

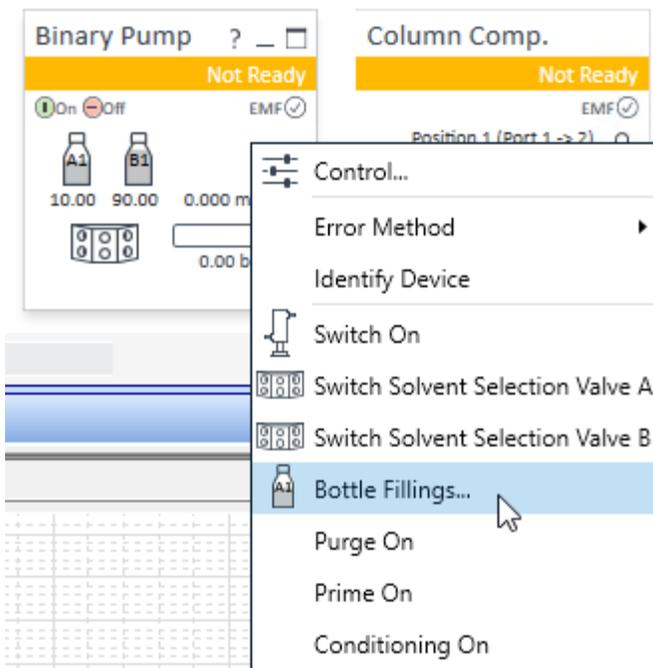
Q-TOF User's Booklet

- Check that there is enough solvent in the bottles.

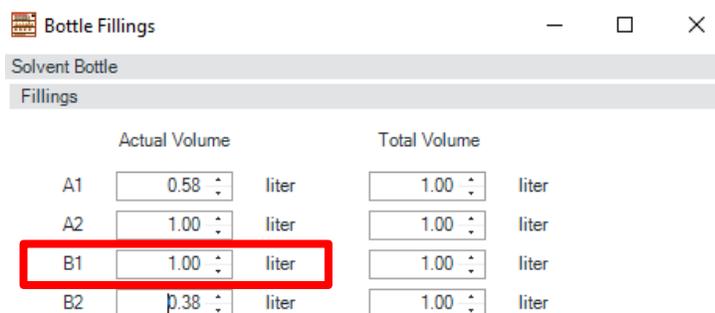


If the acetonitrile bottle is low on solvent, please replace the bottle with one of the spare full bottles of acetonitrile. Then update the solvent level in the software so that the data system will know how much solvent is available.

To do this (while the data acquisition software is running), right-click the Binary Pump status box, and you will see a menu. Click on “Bottle Fillings...”



You will see a dialog box. Set the volume for bottle B1 to 1.00 Liter, and click on Ok.



- Install your HPLC column

You can either use one of the columns on the instrument, or you can use your own column.



↑ The high pressure side, or front of the column, goes on the left side.

↓ The low pressure, or exit end of the column, goes on the right.

Screw the front of the column into the high-pressure fitting until you just feel the column bottom out. Do not over-tighten. Just light pressure. Then put the lever down.

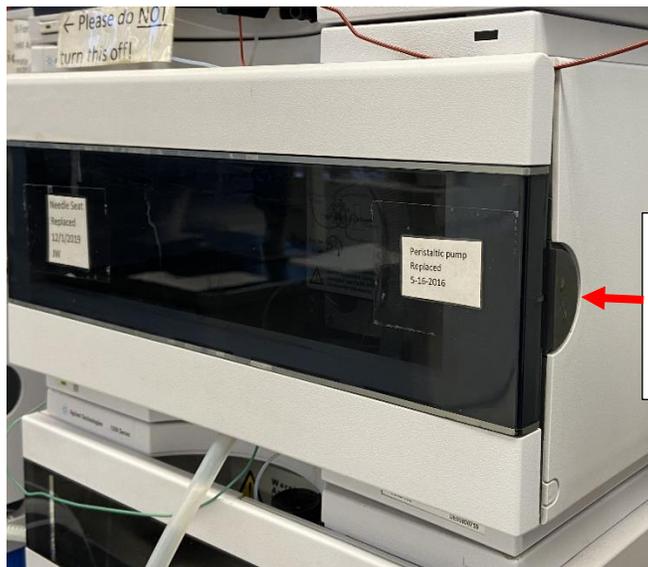


Then screw the plastic fitting into the exit end of the column (this fitting should be tight):



- Put your samples into the Autosampler

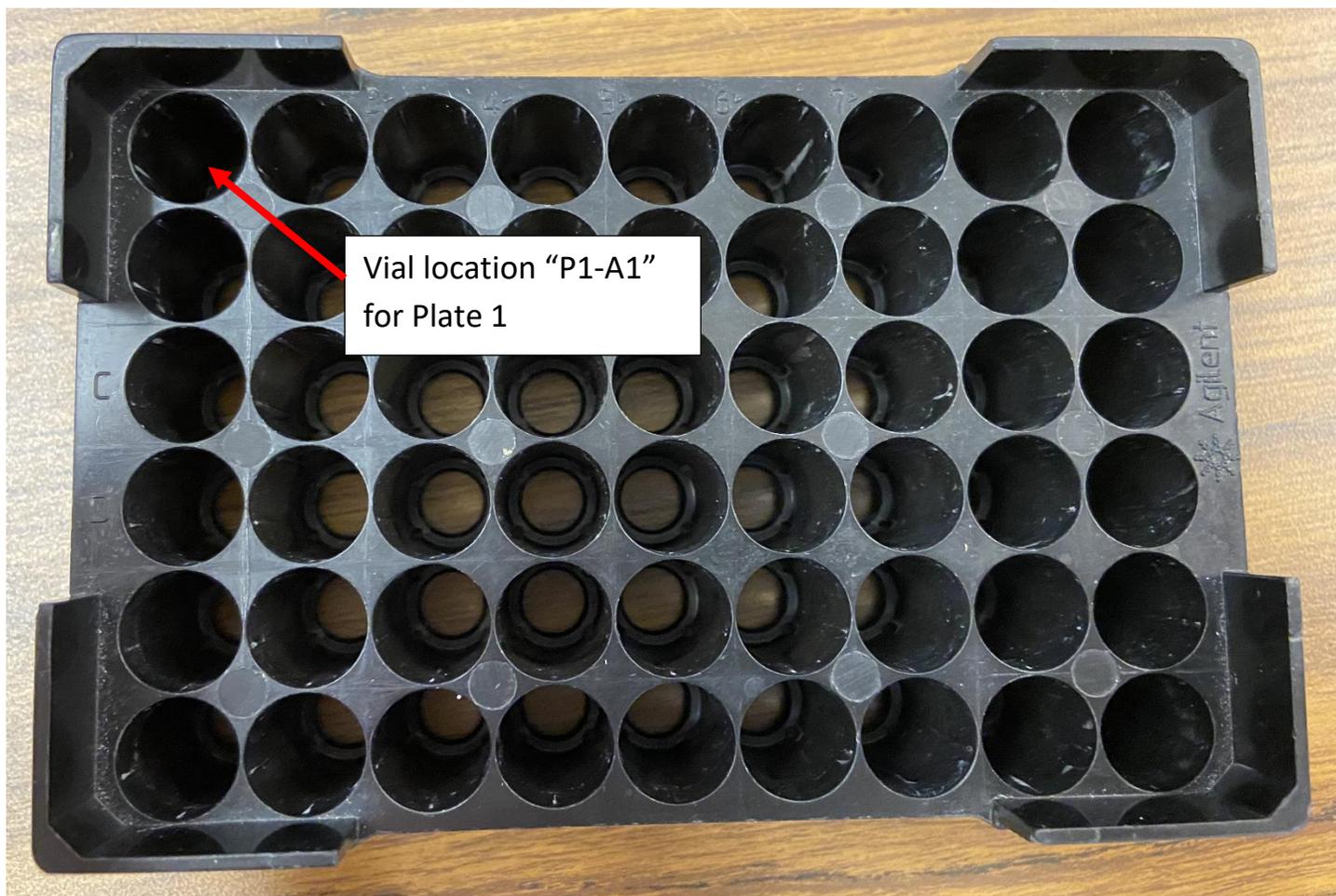
To open the autosampler door, press the button on the right side.



Push the button on the side of the autosampler to open the door.

The sample plate in front is called "P1" and the plate in back is called "P2".

Each plate has numbers 1 through 9 going across, and letters ABCDEF going down.



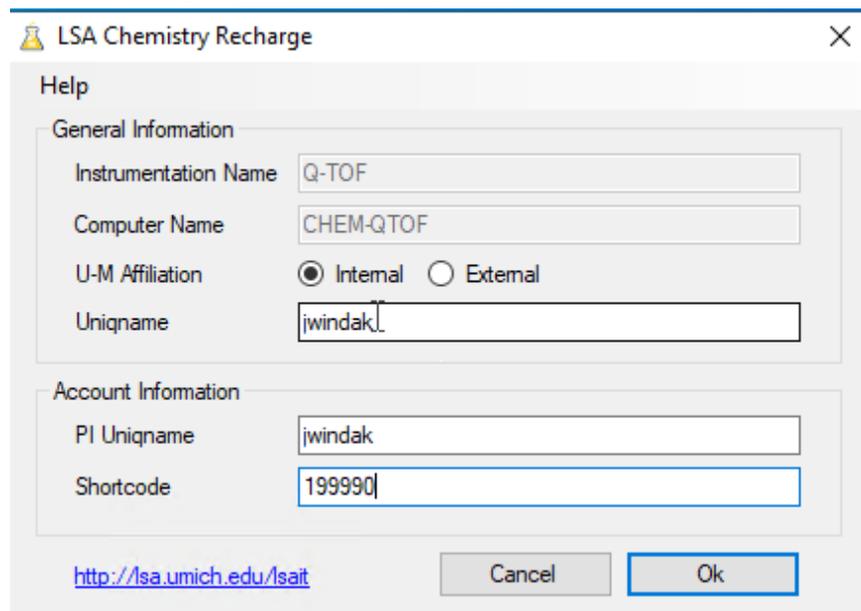
Please note that the autosampler door must be completely closed in order for the autosampler to work. If the door is not latched, the system will not run your samples.

- Log into the Software

Double-click the LSA Chemistry Recharge icon:



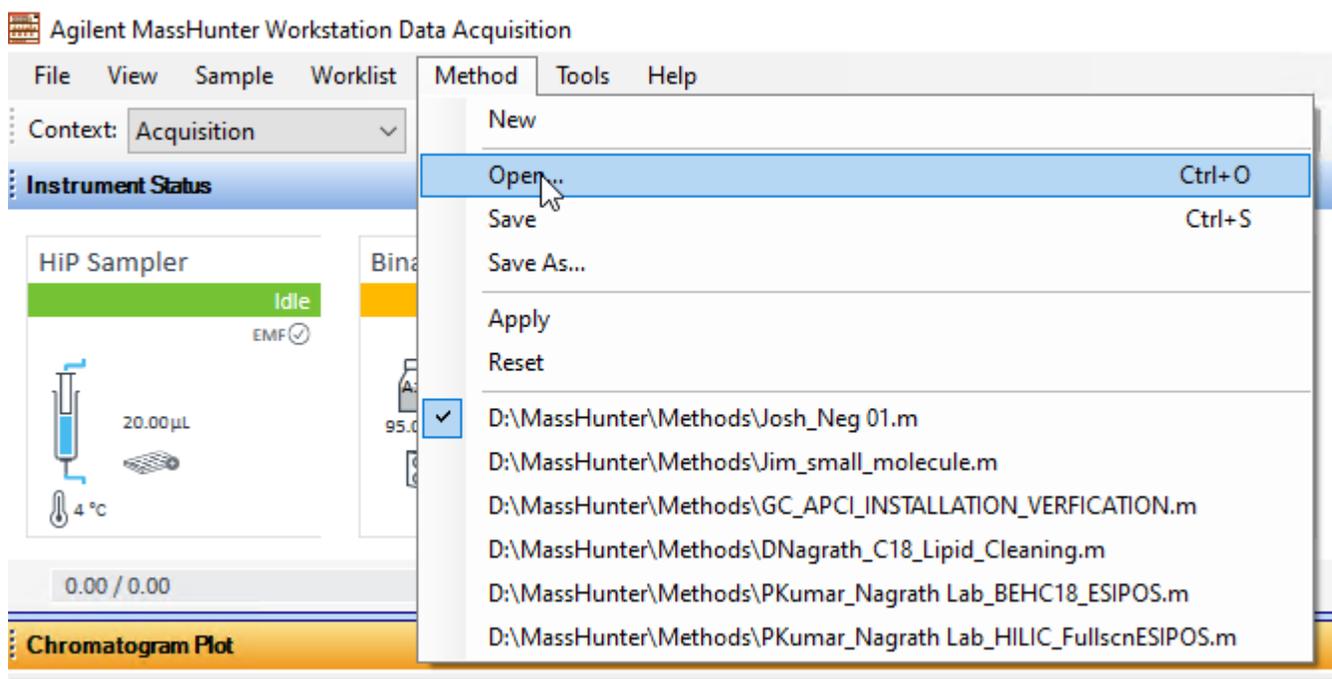
Then enter your username, your PI's username, and your short-code account number:

A dialog box titled 'LSA Chemistry Recharge' with a close button (X) in the top right corner. The dialog box is divided into two sections: 'General Information' and 'Account Information'.
In the 'General Information' section, there are four fields: 'Instrumentation Name' with the value 'Q-TOF', 'Computer Name' with the value 'CHEM-QTOF', 'U-M Affiliation' with radio buttons for 'Internal' (selected) and 'External', and 'Uniqname' with the value 'jwindak'.
In the 'Account Information' section, there are two fields: 'PI Uniqname' with the value 'jwindak' and 'Shortcode' with the value '199990'.
At the bottom left, there is a URL: <http://lsa.umich.edu/lsait>. At the bottom right, there are two buttons: 'Cancel' and 'Ok'.

