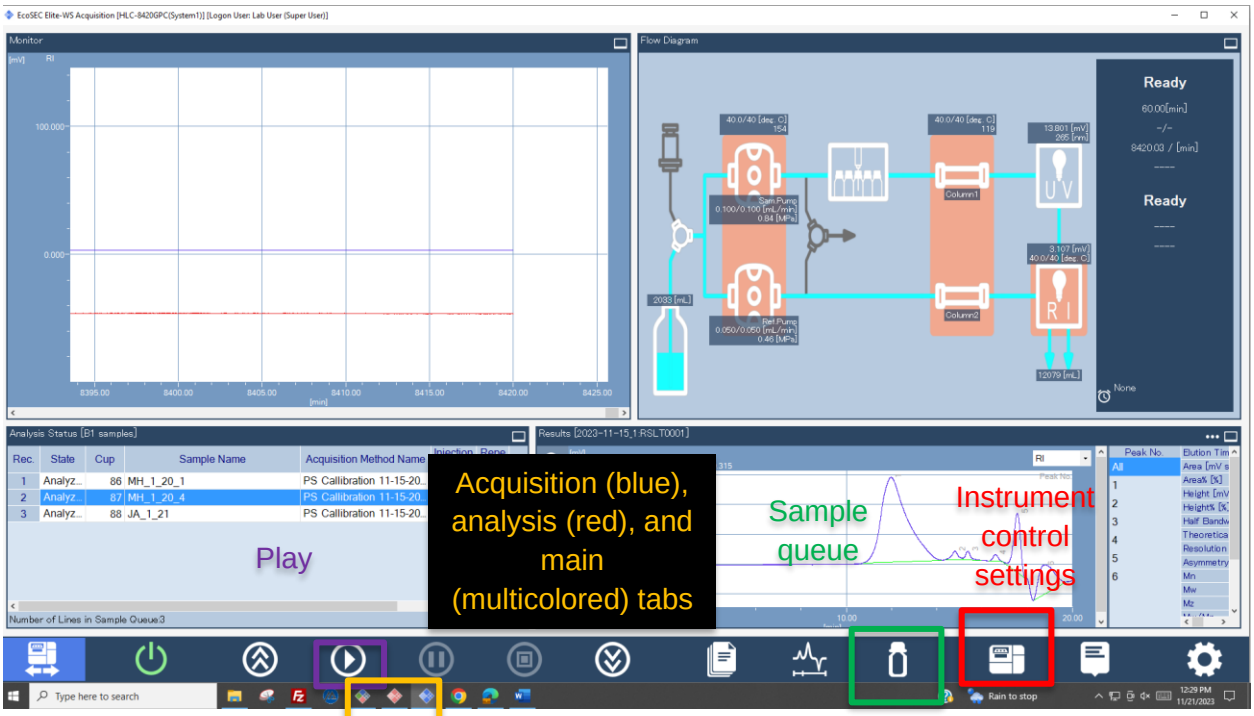
Chloroform SEC

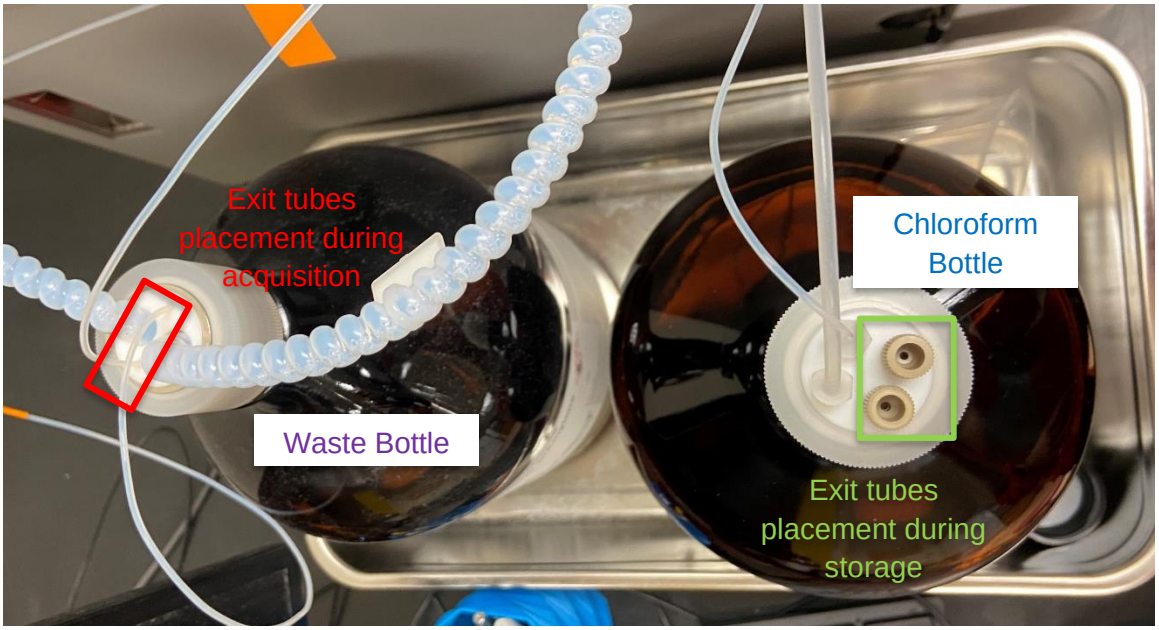
Date: 11/9/2023

Important notes:
Typical pressure at 0.4 mL/min flow rate: Reference: 2 Sample: 3
Typical pressure at 0.1 mL/min flow rate: Reference: .5 Sample: .9
If pressure is high, there might be a clog
