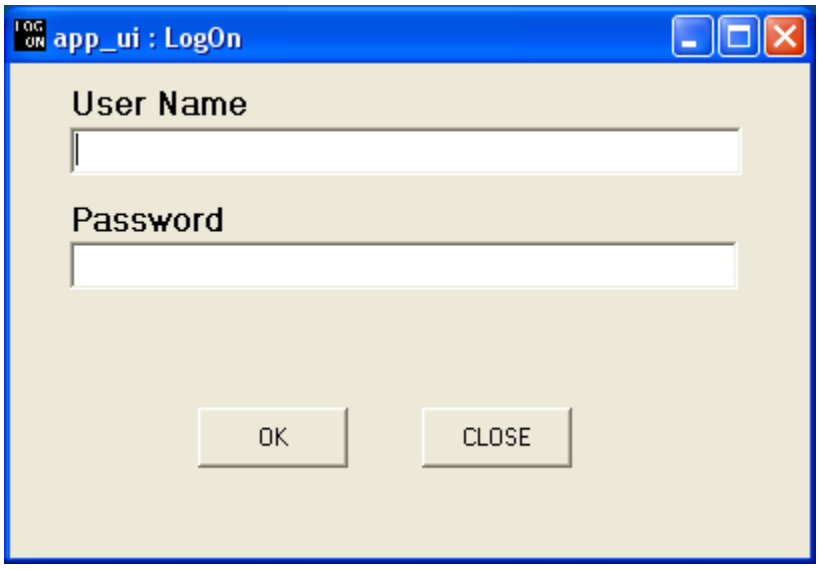
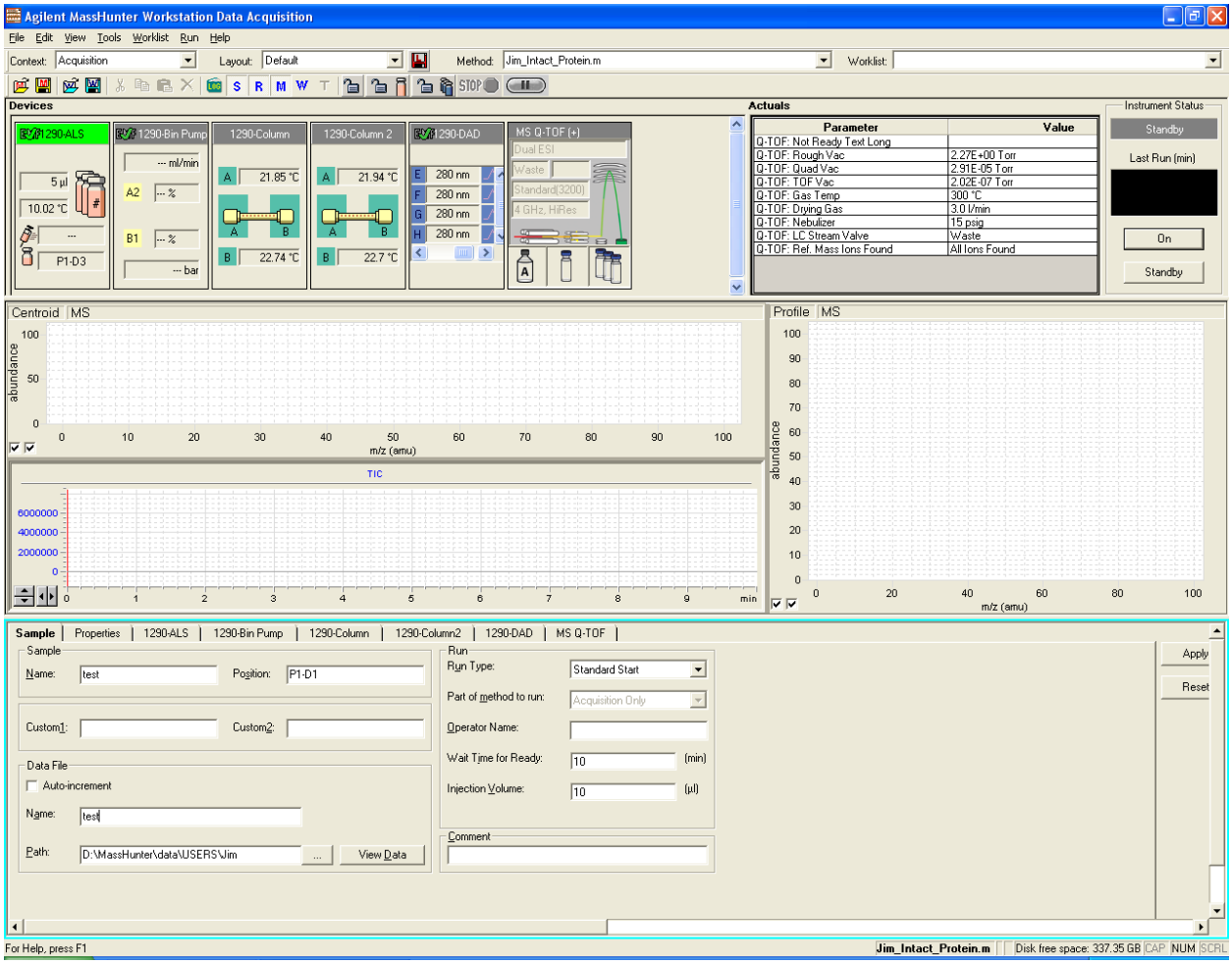
Q-TOF User's Booklet

