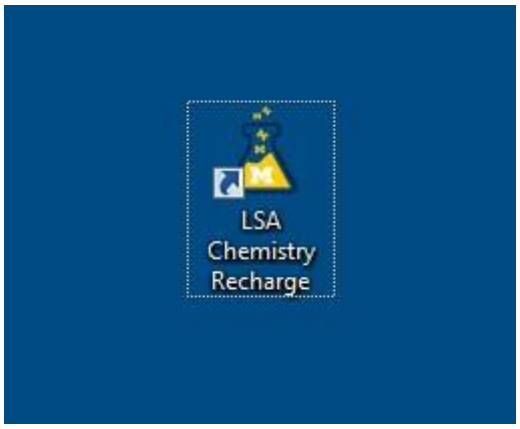
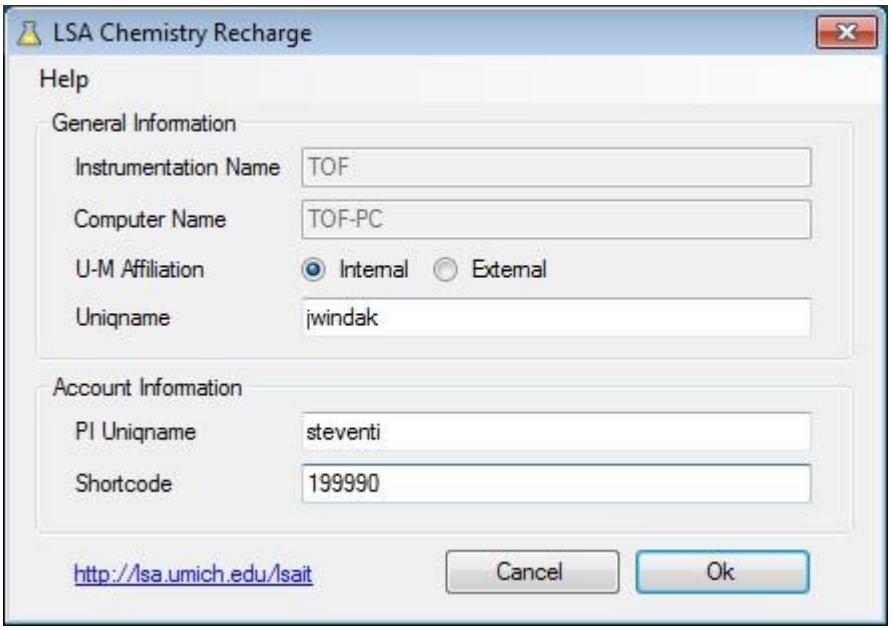
Agilent 6230 TOF User's Booklet

- Double-Click the LSA Chemistry Recharge icon



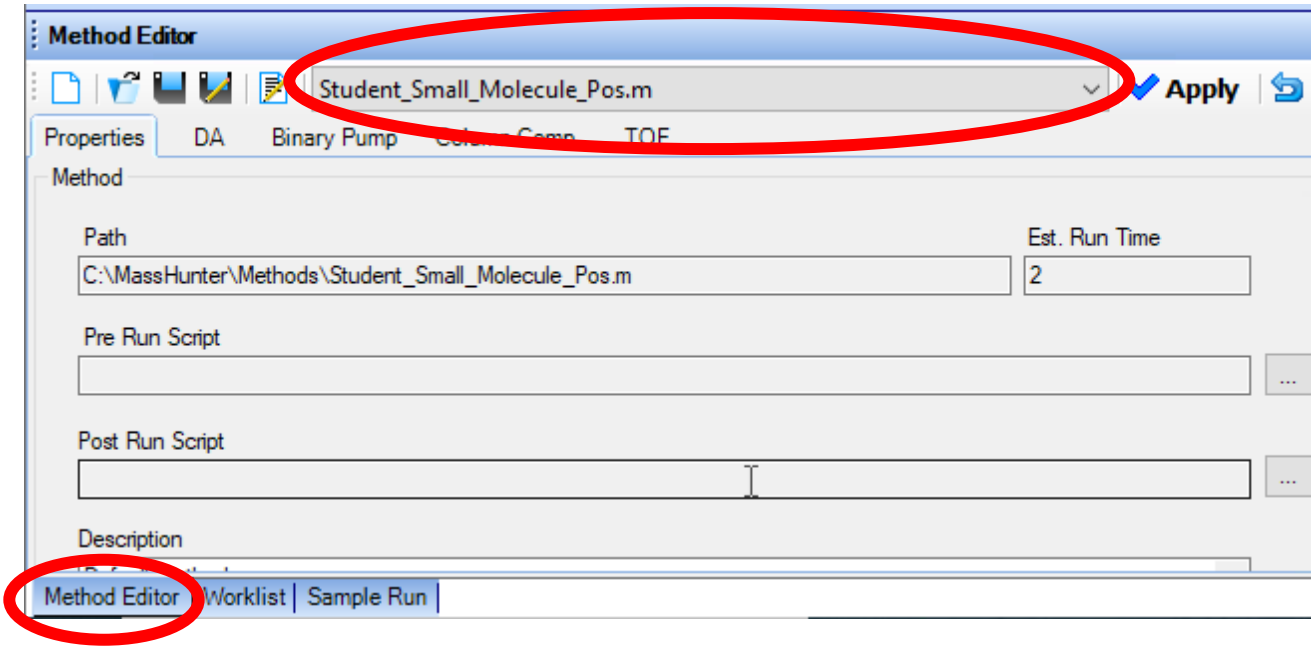
- Type in your uniquename, your PI's uniquename, and your shortcode, and click OK