Chloroform either needs to be HPLC grade with amylene or 0.25 % triethylamine needs to be added (10 mL for 4 L chloroform bottle)

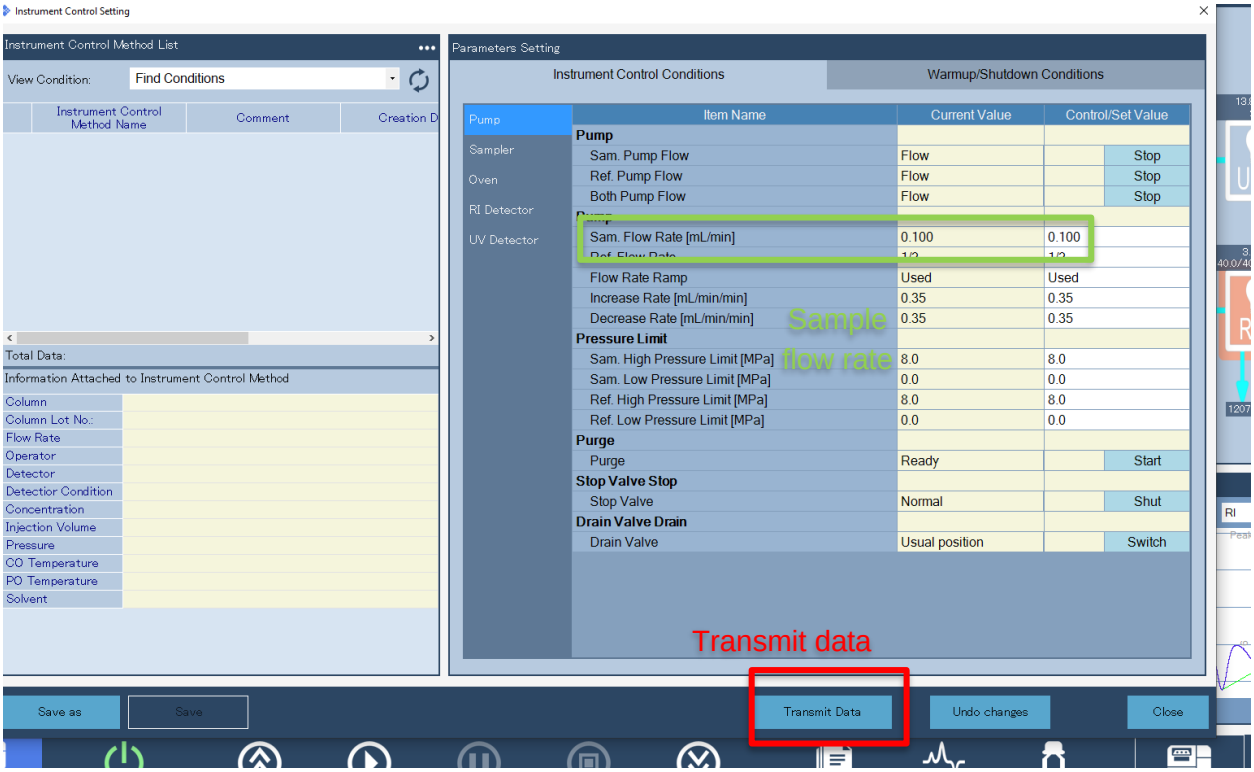
Running a sample:
Shown below is the main acquisition tab (blue icon) that will be used for running a sample. The main buttons are pointed out.



