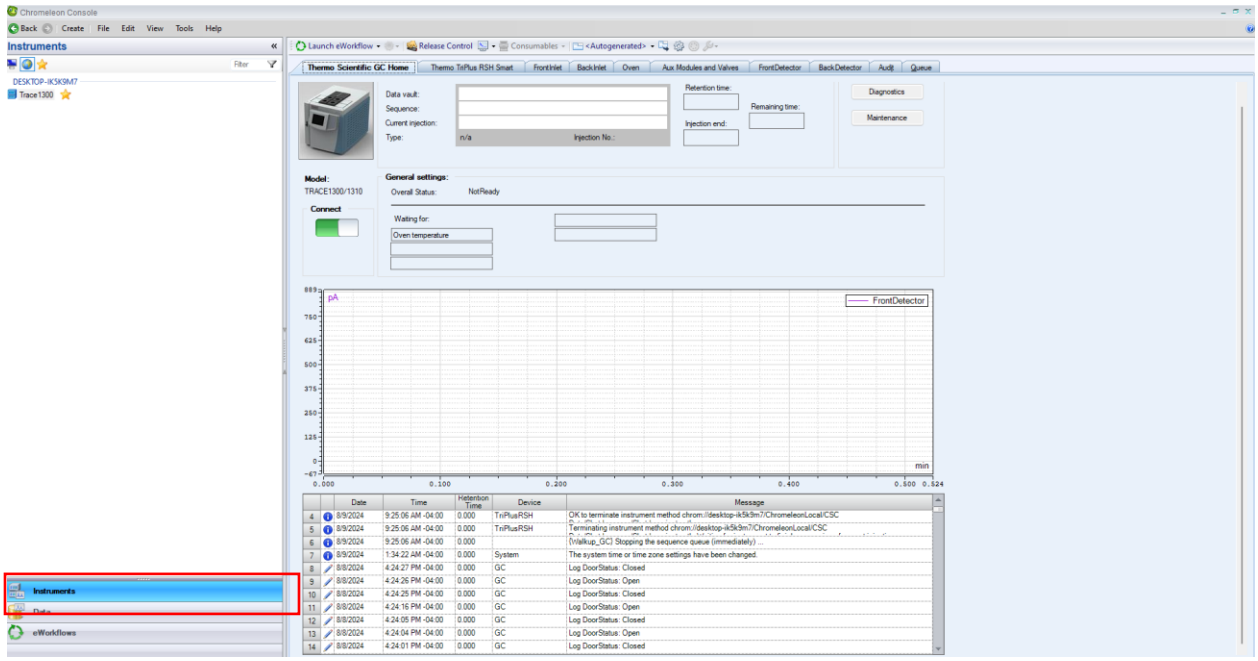
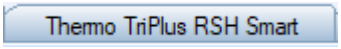
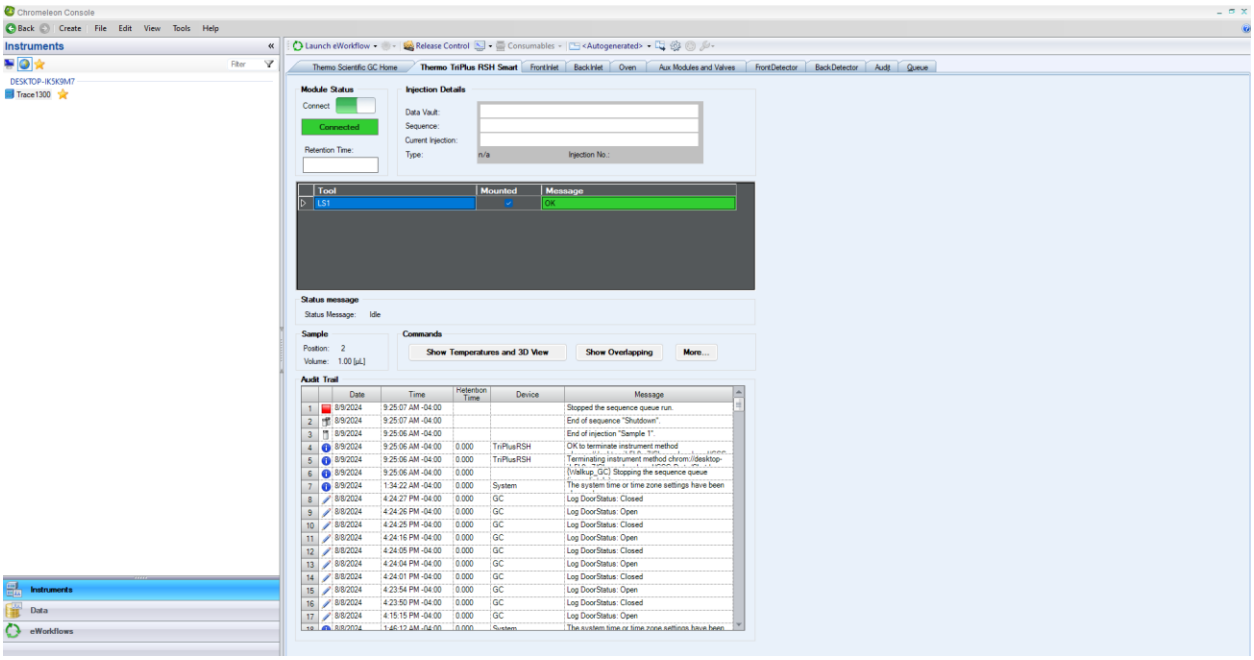
Instructions for GC Operation using front inlet and FID:

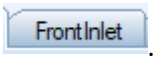
1) Log into the computer if necessary prior to the PPMS login. The username and password are:
   username: Walkup_GC
   password: Walkup_GC
2) Turn on all gas cylinders (hydrogen, to your left, helium, behind you, and air, along the right wall). Note you only need to open the valve on top of the tanks, the regulators should not be adjusted.
3) Open the Chromeleon 7 software (on desktop)
4) The screen should look something like this. If not, find the “Chromeleon Console” window and click on “Instruments” in the lower left corner of the screen. If the green “Connect” switch is red/off, try turning it on. If it does not turn green and stay green, ensure the GC is powered on using switch on the upper rear of the instrument or contact CSC staff.

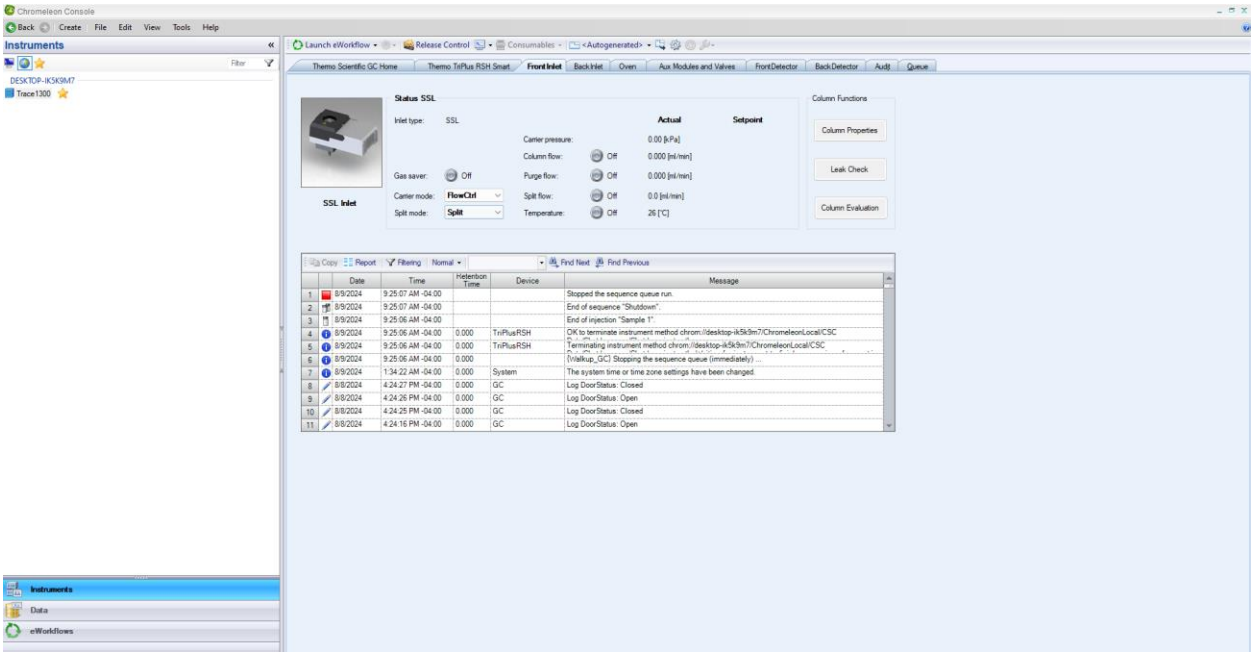


5) Click on “Thermo TriPlush RSH Smart” 

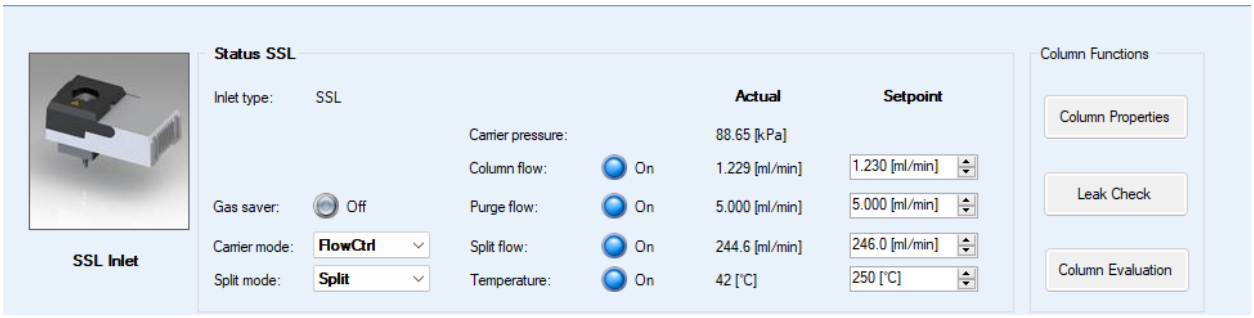
. The screen should look like this. Again, ensure the green “Connect” switch is green/on. If it is red/off, try turning it on. If it does not turn green and stay green, contact CSC staff for help.

