. In the resulting pop-up, click “Registration” to find calibration data.



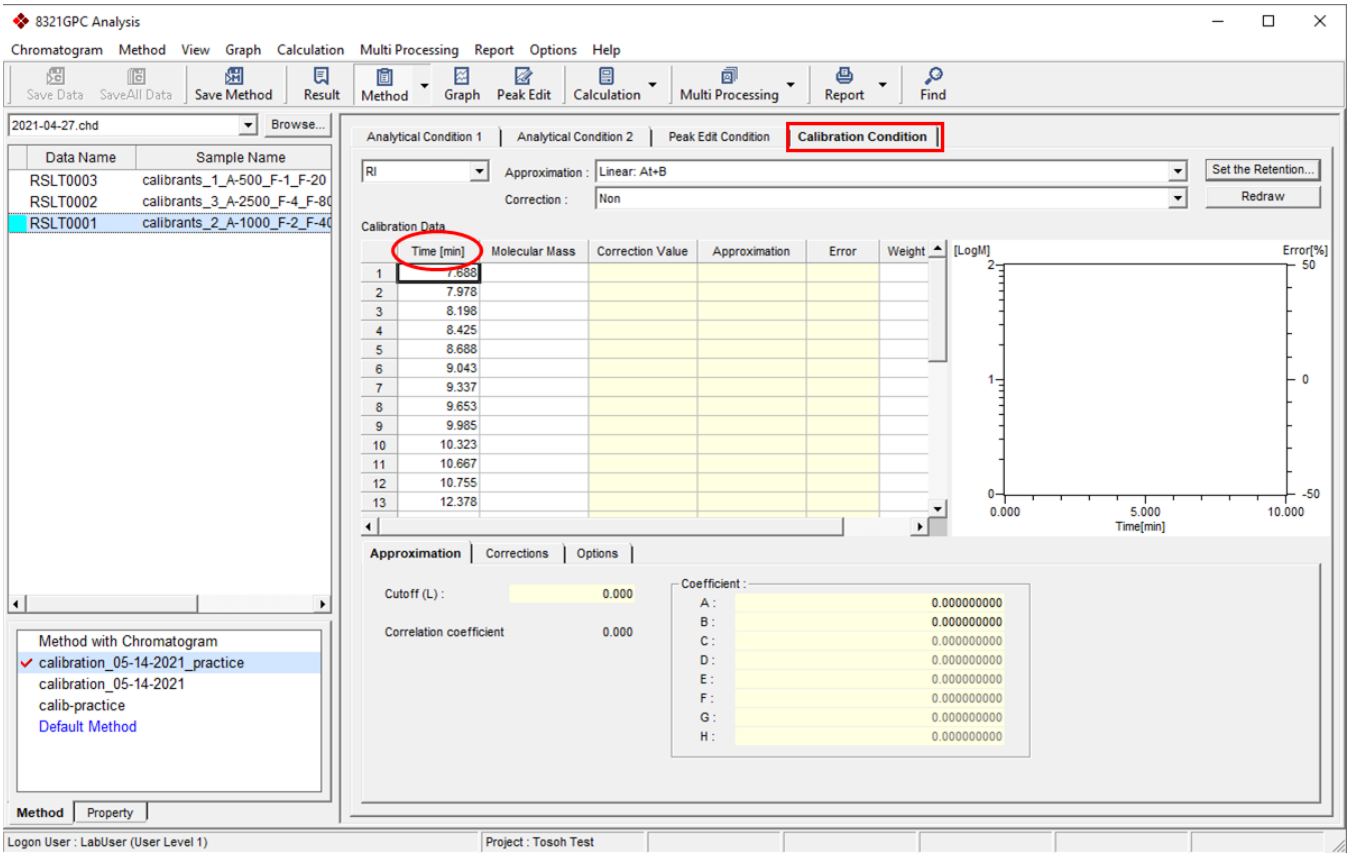

16. Find the calibrant sample runs that you wish to add to the calibration curve. For each sample, click to select it (on the left), then hit apply. Double check that the sample is added to the registration window before adding subsequent samples.