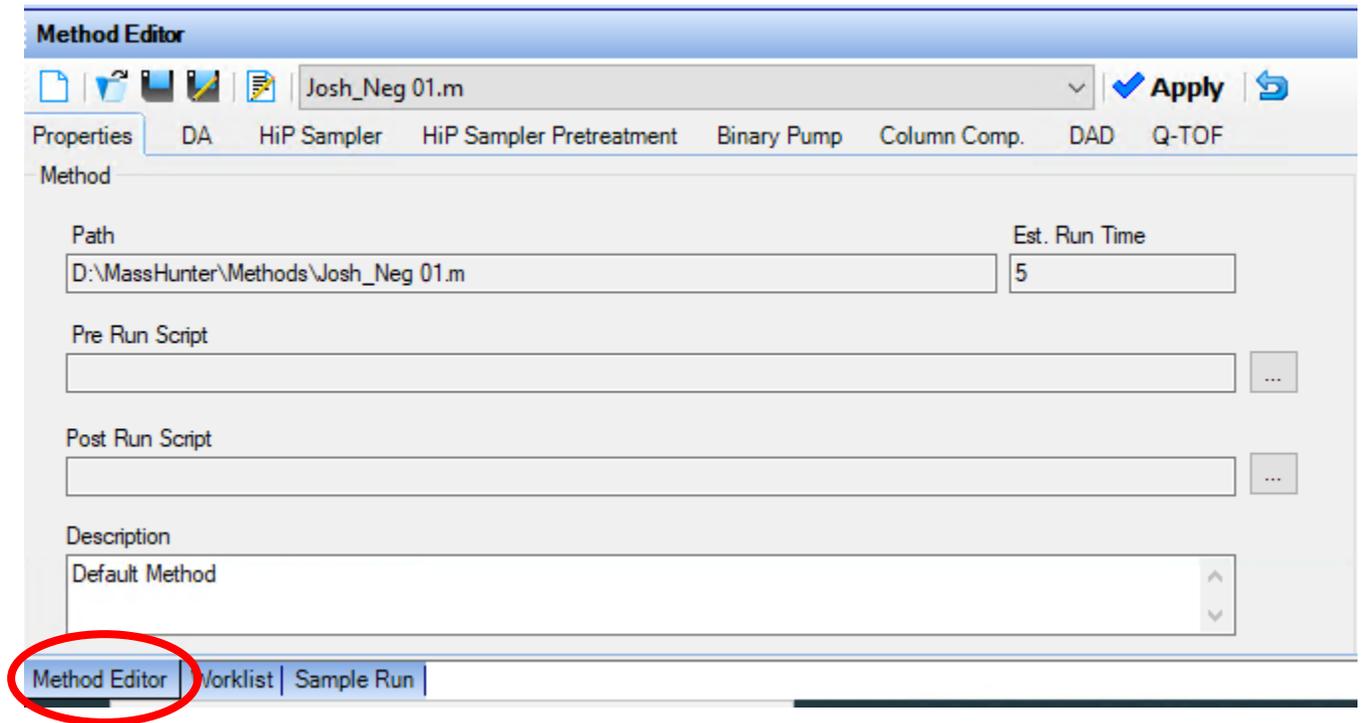
The MassHunter Data Acquisition software will then automatically come up:



After the software comes up, the first step is to open the method that you want to use. Do this by clicking on the Method menu, and then click on Open:

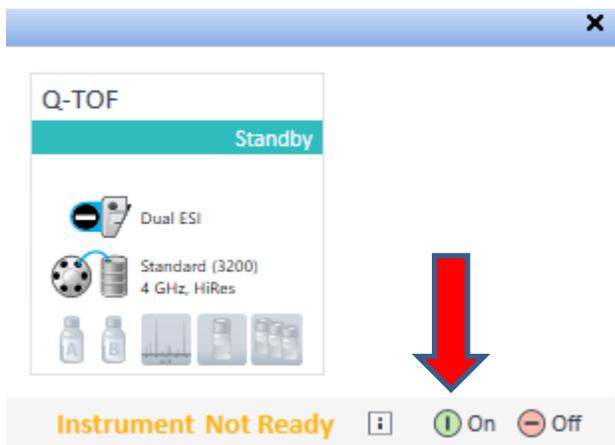


If you want to check what parameters are in your method, or if you want to change anything in your method, click on the “Method Editor” tab near the bottom left side of the screen:

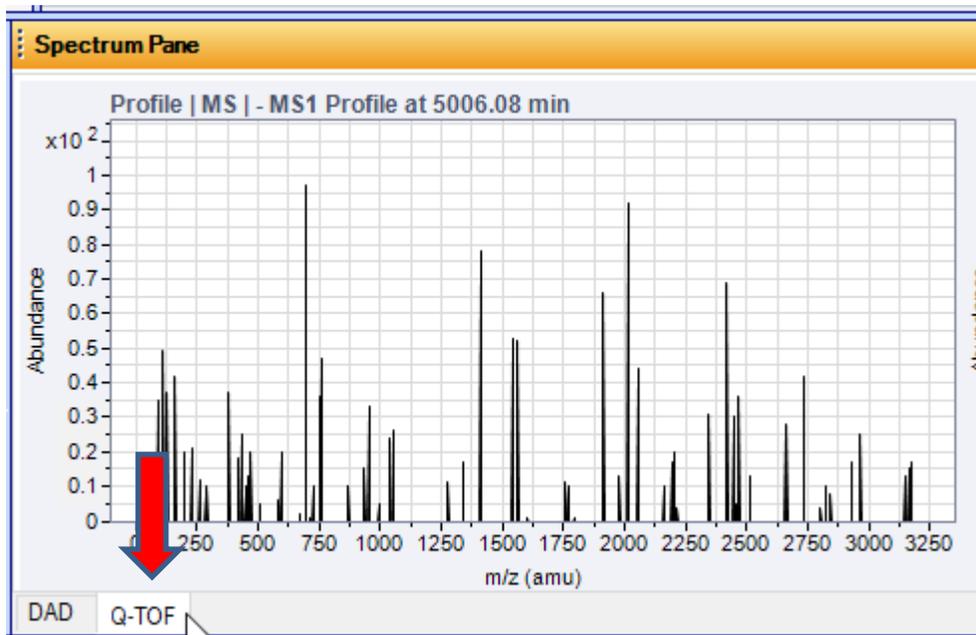


In the method editor, there are tabs for each piece of hardware in the system: The Autosampler, the Binary Pump, the Column Compartment, the UV-VIS Diode Array Detector (DAD), and the Q-TOF.

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

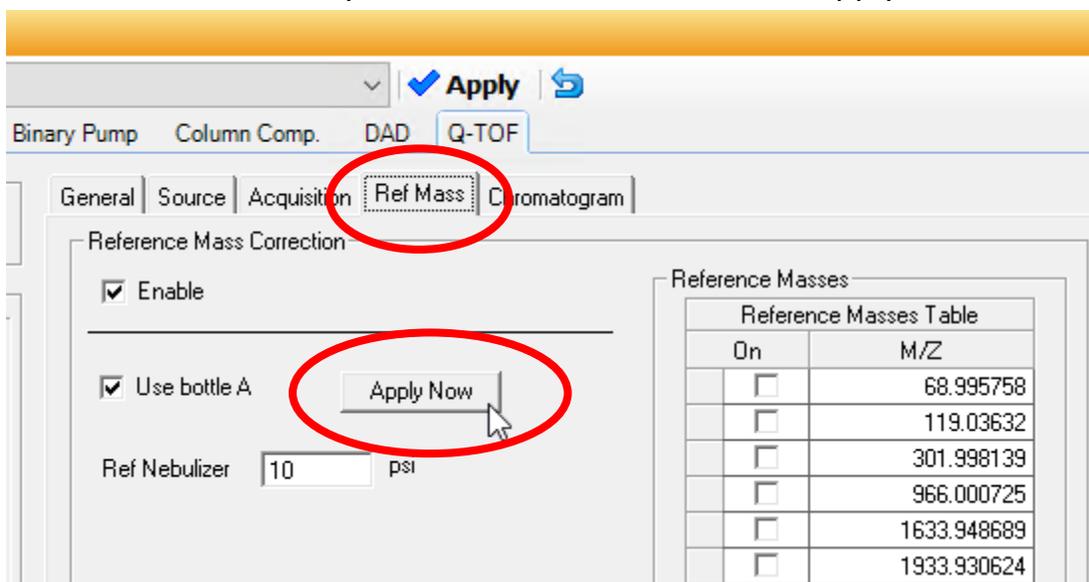


In the mass spectrum window, click on the tab that is labeled “Q-TOF”:

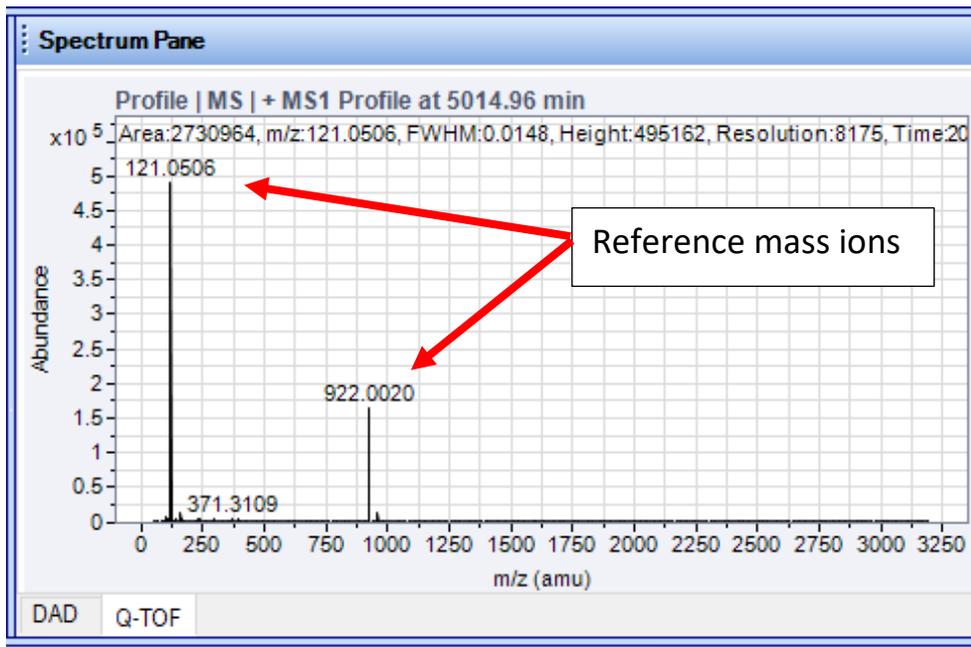


This will show the mass spectra in the window as they are collected in real time.