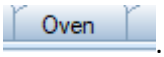


6) Click on “Front Inlet”  . The screen should look like this:

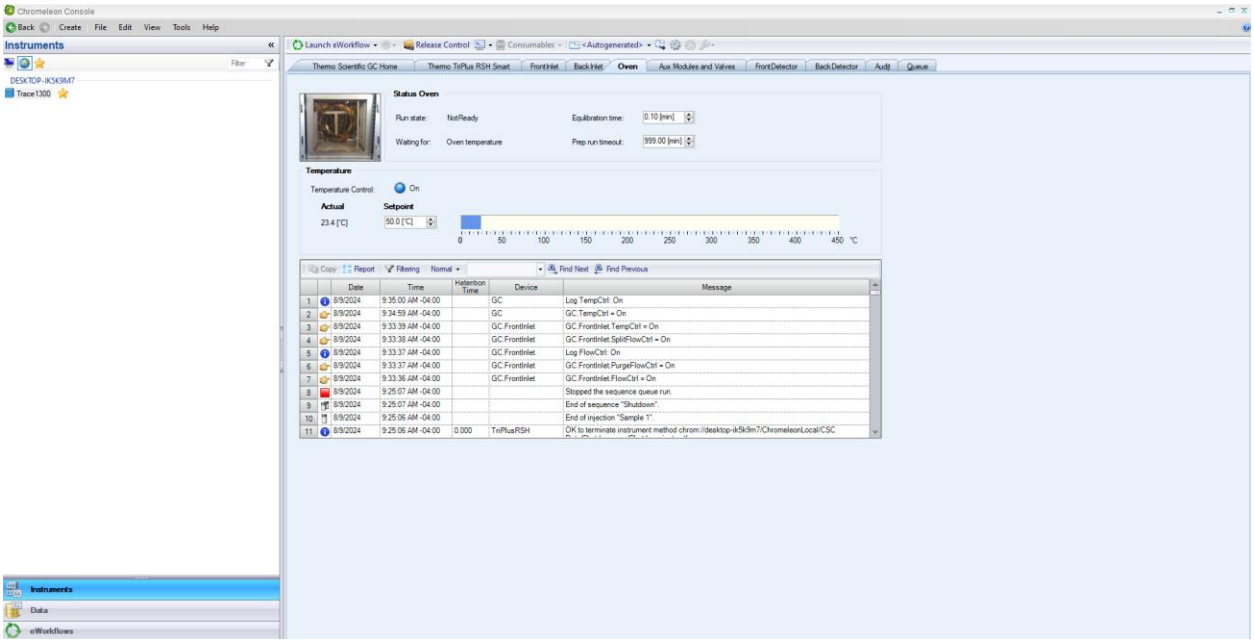


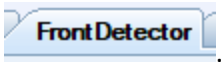
7) Turn on the column flow, purge flow, split flow, and temperature so the screen now looks like this:



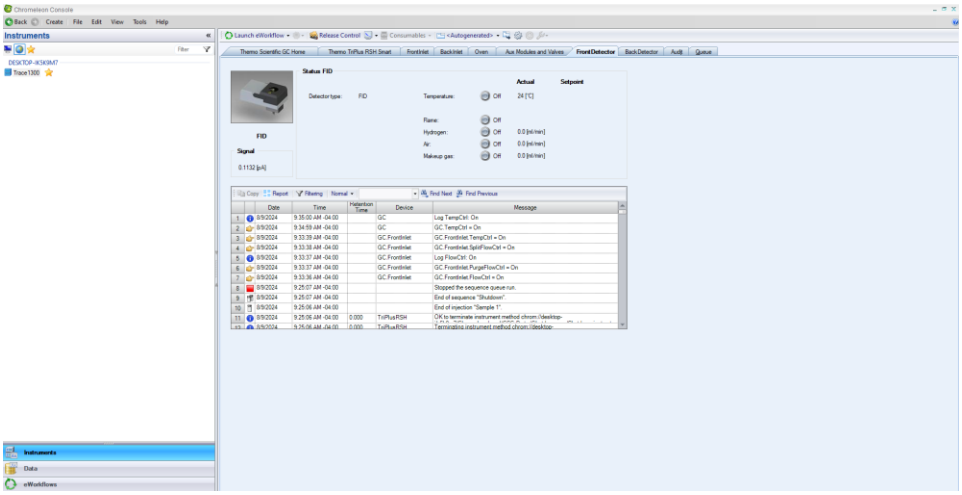
8) Click on "Oven" 

. The screen looks like this. Turn on temperature control.



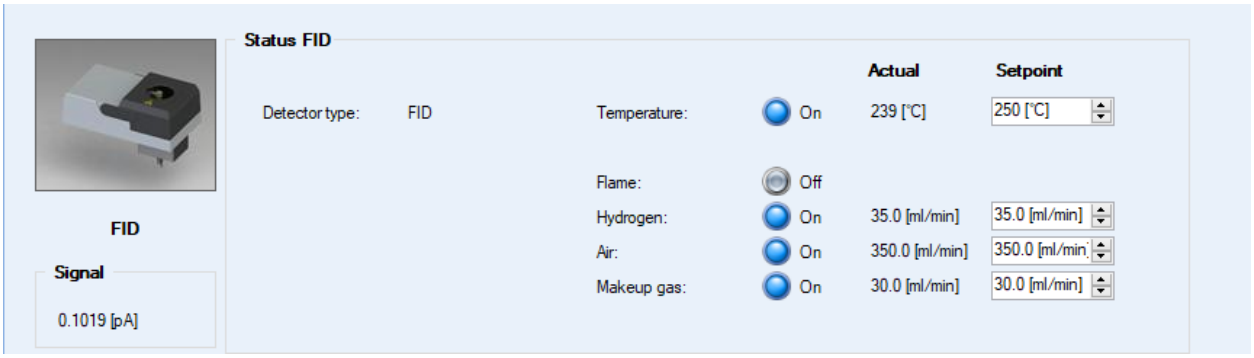
9) Click on "Front Detector" 

. The screen looks like this:

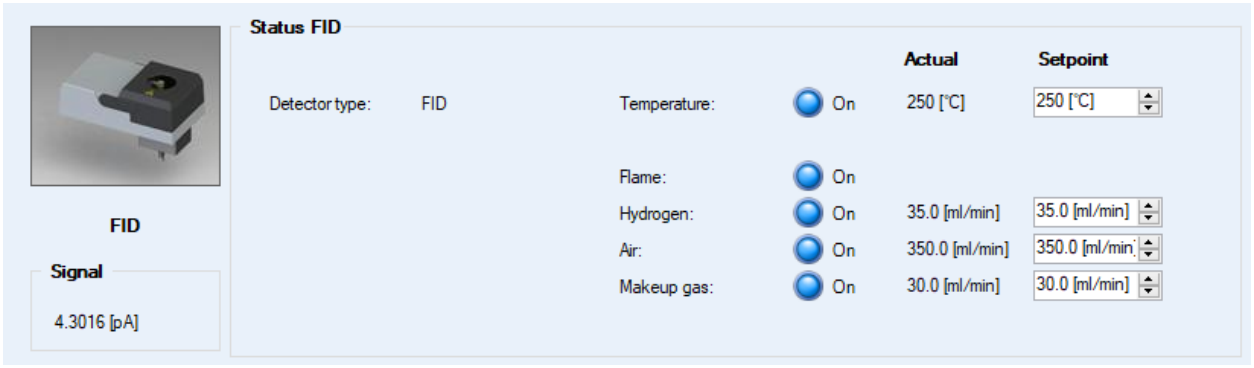


10) Turn on the Temperature, Hydrogen, Air, and Makeup gas. Do not turn on the flame yet. Allow all

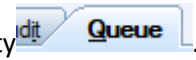
values to equilibrate to their set values. The screen will look like this:



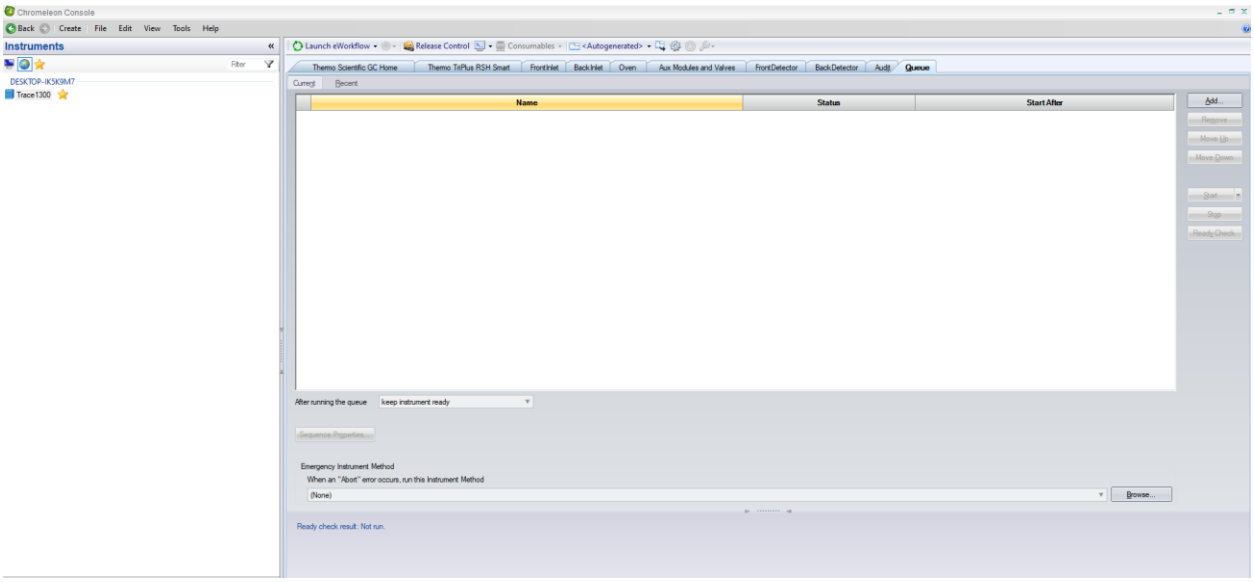
11) Once the temperature has risen above ~200 °C, you can turn on the flame. You should hear a popping sound from the instrument after a short period of time. The screen should look like this:



12) If the “Actual” values of Hydrogen, Air, and Makeup gas are 0 ml/min, an error has occurred in starting the flame. Turn off the flame and all 3 gases, wait one minute, then turn on all gases again. When they have equilibrated to their set points, turn the flame on again. If the flame continues to fail to start, contact CSC staff.

13) Check the Queue is empty 

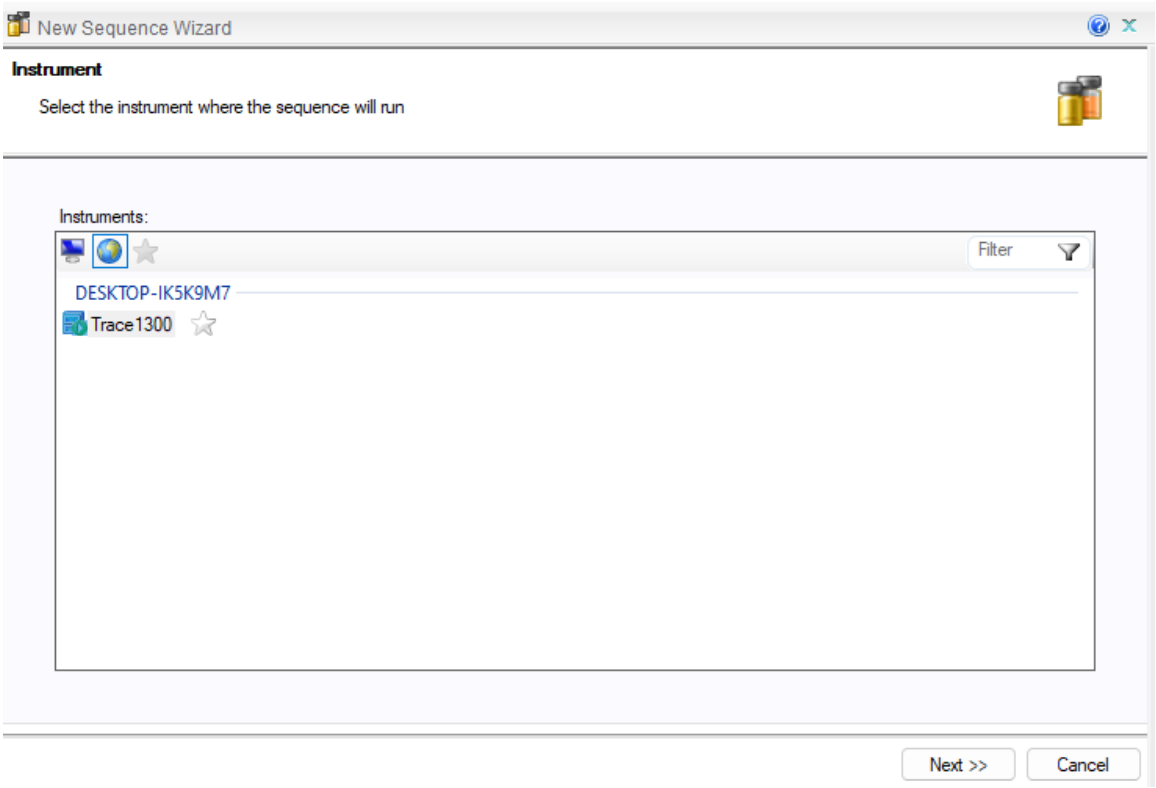
. The screen should look like this:



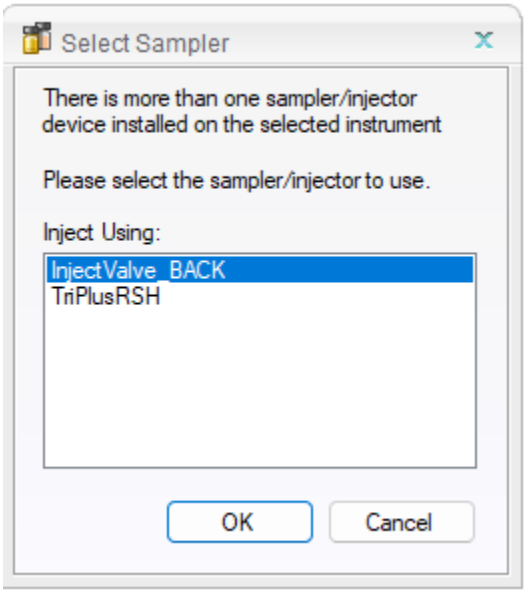
14) If anything is in the Queue, such as a shutdown method, select the line, click “Stop” on the right

side of the screen, and then remove all lines from the queue.

15) You are now ready to set up your sample(s). Click “Create” -> “Sequence”

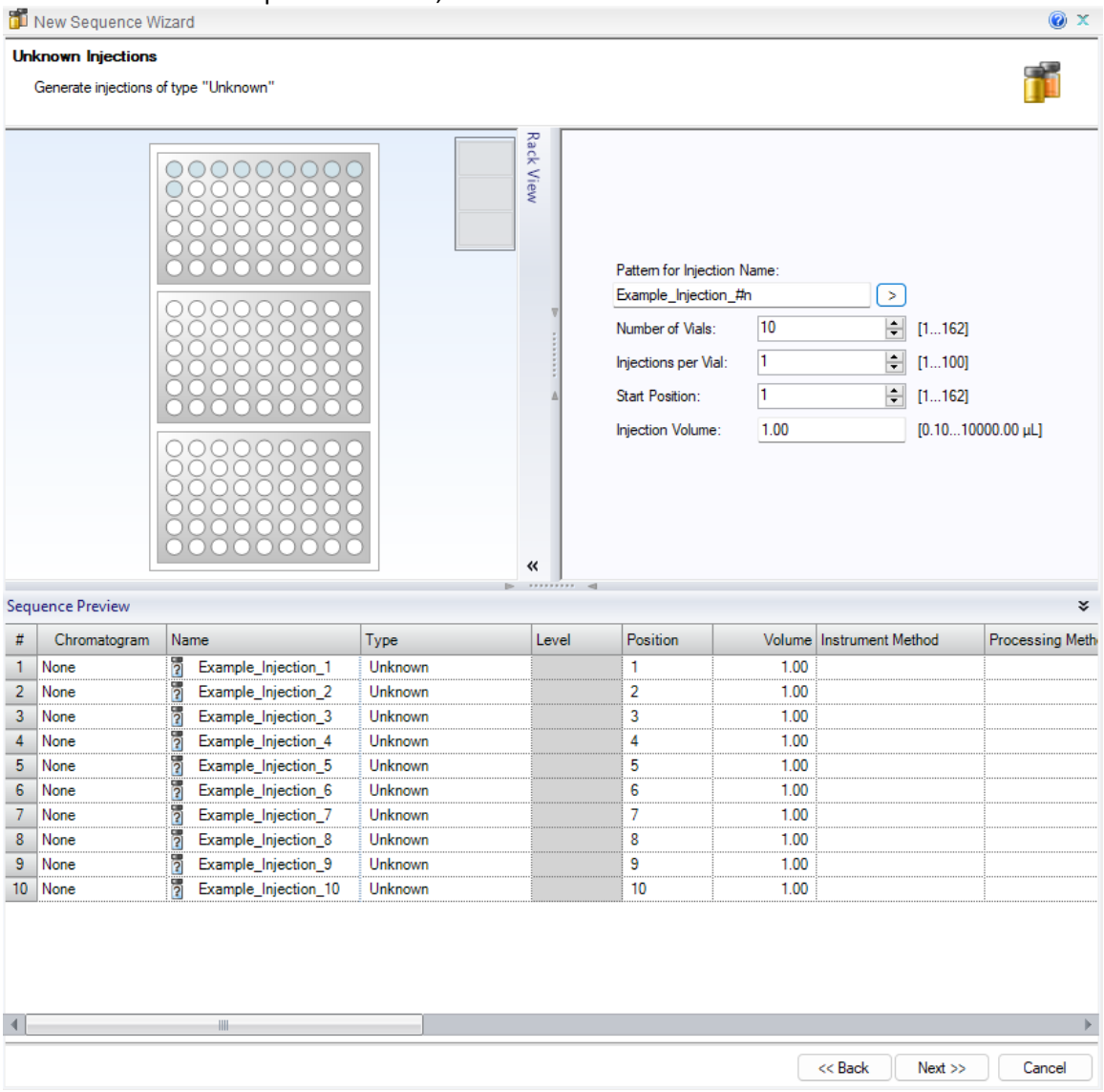


16) Click “Next >>” then “TriPlusRSH” on the window that pops up, then OK:



17) On this screen, you set up your injections. Begin by selecting the start position of your vials on the autosampler, providing a pattern for the injection name, the number of vials, and, if applicable, the number of injections per vial. In general, you should only inject 1 µl unless you are adjusting the split ratio (ask CSC staff if you need to do this). NB: Click > to access a drop-down of auto-populating characters, such as #n, which adds the injection number to the end of

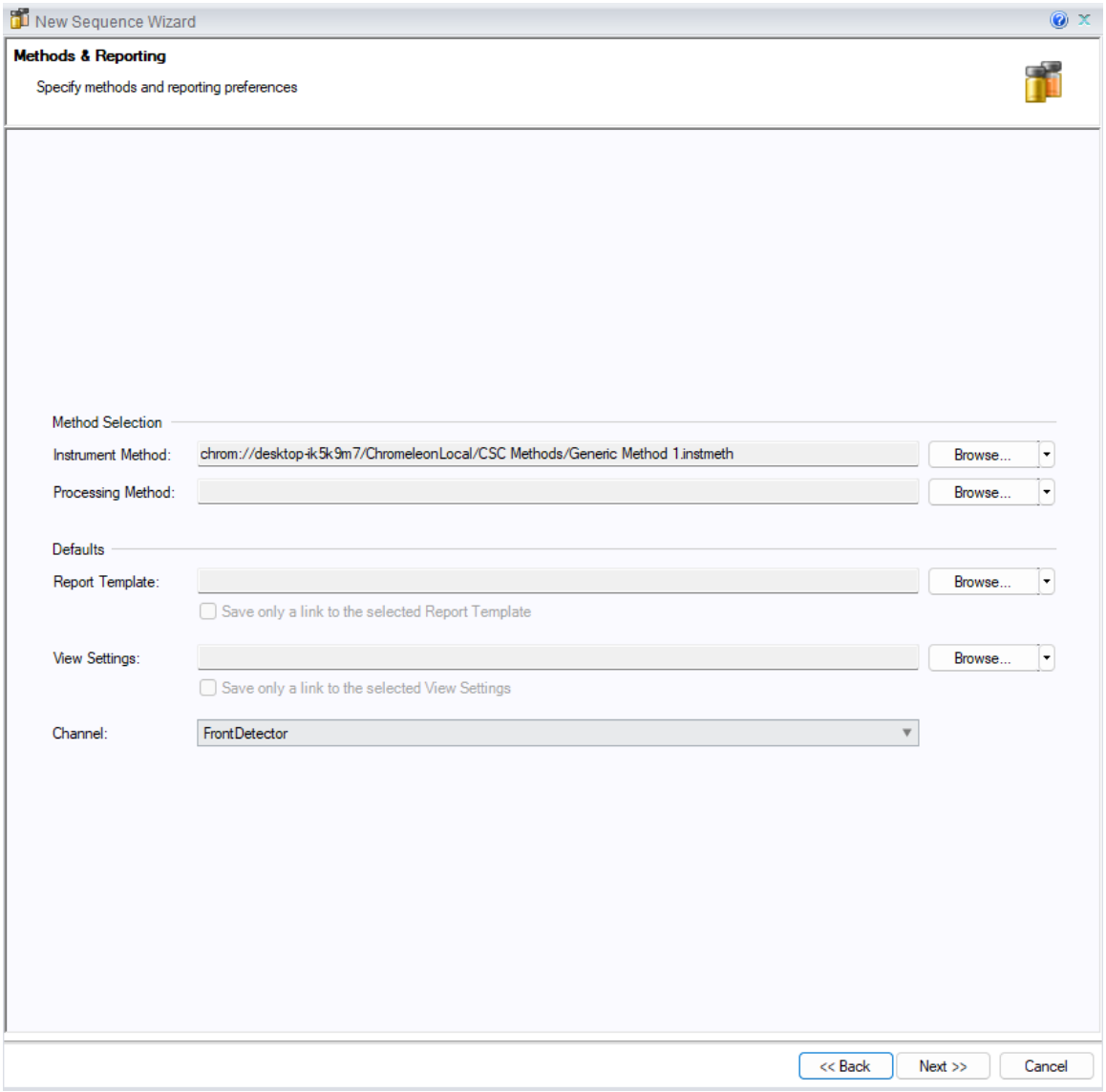
the name for each sample. Once done, click Next.



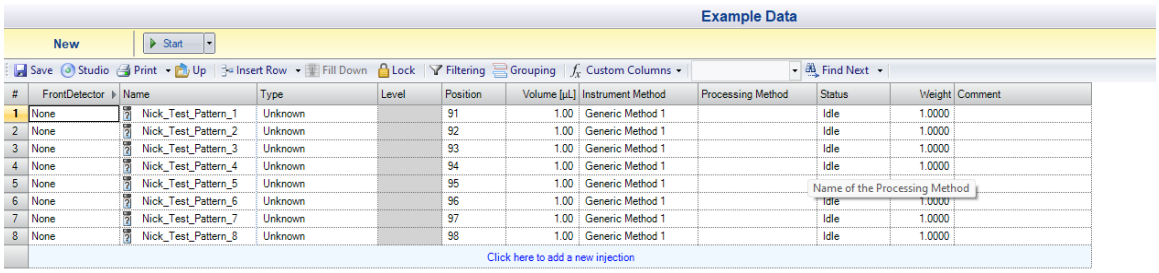
18) Select an instrument method (aka instructions for how the instrument should run the sample) and, if applicable, a processing method (aka instructions for how to work up the data).
    a) For instrument methods, I have pre-programmed several "Generic methods" as a good starting point. See the table below for more information:

Method #	Description
1	50 C for 5 minutes, then 20C/min ramp to 300C, then hold at 300C for 5 min
2	Isothermal at 100C for 30 min
3	40 C for 5 minutes, then 15C/min ramp to 100C, then hold for 5 min
4	50C for 3 minutes, then 25C/min ramp to 300, then hold at 300C for 2 min

19) If you would like to produce your own instrument method, please ask for assistance. Processing methods should be created individually using the process in Appendix 1: Processing methods and calibration curves. If you have one already created, you can apply it here.

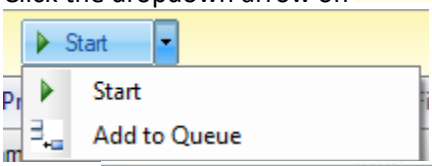


20) The next page is Chromeleon assuming the instrument has a mass spec. It does not. Click Next.
21) Add any comments you would like, such as notebook page numbers, details on what is being injected, etc. Then click Finish.
22) Double check the sample details on the page that shows up (see below). Leave the type "Unknown" for the moment. If you need to add additional samples either now or during the run, click "Click here to add a new injection" and a new line will be added. All fields in this table are editable.



