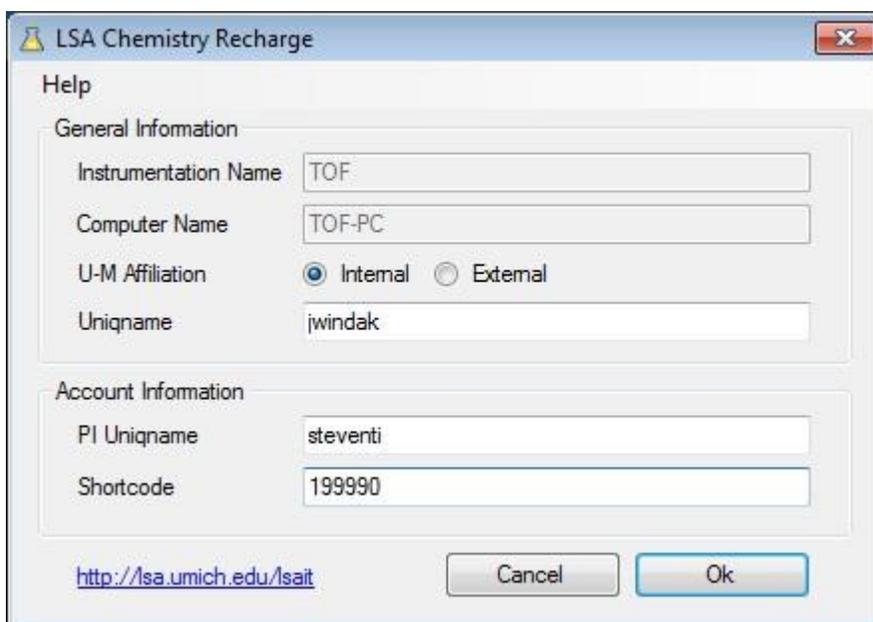


Agilent 6230 TOF User's Booklet

- Double-Click the LSA Chemistry Recharge icon



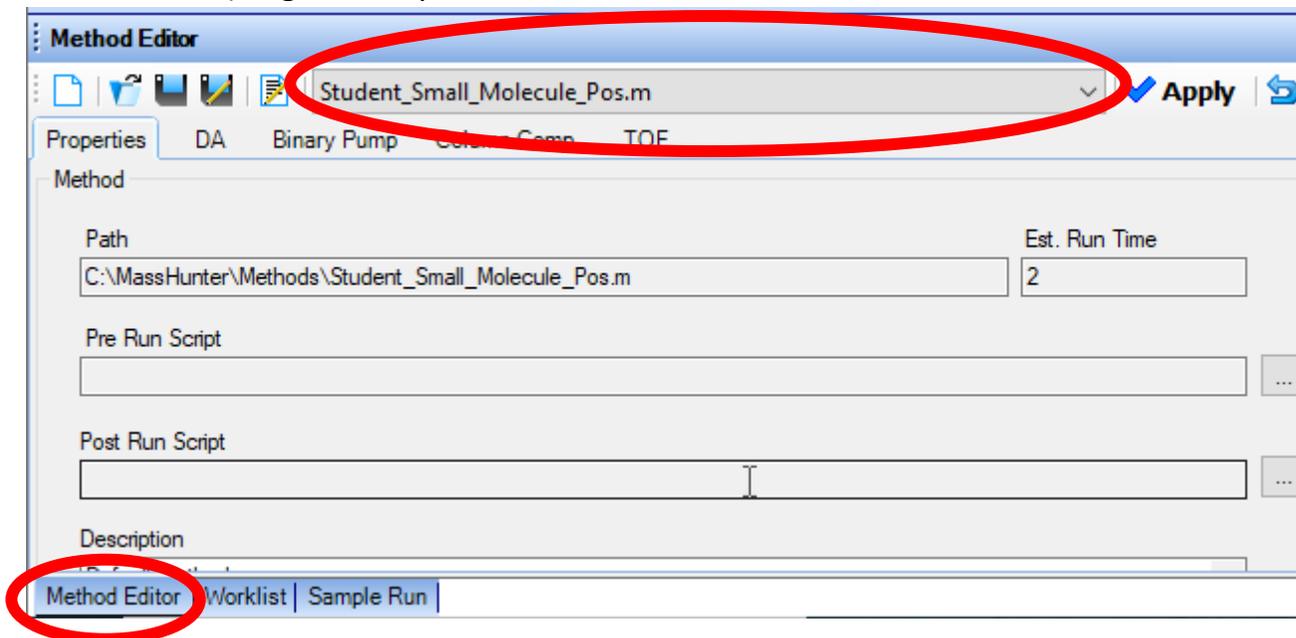
- Type in your uniqueness, your PI's uniqueness, and your shortcode, and click OK

A screenshot of the "LSA Chemistry Recharge" dialog box. The window title is "LSA Chemistry Recharge". It has a "Help" button in the top left and a close button in the top right. The dialog is divided into two sections: "General Information" and "Account Information".
General Information:
Instrumentation Name: TOF
Computer Name: TOF-PC
U-M Affiliation: Internal External
Uniqname: jwindak
Account Information:
PI Uniqname: steventi
Shortcode: 199990
At the bottom, there is a URL <http://lsa.umich.edu/lsait>, a "Cancel" button, and an "Ok" button.

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



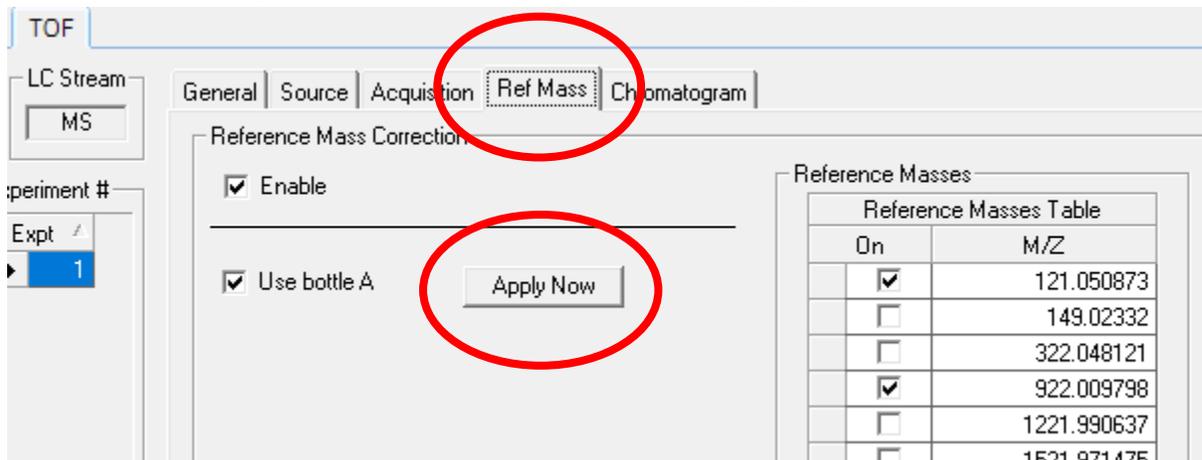
- Click on the tab near the bottom of the screen that is labeled “Method Editor”. Then select the method that you want to use from the drop-down menu. Generally, you will usually use the method called “Student_Small_Molecule_Pos” for relatively small (less than 1500 Da) organics in positive ion mode.



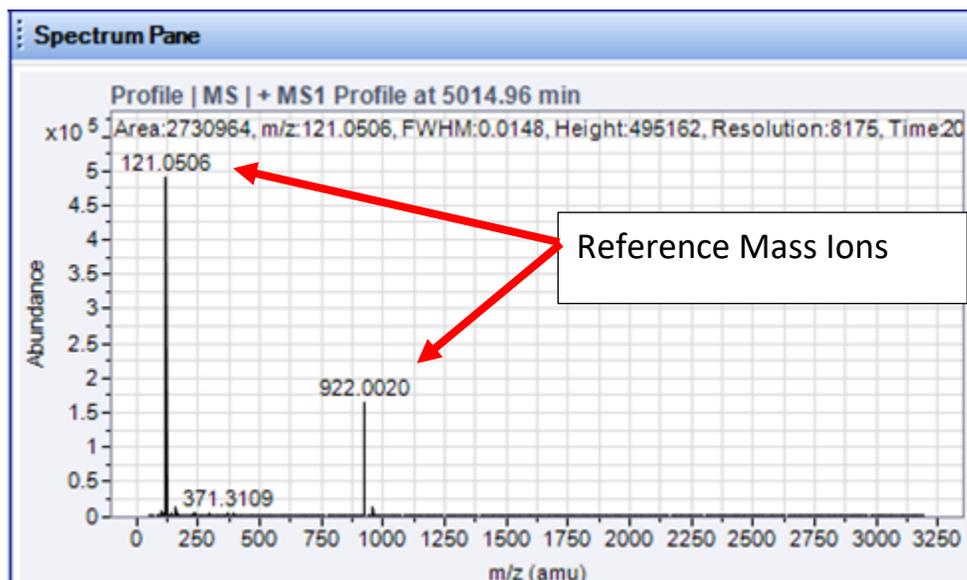
- After you have loaded your method, turn the system On, using the On button:



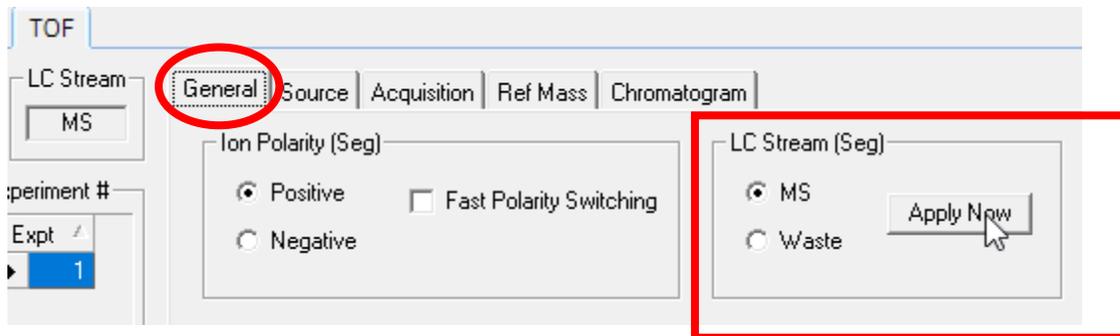
- Go to the method editor, click on the “TOF” tab, and then click on the “Ref Mass” tab. Near where it says “Use Bottle A”, click on the “Apply Now” button.



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.

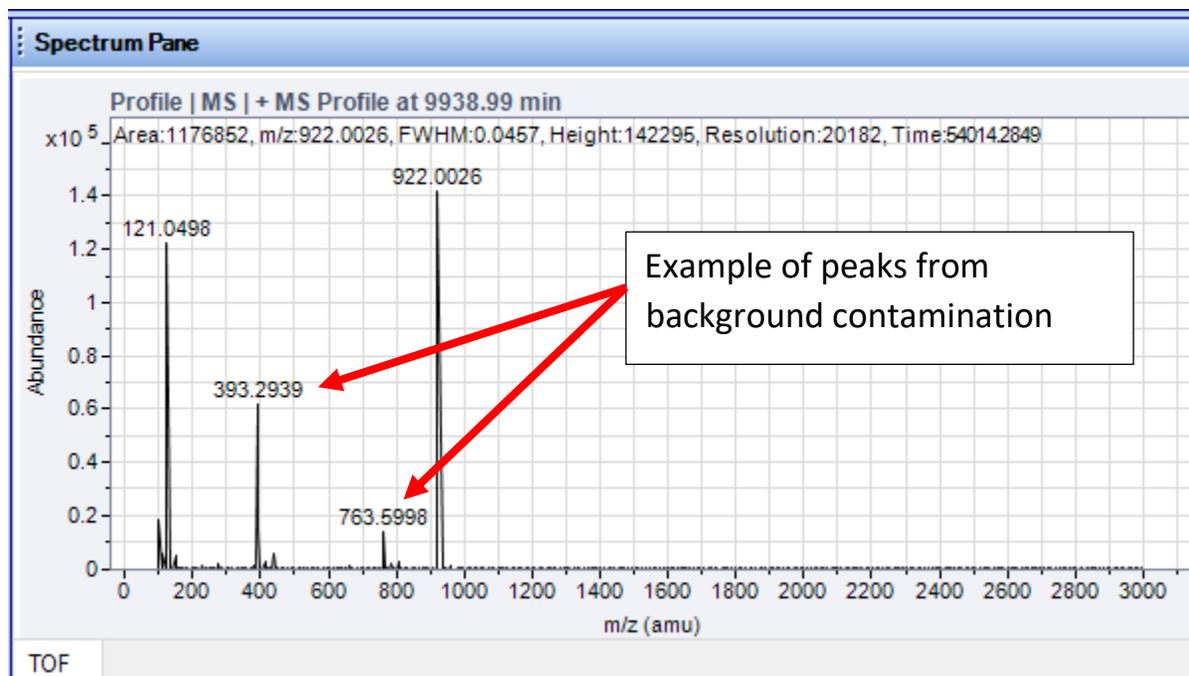


- On the method editor TOF tab, click on the General Tab. Click on “Apply Now” under “LC Stream to MS”.



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

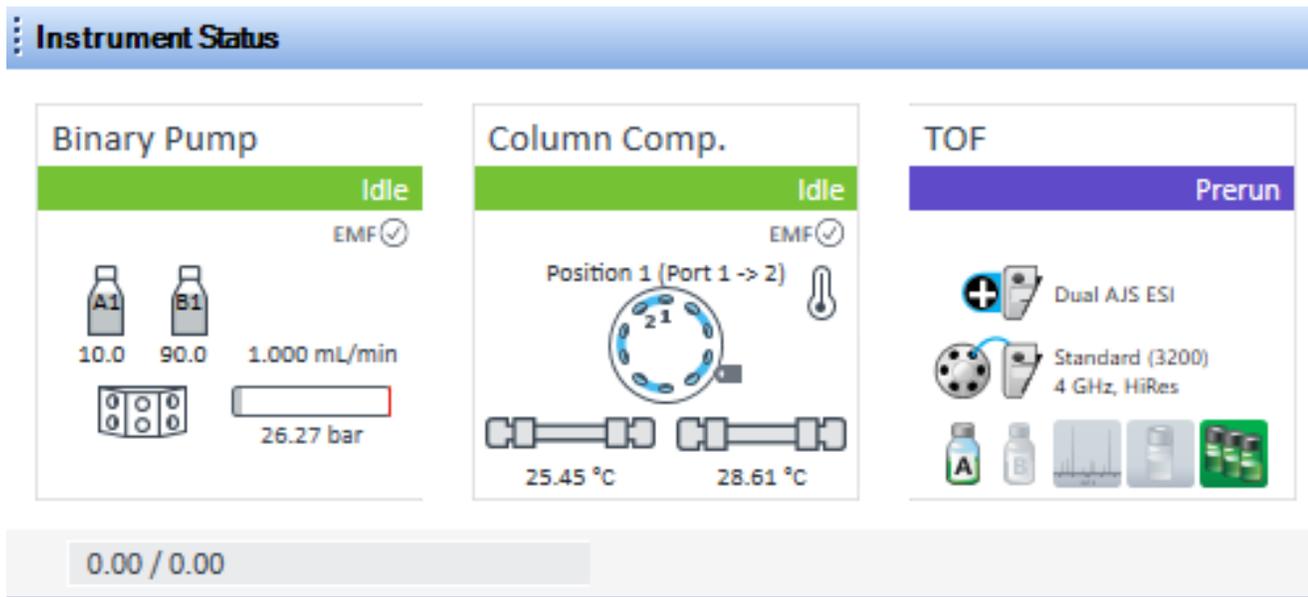
The screenshot shows the 'Instrument Status' panel with three main sections: Binary Pump, Column Comp., and TOF. Each section has a green status bar at the top indicating 'Idle' and 'EMF' (Electromagnetic Interference) is checked. The Binary Pump section shows two bottles labeled A1 and B1 with volumes of 10.0 and 90.0, a flow rate of 1.000 mL/min, and a pressure of 18.37 bar. The Column Comp. section shows a rotary valve in Position 1 (Port 1 -> 2) with temperatures of 25.50 °C and 28.72 °C. The TOF section shows 'Dual AJS ESI' and 'Standard (3200) 4 GHz, HiRes'.

← 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.

The screenshot shows the 'Sample Run' dialog box. At the top left, a box points to the 'Start run button' (a play icon). The 'Sample' section contains fields for Name (filled with 'reserpine'), Position (dropdown set to 'No Injection'), Injection Volume (dropdown set to 'As Instrument'), and a Comment field. The 'Data File' section has a checked 'Auto Increment' box, a Name field (filled with 'resp_neg_R5_2-20-2029.d'), and a Path field (filled with 'E:\Data\jwindak'). A box points to the Name field with the text 'Type filename here ↓'. Another box points to the Path field with the text 'Choose path to data here ↑'. At the bottom, a red circle highlights the 'Sample Run' tab in the navigation bar.

- 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:

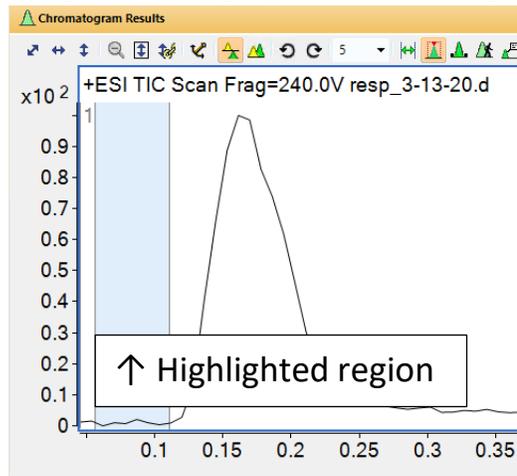


↓ Rotate handle down to start the run.

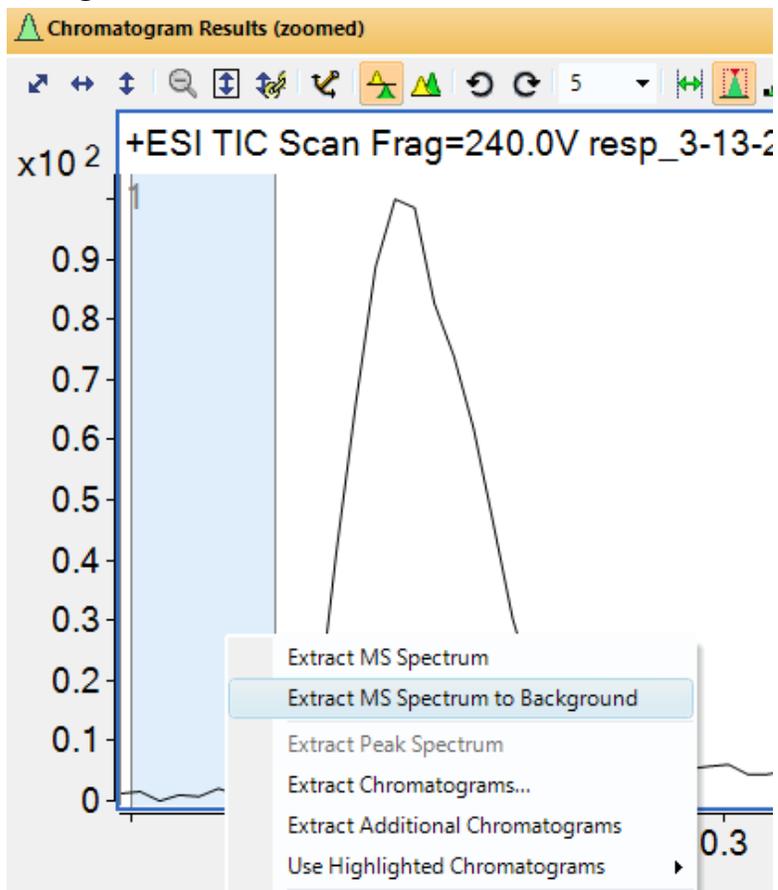
- Remove the syringe and solvent rinse the syringe.
- When the run is completed, you can process the data.

How to Process the Data

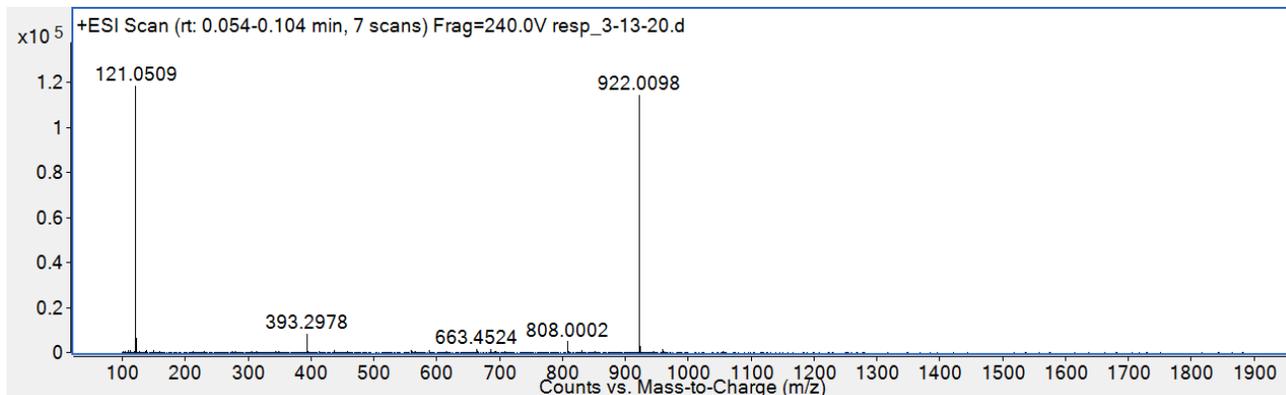
- Open the Qualitative analysis program (unless it is already open).
- Open your data file.
- Select a region of the chromatogram before the peak for a background spectrum. Do this by left-clicking and dragging across the region.



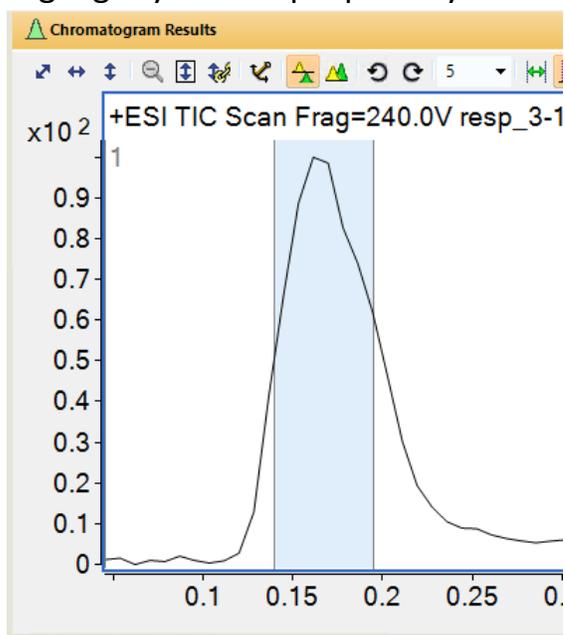
- Right-click in the region to obtain a menu. Left click on "Extract MS Spectrum to Background":



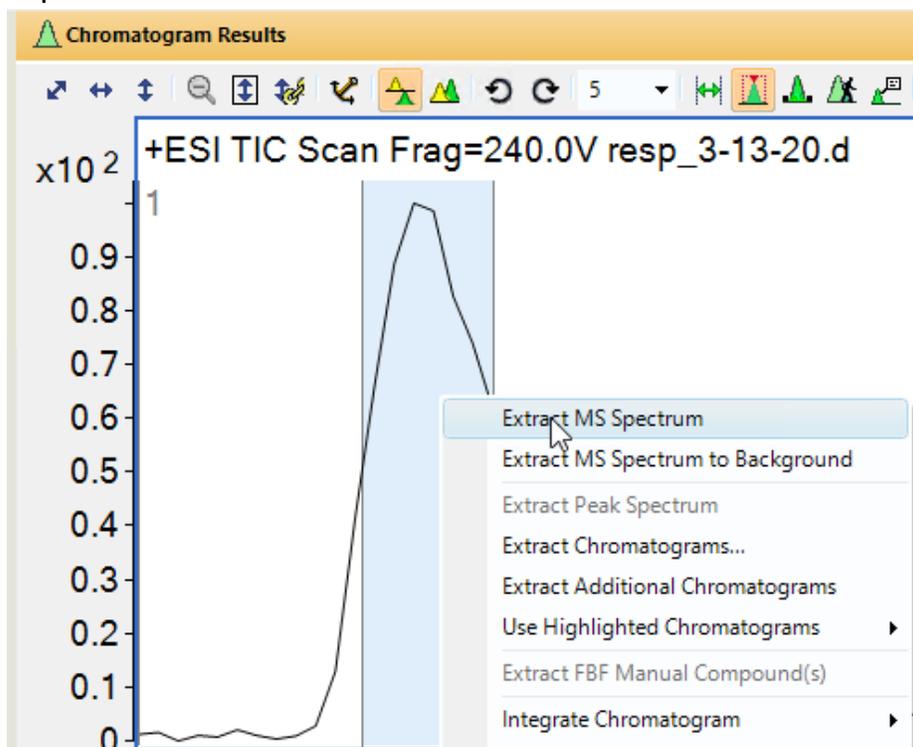
The background spectrum will have the two reference mass peaks present. They should have the correct exact mass values, which are 121.0509 and 922.0098



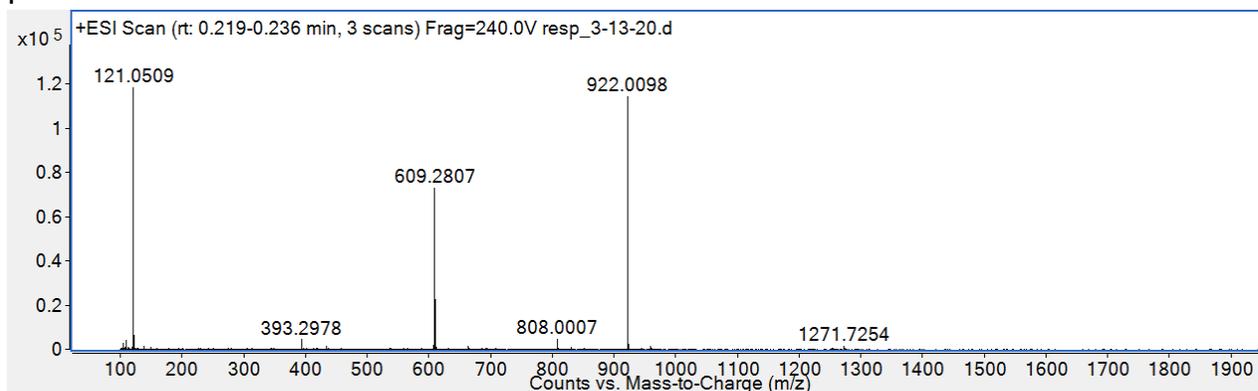
e. Highlight your sample peak by left-clicking and dragging across it.



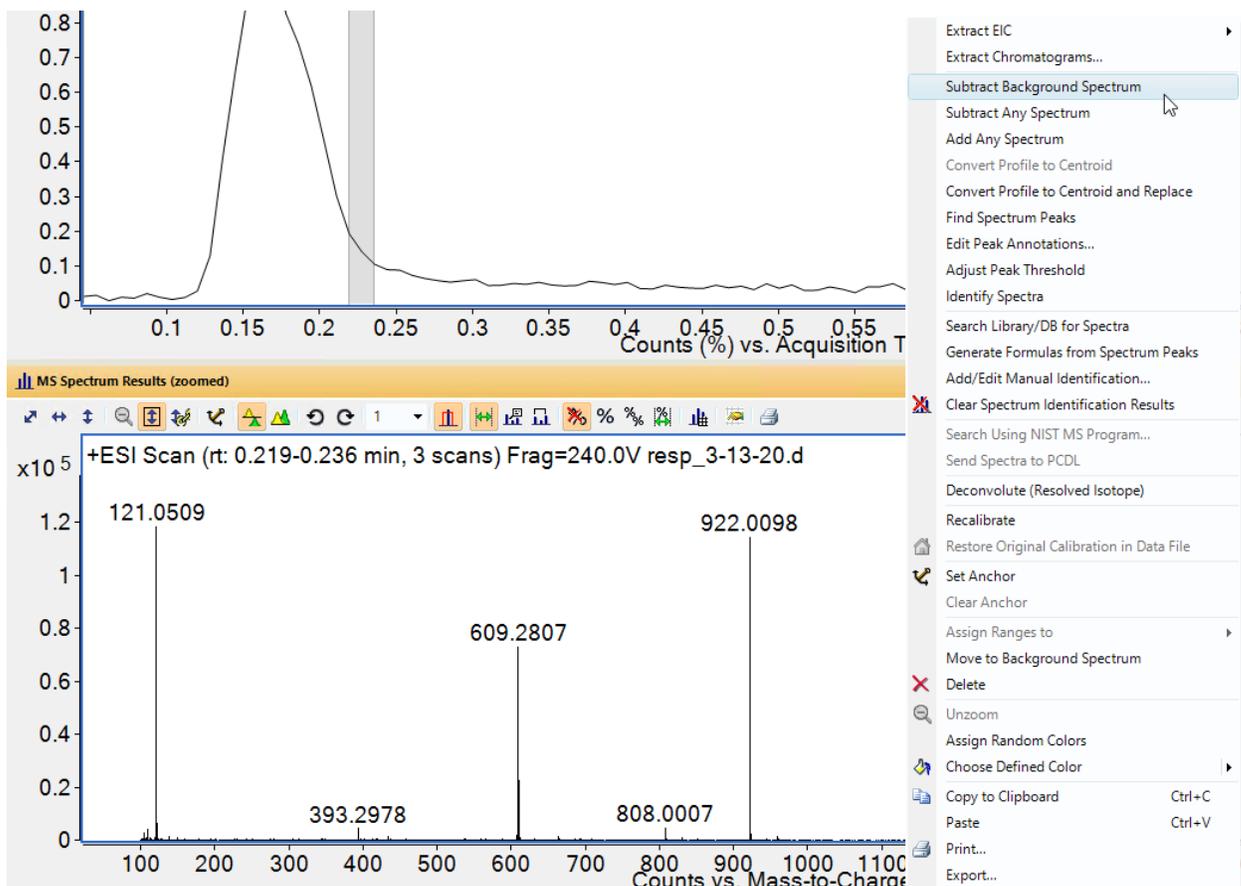
- f. Right-click on the selected region to obtain a menu. Left click “Extract MS Spectrum”:



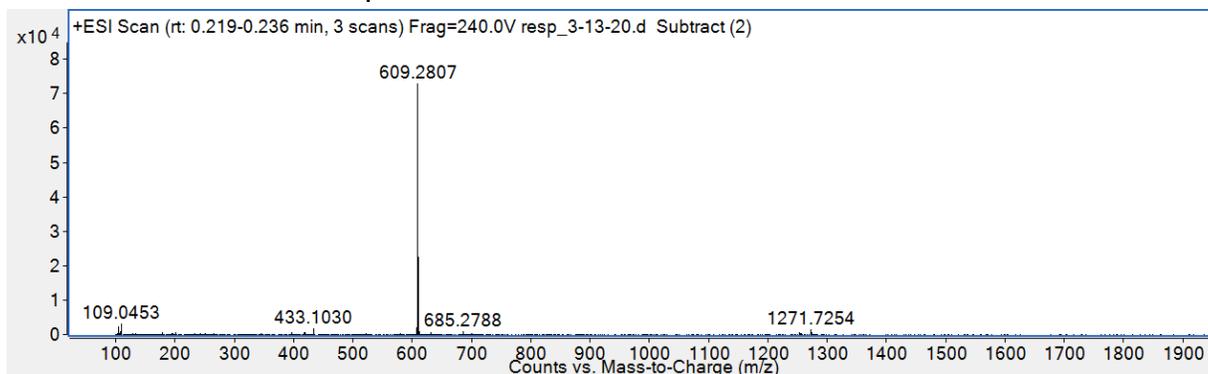
The mass spectrum will show your sample peaks as well as the reference mass peaks.



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



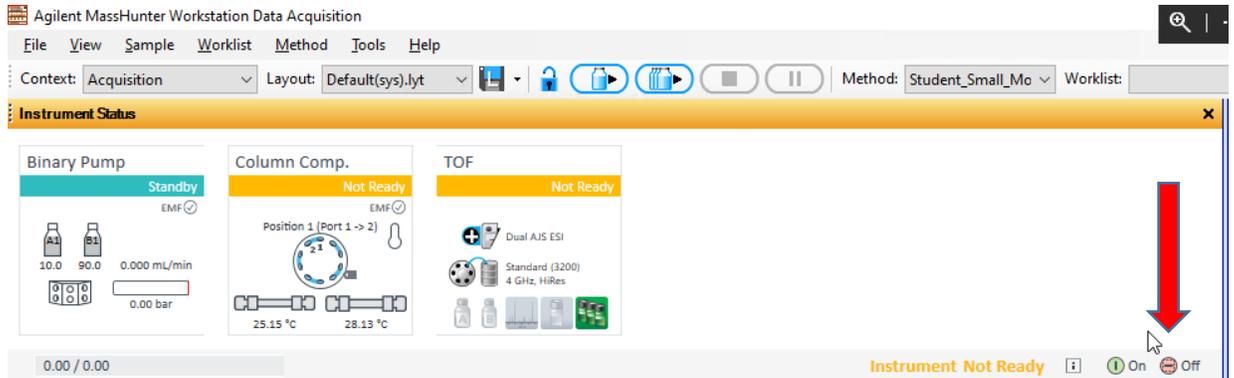
Here is the subtracted spectrum:



- 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 Yes.