1. Move the exit tubing (smallest tubes) from the chloroform bottle to the waste bottle as shown below. This way the waste coming the instrument will go into the waste bottle. We typically recirculate the solvent while the instrument is in storage, however, we do not want to do this when a sample is running since we don't want to recirculate samples.

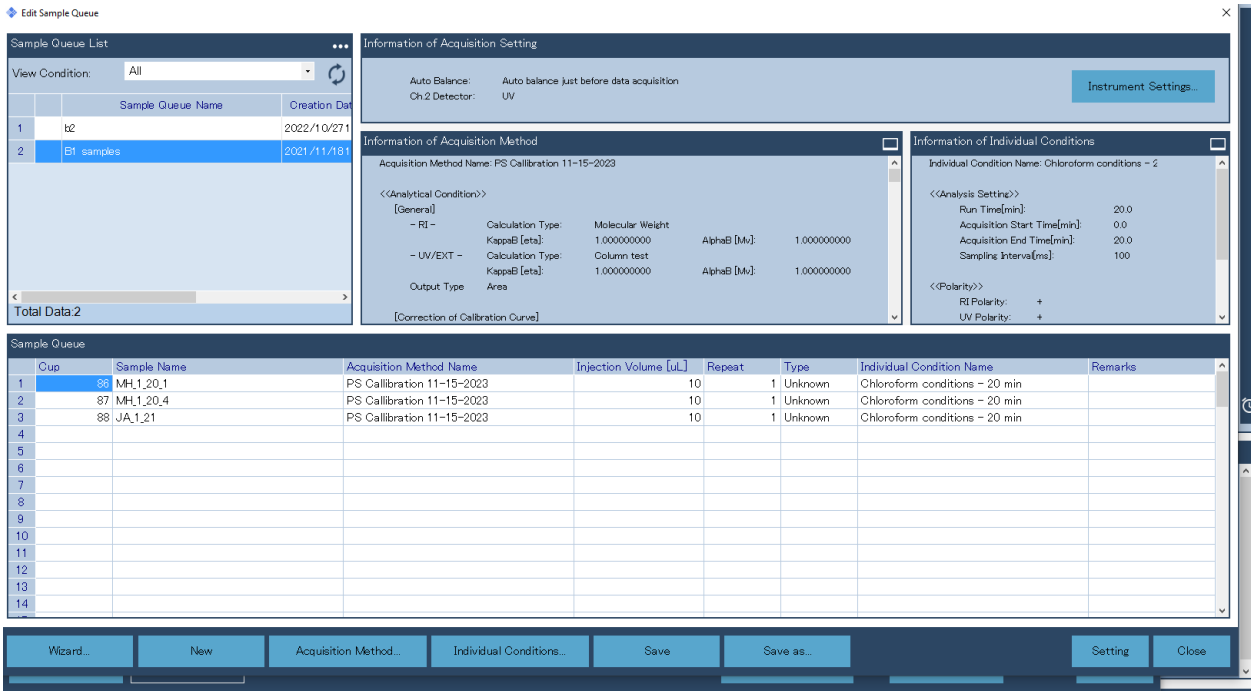


2. The flow rate need to be increased from 0.1 to 0.4 ml/min. Click on the instrument icon on the bottom of the screen to pull up the *instrument control settings* page. Then change the sample flow rate to 0.4 and click *transmit data* to change it on the instrument. The baseline will need to settle for 1-3 hours before you can run a sample on the instrument.