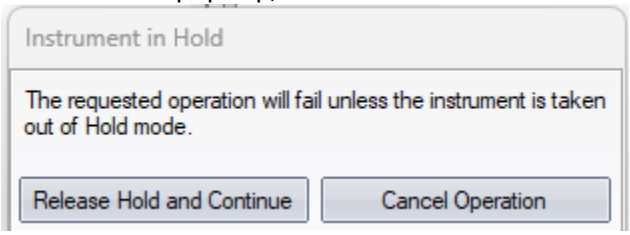
23) Click the dropdown arrow on 

and click "Add to Queue

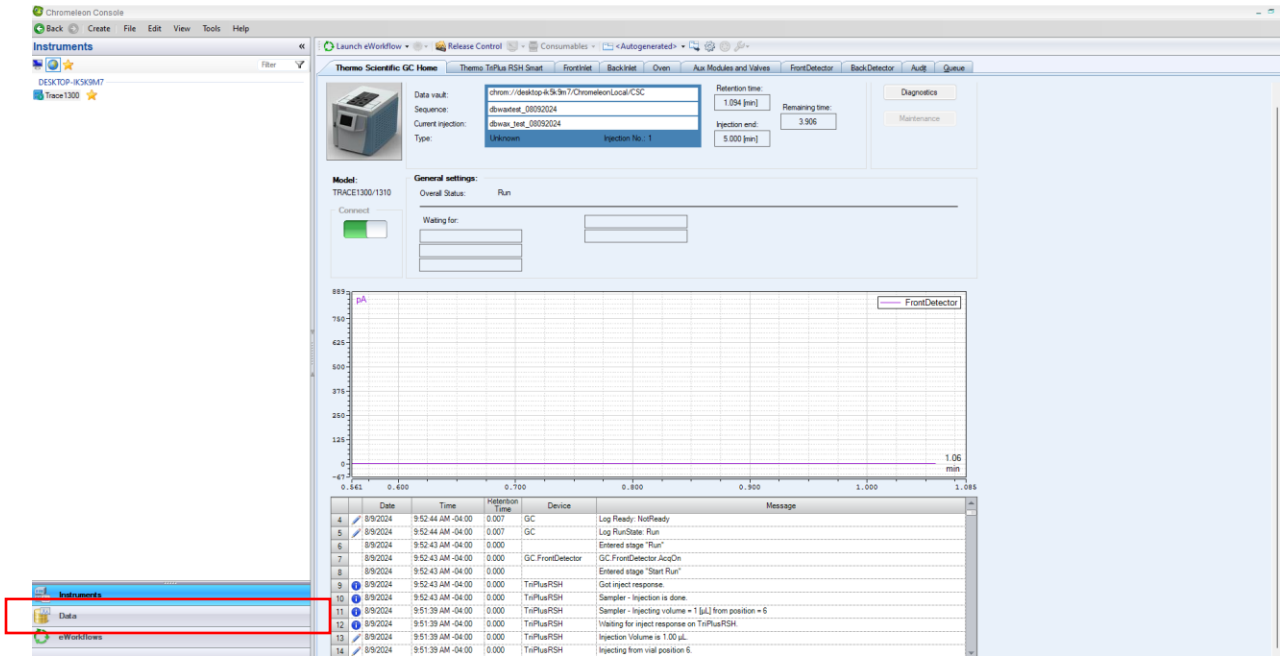


24) Click on 

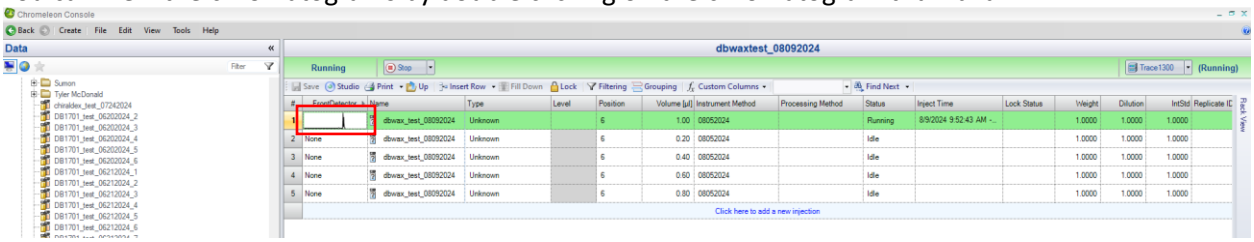
25) Click on the "Queue" tab at the top
26) If nothing is running, click "Start". If samples are already running, your samples will run when the others are finished.
27) If this window pops up, click “Release hold and continue”



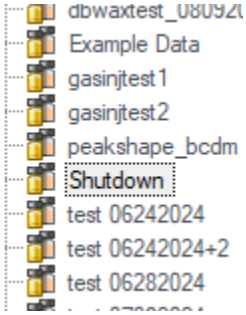
28) You can view the data live in the “Thermo Scientific GC Home” tab. When the run is finished, click “Data” in the lower left corner of the screen and navigate to your sequence.



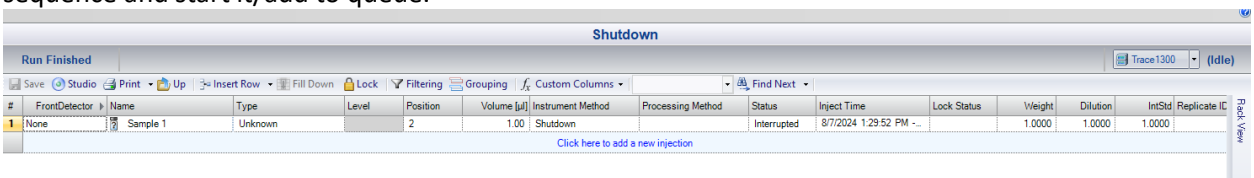
29) You can view the chromatograms by double clicking on the chromatogram thumbnail



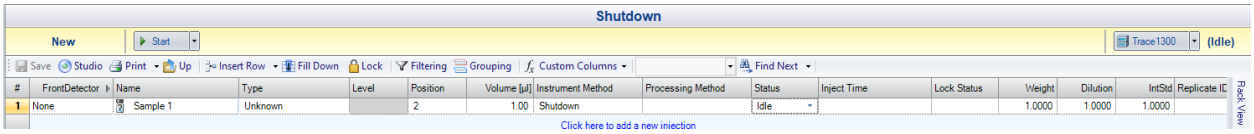
30) To shutdown the instrument, find the sequence called “Shutdown” in the data window



31) Double click to open. If the screen looks like this, change the “Status” to “Idle”, then save the sequence and start it/add to queue.



32) When the screen looks like this, it is ready to add to queue. Run this sequence, then turn off all gas cylinders for the instrument (hydrogen, helium, and air) and log out of the computer. If any warnings come up when you run the shutdown sequence, simply click ignore and continue.



Congratulations, you've run your GC samples! See the appendix for more information about data processing and building calibration curves.

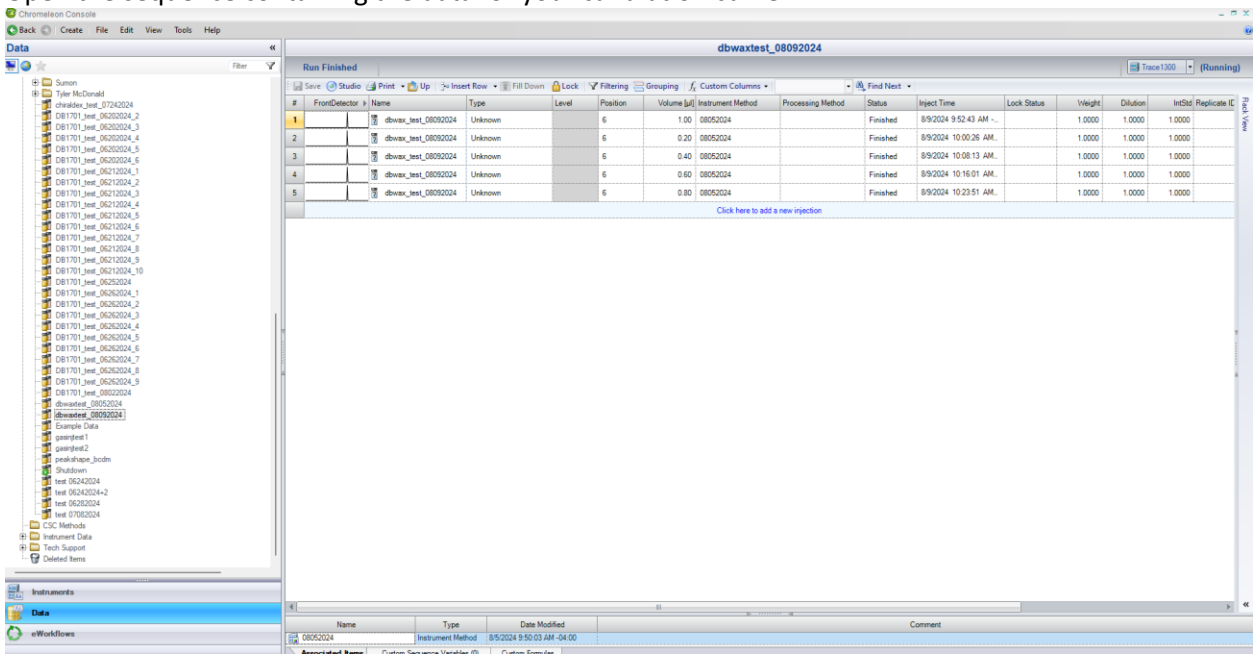
Building a processing method and calibration curve:

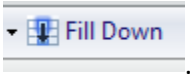
Prior to building a calibration curve, you must consider how you would like to build the curve. There are two main strategies for how to physically acquire the data for a calibration curve: 1) inject the same sample multiple times, but inject different volumes each time; or 2) inject the same amount of different solutions, each containing a different amount of your analyte. There are benefits and drawbacks to each:

Benefits and Drawbacks to Methods of Building Calibration Curves	
Injecting one solution at different amounts	
Benefits	*Drawbacks*
Faster standard preparation – only need one	Susceptible to human error: mess up one sample, the entire curve must be thrown out
Random error based on concentration of single sample and automated injector (easier to control)	Autosampler may or may not be designed to inject all volumes you want (GC syringe is 10 µl, may struggle to inject <0.5 µl reliably)
Can easily rerun standard with every sequence if needed	Overlapping peaks may be harder to "calibrate around" with a nonzero intercept. For example, if the tail of your peak overlaps with solvent, using this method, the solvent peak will get bigger with the analyte peak. If you make multiple samples, the analyte peak will get larger while the solvent peak stays the same size.
Random error in injection volume can be easily measured by repeated injections	Sometimes conceptually harder to understand – your calibration curve isn't "concentration", it's "amount of analyte detected" on the x-axis.
Injecting multiple solutions at the same amount	
Conceptually easier to build	Takes more human preparation time
Random error in standard preparation arises from differences between samples, injection volume can be measured by repeated injections	Random error gets multiplied by additional human-based measurements
Easier to build calibration curve if you have overlapping peaks	May take longer to run your curve if you want to run it every time
Autosampler is designed to inject exact volume you require	Uses additional vials, solvent, materials, etc.
Less susceptible to human error, depending on how you prepare the samples	