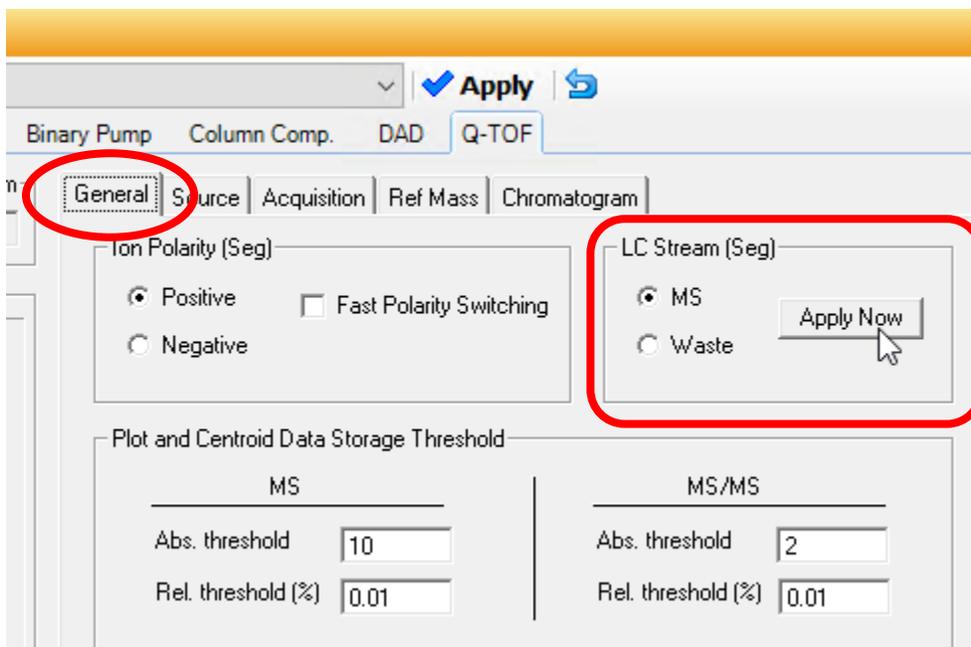
In the method editor, click on the “Q-TOF” tab, and then click on the “Ref Mass” tab. Then, near where it says “Use Bottle A”, click on the “Apply Now” button.



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.

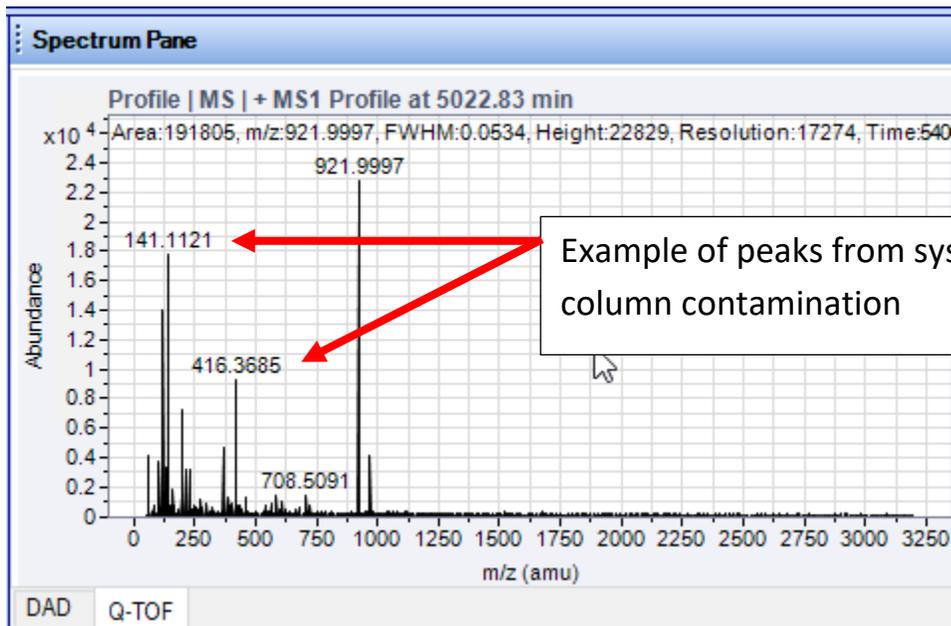


Next, under the Q-TOF tab, click on the “General” tab. Where it says LC Stream to MS, click on “Apply Now”



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 or on the HPLC column, or in the solvents.



- Set Up Your Sample Worklist

Now the system is ready to run your samples. To run a batch of samples, you will need to set up a Worklist. Click on the “Worklist” tab near the bottom left side of the screen:

	<input checked="" type="checkbox"/>	Sample Name	Sample Position
1	<input checked="" type="checkbox"/>	ARA-4I-pdt	P1-B5
2	<input checked="" type="checkbox"/>	ARA-4-COOH-pdt	P1-B6
3	<input checked="" type="checkbox"/>	ARA-4CF3-aMeBMe-p	P1-B7
4	<input checked="" type="checkbox"/>	ARA-4-Ph-pdt	P1-B8
5	<input checked="" type="checkbox"/>	ARA-4OCF3-pdt	P1-B9
6	<input checked="" type="checkbox"/>	ARA-4CF3-NH2	P1-C1

Method Editor **Worklist** Sample Run

To set up the worklist, you will need to enter one row for each sample that you want to run. In each row, you need to specify the Sample Position, which is where you placed the sample in the autosampler.

	✓	Sample Name	Sample Position
1	✓	ARA-4I-pdt	P1-B5
2	✓	ARA-4-COOH-pdt	P1-B6
3	✓	ARA-4CF3-aMeBMe-p	P1-B7
4	✓	ARA-4-Ph-pdt	P1-B8
5	✓	ARA-4OCF3-pdt	P1-B9
6	✓	ARA-4CF3-NH2	P1-C1

You will also need to specify a Method to use for running the sample. To choose your method, click on the cell for method, and a drop-down menu will appear letting you choose a method from the methods folder. If your method is located elsewhere, you can scroll down to the bottom of the list and click on “Other...”, and it will let you browse to wherever your method is located.

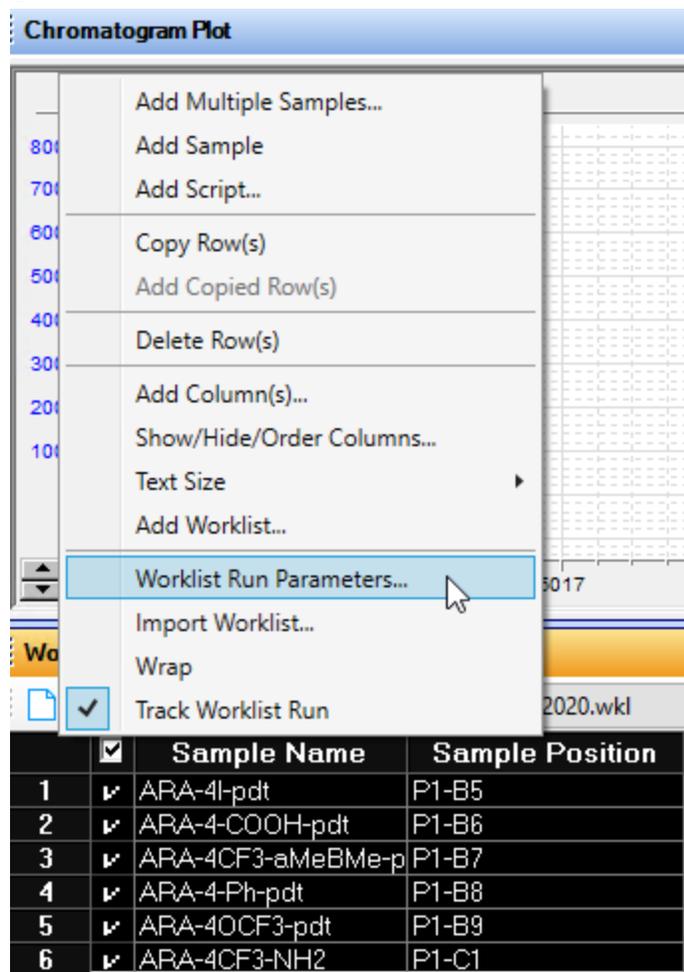
	✓	Sample Name	Sample Position	Method
1	✓	ARA-4I-pdt	P1-B5	Jim_small_molecule.m
2	✓	ARA-4-COOH-pdt	P1-B6	Zack positive method test for dis
3	✓	ARA-4CF3-aMeBMe-p	P1-B7	Zajda_leaching_neg.m
4	✓	ARA-4-Ph-pdt	P1-B8	Zajda_leaching_pos.m
5	✓	ARA-4OCF3-pdt	P1-B9	zEDS_Intact_Protein.m
6	✓	ARA-4CF3-NH2	P1-C1	zILO_Intact_Protein.m
				zLD_Intact_Protein.m
				z_CAP_Intact_Protein.m
				Other...

You will also need to enter a data file name. If you click on the Data File cell, you will see an icon that looks like , and if you click on it, it will let you browse to wherever you would like to put the data.

Data File	Inj Vol (µl)
D:\MassHunter\Data\USERS\Jim\Anthony Robert Allen\ARA-4I-pdt_ESI_3-19-2020.d	1
D:\MassHunter\Data\USERS\Jim\Anthony Robert Allen\ARA-4-COOH-pdt_ESI_3-19-2020.d	0.2
D:\MassHunter\Data\USERS\Jim\Anthony Robert Allen\ARA-4CF3-aMeBMe-pdt_ESI_3-19-2020.d	0.2
D:\MassHunter\Data\USERS\Jim\Anthony Robert Allen\ARA-4Ph-pdt_ESI_3-19-2020.d	0.2
D:\MassHunter\Data\USERS\Jim\Anthony Robert Allen\ARA-4OCF3-pdt_ESI_3-19-2020.d	0.2
D:\MassHunter\Data\USERS\Jim\Anthony Robert Allen\ARA-4CF3-NH2_ESI_3-19-2020.d	0.2

You can also specify an injection volume for each sample. The allowable range is from 0.1 ul to 20 ul. If you do not specify an injection volume, the system will use the default value that is in your method.

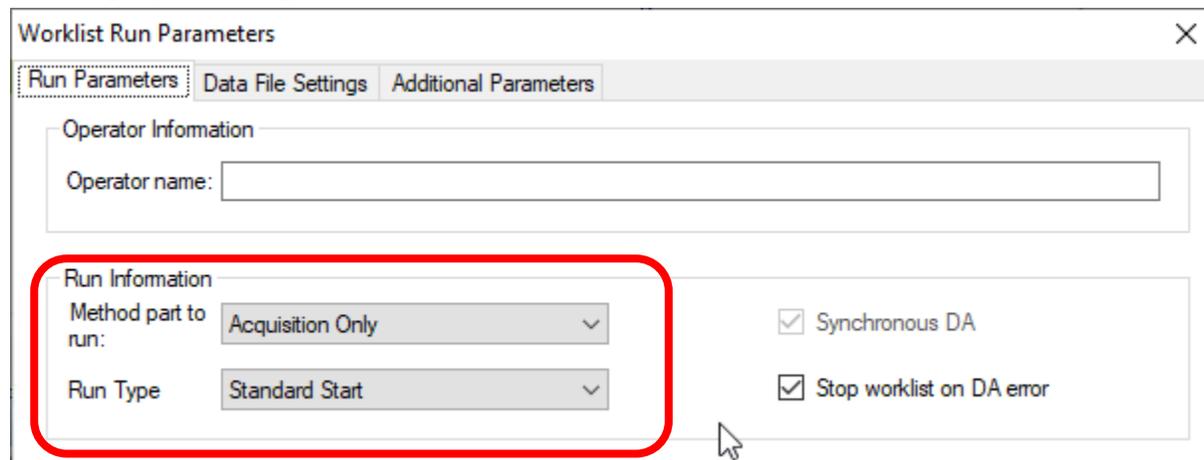
After the worklist table is filled out, Right-click on the upper right corner of the table to get a menu. Click on Worklist Run Parameters...