Enter your username and password to login. After you login, the data acquisition program will automatically start.



This data acquisition window will come up after the program has loaded:



Depending upon your application, please go to the appropriate page in this booklet.

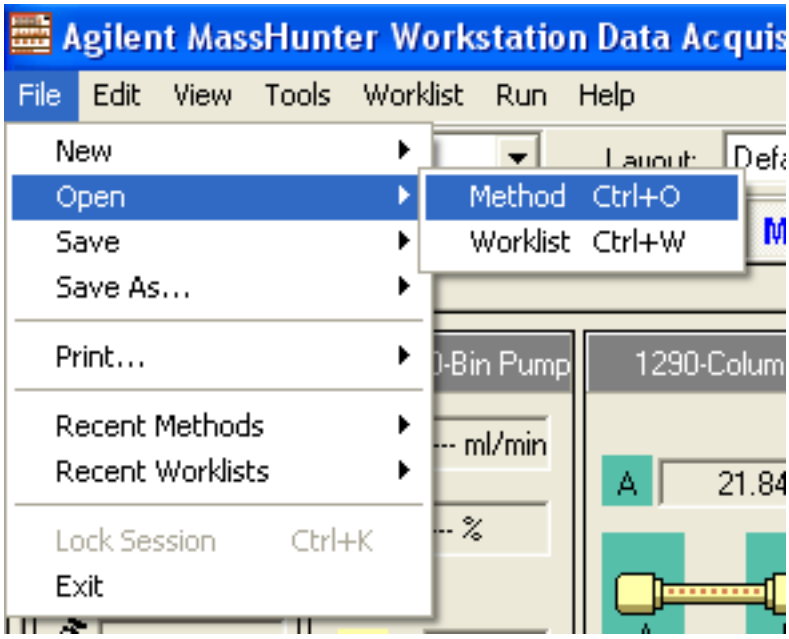
For intact proteins, please go to page 3.

For peptides, please go to page 16.