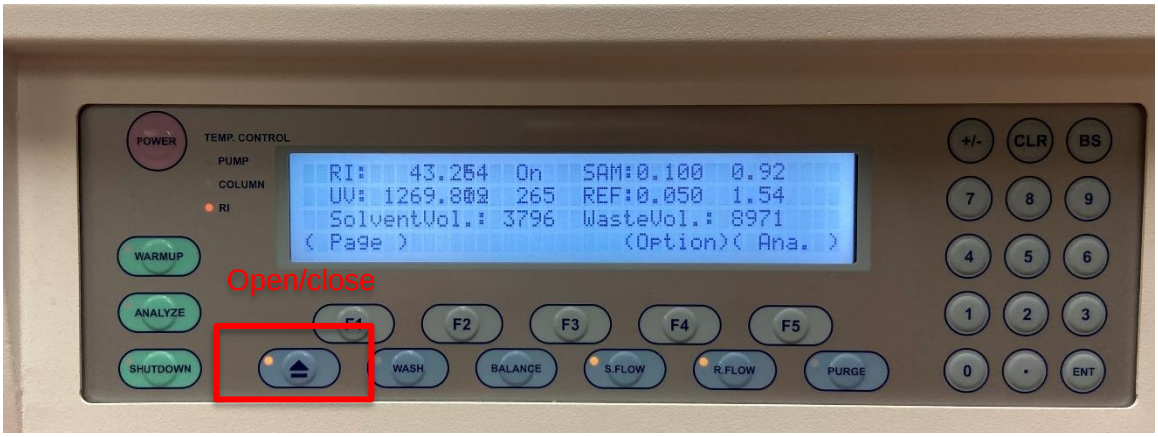


3. Click on the vial icon to pull up the *sample queue* page. Here you can type in the sample name, choose the cup (which number you will put your vial in), acquisition method (which calibration queue to use), the injection volume (typically 10 uL), type (unknown unless doing a calibration curve) and the conditions (typically there will be one optimized

one that you can choose). You can add multiple samples by adding this information to multiple rows. Next click save.



4. Put your sample in the cup by pressing the open/close button on the instrument for a few seconds to open the sample drawer. Close the sample drawer by pressing the same open/close button.

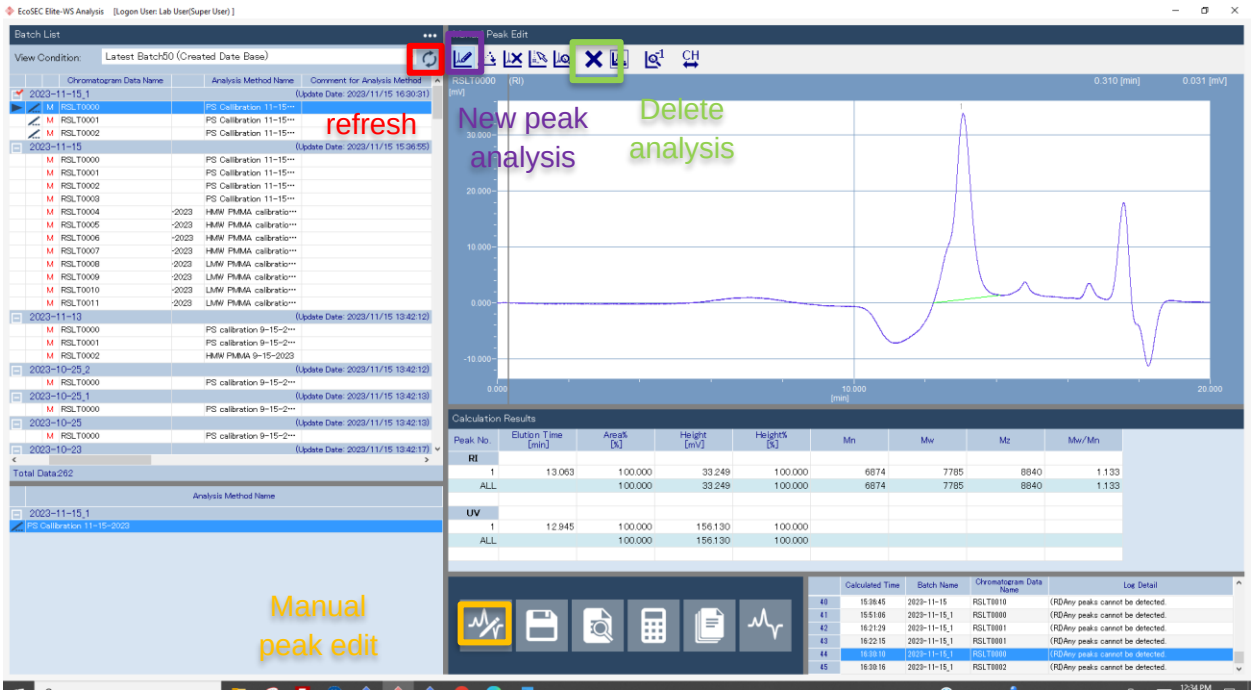


5. Press play on the main acquisition screen to start the sample run.
6. Once your sample or samples are done running, change the flow rate back to 0.1 mL/min as described in 2 (make sure to transmit data!) and move the exit tubes back to the chloroform bottle so the solvent is recirculated.

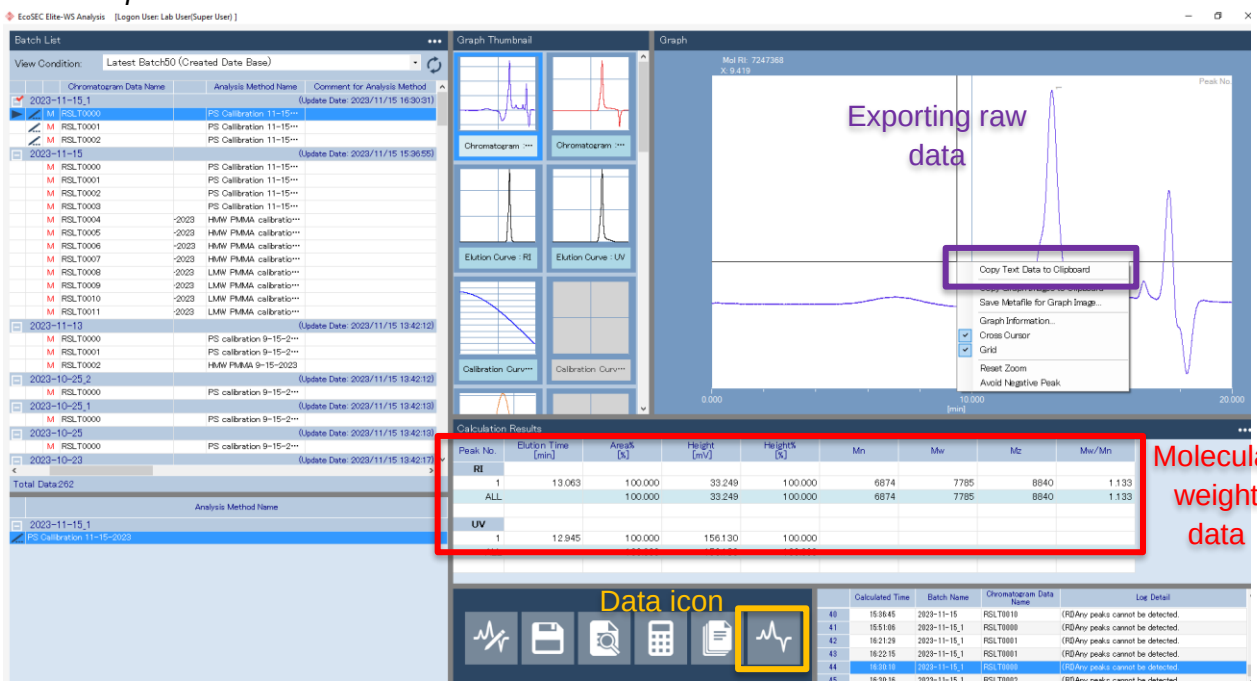
Analyzing a sample:

1. Once your sample is done running, you can analyze it on the Analysis tab (red icon). First, you will need to open the sample up by clicking on the sample name. If it does not appear, but the run is finished, you might need to refresh.
2. Click on the manual peak editing icon. Delete the analysis that the software does automatically by clicking on the X icon. (usually it is wrong). Select your new peak by

clicking on the new peak analysis icon and drawing a line between the two baselines surrounding your peak.

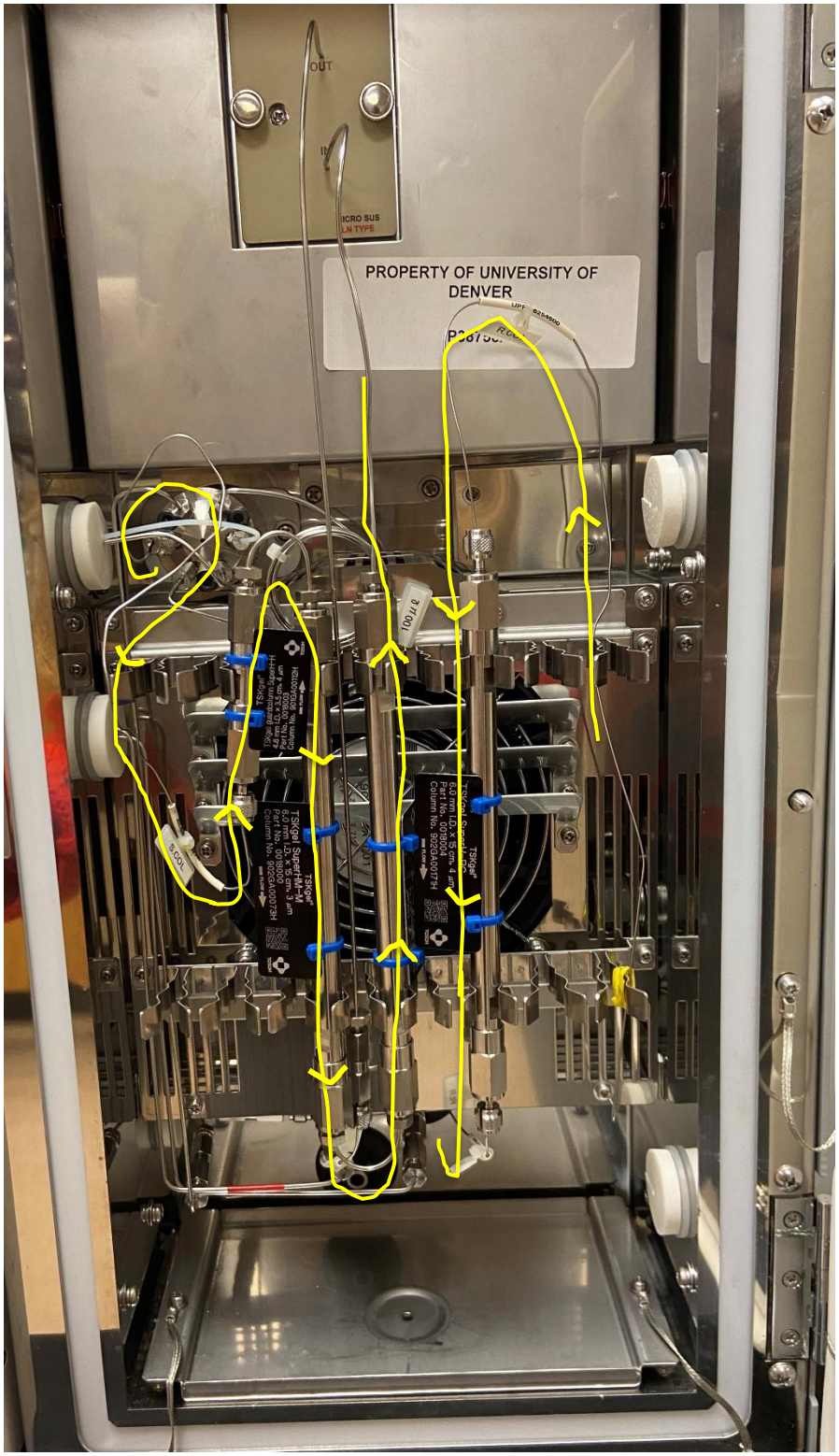


3. Export data by clicking on the data icon. Molecular weight data using the calibration curve chosen is displayed in the table and can be copied and pasted. Raw data can be copied to the clipboard by right clicking on the graph and selecting *copy text data to clipboard*.



Troubleshooting:

If the instrument is clogged (high pressure) the best plan is to reverse the column direction and dispose of the solvent coming out. An image below shows how the flow rate goes normally for both the sample right (left) and reference columns (right).



To reverse flow for the sample columns: Tube 1 needs to connect to column outlet 2. Tube 2 stays disconnected and column inlet 1 goes into a waste container. Run this for 2+ hours (likely overnight).
To reverse flow for the reference cell: Tube 3 needs to connect to column outlet 4. Tube 4 stays disconnected and column inlet 3 goes into a waste container. Again, run this for 2+ hours (likely overnight).
All of these connections are finger twists except the connection between column outlet 2 and tube 2 which needs to be tightened with 2 wrenches (one on each connection).
Note: As of November 2023, the reference column is flowing backwards due to a clog that only goes forward (unclear why).