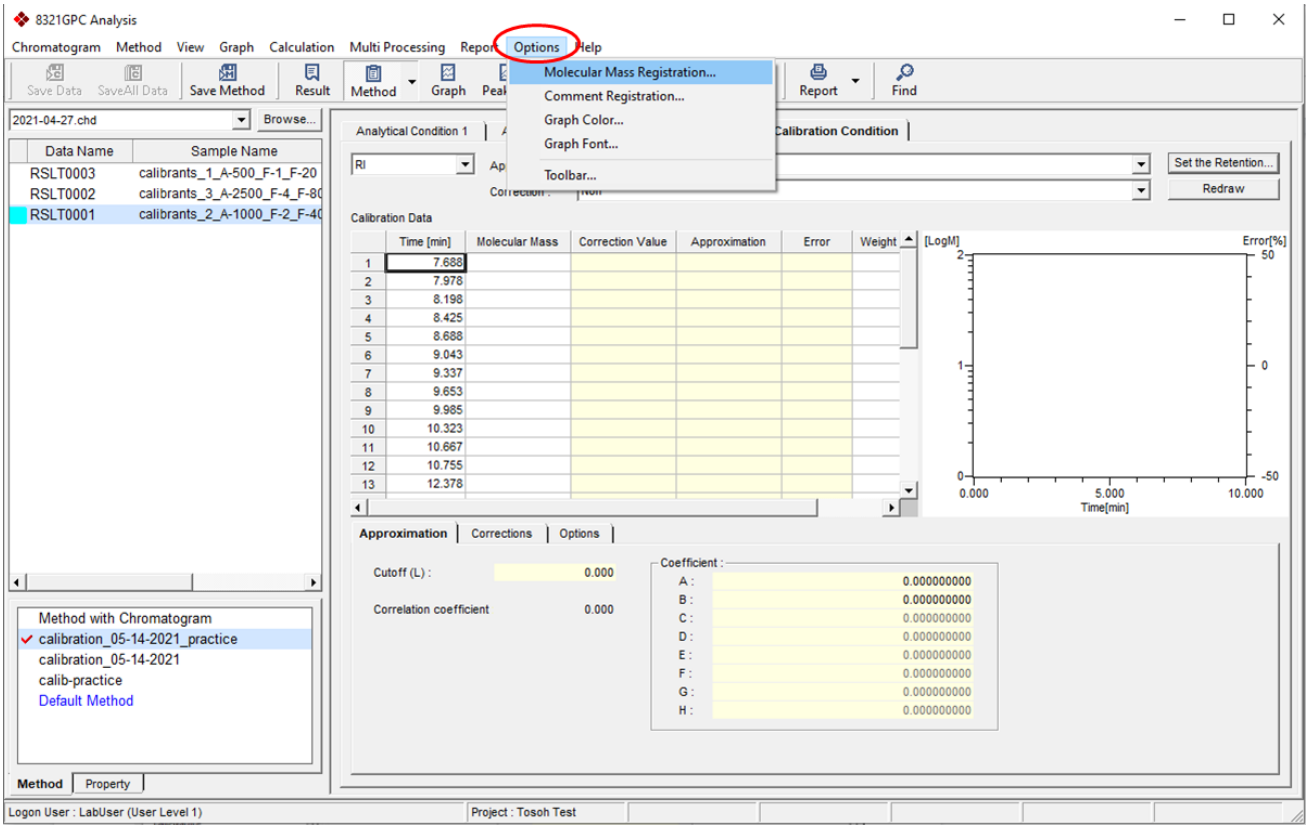
17. Once you have added all of the calibration samples you desire, click “close.” In the registration window, click “Create from Another Data.” The word “completed” will auto-populate the right hand column. Click “Close.”



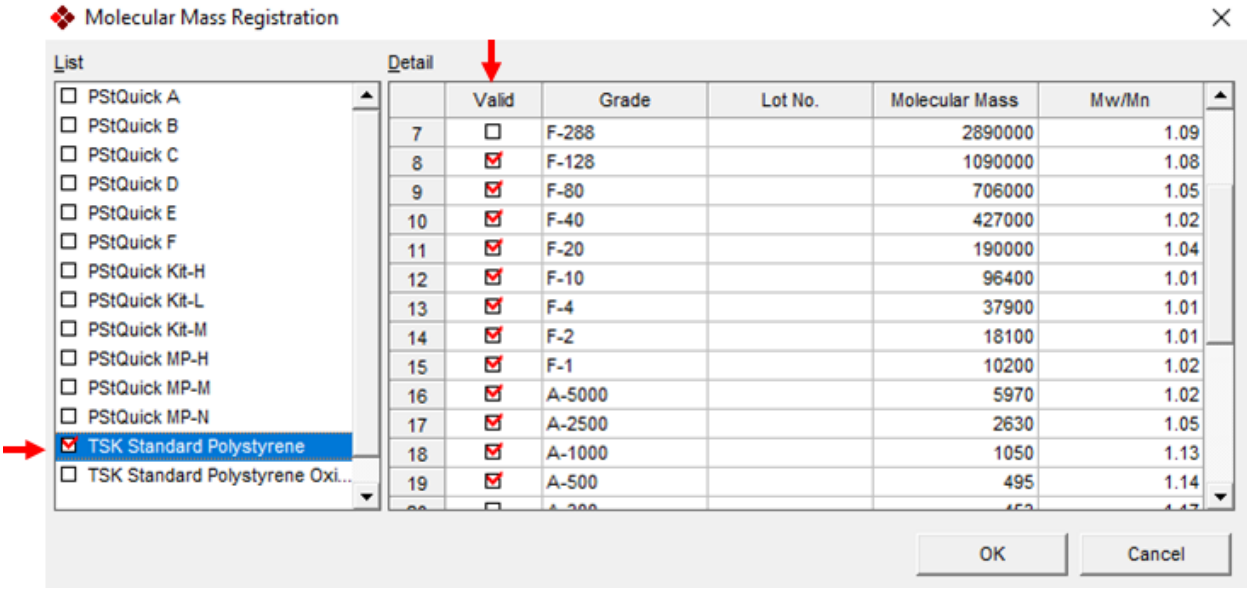

18. The retention times from all of your calibrants should appear in the “Time” column under the “Calibration Conditions” tab.