The screenshot shows a software interface with a 'Chromatogram Plot' window. A context menu is open over a table, with 'Worklist Run Parameters...' selected. The table below is as follows:

	<input checked="" type="checkbox"/>	Sample Name	Sample Position
1	<input checked="" type="checkbox"/>	APA-4I-pdt	P1-B5
2	<input checked="" type="checkbox"/>	APA-4-COOH-pdt	P1-B6
3	<input checked="" type="checkbox"/>	APA-4CF3-aMeBMe-p	P1-B7
4	<input checked="" type="checkbox"/>	APA-4-Ph-pdt	P1-B8
5	<input checked="" type="checkbox"/>	APA-4OCF3-pdt	P1-B9
6	<input checked="" type="checkbox"/>	APA-4CF3-NH2	P1-C1

This will bring up a dialog box:



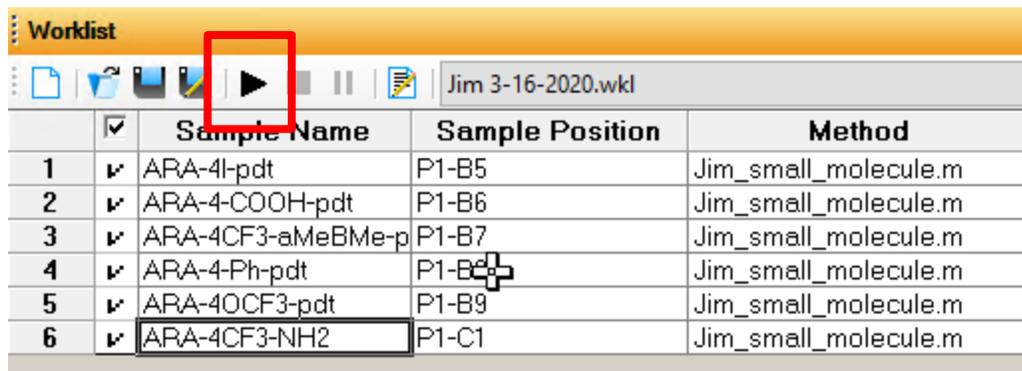
The 'Worklist Run Parameters' dialog box is shown with three tabs: 'Run Parameters', 'Data File Settings', and 'Additional Parameters'. The 'Run Parameters' tab is active. The 'Operator Information' section has an 'Operator name' field. The 'Run Information' section is highlighted with a red box and contains:

- 'Method part to run:' dropdown menu set to 'Acquisition Only'
- 'Run Type' dropdown menu set to 'Standard Start'
- Checkboxes for 'Synchronous DA' and 'Stop worklist on DA error', both of which are checked.

Make sure that "Method part to run:" is set to "Acquisition Only", and "Run Type" is set to "Standard Start".

After your Worklist is set up, you can save your worklist if you wish, by clicking on the Worklist menu and clicking on Save As.

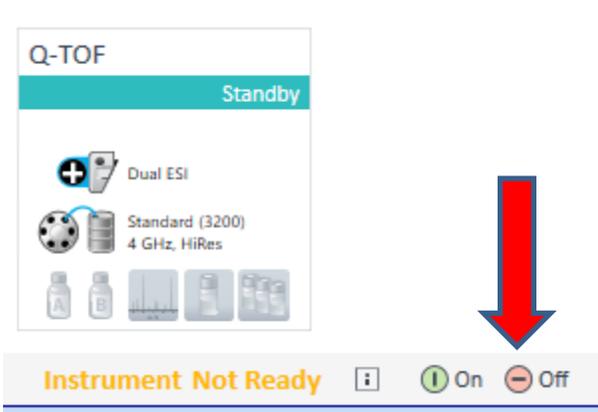
The worklist can be started by clicking on the  icon:



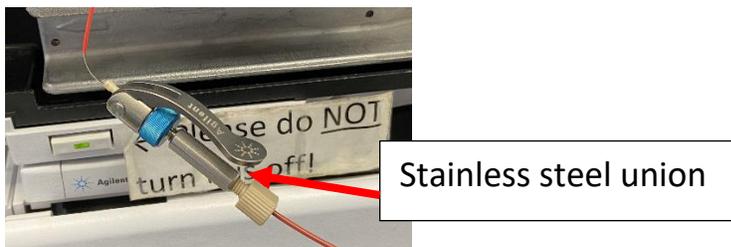
	<input checked="" type="checkbox"/>	Sample Name	Sample Position	Method
1	<input checked="" type="checkbox"/>	ARA-4I-pdt	P1-B5	Jim_small_molecule.m
2	<input checked="" type="checkbox"/>	ARA-4-COOH-pdt	P1-B6	Jim_small_molecule.m
3	<input checked="" type="checkbox"/>	ARA-4CF3-aMeBMe-p	P1-B7	Jim_small_molecule.m
4	<input checked="" type="checkbox"/>	ARA-4-Ph-pdt	P1-B8	Jim_small_molecule.m
5	<input checked="" type="checkbox"/>	ARA-4OCF3-pdt	P1-B9	Jim_small_molecule.m
6	<input checked="" type="checkbox"/>	ARA-4CF3-NH2	P1-C1	Jim_small_molecule.m

- When the samples have finished running, you can shut down the system

To shut the system down, first put the instrument in Standby by clicking on the Off button:



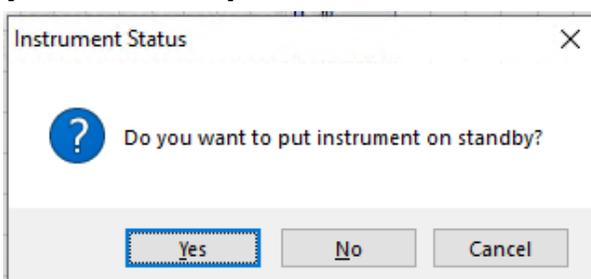
Next, remove your HPLC column from the column compartment, and in its place, install the stainless steel union.



Remove your samples from the sample compartment.

Lastly, 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.



How to Process Data with the Qualitative Analysis Software

To launch the data processing software, click on the  icon at the bottom of the screen.

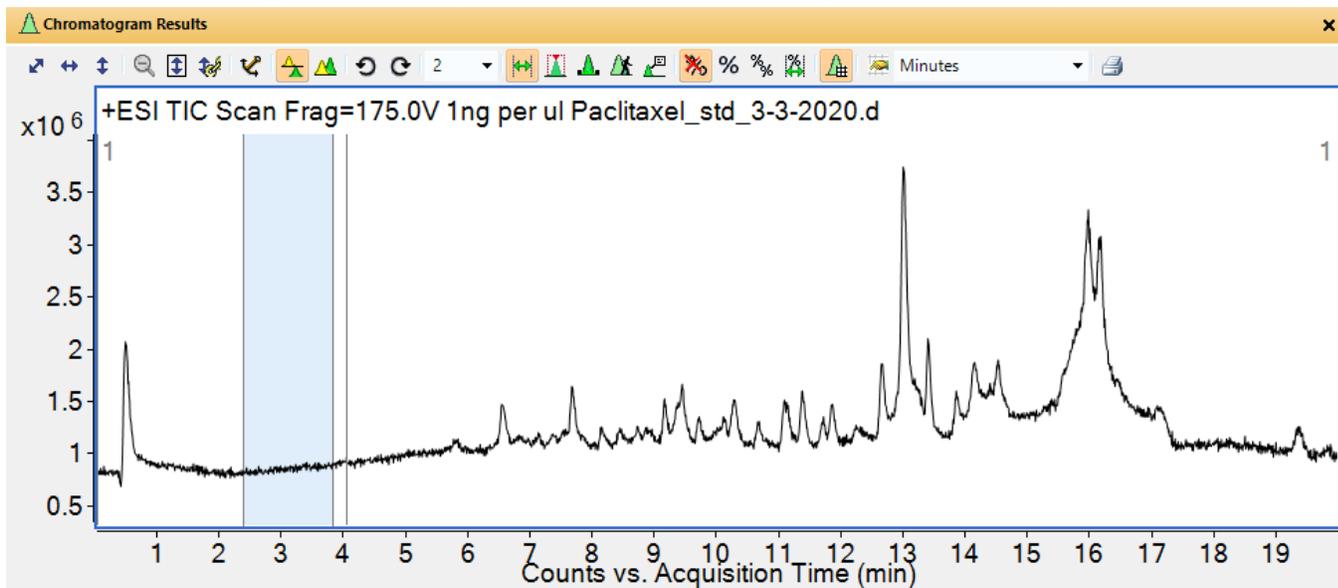
The Qualitative Analysis program will come up:



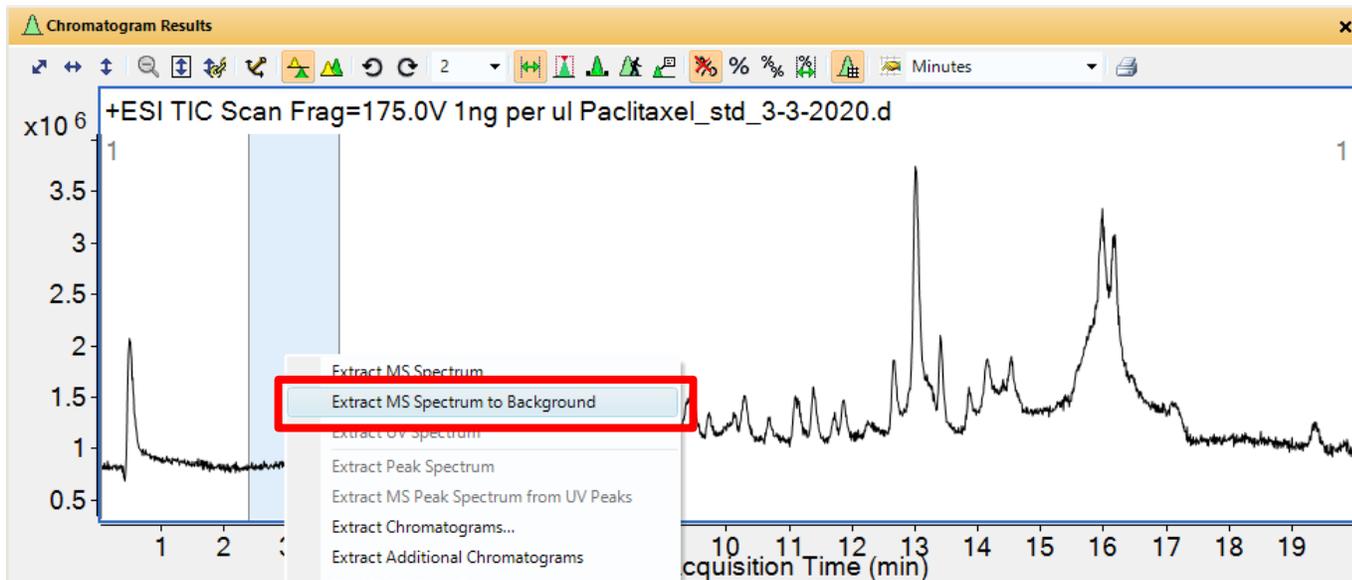
After the program launches, it will ask you to open a data file. If the program was already running, you can click on File and Open to open a data file, or you can click on the  icon near the upper left side of the screen.

After your data file has been opened, you will see a chromatogram.

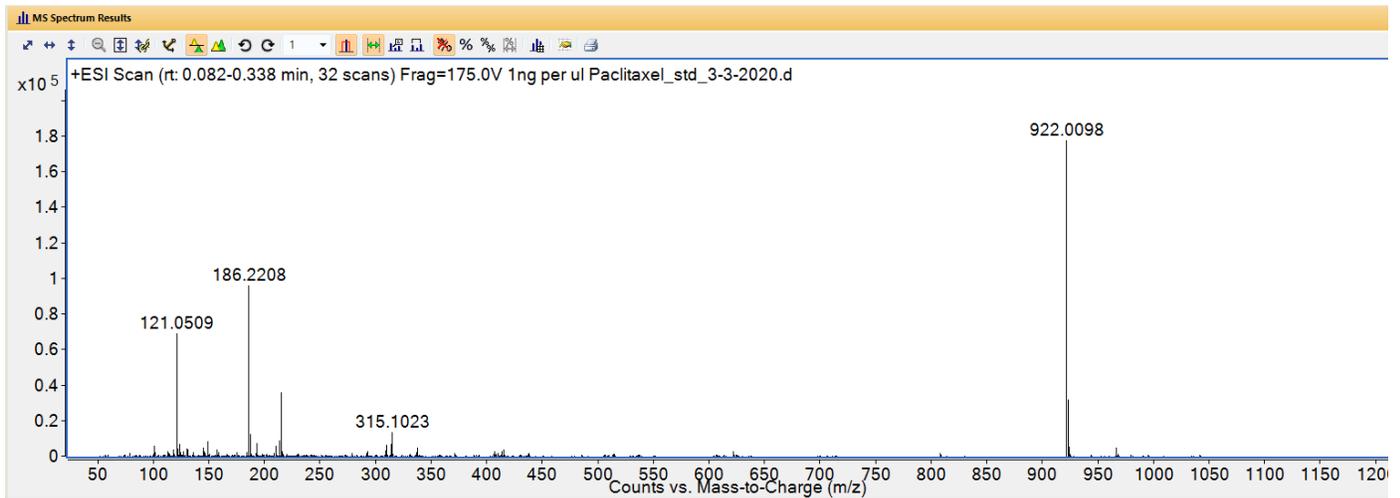
Left-click and drag to select a portion of the chromatogram where no peaks are eluting.