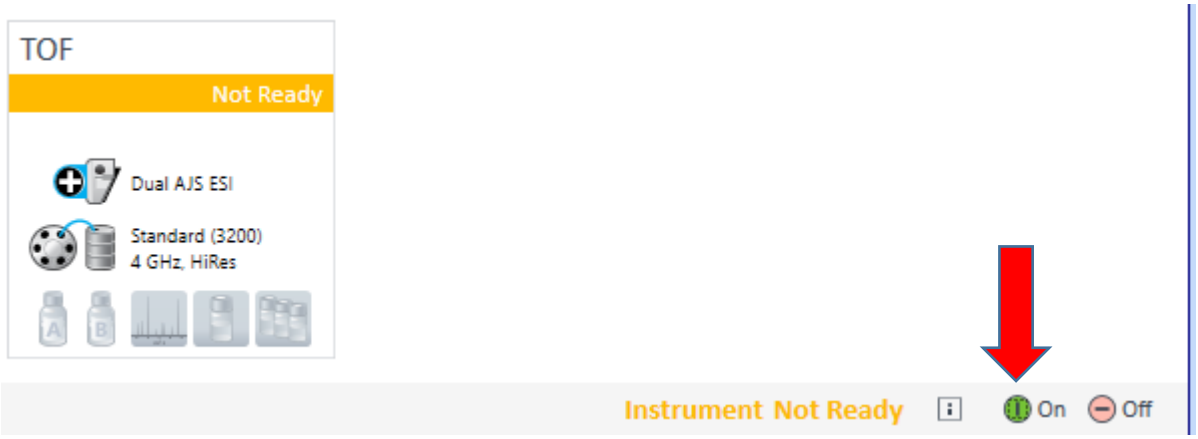
The MassHunter Data Acquisition program will come up automatically after you login

- Click on the tab near the bottom of the screen that is labeled “Method Editor”.

Then select the method that you want to use from the drop-down menu. Generally, you will usually use the method called “Student_Small_Molecule_Pos” for relatively small (less than 1500 Da) organics in positive ion mode.

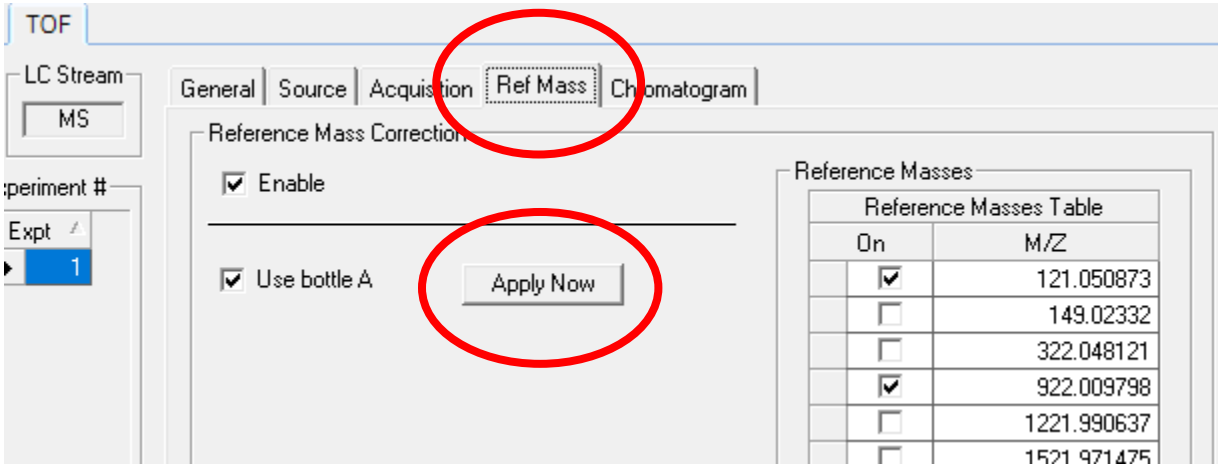


- After you have loaded your method, turn the system On, using the On button:

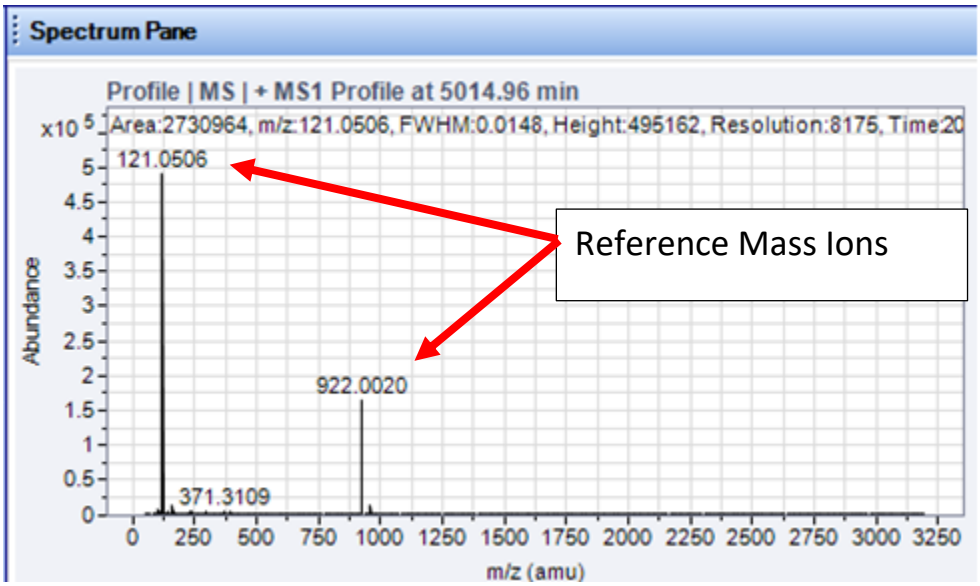


- Go to the method editor, click on the “TOF” tab, and then click on the “Ref Mass” tab.