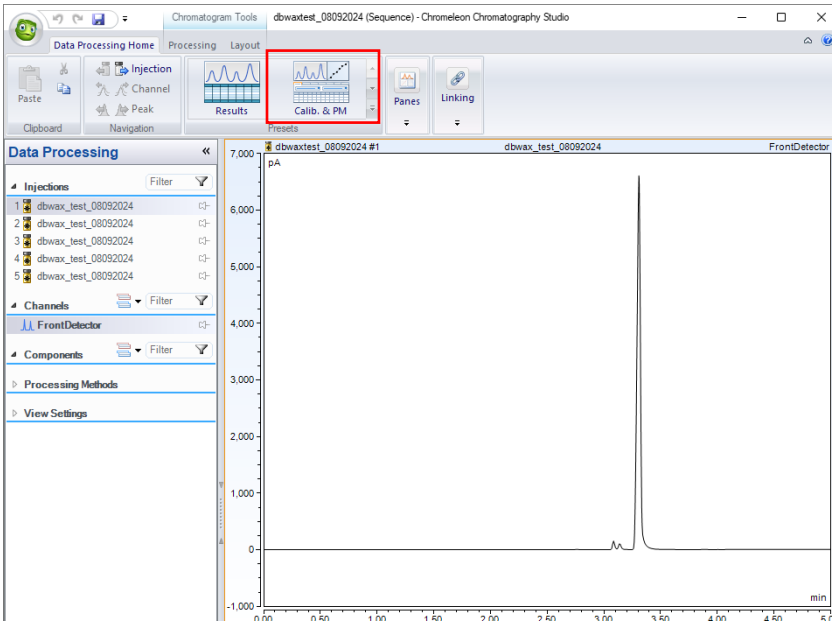
Here, I'll describe how to build the calibration curve using the first method, injecting different amounts of the same solution multiple times. At the end, I'll briefly describe how to change things in the software to accommodate the second method.

1) Open the sequence containing the data for your calibration curve:

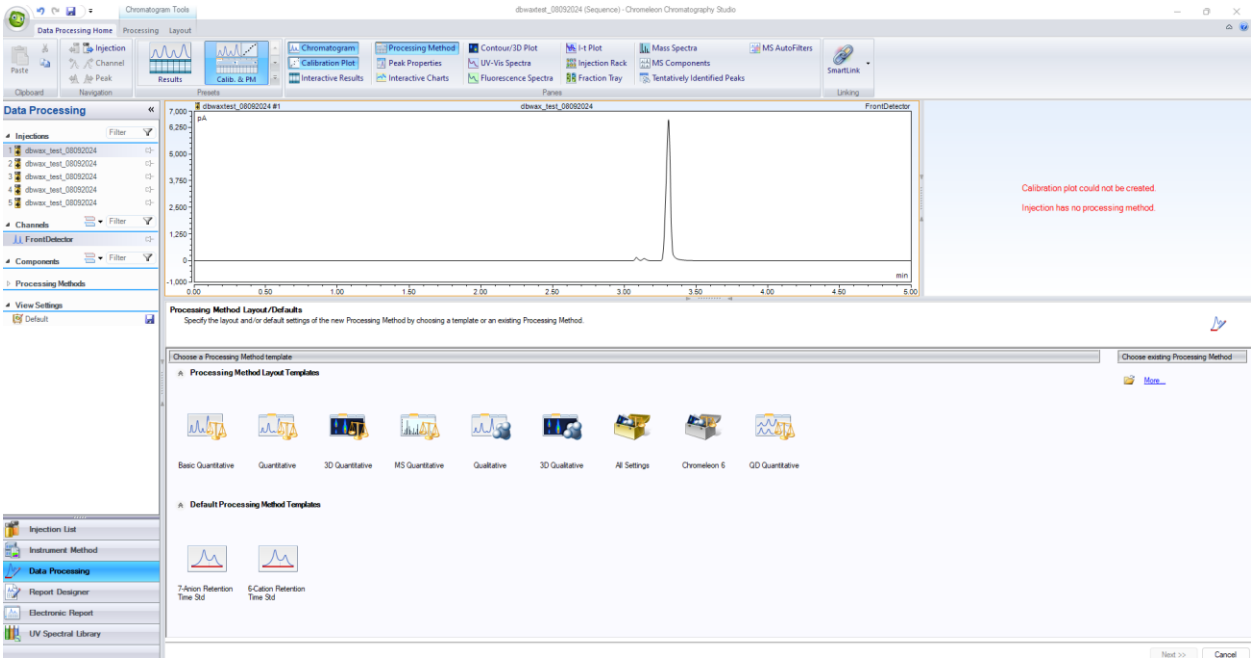


2) Change the "type" of the first injection to "Calibration standard" and click "Fill Down" 

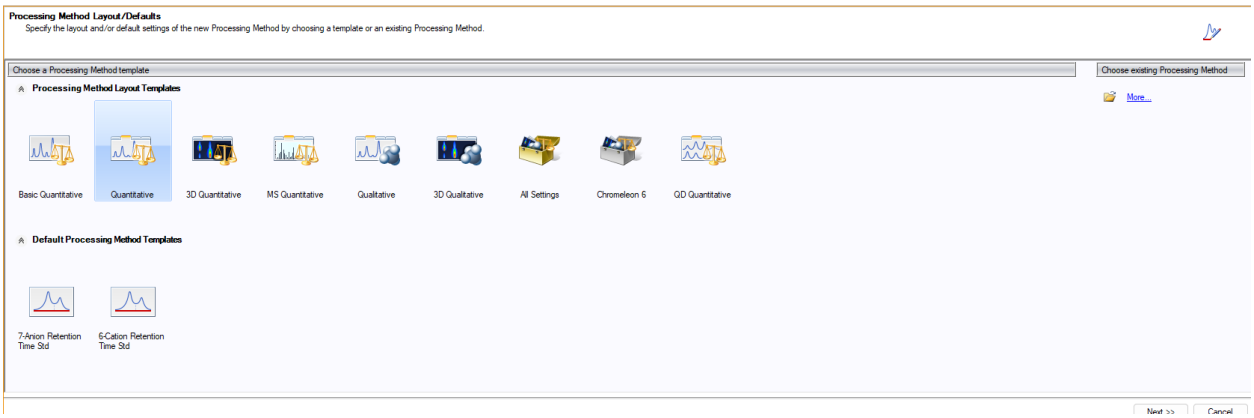
. Then, set the Level of all injections to "1" for all injections of a solution of the same concentration. If every injection is a solution of a different concentration, you will need to set every line to a different level.
3) Open the first injection by double-clicking on the chromatogram thumbnail
4) In the window that opens, find the "Presets" section of the ribbon and click "Calib. & PM". If it isn't visible, click the dropdown arrow on the Presets box.



5) The window now looks like this:

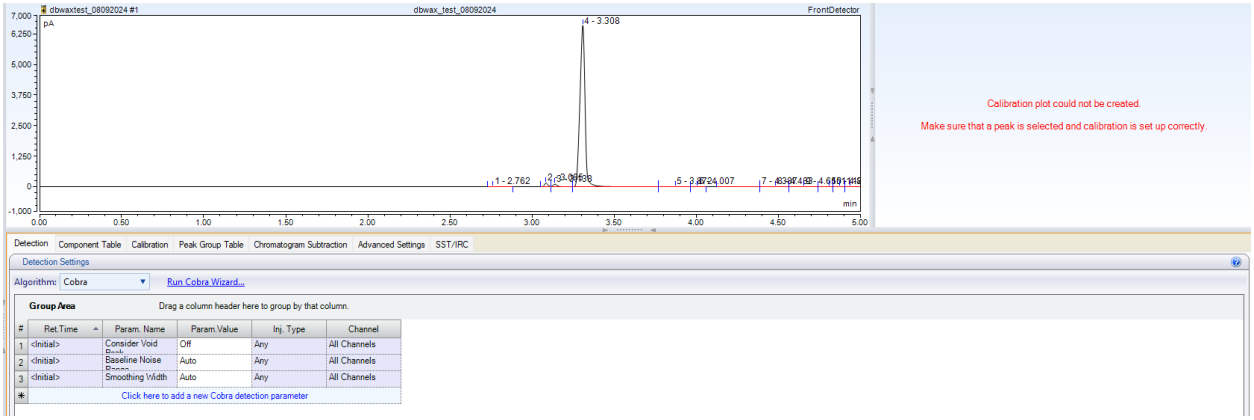


6) You will want to build a “Quantitative” processing method, so click on that. NB: “Basic Quantitative” is good if you just want integrations, but makes it difficult if not impossible to build a calibration cuve. I find it has limited utility. Select Quantitative, then click next.

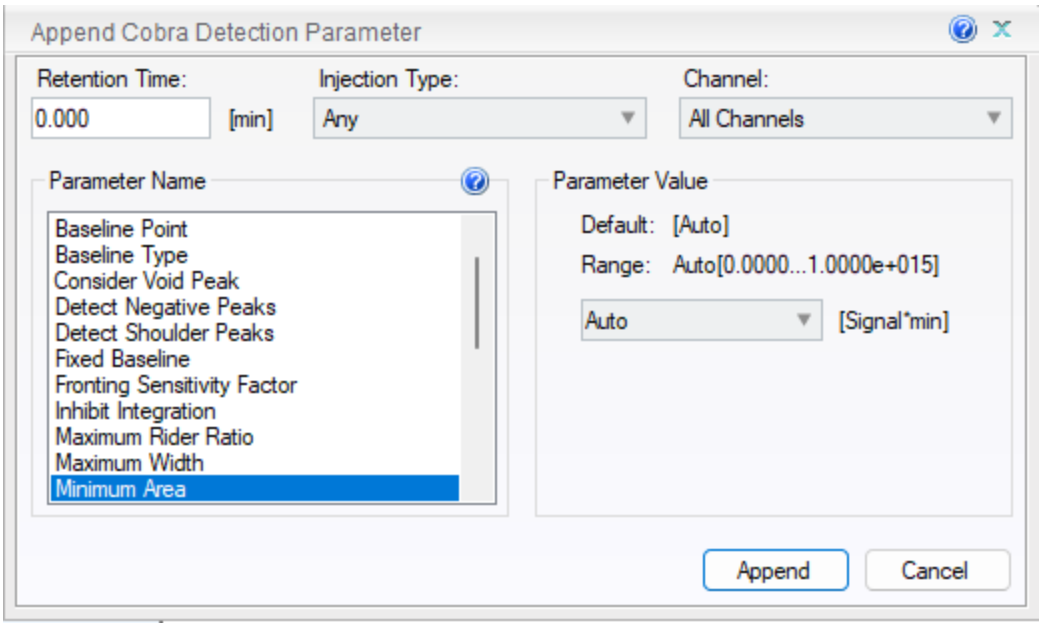


7) Provide a name for the method, and assign the new processing method to all injections of the sequence:

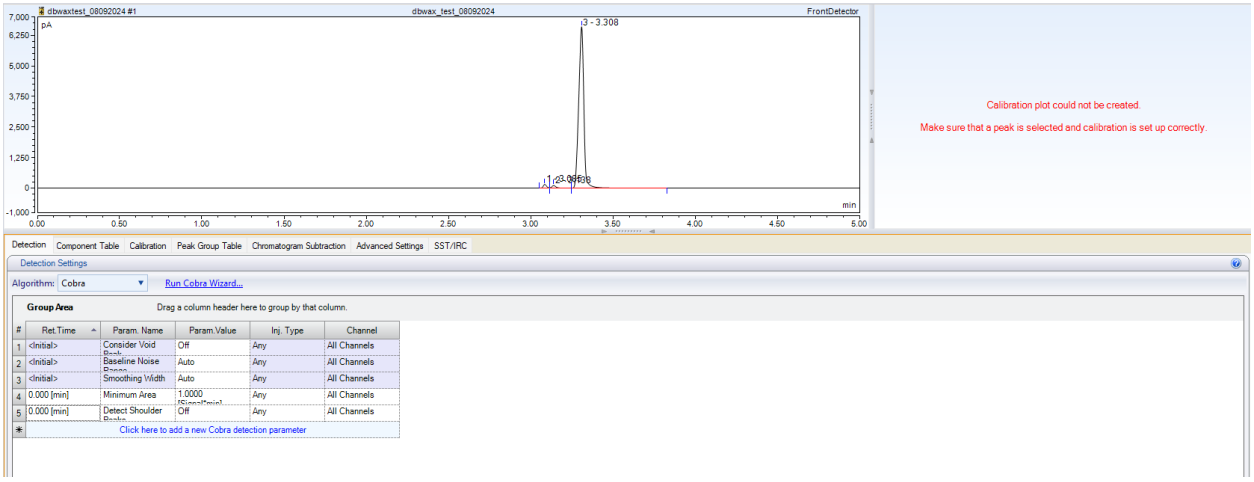
8) The software will automatically integrate the chromatogram using its default detection algorithm (Cobra) that is quite terrible, although for this chromatogram it doesn't look too bad. Go to the "Detection" tab:



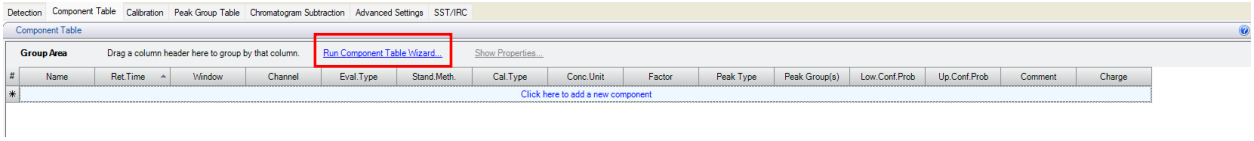
9) Click on "Click here to add a new Cobra detection parameter"



10) This window will allow you to add minimum areas for peak detection, set windows for integration, etc. I highly recommend you add at least one parameter where you go to "Detect Shoulder Peaks" and select "off", then click "append". Other than that, apply whatever integration settings you feel are appropriate for your sample. My chromatogram and detection parameters look like this:

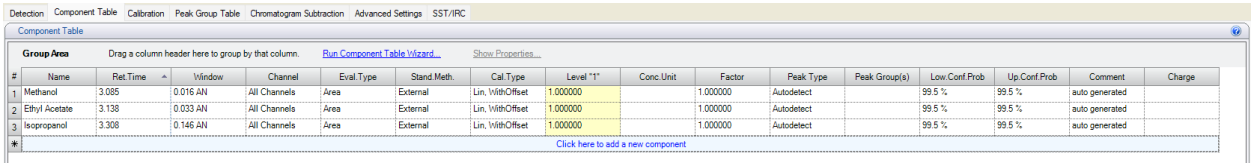


11) Go now to "Component table" and click on "Run component table wizard":

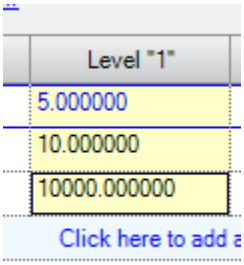


12) The wizard will walk you through picking which peaks are important, aka the "Components" of your sample you wish to quantify. It is okay to have too many components. For example, if the software picks the solvent peak as a component, that will not cause a problem, we will just not build a calibration curve for it. Even if you just use the defaults, this will do a fairly good job.
13) On the "Review" window, you can name all of the components, then click Finish:

14) Your window now looks like this:

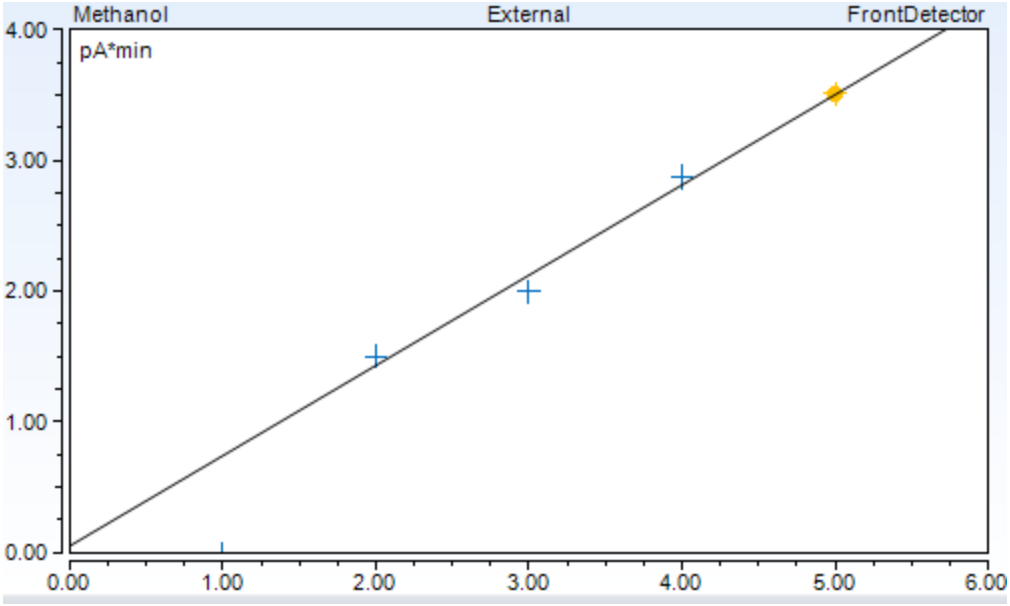


15) In the “Level “1”” Column, put the <u>amount of each analyte in that injection</u>. If you’ve injected 1 µl, you can assign this as the concentration. If you’ve injected less, put the amount in mmols (or µmols) that was injected. The software will adjust the calibration curve for the different volumes of solvent that were injected. I’ve added arbitrary numbers to mine:

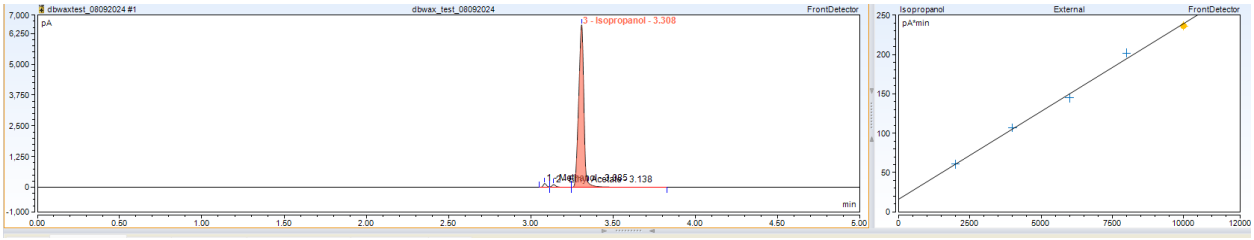


NB: If you’re using the second method and injecting different solutions of different concentrations, there will be a column for every level. Put in the concentration of each analyte at each “Level” into the table. This will build the calibration curve from those points.

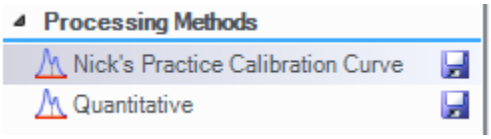
16) The upper left corner should now have a calibration curve for your first component:



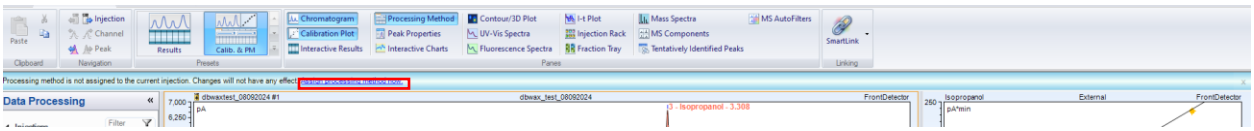
17) To view calibration curves for other components, click on the peak corresponding to each component on the chromatogram at the top of the screen



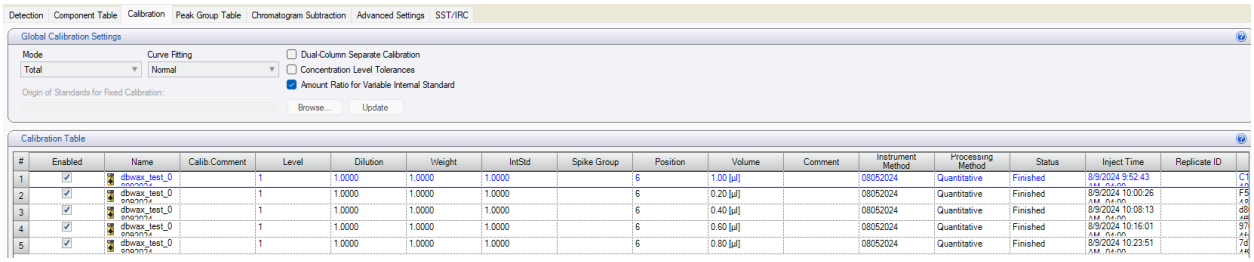
18) Remember to save your processing method by clicking the  in the upper left corner of the screen.
19) Your calibration curve is now built. In order to apply it, open any sequence of data you have. If you added the processing method when setting up the sequence, skip to step 25. Otherwise:
20) Click on  in the upper left corner of the screen, then Add -> Processing method
21) Navigate to the sequence that contains your calibration curve processing method, open it, select the method, and click Add
22) Along the left side of the screen, in the “Data Processing” pane, find “Processing methods”, where that new processing method has been added:



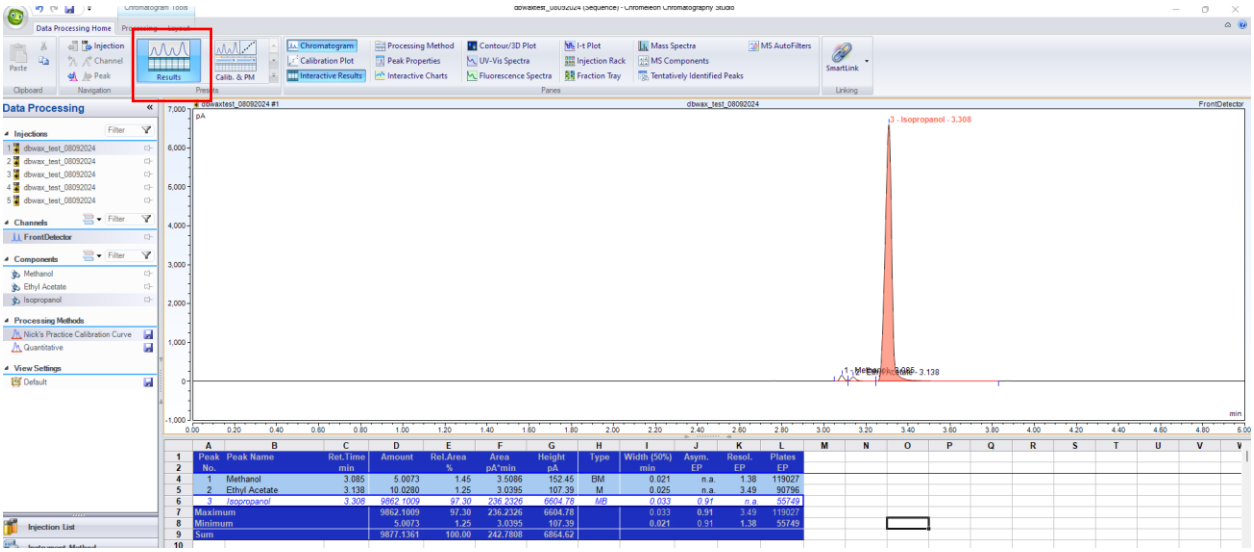
23) Click on the processing method (here, “Quantitative” is the new one I loaded for example purposes)
24) A blue banner will appear at the top of the screen. Click “Assign processing method now” and assign it to all injections of the sequence you wish to quantify.



25) Go to the “Calibration” Tab. Where it says “Mode”, select “Fixed”



26) In “Origin of Standards for Fixed Calibration”, Click browse and select the sequence that contains your calibration curve standards.
27) The calibration curve you created is now fully applied to the sequence of data. You can go back up to the Ribbon and in the “Presets” box select “Results”

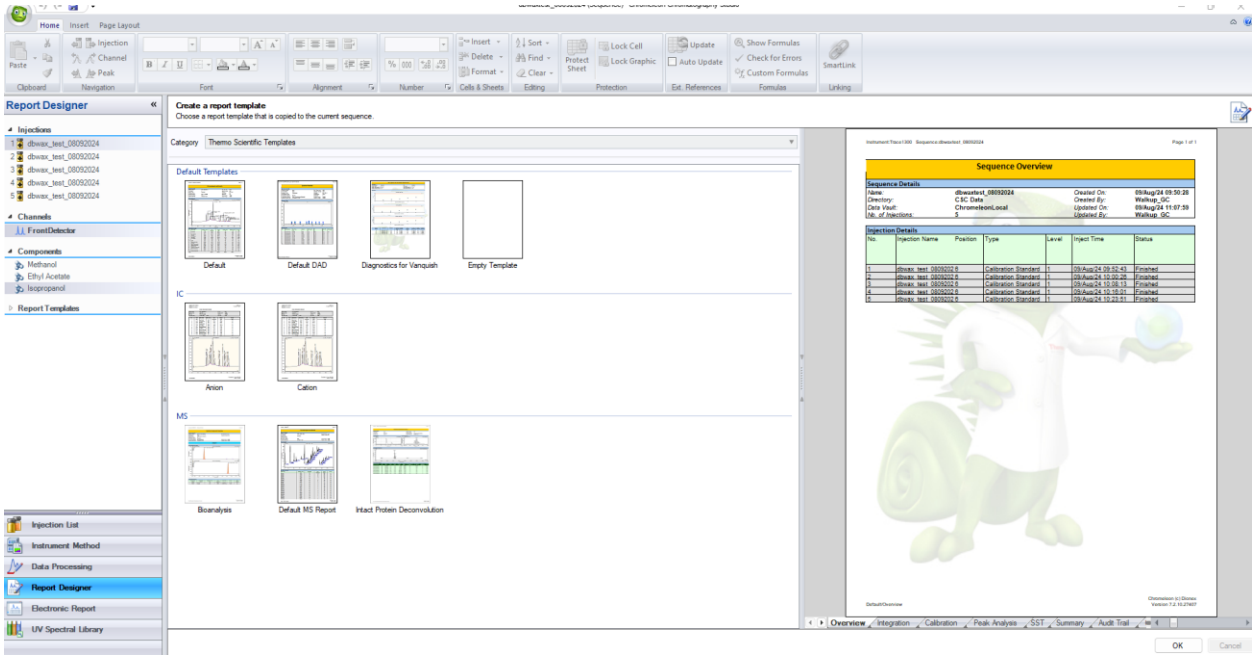


28) The table at the bottom of the screen provides all found components, the peak names, their retention times, and the “Amount” is the read-off from the calibration curve. If you were able to put in concentrations into the level column(s) in the component table, these amounts will be concentrations. If you put in mmols, these values will have those units.

How to report/export data:

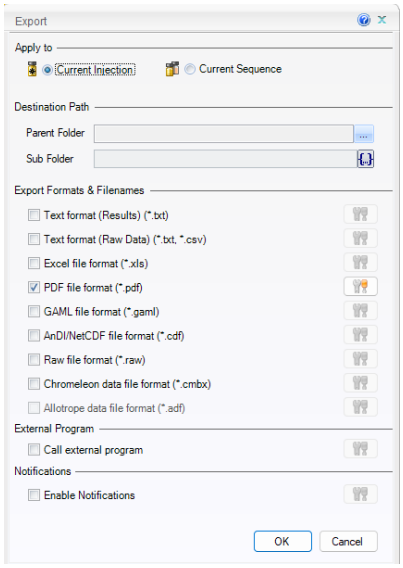
After building and applying a processing method and calibration curve, you might find yourself wanting to create a PDF report of the data for your electronic lab notebook or you might just want the data exported so you can work with it on your own computer.

1) In the Chromeleon Studio window (Accessed by double-clicking the chromatogram thumbnail in an injection sequence, or the window open when you built a processing method), select “Report Designer”:



2) The default report is probably adequate for most users. As staff if you want to change what is in the report. Select your desired report, then click OK.

3) You will now get a preview of the report. Click on  , then “Export” to get this screen:



4) You can export a report for either a single injection or for the entire sequence. The PDF file will provide a nice PDF that contains all of the information about the samples, the integrations, the calibration curves used, and amounts of material found in each sample. The excel file format exports almost exactly what was on your screen, a spreadsheet with tabs and all of the data I just mentioned. Possibly the most useful option is the ".cdf" file format. This chromatography data file is fairly universal and can be opened by most chromatography software. Notably, it can also be opened by MNova to integrate, process data, and generate chromatogram figures on your own computer.