Then right-click in that region, and you will see a menu. Click on “Extract MS Spectrum to Background”

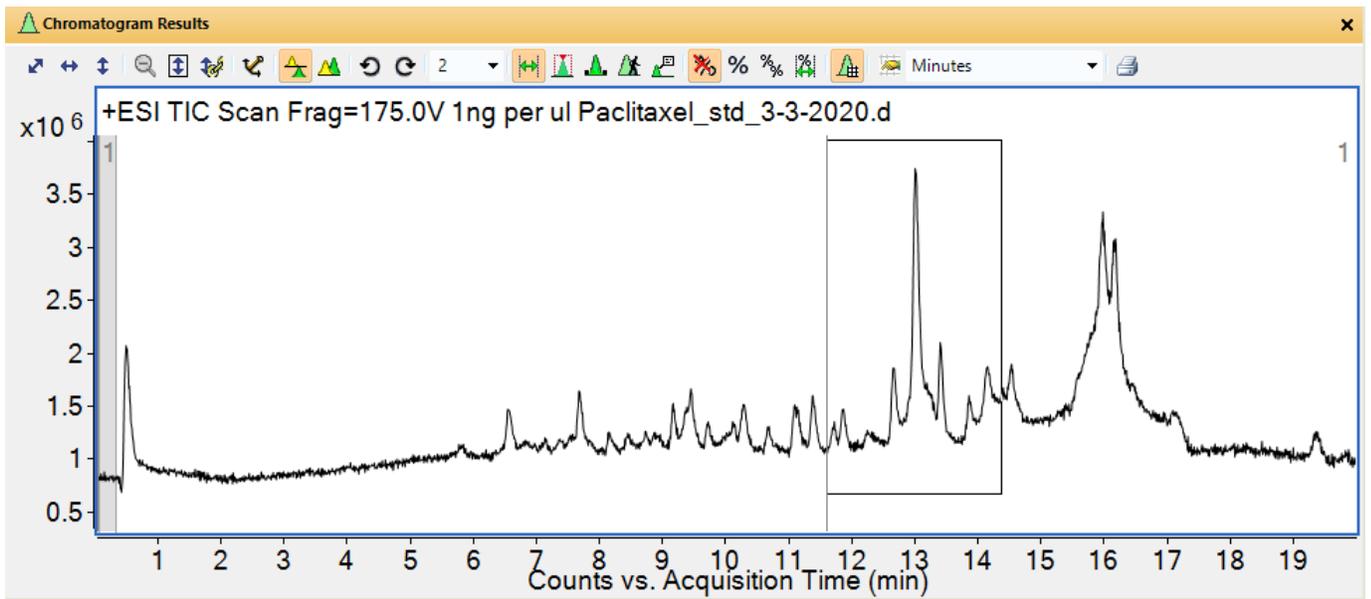


This will display and define a background spectrum:

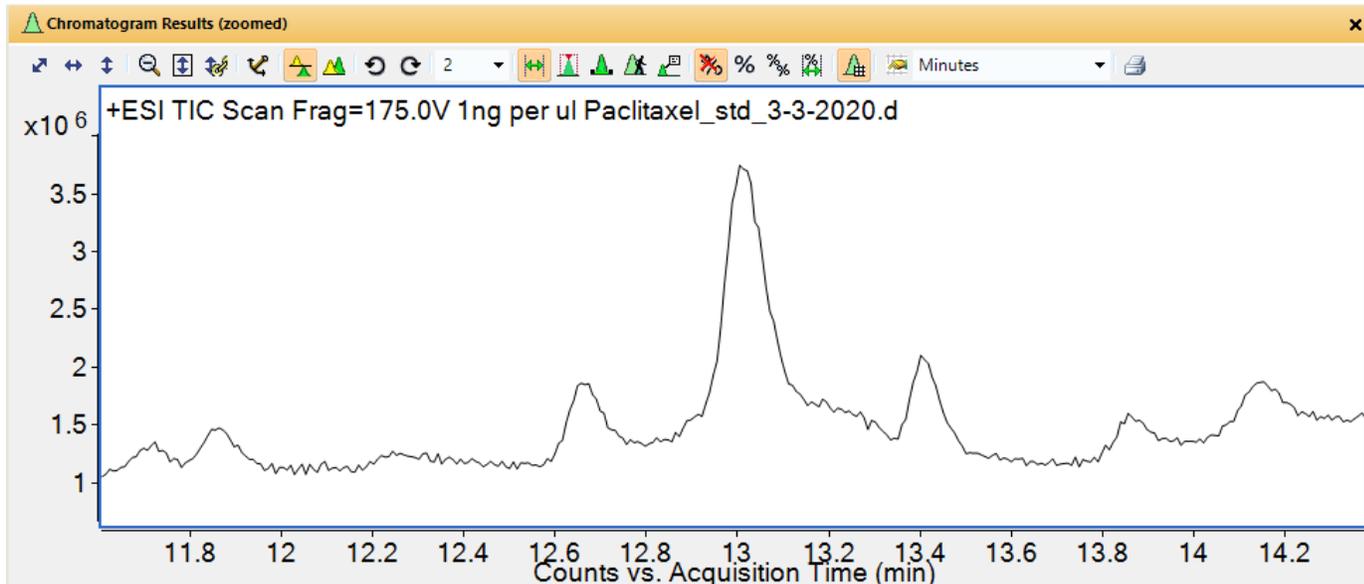


You should see the reference mass ions in the background spectrum, and they should be the correct exact mass. In positive ion mode, the exact m/z values are 121.0509 and 922.0098. In negative ion mode, the exact m/z values are 112.9856 and 1033.9881.

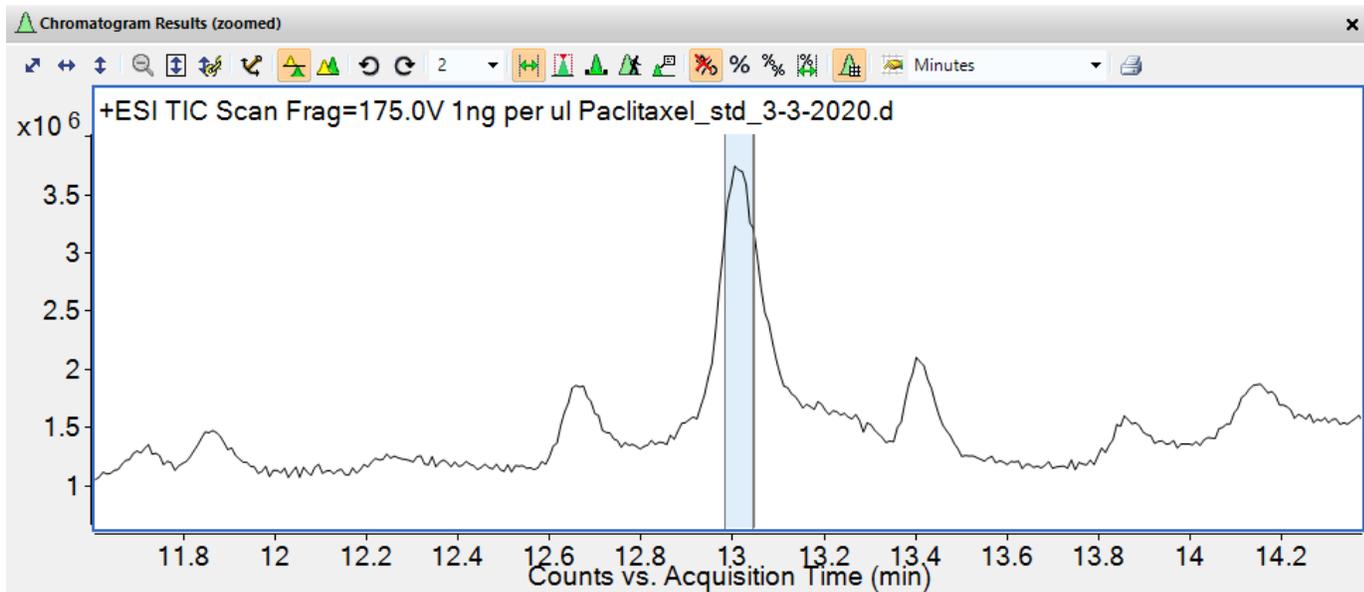
If you want to zoom into a portion of the chromatogram, you can hold down the **right** mouse button and drag a box:



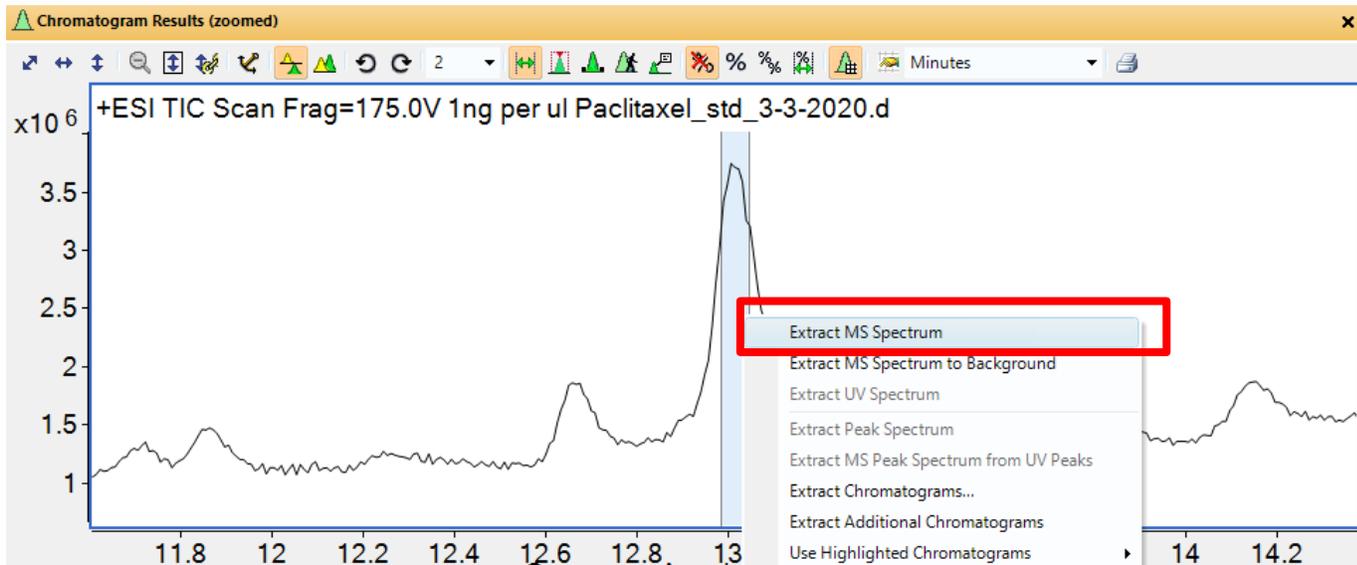
When you release the right mouse button, the chromatogram will zoom into the area of the box:



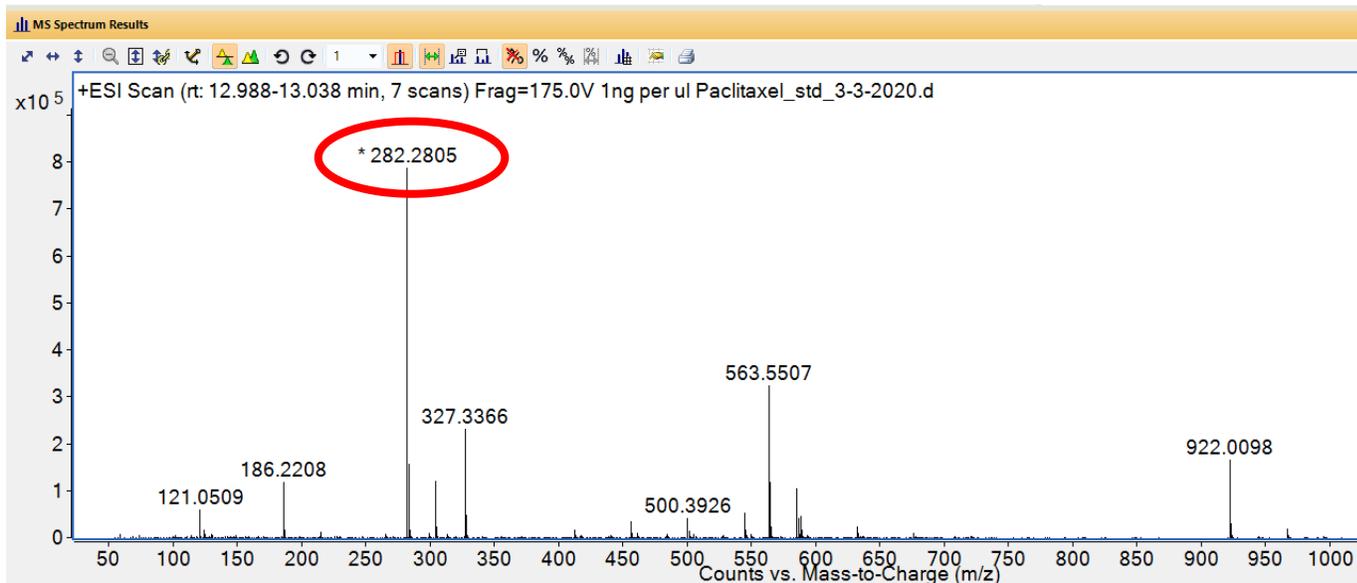
You could then select a peak, by left-clicking and dragging



If you right-click on the spectrum, you will see a menu. Click on Extract MS spectrum:

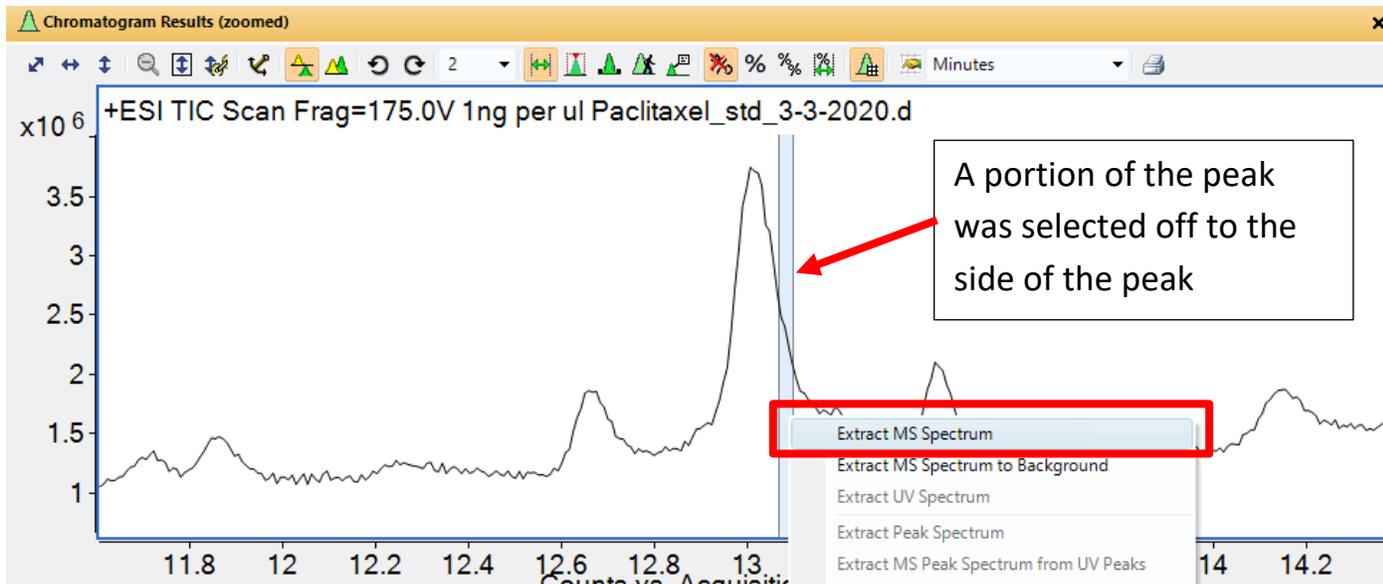


You will see the mass spectrum. Notice that there is an asterisk next to m/z 282.2805.

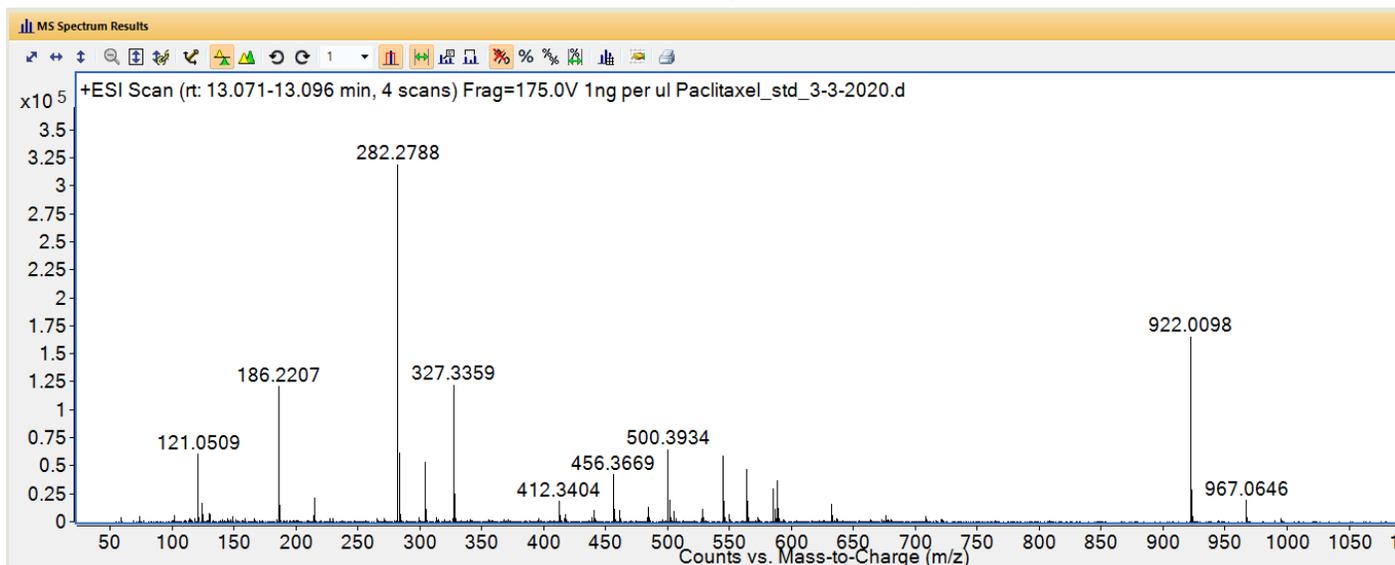


The asterisk means that this m/z peak is saturated. The signal was too large, because the sample was too concentrated. When this happens, the m/z value will not be as accurate as it could be.

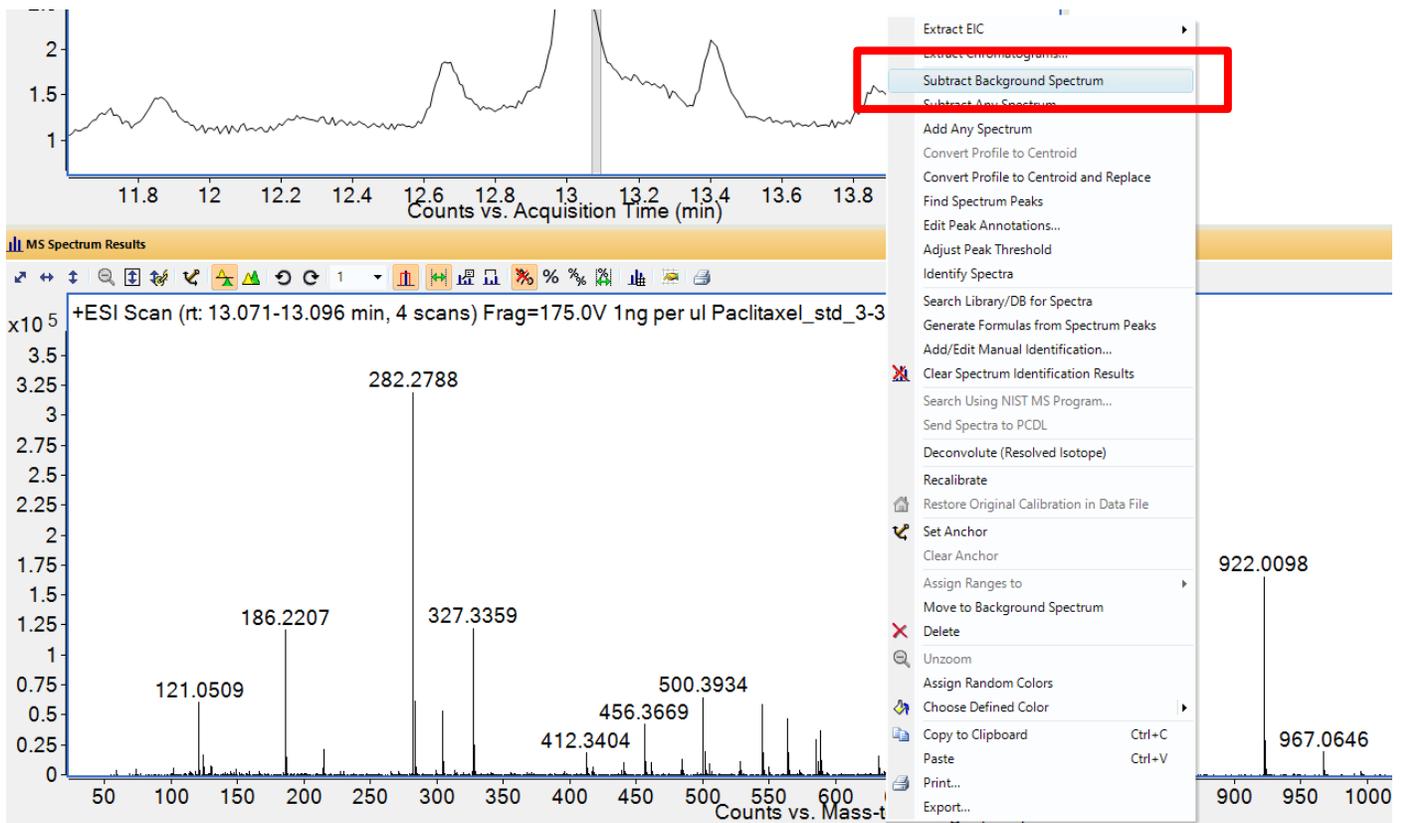
To fix this problem, we could select a portion of the chromatogram where the peak was tailing away, and less concentrated:



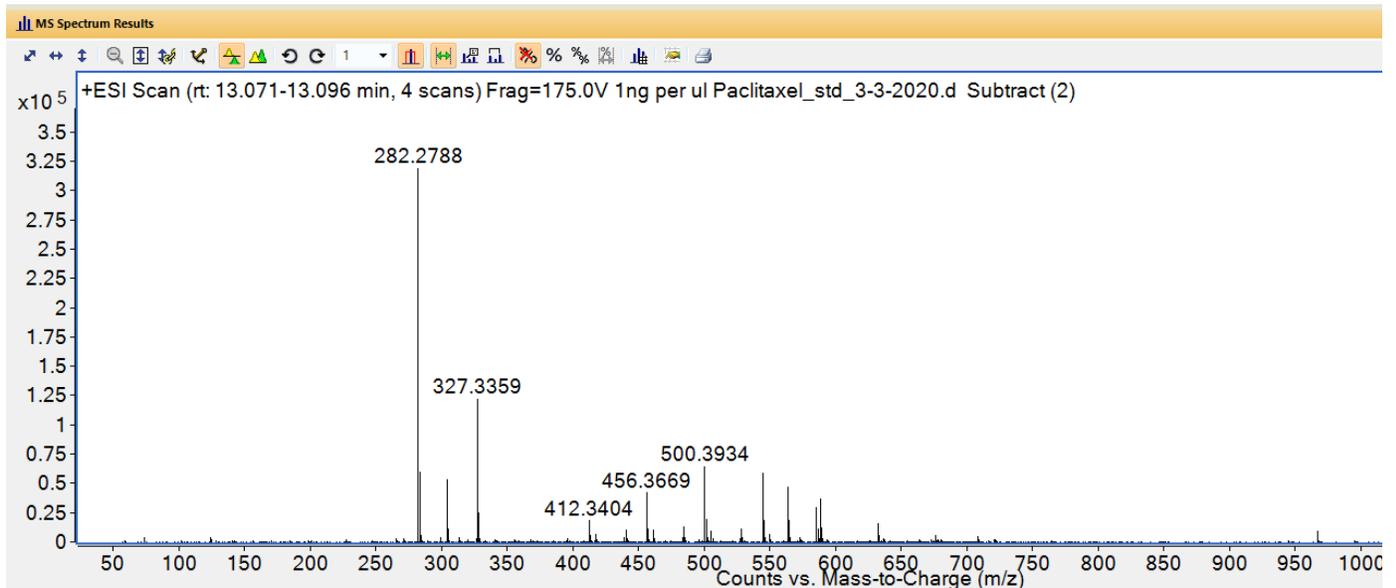
Right-click to get a menu, and click on “Extract MS Spectrum”. The resulting spectrum in this case is not saturated (no asterisk next to any m/z values):