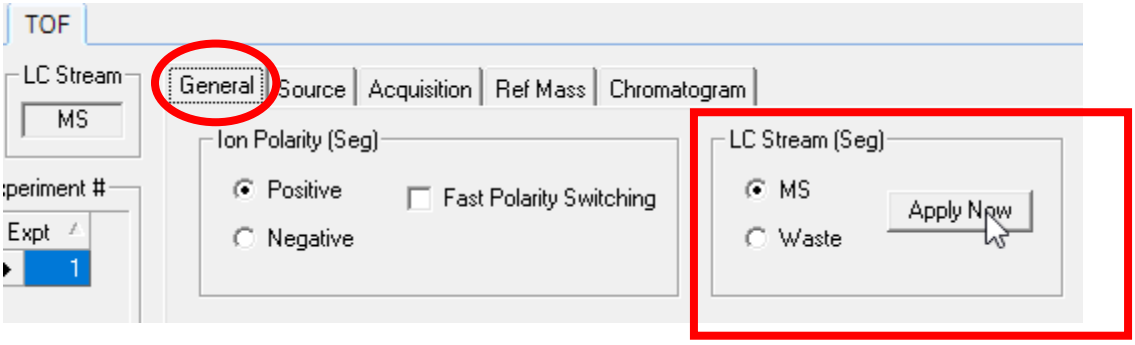
Near where it says “Use Bottle A”, click on the “Apply Now” button.



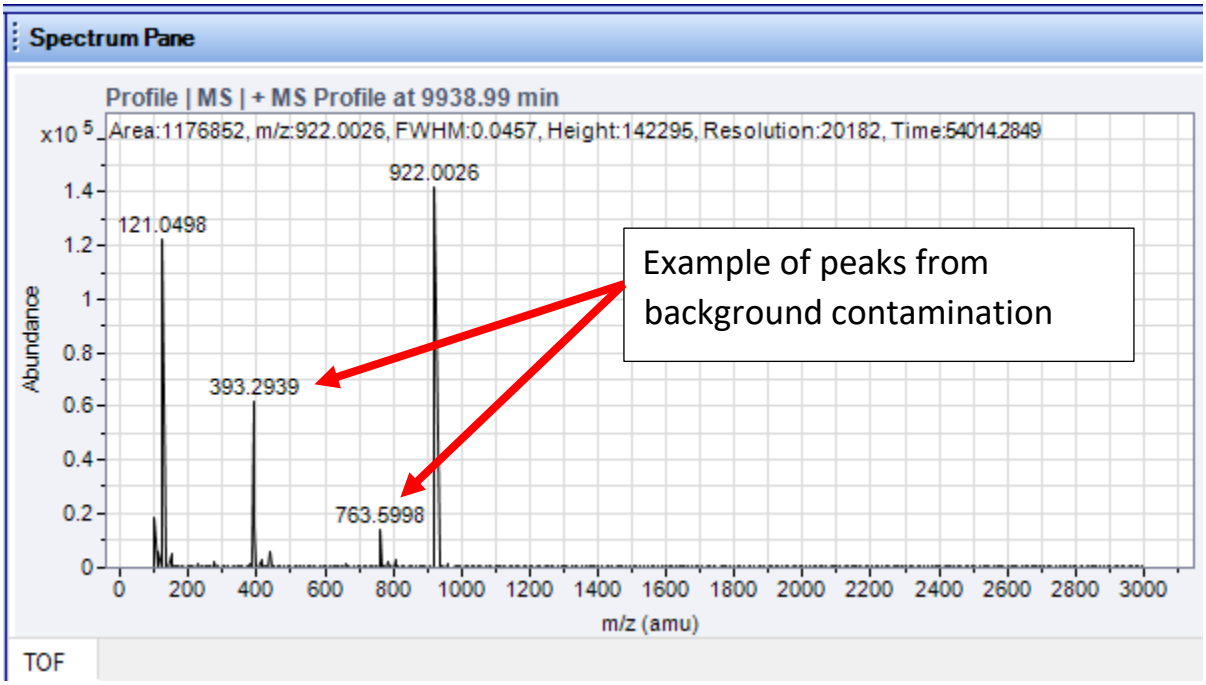
This will send the Bottle A reference mass solution into the mass spectrometer.
You should then see the reference mass ions in the mass spectrum window.
In positive ion mode, these reference ions will be m/z 121 and m/z 922.
In negative ion mode, these reference ions will be m/z 112.9 and m/z 1034.



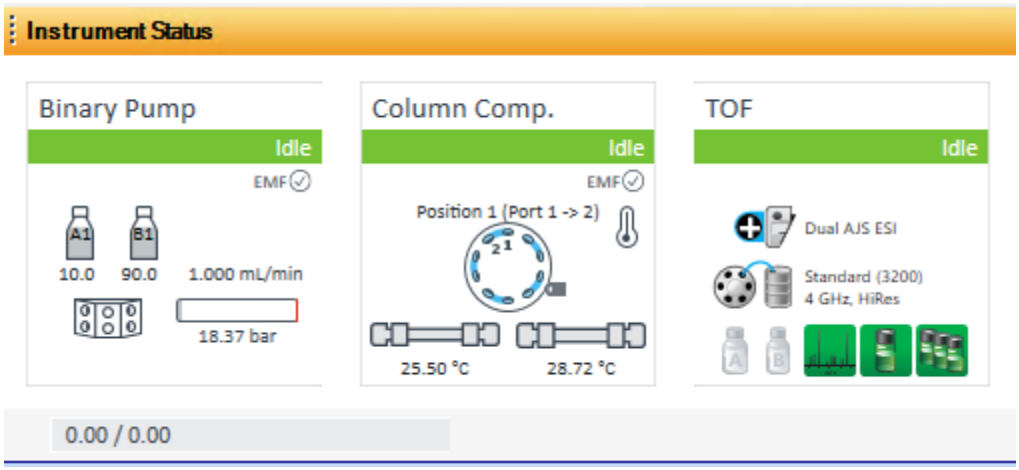
- On the method editor TOF tab, click on the General Tab. Click on “Apply Now” under “LC Stream to MS”.



This will send the HPLC solvent flow into the mass spectrometer.
After that is done, what we would ideally like to see in the mass spectrum window is only our reference mass ions. If we see other peaks that are not supposed to be there, then there could be background contamination in the system from previously run samples that were too concentrated.

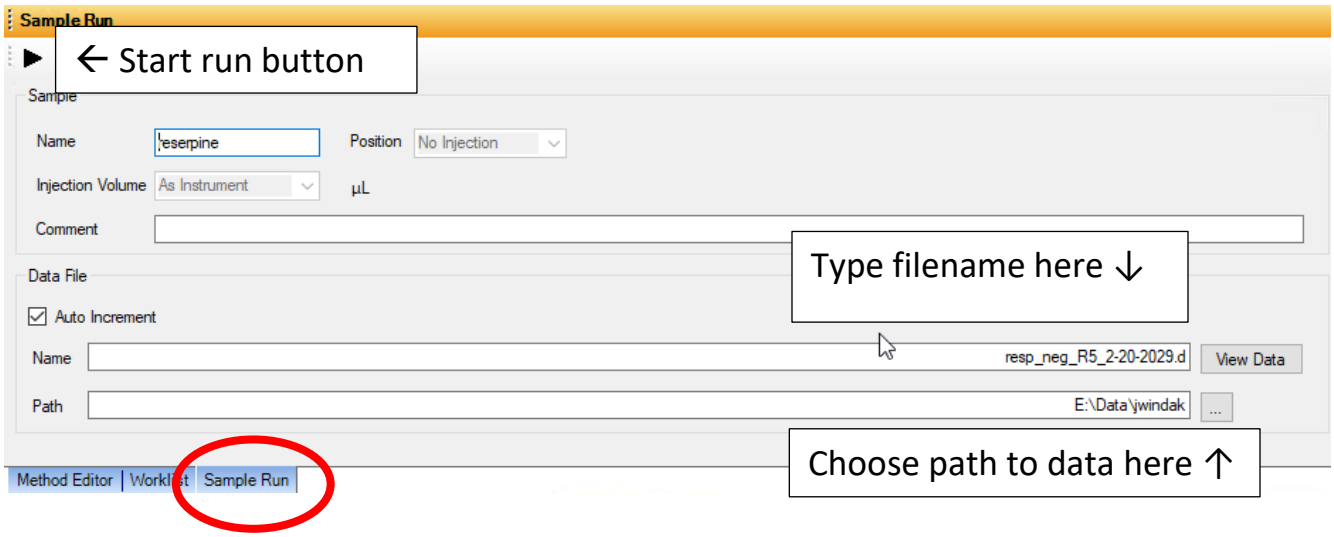


- Wait for the TOF status to turn from Yellow to Green, indicating that it is ready to use



← This bar will turn green when the instrument is ready.

- When the TOF is ready, click on the Sample Run tab near the bottom of the screen. Choose where to put the data, type in a filename, type in a comment (optional), then click on the ▶ button to start the run.

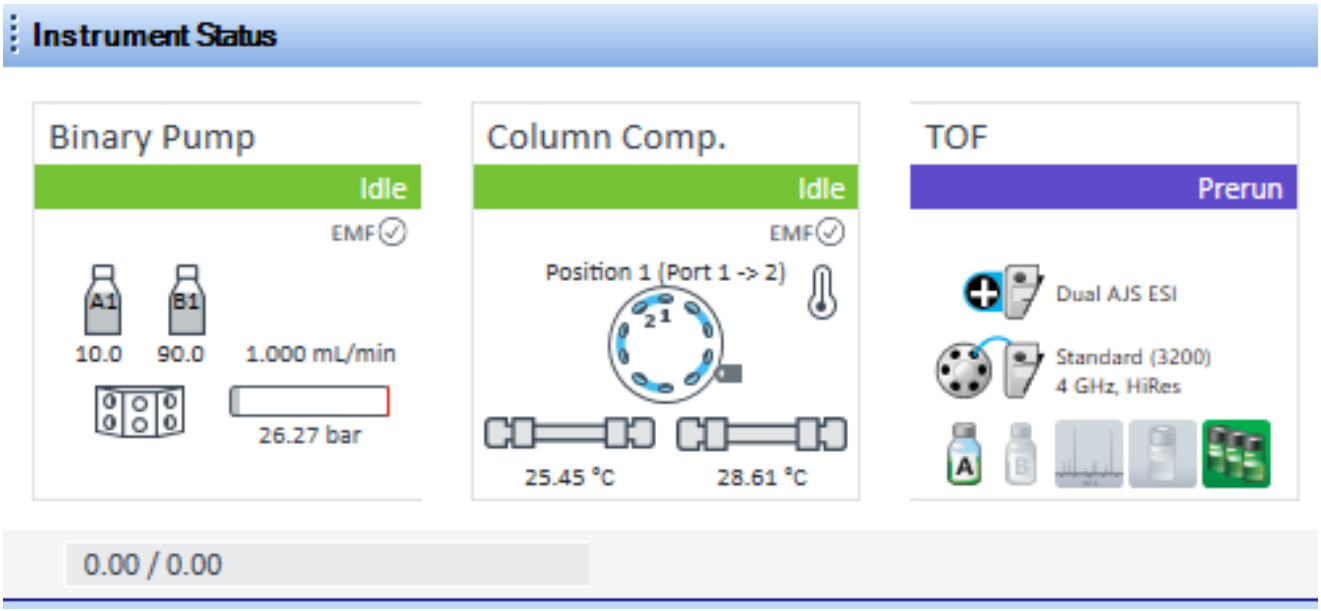


← Start run button

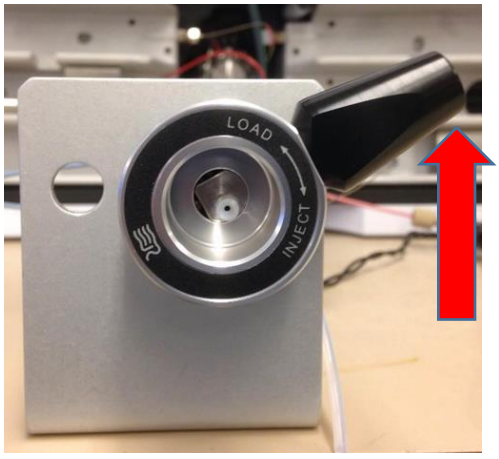
Type filename here ↓

Choose path to data here ↑

- After you have clicked on the ▶ button, the TOF status should change to a dark blue-purple color, for the Prerun state:



- Make sure that the injector handle is up in the Load position:



Please note:

Your samples need to be in the low micro-molar concentration range!
Do not run samples that are more concentrated than this!

- Rinse the syringe with clean solvent. Then, draw some sample solution into the syringe (around 50 ul). Insert the syringe into the injector valve as far in as it will go, and inject your sample solution into the valve (while the handle is still in the Load position).

↑ Handle still up in Load position while injecting the sample solution.

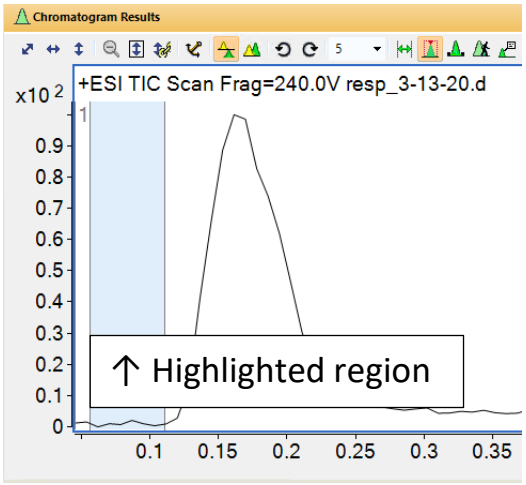
- After you have injected the sample solution, rotate the handle down to the Inject position to start the run:

↓ Rotate handle down to start the run.

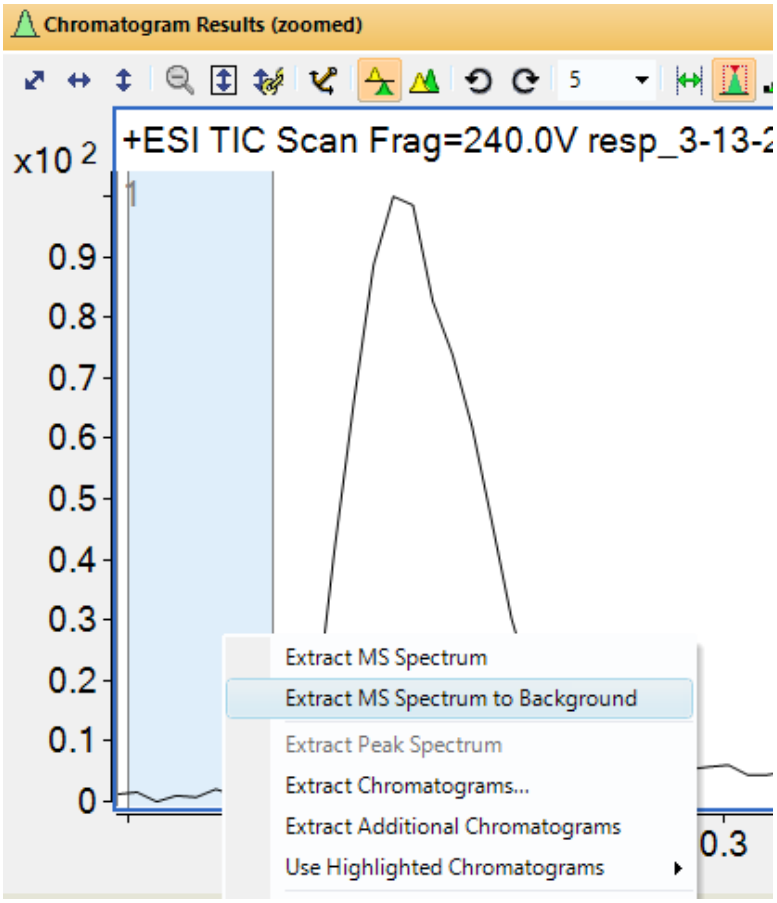
- Remove the syringe and solvent rinse the syringe.
- When the run is completed, you can process the data.

How to Process the Data

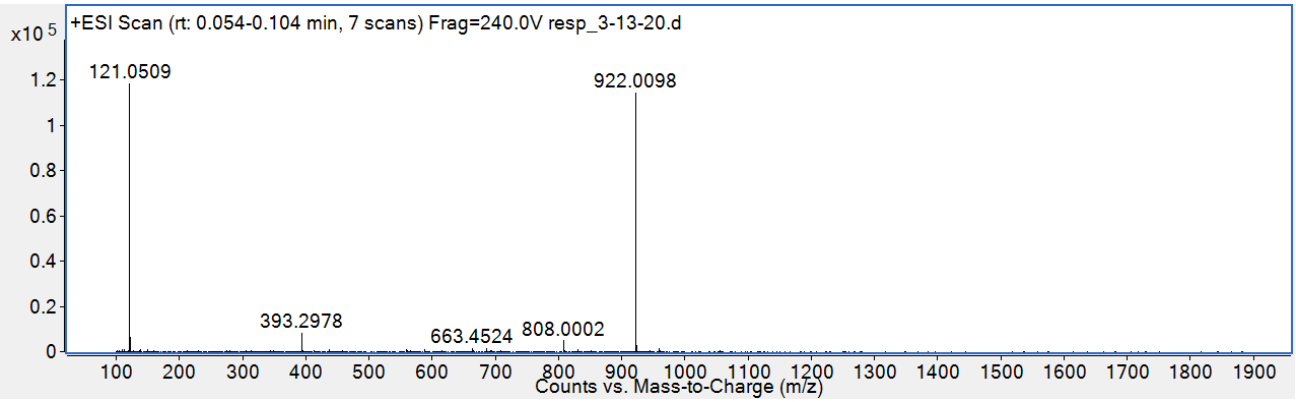
a. Open the Qualitative analysis program (unless it is already open).
b. Open your data file.
c. Select a region of the chromatogram before the peak for a background spectrum. Do this by left-clicking and dragging across the region.



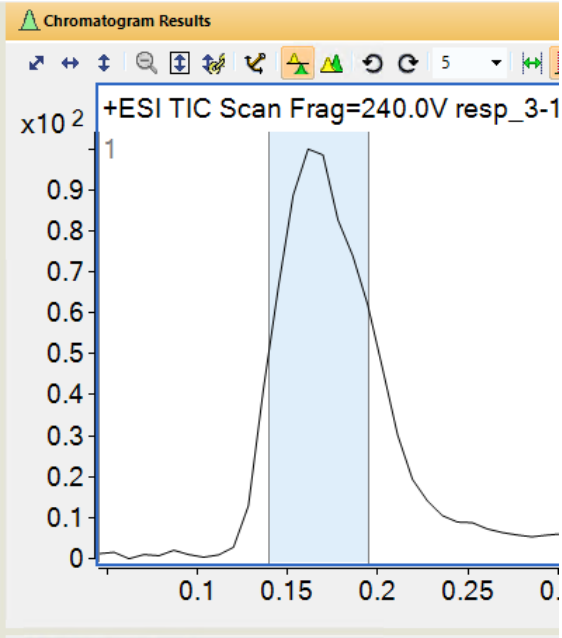
d. Right-click in the region to obtain a menu. Left click on "Extract MS Spectrum to Background":



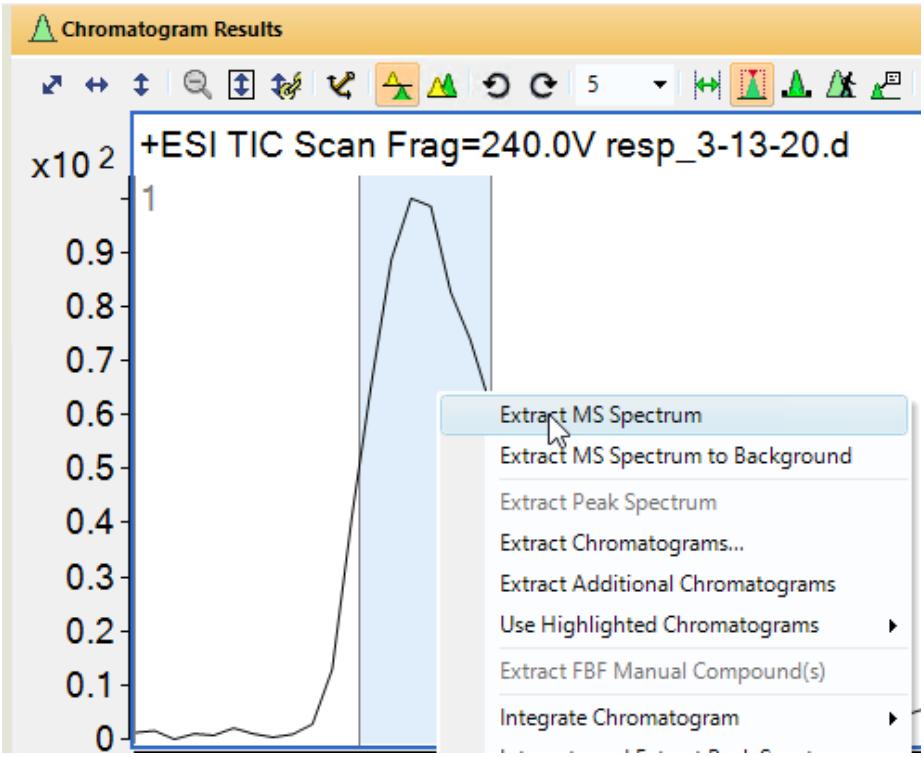
The background spectrum will have the two reference mass peaks present. They should have the correct exact mass values, which are 121.0509 and 922.0098



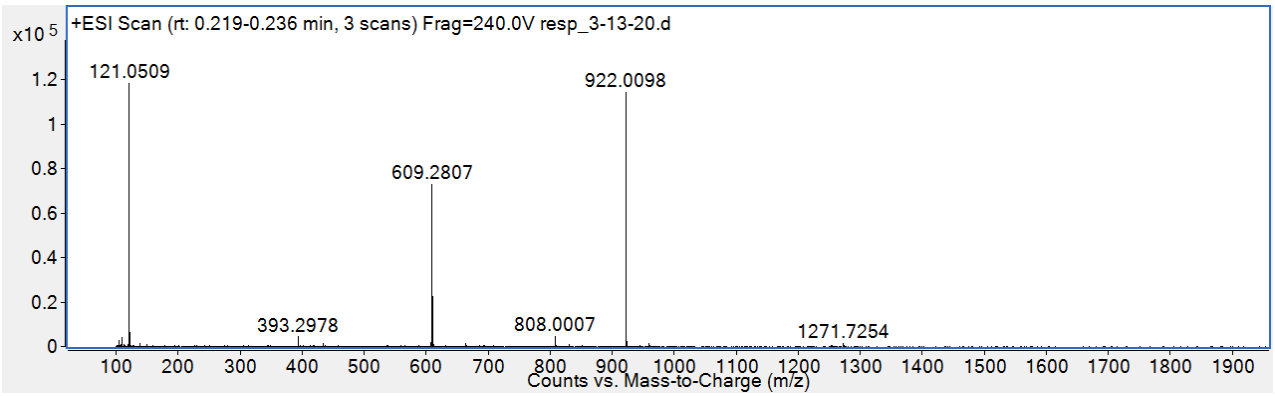
e. Highlight your sample peak by left-clicking and dragging across it.



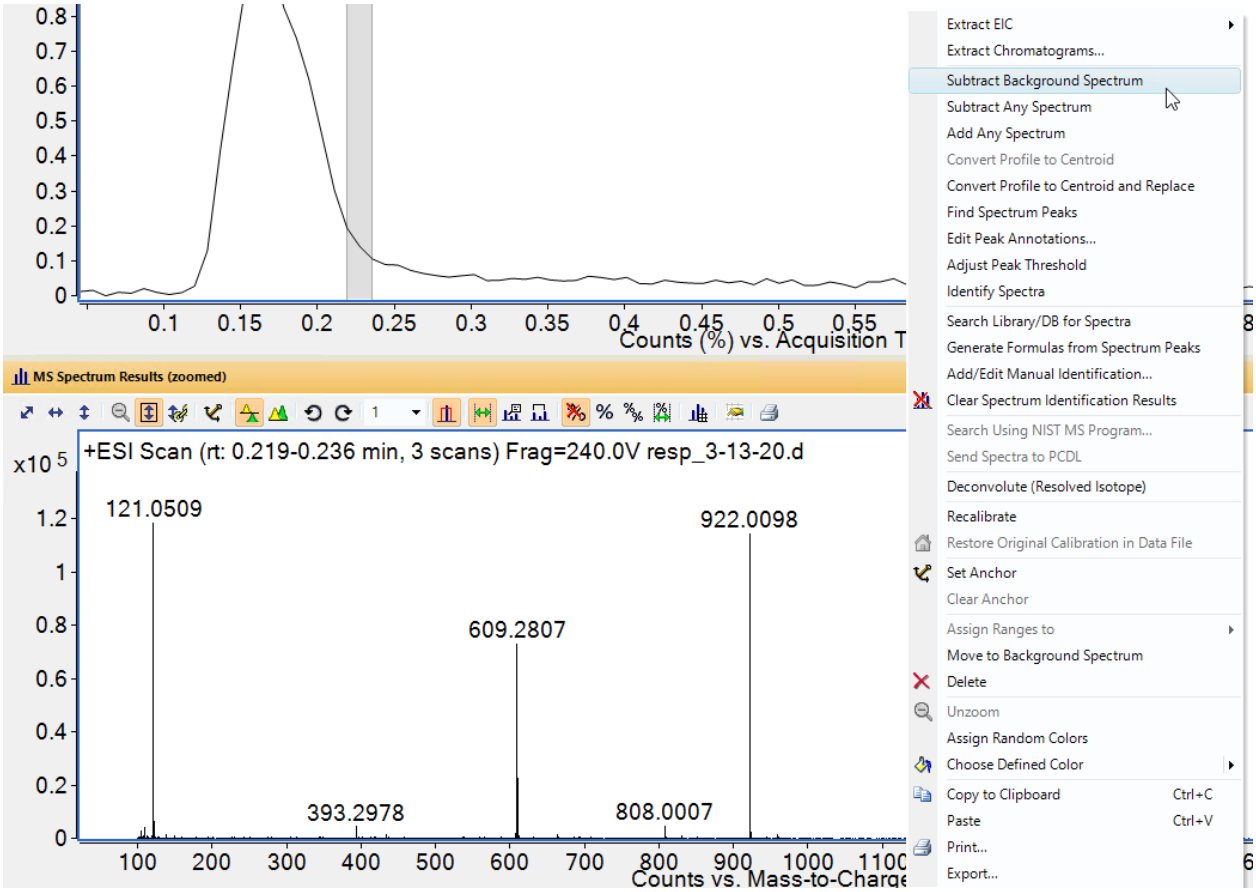
f. Right-click on the selected region to obtain a menu. Left click “Extract MS Spectrum”:



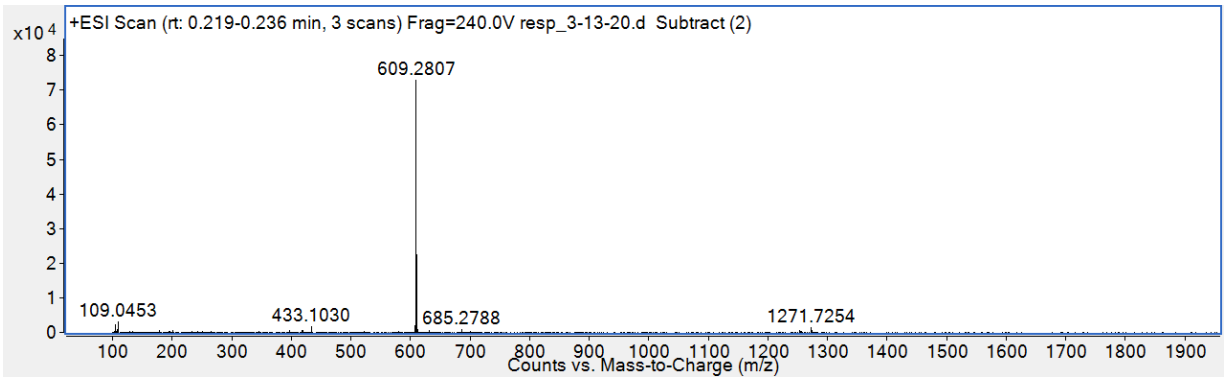
The mass spectrum will show your sample peaks as well as the reference mass peaks.



g. Subtract the background spectrum from your sample spectrum, by right-clicking the **spectrum** to get a menu. Then left-click "Subtract Background Spectrum"



Here is the subtracted spectrum:

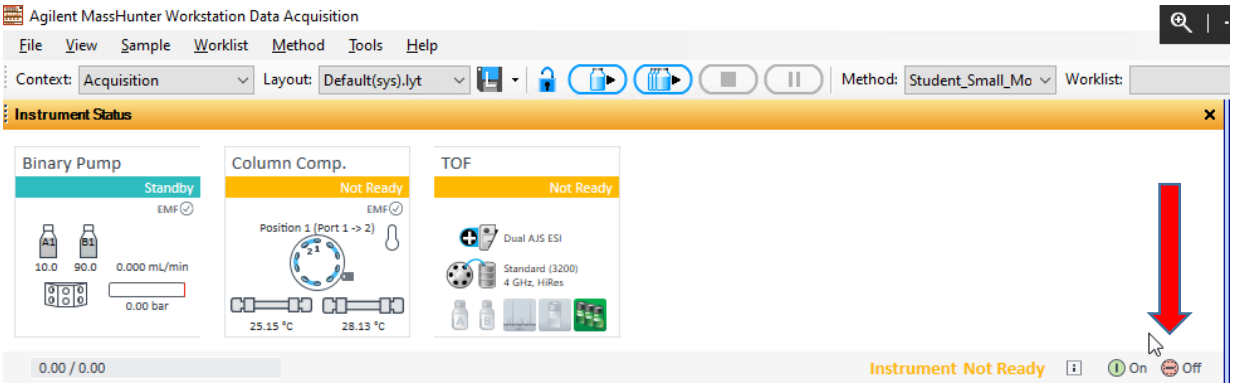


h. For printing, the spectrum can be copied and pasted into Microsoft Word.

i. To obtain a mass list, right-click the spectrum to get a menu, and then left-click "MS Spectrum Peak List 1"

How to Shut Down the Instrument When Finished

Click on the Off Button on the Data Acquisition page:



Close the MassHunter data acquisition software. This will close out your account and it will stop adding up time that you are billed for.

Important: When you close the software, it will always ask whether or not you want to put the instrument in Standby. Always click on <u>Yes.</u>