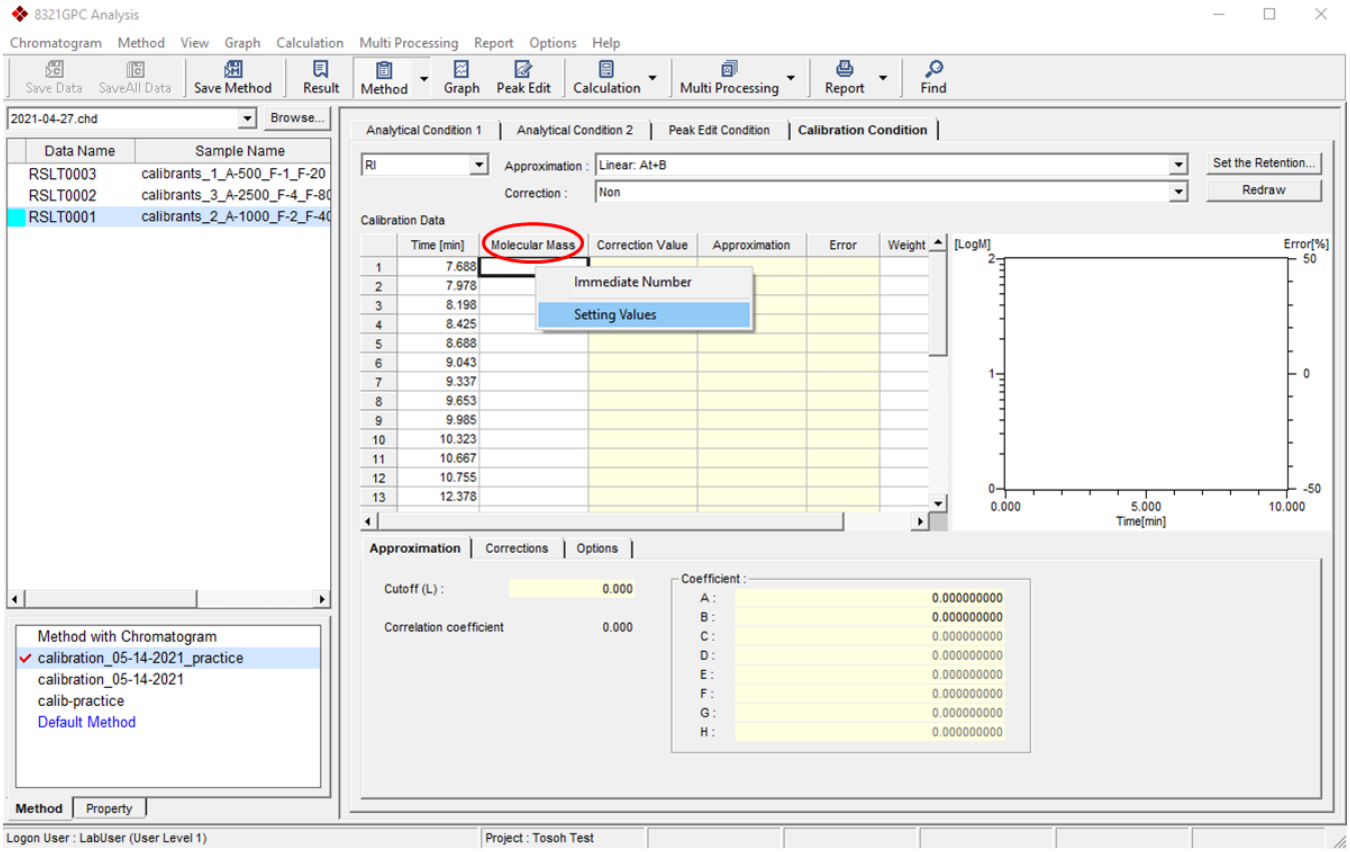
19. From the top toolbar, click “Options” and select “Molar Mass Registration” to open a database of standard data.



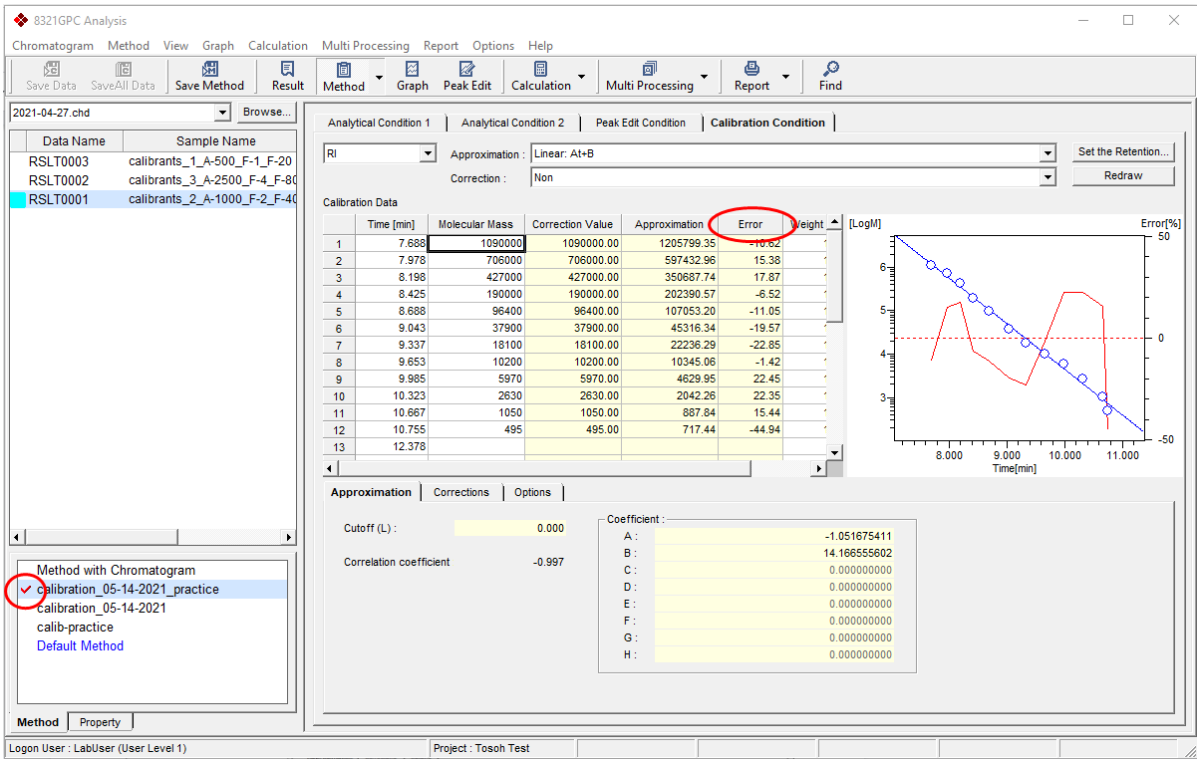

20. Select the appropriate group of standards from the list on the left. Our lab uses “TSK Polystyrene Standards.” In the “valid” column near the center, click each standard that was used to generate the calibration curve. Selected standards will have a red check mark. Click “OK.”



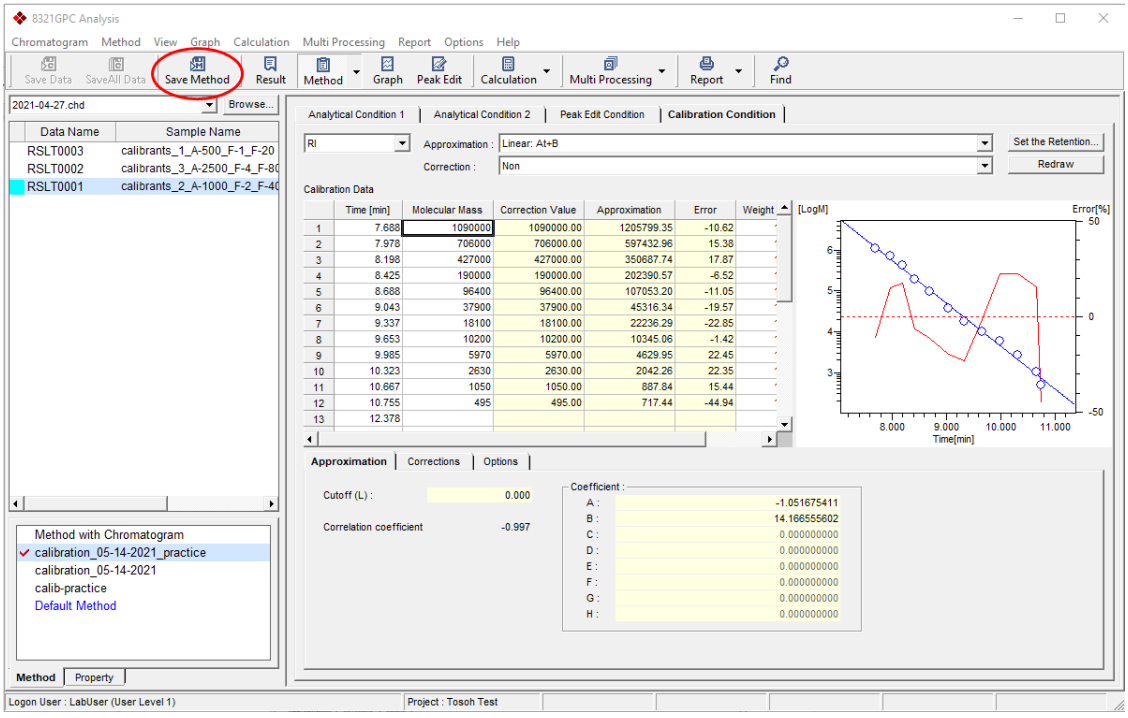
21. Double click on the first box in the “Molecular Mass” column and select “Setting Values” from the resulting dropdown menu.



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.




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



# 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