You can subtract the background spectrum from this spectrum, by right-clicking anywhere on the spectrum. You will see a menu. Click on “Subtract Background Spectrum”:

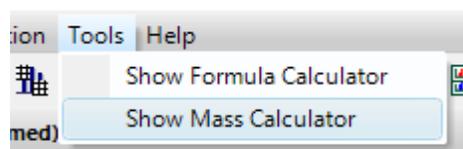


The resulting spectrum will have the background peaks subtracted out:

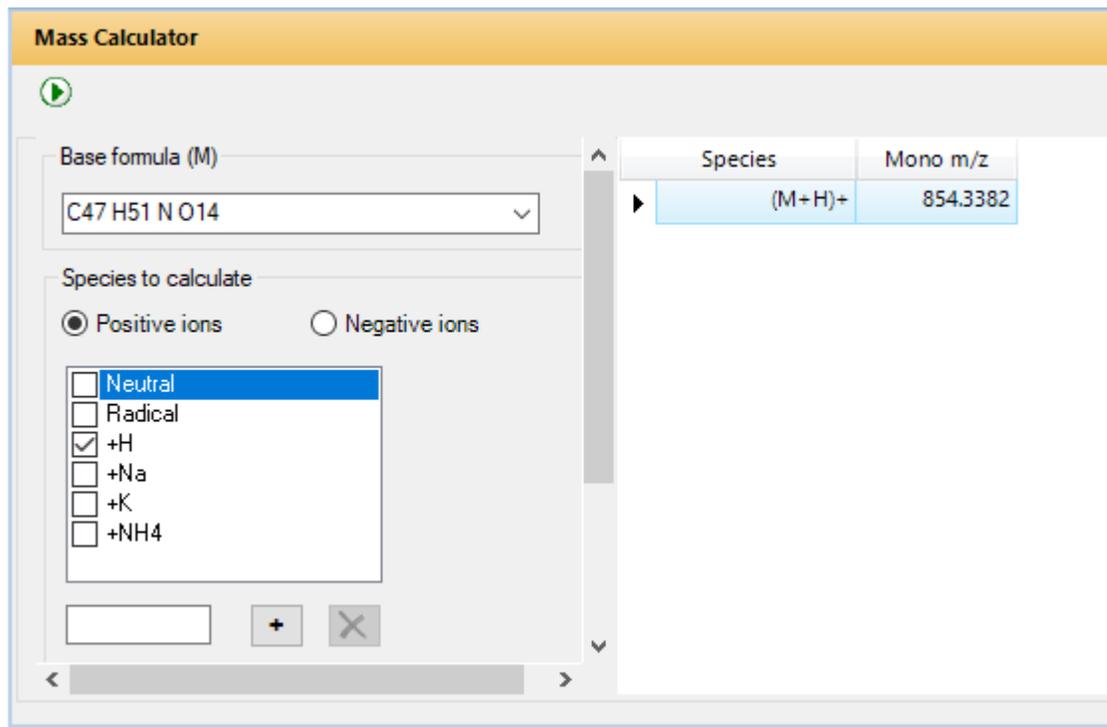


How to use the mass calculator utility:

Click on the tools menu, and click on "Show Mass Calculator":



You will see the Mass Calculator window. You can enter a molecular formula, and choose what ion m/z values to calculate (such as $[M+H]^+$), and then click on the  button and it will do the calculation.

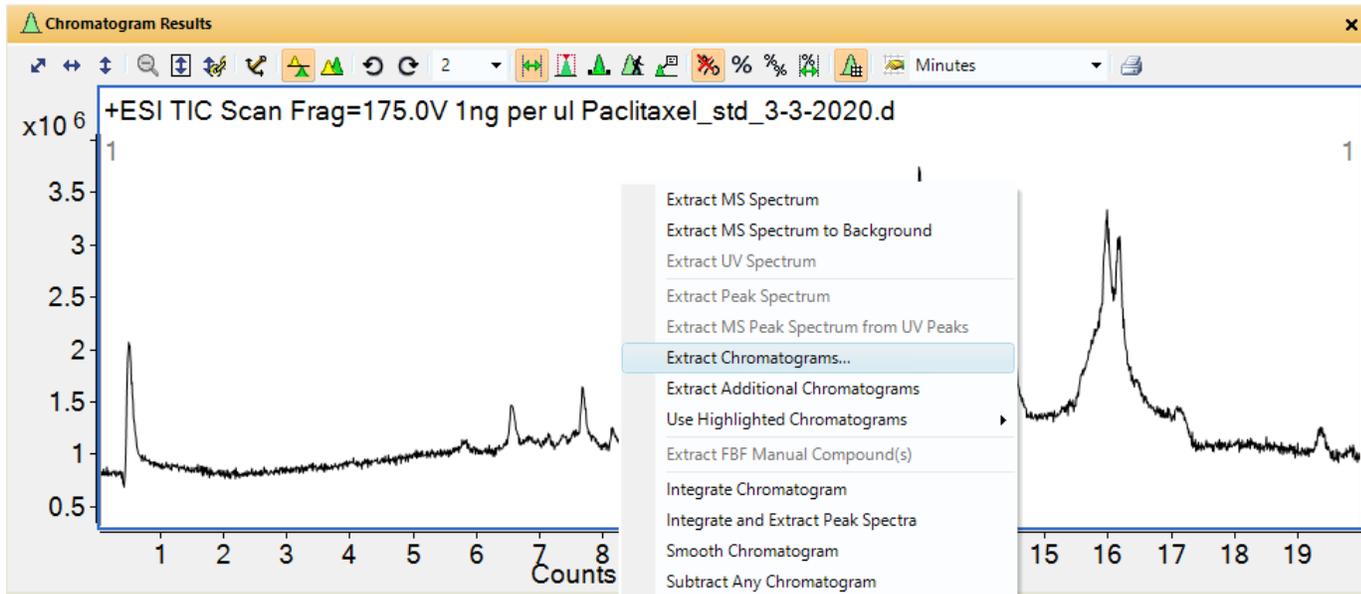


How to display an Extracted Ion Chromatogram (EIC)

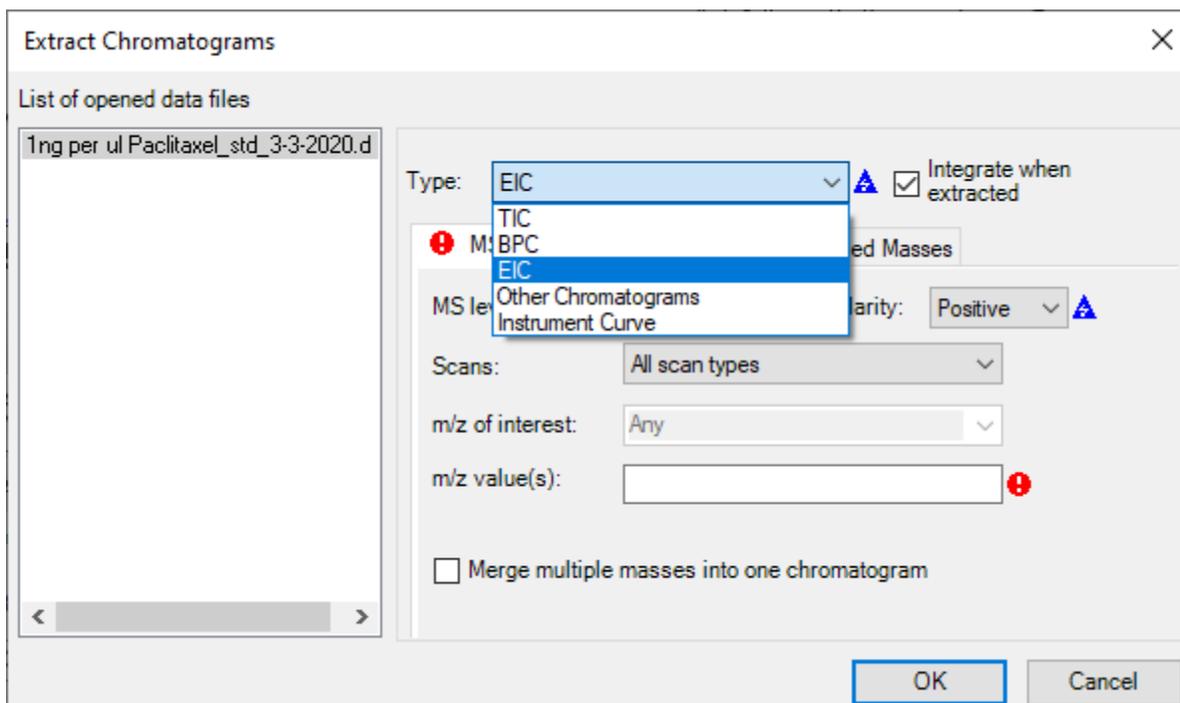
It is sometimes useful to display a chromatogram showing only one particular m/z value. This is called an Extracted Ion Chromatogram or EIC.

This could be useful to find where a specific compound with a particular m/z value is eluting in the chromatogram. It could also be used to quantify that compound by integrating the area under the peak.

To generate an EIC, right-click the chromatogram. Then left-click “Extract Chromatograms...”



You will see a dialog box. Click on the drop-down menu for chromatogram type, and select EIC:



Type in the m/z value that you want in the m/z value(s) field:

Extract Chromatograms

List of opened data files

1ng per ul Paclitaxel_std_3-3-2020.d

Type: EIC Integrate when extracted

MS Chromatogram Advanced Excluded Masses

MS level: All Polarity: Positive

Scans: All scan types

m/z of interest: Any

m/z value(s): 854.3382

Merge multiple masses into one chromatogram

OK Cancel

Under the “Advanced” tab, put in the mass tolerance that you would like. A tolerance of 30 ppm of mass works well:

Extract Chromatograms

List of opened data files

1ng per ul Paclitaxel_std_3-3-2020.d

Type: EIC Integrate when extracted

MS Chromatogram **Advanced** Excluded Masses

Fragmentor: Any Ionization: Any

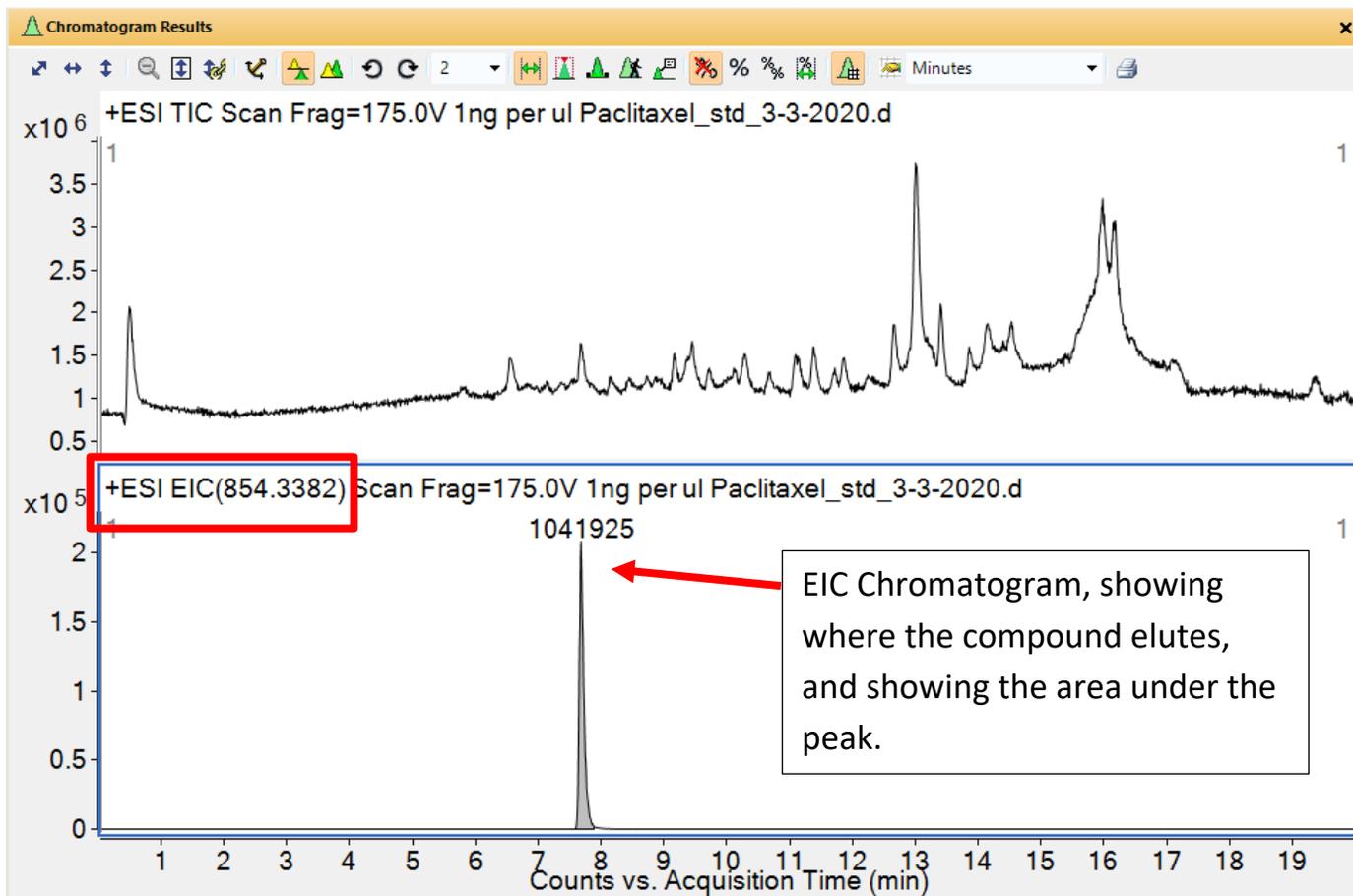
Collision energy: Any

Single m/z expansion for this chromatogram

Symmetric (ppm) ± 30

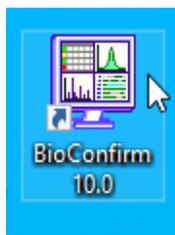
OK Cancel

Then click on OK, and it will display the EIC chromatogram:



How to Process Intact Protein Data Files

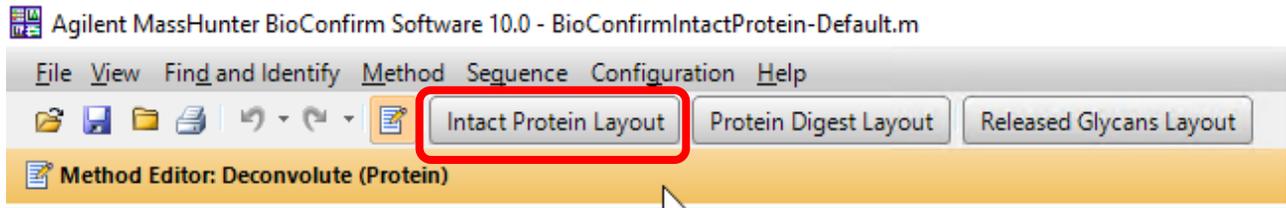
First, launch the Bioconfirm data processing program, by double-clicking the Bioconfirm icon on the desktop:



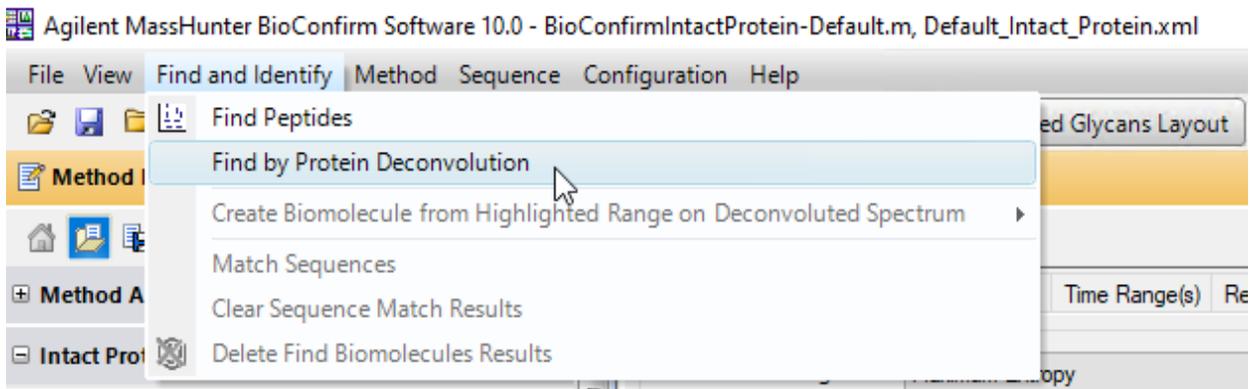
Alternatively, you can click on the  icon on the taskbar near the bottom of the screen.

After Bioconfirm comes up, it will ask you to open a data file. If the program was already open, you can open your data file by clicking on File and Open, or by clicking on the  icon near the upper left corner.

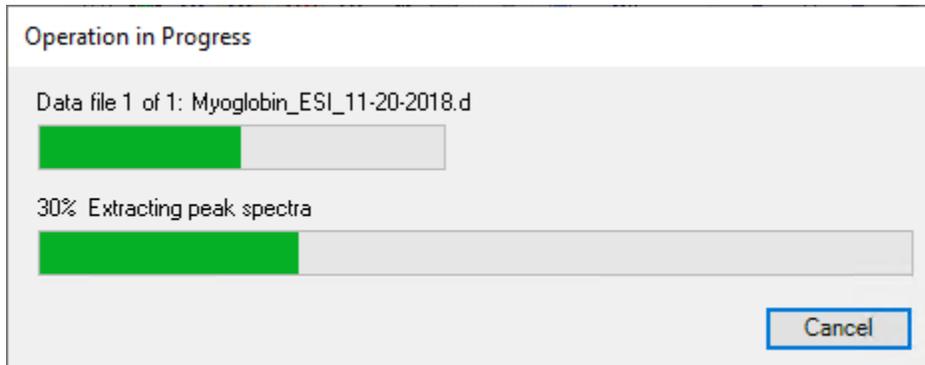
After your data file is open, click on the “Intact Protein Layout” button near the top of the screen:



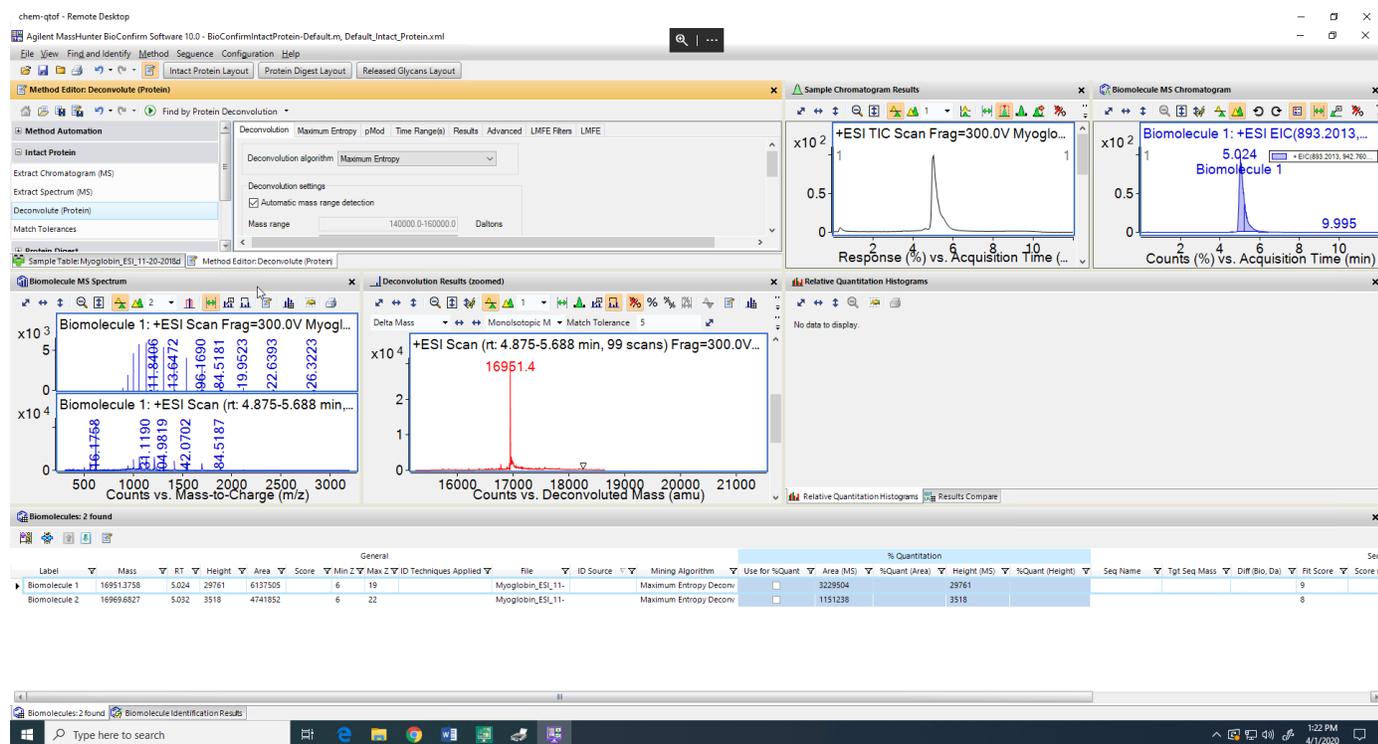
To find intact proteins in the data file, click on the “Find and Identify” menu, and then click on “Find by Protein Deconvolution”



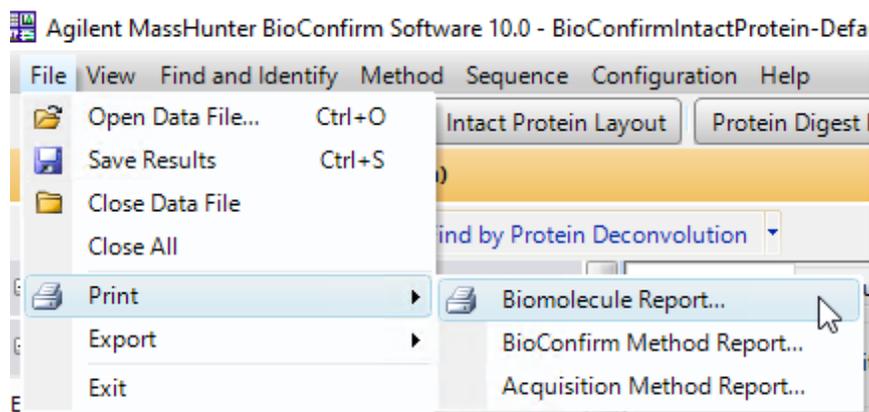
This process may take several minutes. You will see a progress bar like this:



When it is finished, you will see a list of proteins found:



To generate a report, click on File and Print, and Biomolecule Report....



You will see a dialog box which will allow you to either print or save or preview the report:

Print Biomolecule Report

List of opened data files:
Myoglobin_ESI_11-20-2018.d

Report contents
 All results Separate report per data file
 Only highlighted results

Print report
 Print report
 Printer name: <Default>
 Print preview
 Page size: A4 Letter

Save report
 Save report ▲
 Inside data file's reports subdirectory
 At specified directory:
 D:\MassHunter\reports
 If report file already exists
 Overwrite existing report
 Auto-generate new report file name

OK Cancel

It will then display the report as a pdf:

