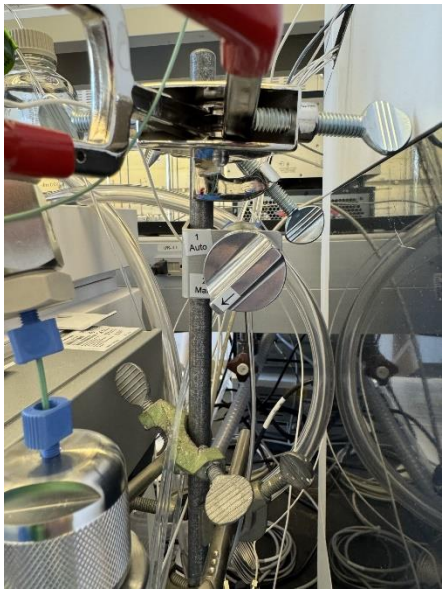
Agilent 1260 Prep LC User Guide

As an introduction, there are a few items to go over for prep LC. Prep LC is a great way to purify organic molecules that you struggle to purify by normal phase chromatography. The system in the CSC is currently equipped with only one type of column: a 250mm C18 column. The system consists of a pump (capable of delivering up to 50 ml/min, but in practice you should never use flow rates higher than ~25ml/min on the equipped prep column), a combined autosampler/fraction collector, diode array detector, two columns (a 20 mm diameter prep column and 4.6 mm diameter analytical column), and an analytical/prep switching valve. An additional valve has been added to facilitate switching between using the autosampler and using a manual injection valve.

Prior to use, you should prepare your sample. For ideal chromatography, your sample will be dissolved in whatever your starting mobile phase is. As an example, if your gradient starts at 95% water/5% acetonitrile, you should have your sample dissolved in a 95/5 mixture of water and acetonitrile. The closer you can get to this as your solvent, the better. Lipophilic samples that are completely insoluble in any water/acetonitrile mixtures and require pure acetonitrile should be approached with extreme caution and may require special handling or considerations. Alteratively, consider using the preparatory SFC when it becomes available.

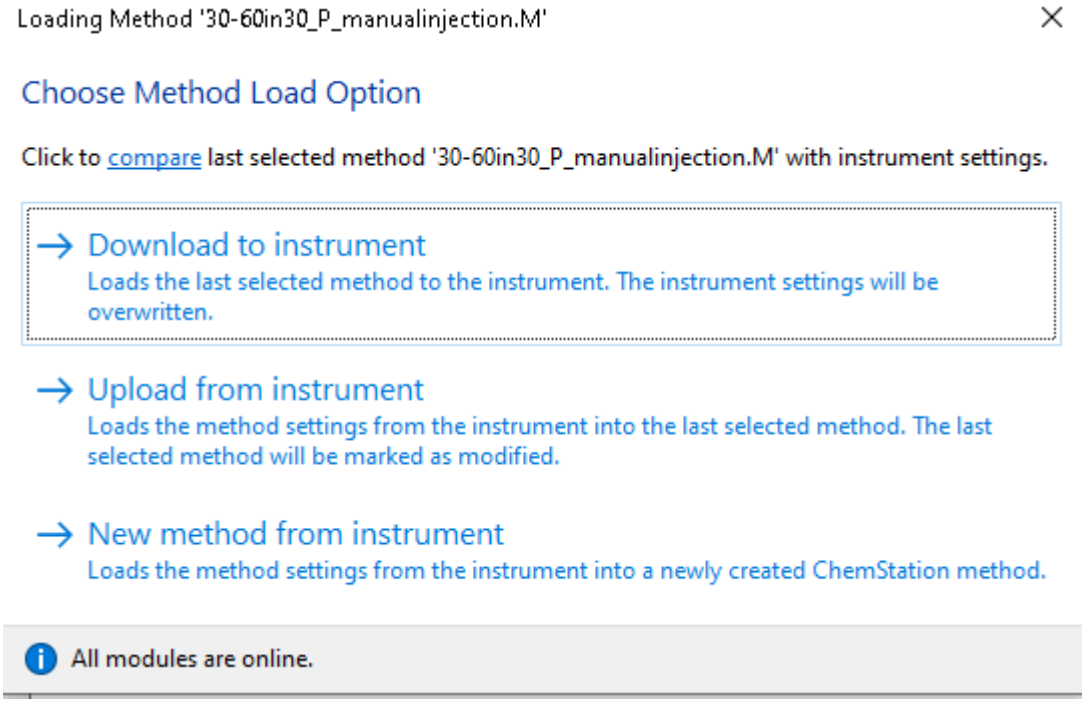
Once the sample is ready, you must first make a crucial decision, whether to use the autosampler or the manual injection valve. Turn the valve to make your selection *prior to turning on the pump!* The manual injection valve will provide slightly higher recoveries, especially if you are unable to use high recovery vials for the autosampler.



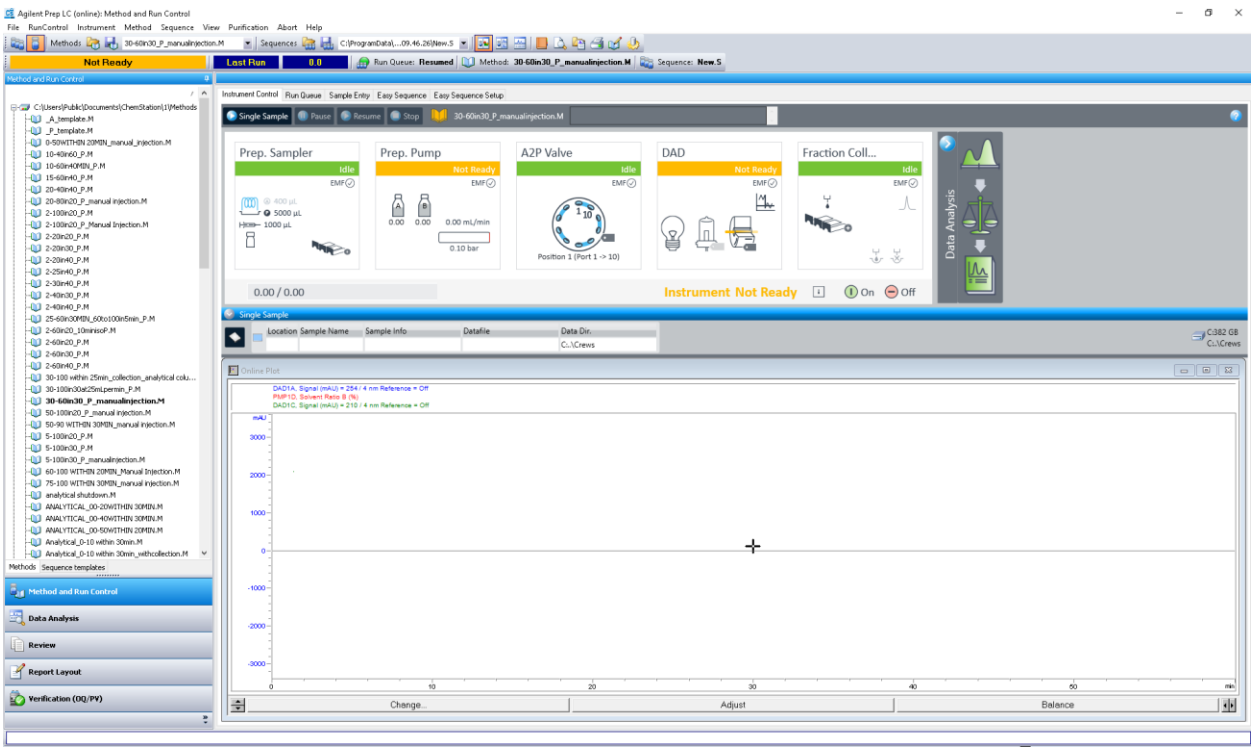
Once you have the autosampler vs. manual injection valve set, you can move on to preparing to run your sample with these instructions:

1) Log into the computer. The computer login has the username Yale and the password is yale
2) Log into PPMS with your netID and password
3) Open “Agilent Prep LC (online)” from the desktop. Note that the offline version is only for data processing

4) During startup, you'll receive the below prompt asking whether you want to use the method loaded on the instrument or the one on the computer. Select "Download to instrument" for the simplest startup.



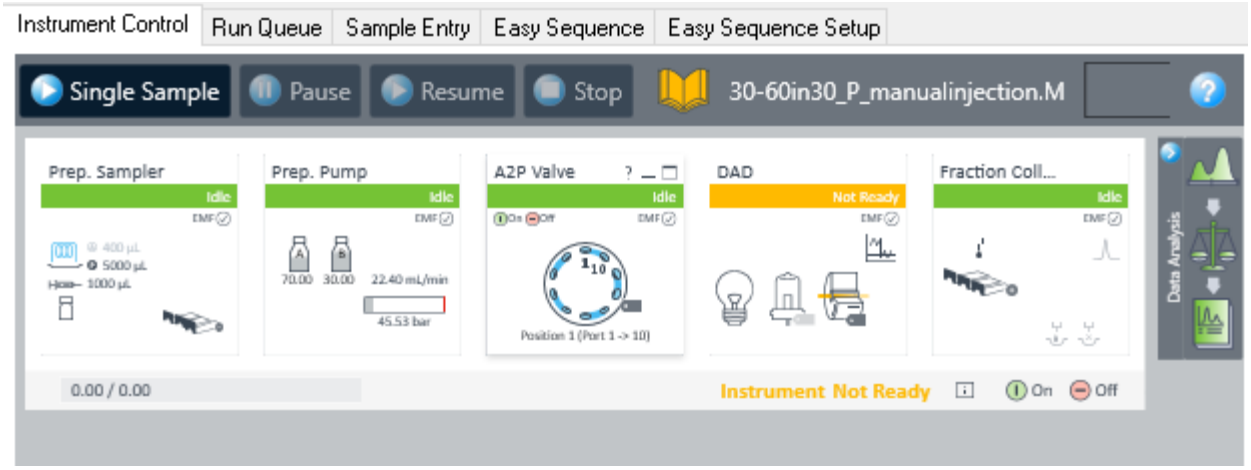
5) The screen should now look like this:



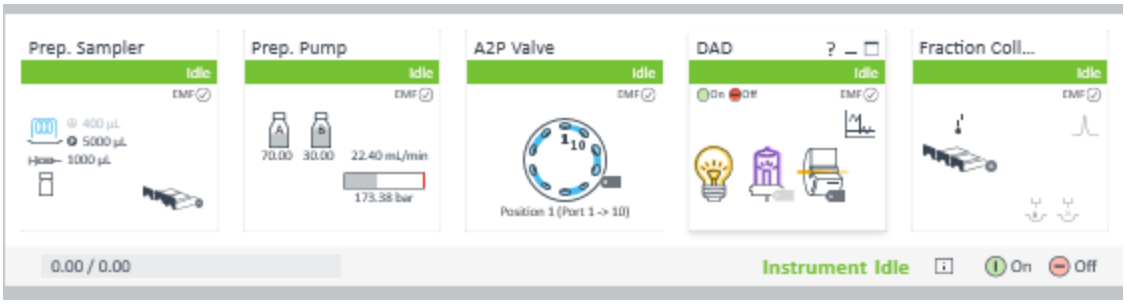
6) You must now get the instrument ready to run a sample. Begin over on the left panel of the screen where a list of methods is available. If possible, find one that reflects the gradient you wish to run. "_P" indicates a method for a prep column, while "Analytical_" indicates a method

for the analytical column. Similarly, “manualinjection” indicates the method is for using the manual injection valve. For our purposes, I will use “30-60in30_P_manualinjection”. Select by double-clicking. If you need to make a new one, select the one closest to what you want to do, and we will edit it later.

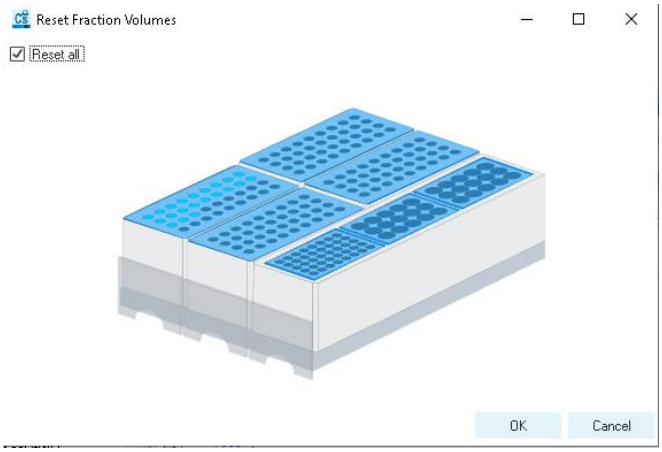
7) Now you should start the instrument running. Hover your cursor over the “Prep. Pump” box, and on/off buttons will appear. Select “on” to start solvent flow through the column.



8) Turn on the lamps on the DAD by right clicking in the white space of the DAD box and selecting “Turn on” for both lamps (note, the green “on” button will not turn on both the lamps).

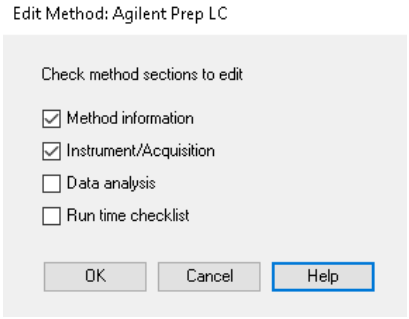


9) We also need to make sure the Fraction Collector does not have a memory of old fractions that have been removed from the system. Right click in the white space of the Fraction Collector box and select “Reset Fraction Volumes”. Select “Reset all”, then click OK.

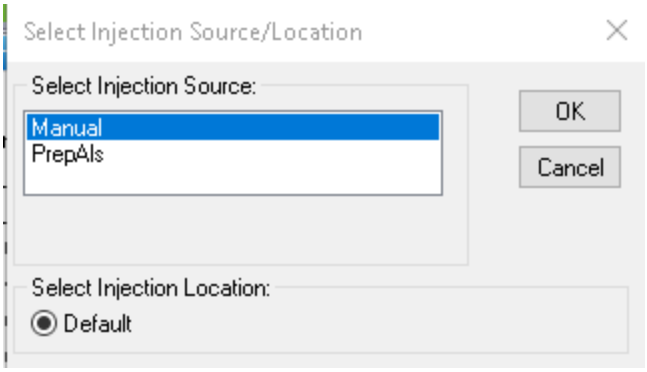


10) You should make sure the method you have reflects the gradient you want to run. Alternatively, if the gradient you want wasn’t available, you need to make it. At the top of the screen, click

"Method" -> "Edit Entire Method". Click "OK".

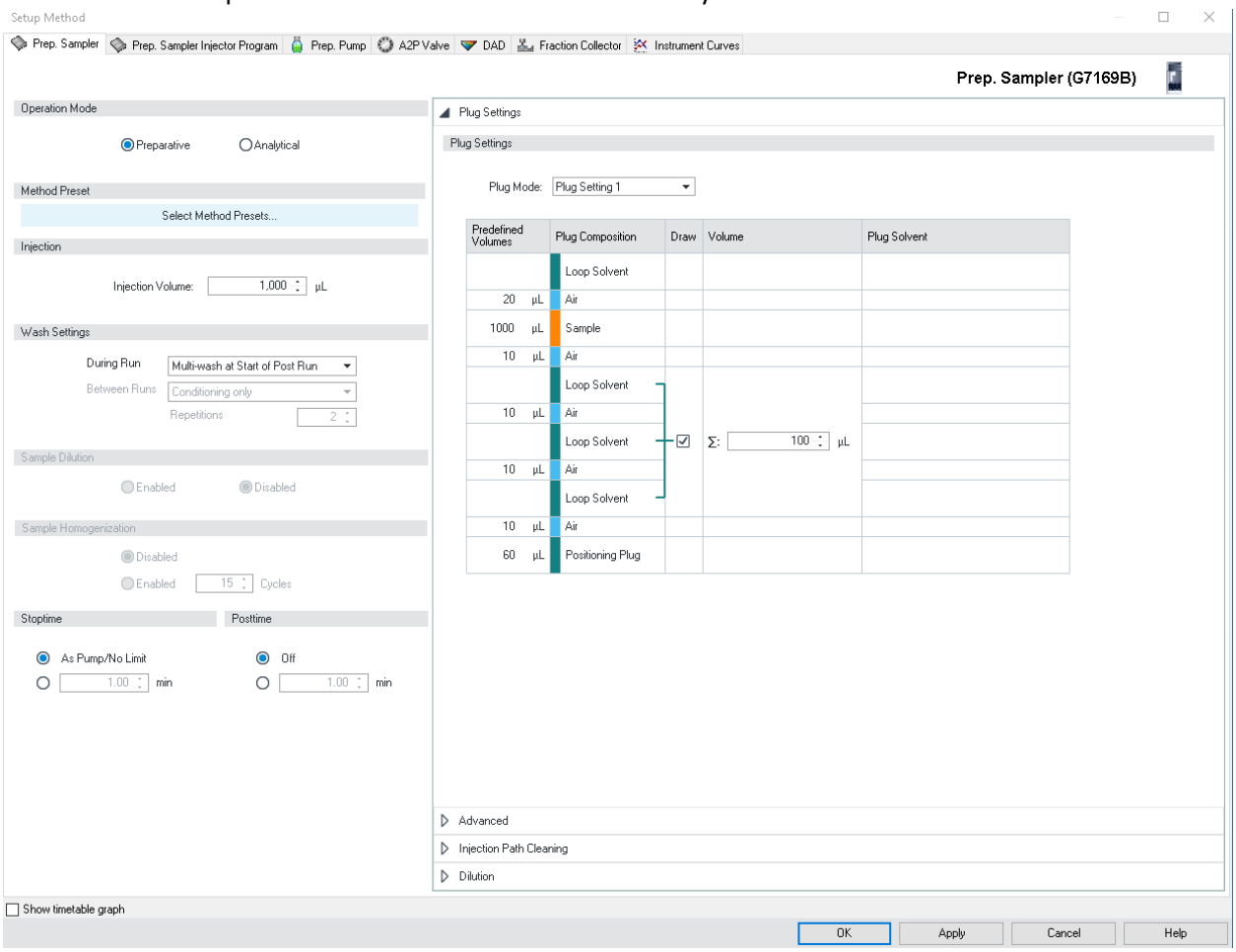


11) Select whether the method you want to use will use the Manual or Prep AIs (aka autosampler)



12) On the first tab, ensure that the correct Operation Mode (Preparative or Analytical) is selected. Regardless of whether you are using the autosampler or not, this selection must be correct to

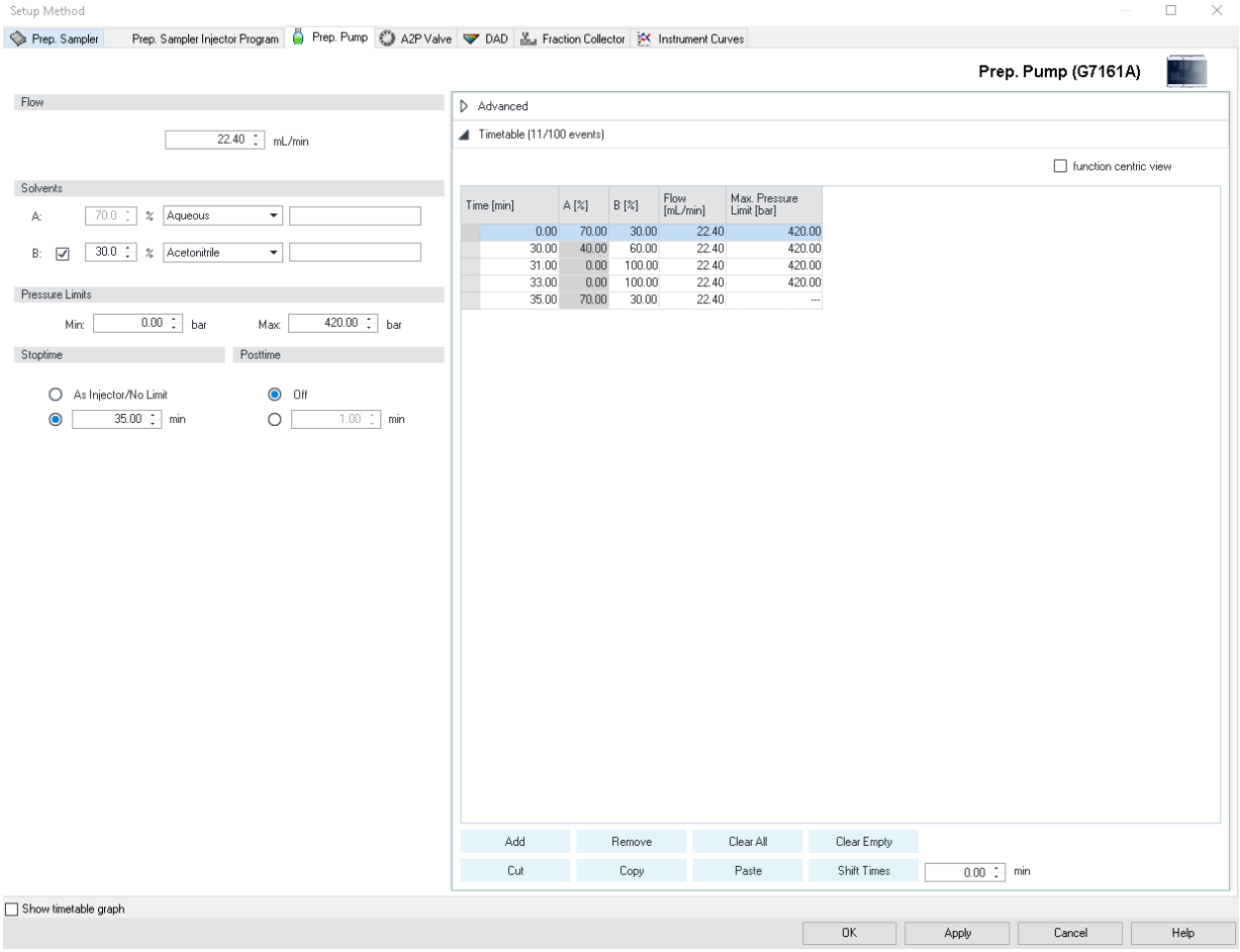
allow fail-safes for pressure and flow rate to be set correctly.



a. If you are using the manual injection valve, you can go to the next tab. Skip “Prep. Sampler Injector Program” and go straight to “Prep. Pump”
b. If you are using the autosampler, ensure your injection volume is set on this screen. Unless you have good reason to change it or a sample with unusual solubility concerns, leave the plug Mode set to Plug Setting 1. Once finished, go to the “Prep Pump” tab, skipping “Prep. Sampler Injector Program”.

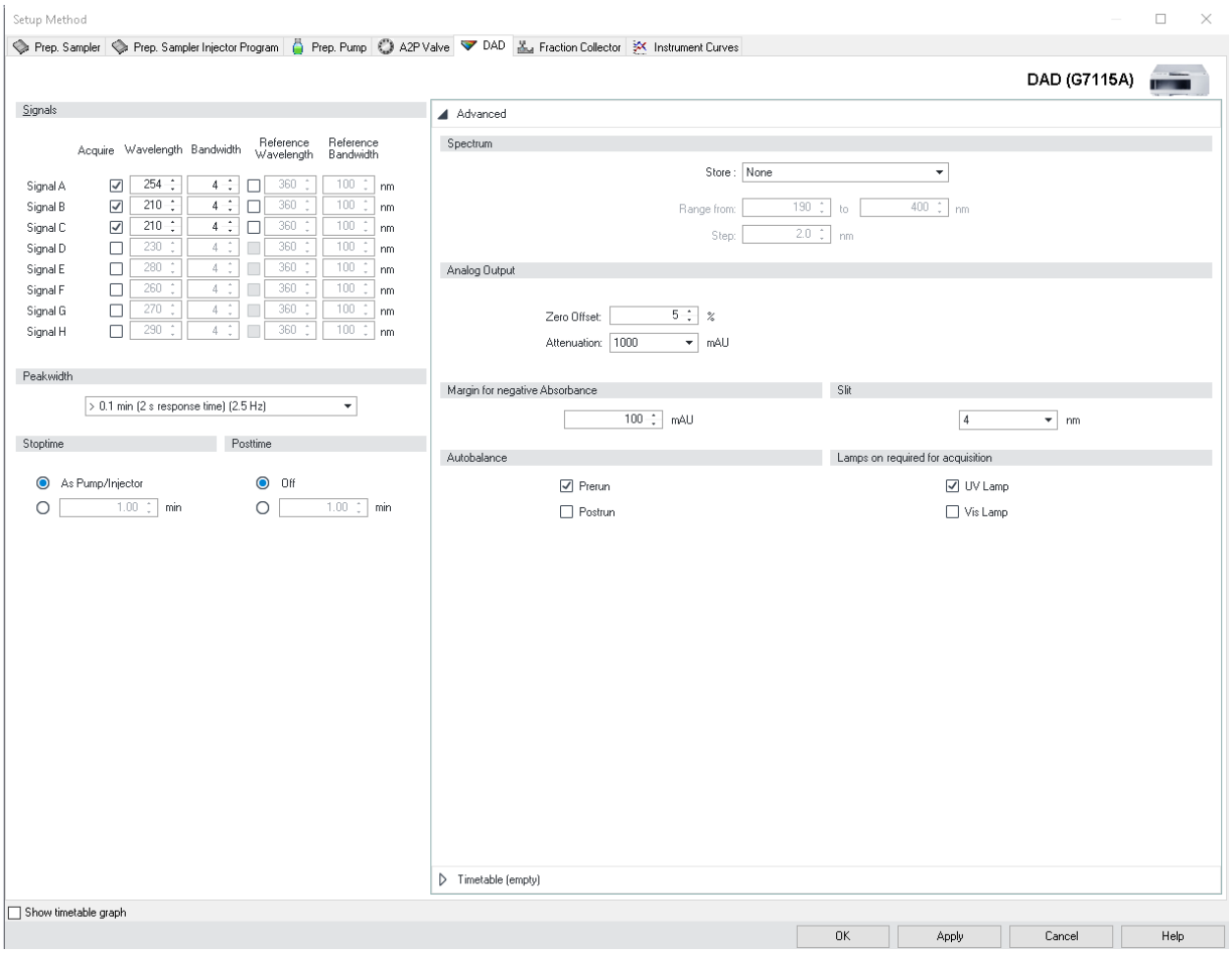
13) Now you need to program your gradient information. On the left panel, input initial conditions for your method such as flow rate and starting mixture of solvents. Flow rates for analytical methods should not need to go over 1.5 ml/min and for preparative methods 25 ml/min. Also set the stop time of the method (the total length needed for data acquisition). Do not change

the pressure limits above 420 bar.



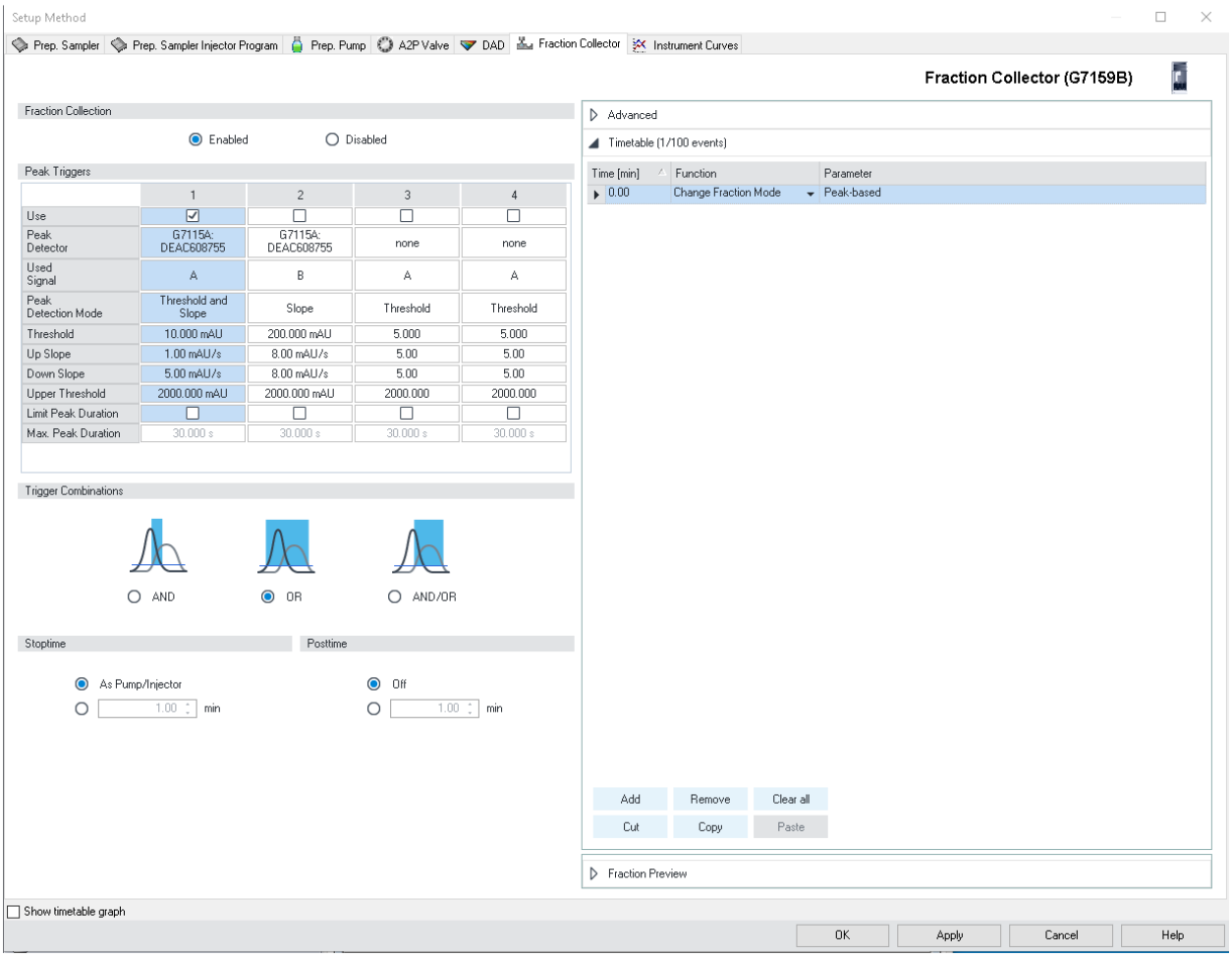
14) Build your solvent gradient using the table on the right. Note that all methods should have at least 2 minutes of run time held at 100% acetonitrile to flush the column. If your sample is highly retained, you should have at least 5 minutes of flush time.
15) In the “A2P Valve” tab, make sure the valve position matches the column you wish to use (Position 1 for preparative, Position 2 for analytical).
16) In the DAD tab, provide settings for the diode array detector. In particular, provide specific wavelengths where you want 2D chromatograms recorded and/or that you want to use for

fractionation.



17) On the “Fraction Collector” tab, ensure first that if you want to collect fractions, “Enabled” is checked. If “Disabled” is checked, you will not be able to do anything to collect your material

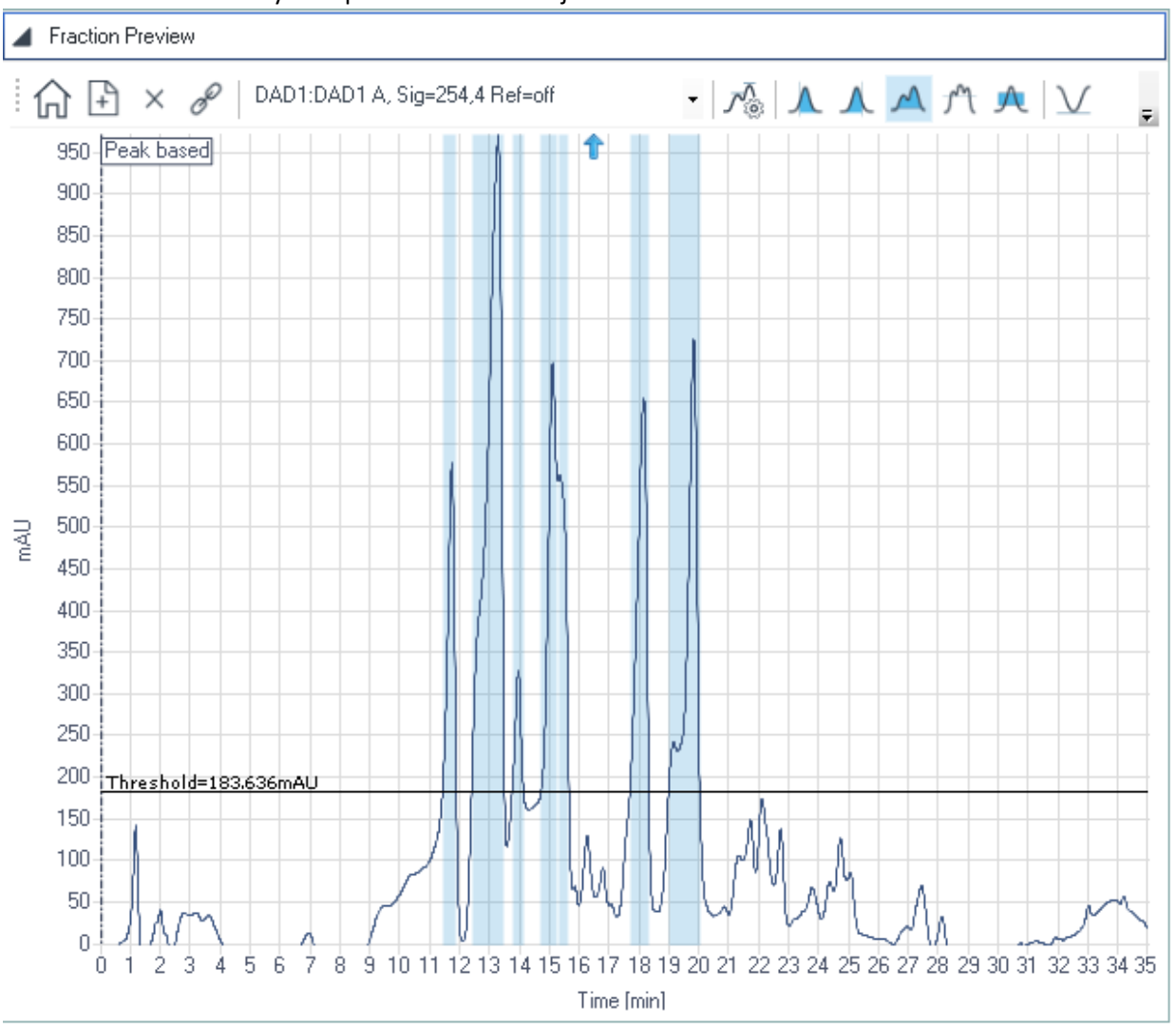
once the method is started!



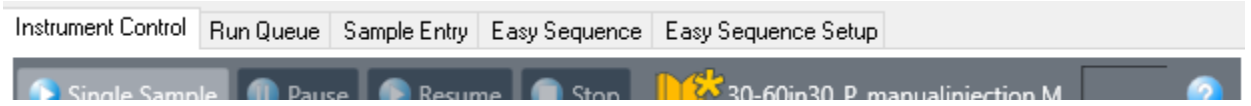
18) Use the table on the left to select which signal(s) you want to use for fraction collection (letter values are matched to signals in the table under the DAD tab). Also select detection mode (using threshold, slope, or both to trigger collection) and values for these modes. If using multiple signals, select whether you want And, Or, or And/Or combinations. You can use the table on the right to change between not taking fractions, using detector based triggers (aka “Peak-based”) or collecting time or volume slices.
19) Under the “Advanced” tab on the right, you can set a max fill volume per location (make sure this is smaller than the volume of the collection vessel!).
20) Under the “Fraction Preview” tab, you can load a reference chromatogramby clicking  and navigating to the proper chromatogram.

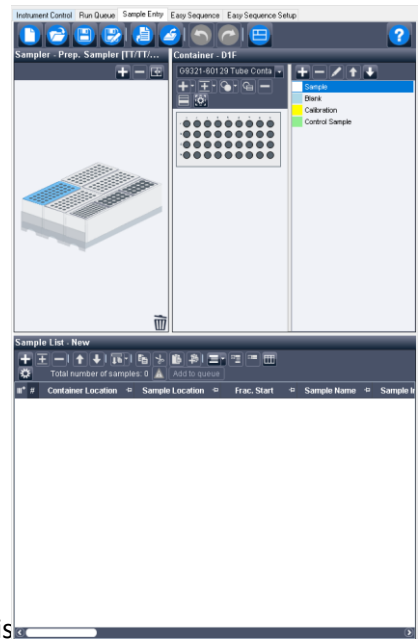
21) Now you can use these buttons () to interactively determine fraction collection parameters. Once you set them, the software will highlight in blue where it expects to

collect fractions should you repeat an identical injection to the reference.

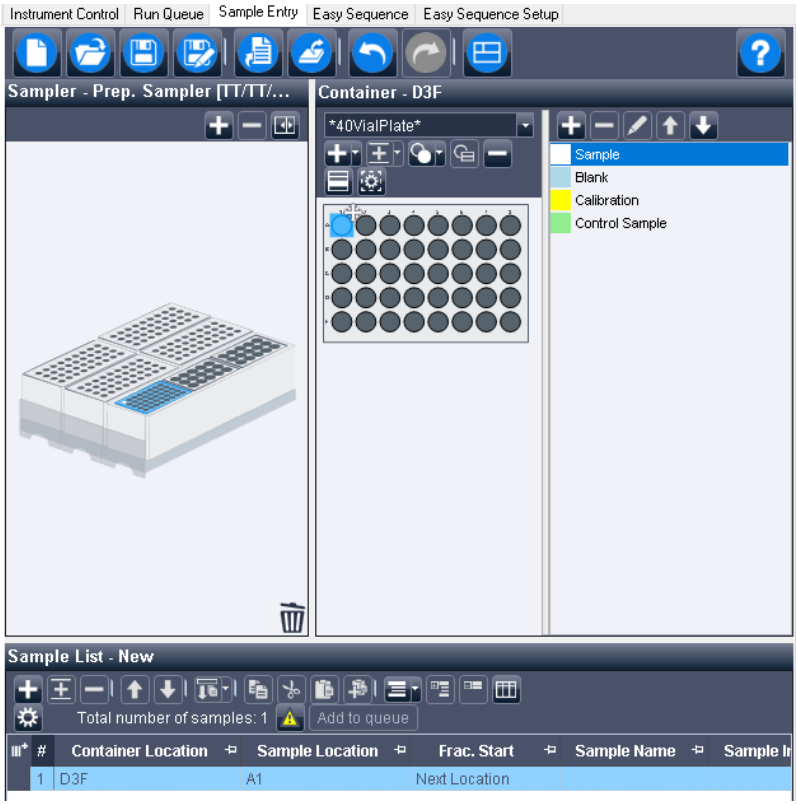


22) This is the last thing you need to change in the method. You can now click “OK” at the bottom.
23) Click File -> Save as -> Method to save the changes to your method, or to save your new method.
24) You now need to queue your sample(s). There are two ways to do this. The first I recommend for those using the autosampler. The second I recommend for those using the manual injection valve.
    a. For the first (autosampler) method, click “Sample entry” near the top of the screen.



i. The screen should look like this 

ii. On the autosampler bed, select the position of your sample to input interactively, first selecting the container on the left, then the position in the middle. Double clicking a vial location will add a row to the sample list at the bottom



iii. Select the fraction start location (the three dots will let you select this interactively), provide a sample name, a method name (the three dots, again, will let you select this from a list interactively), and provide again the injection

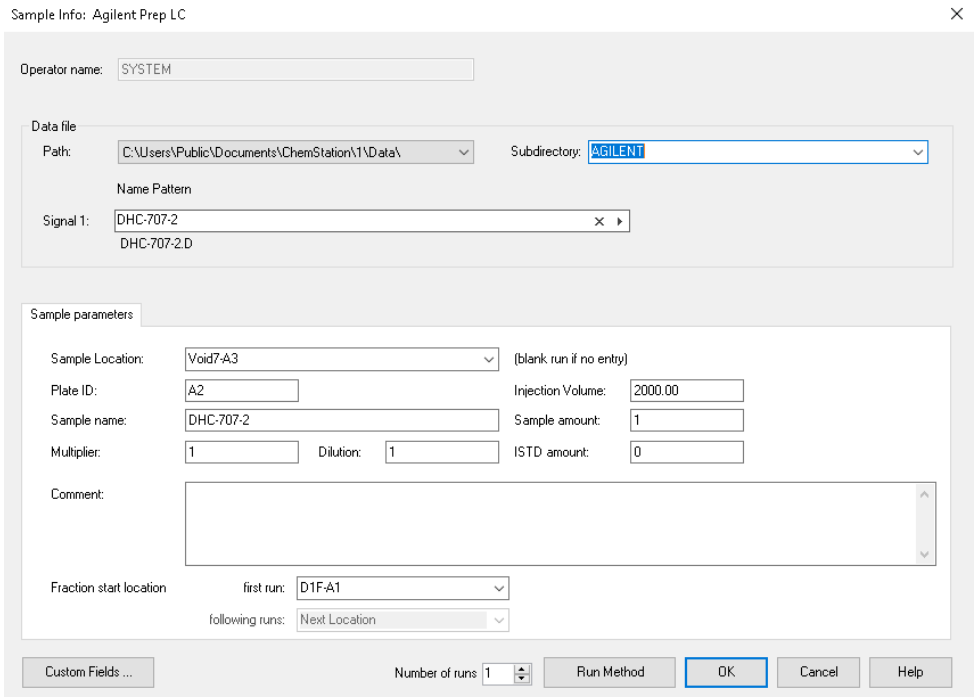
volume. All other information in the list is rather unnecessary unless you are looking to do analysis. If you are looking to do this, you are likely using the wrong system!

iv. Add in all remaining samples you wish to run, including multiple injections from the same vial. If doing this, you may want to select “Pooling” as your fraction start location for all subsequent injections of the same vial.

v. Click “Add to Queue” to start the first run

b. For single samples, double click the blue rectangle in the “Single Sample” Bar

i. Put in all relevant sample information into this window. Select the fraction start location interactively by selecting the drop down arrow on “first run”

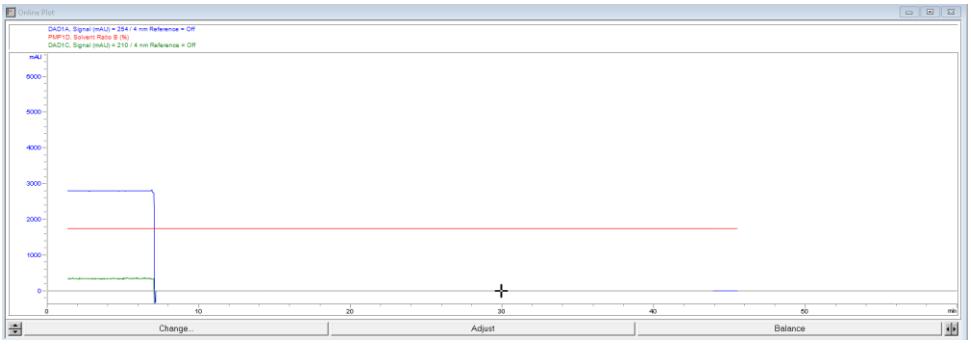


ii. Click “Run Method”

iii. You can now place your sample in the sample loop using a syringe and one of the provided, Hamilton blunt tip needles. Prior to putting your sample in the loop, I recommend flushing the needle and loop with acetonitrile then water to clean out any residual material.

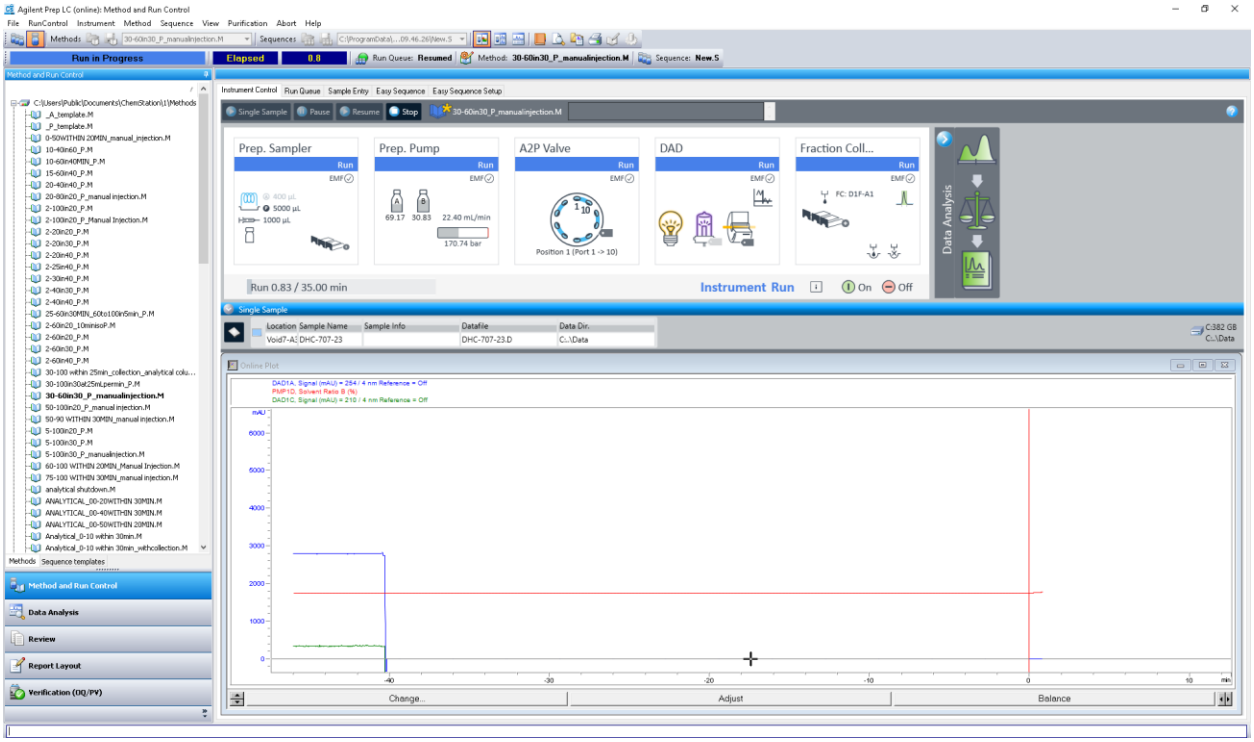
iv. When running a manual injection, you will need to manually zero (balance) the detector. After Selecting run method and placing your sample in the loop,

double click on what channel you are using for fraction collection in the online plot, such as 254 or 210 nm. If it is not there, use the “change” button to add it. Once selected, click “Balance” to zero that channel.



v. Once this is done, rotate the handle on the manual injection valve clockwise. Ensure to do this quickly, in one rapid motion, all the way until the valve stops moving. This is imperative for introducing the sample loop into the instrument flow path without incident and starting the instrument properly.

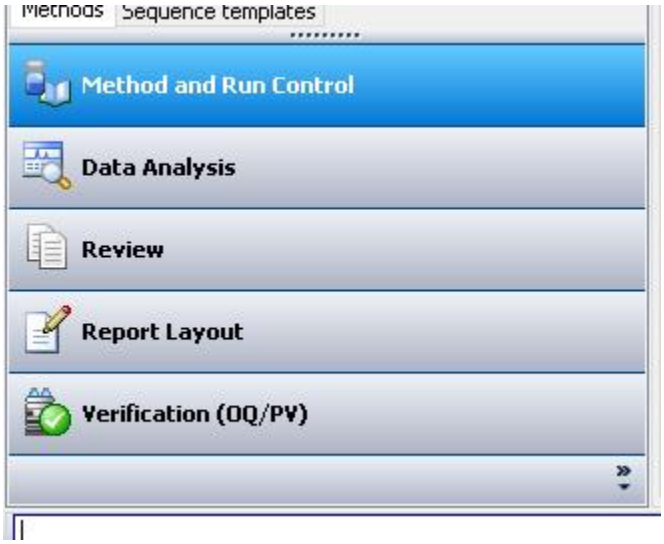
25) Regardless of what method you chose to use, your sample should now be running.



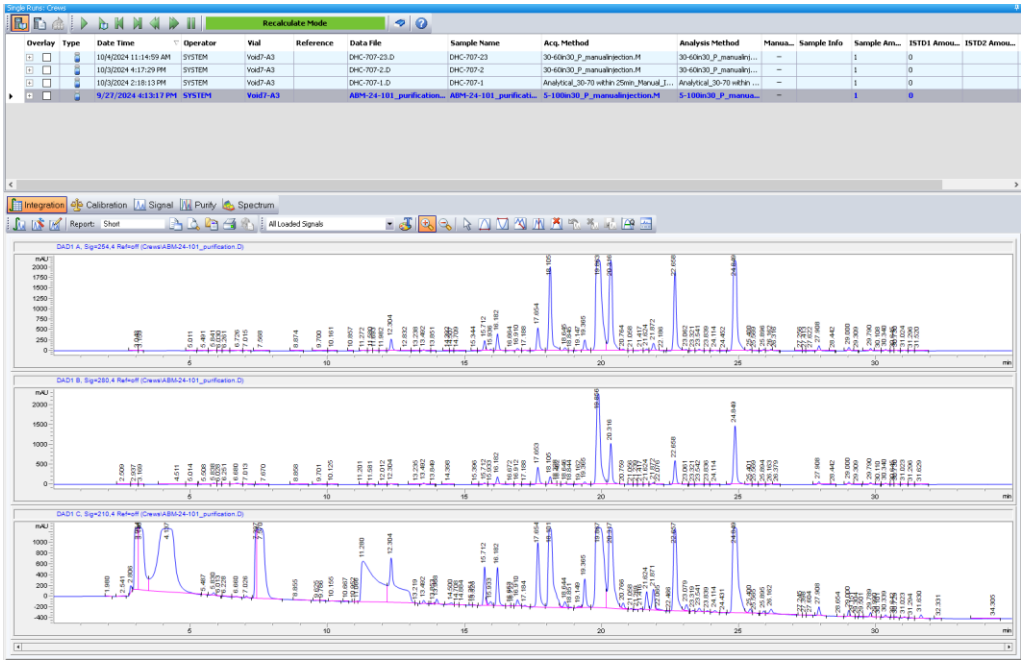
26) If for any reason you become concerned the automatic fraction collection criteria of the method is inadequate, the two icons ( ) in the fraction collector box will let you manually start (left) and stop (right) fractions. This will still be subject to the max volume per container limit you put in the method.

27) Once all samples are run, you will get a PDF of the chromatograms highlighting which fractions correspond to which parts of the chromatogram. Save this, or print it.

    a. If you accidentally close this, or want to see it again later, click on “Data Analysis” in the lower left corner of the screen.



b. Navigate to your sample using the pane at the left to find sequences or folders of single runs, then double clicking the appropriate line at the top.



c. Click “Report” at the top of the screen, then “Print report” to bring back the original default report. This report should have all information you need, but it is also available interactively when the sample is open.

28) Back in the Method and Run Control Window (the first one you have open in this guide), you now need to shut down the instrument. Turn off the pump similar to how you turned it on (of course, now you must select “Off”, not “On”, but I really hope you already knew that). Similarly, turn off the DAD.
29) Make sure to take ***all fractions*** with you no matter whether you want them or not. I do not want them. I have no use for them. If you want, you can add them to the CSC’s waste containers.
30) Close the software and log out.

Congratulations, you have finished the guide on how to run Prep LC samples. Best of luck on all purifications moving forward!