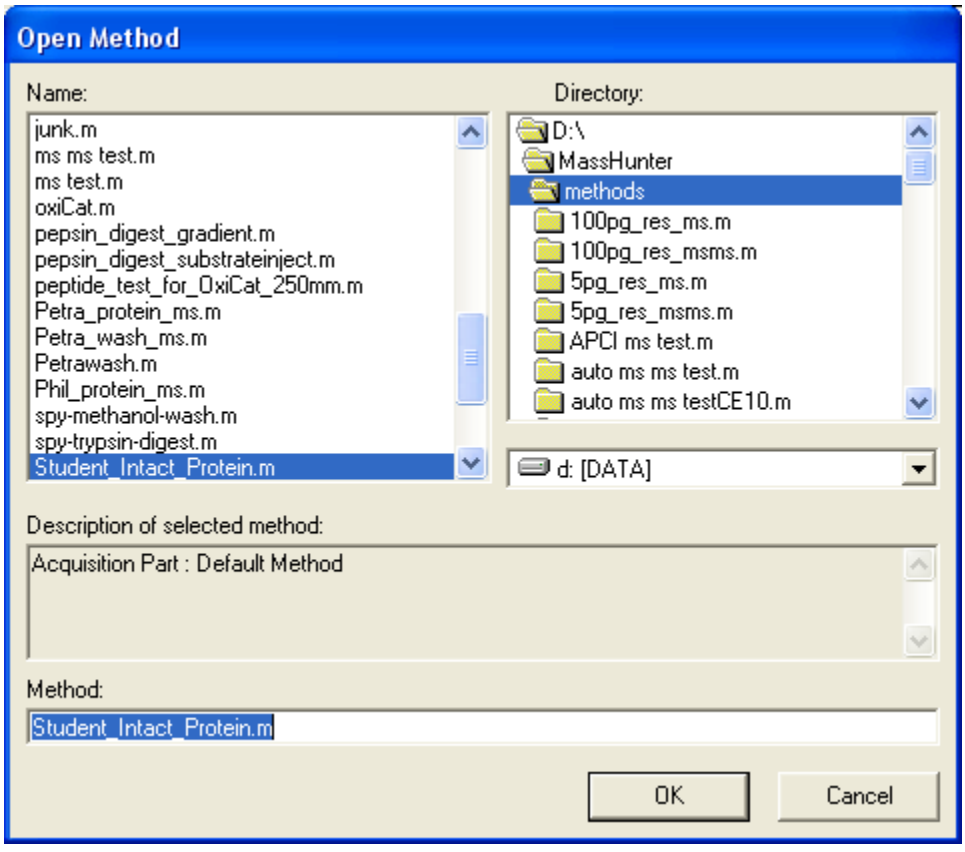
For small molecules, please go to page

Intact Proteins

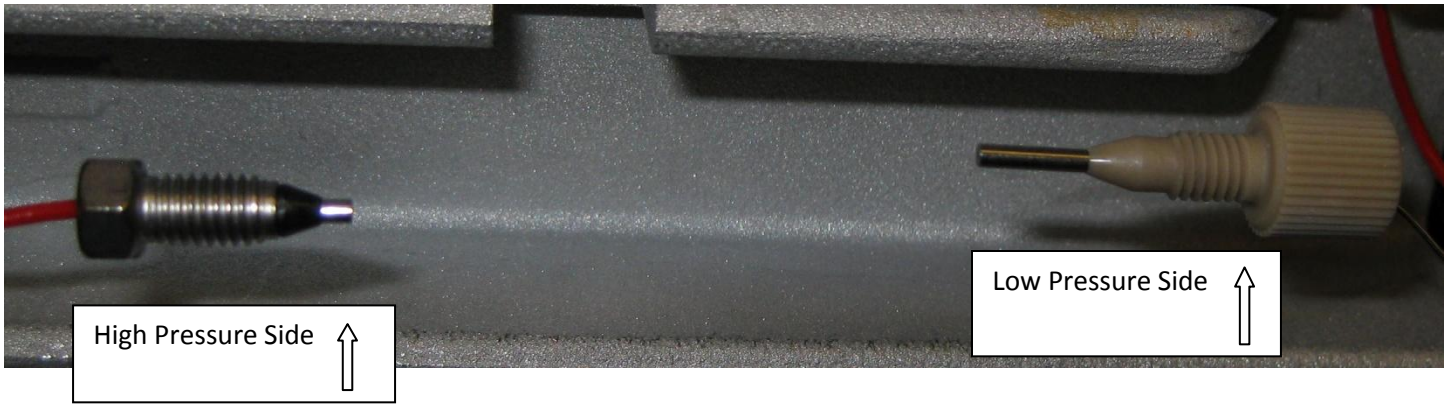
1) Go to the File menu, and click on Open Method…..

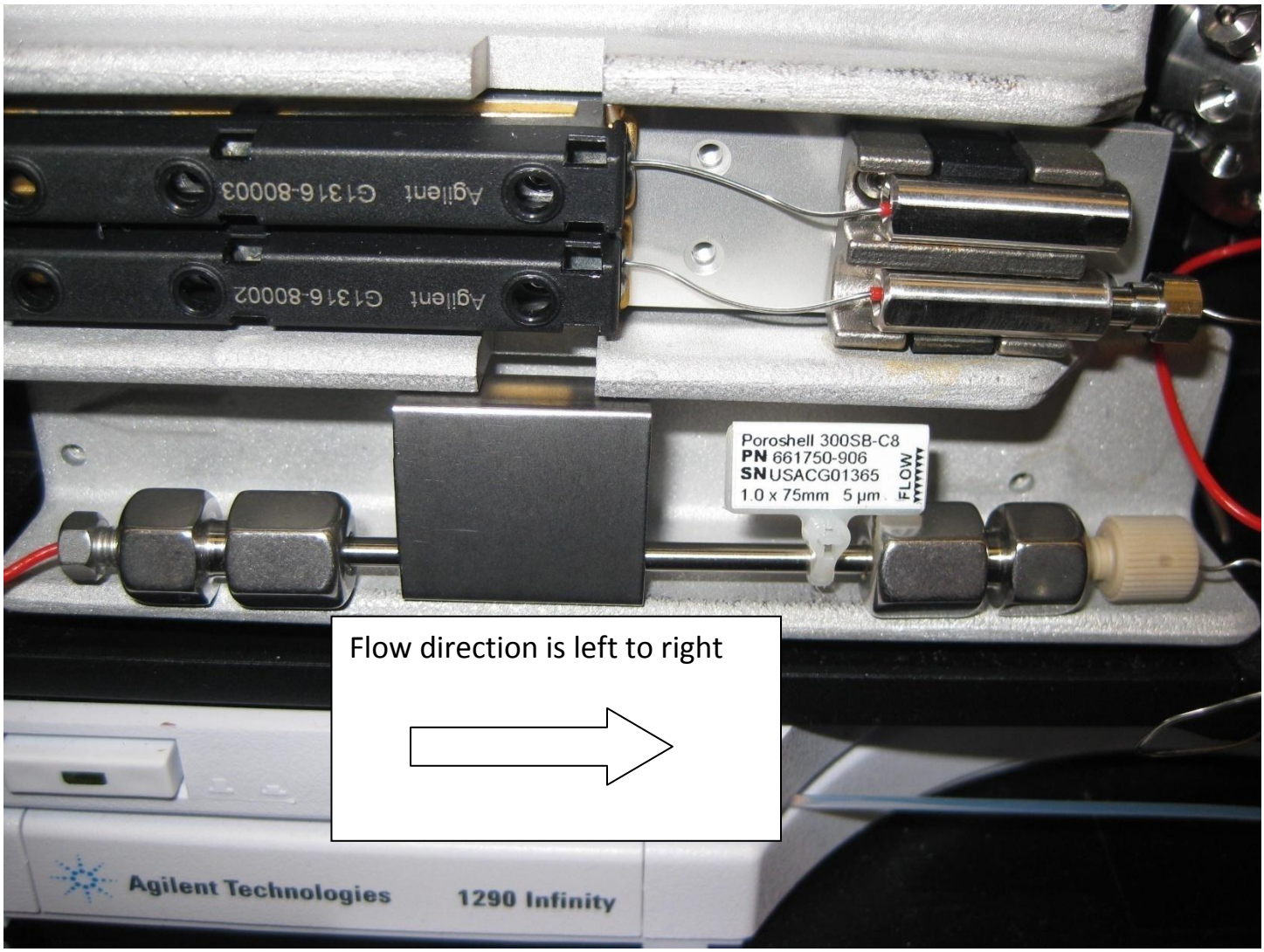


2) Choose the method called “Student_Intact_Proteins”.

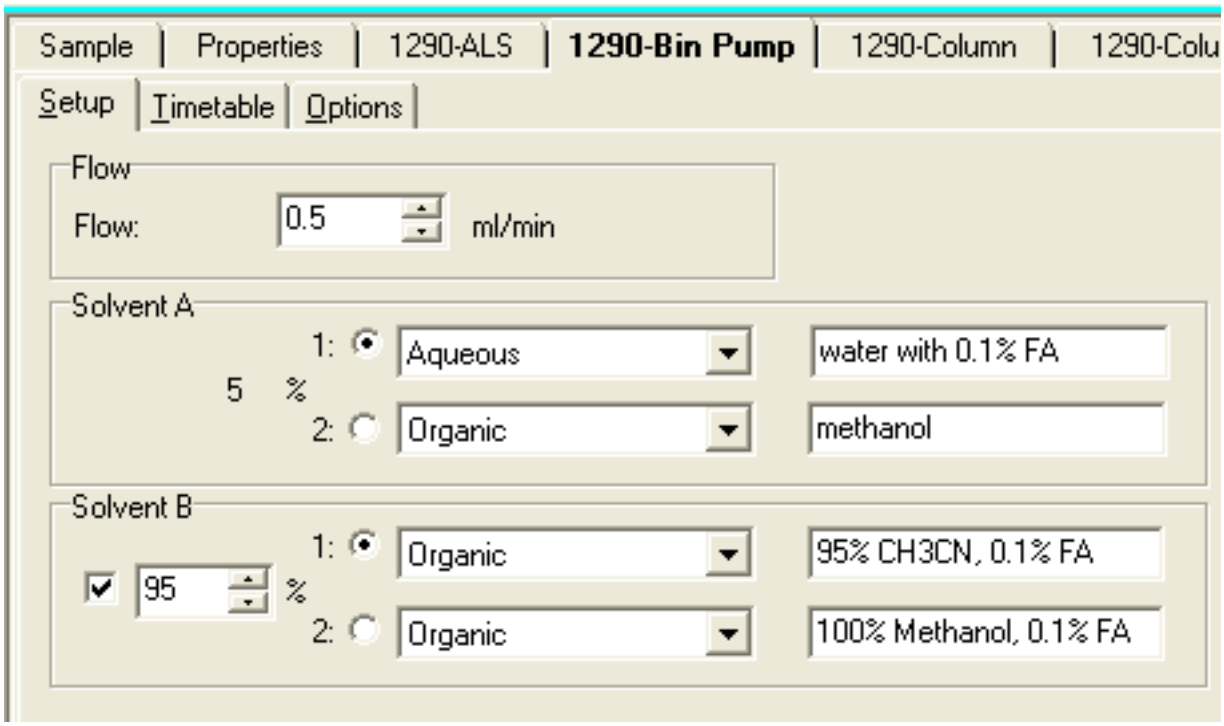


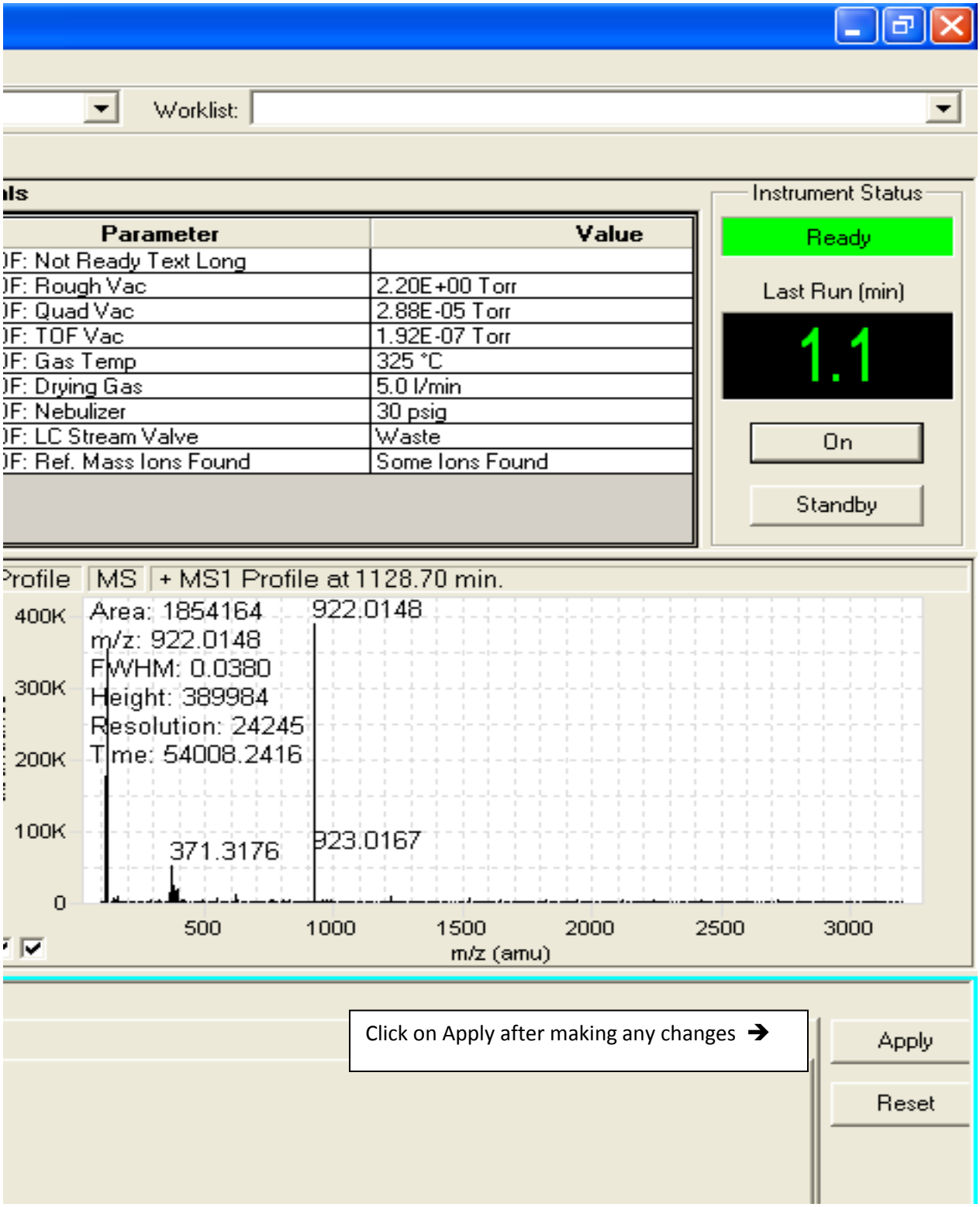
3) Install the Poroshell 300SB-C8 column. Note that the flow direction is left to right. The left side is the high pressure side, and requires a nut and ferrule. The low pressure side on the right only requires a one-piece PEEK fitting.



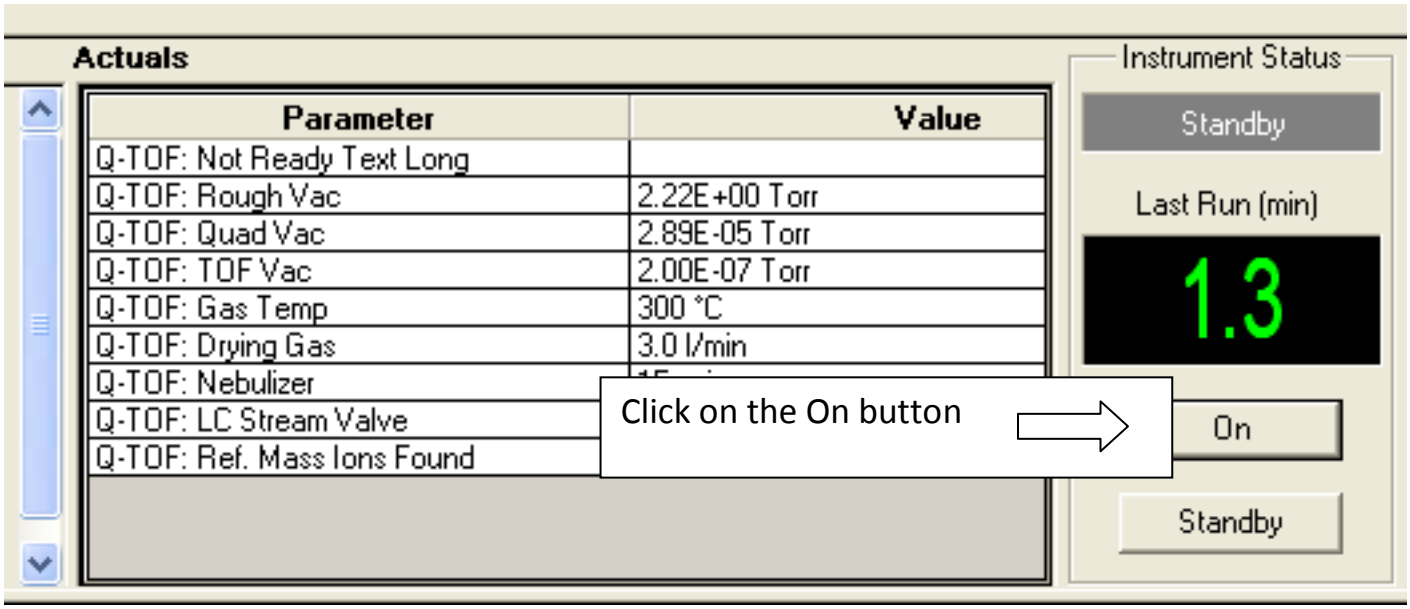


4) Set the initial solvent flow to 95% acetonitrile with 0.1% formic acid, and 5% water with 0.1% formic acid, at a flow rate of 0.5 ml/min, using the “1290-Bin Pump” tab on the method editor. Click on the “Apply” button.

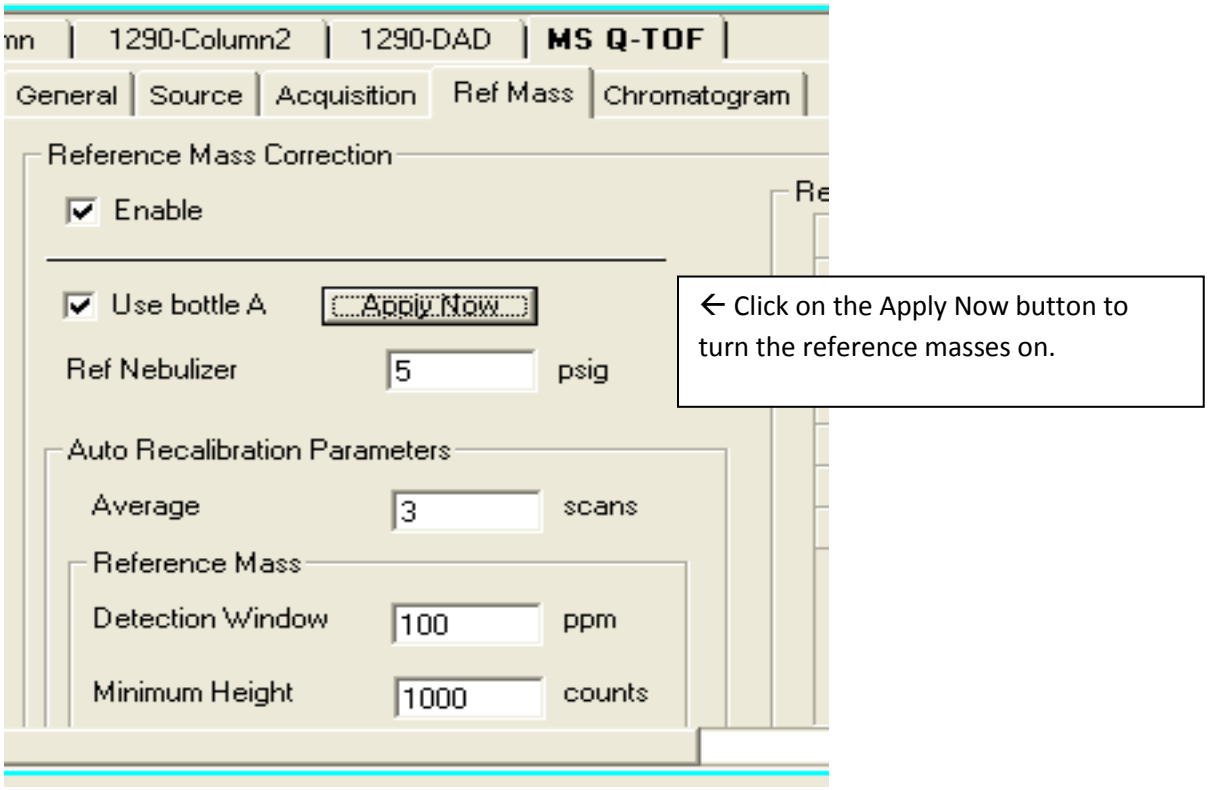



Worklist:
Instrument Status
Ready
Last Run (min)
1.1
On
Standby
Parameter
Value
OF: Not Ready Text Long
OF: Rough Vac
2.20E+00 Torr
OF: Quad Vac
2.88E-05 Torr
OF: TOF Vac
1.92E-07 Torr
OF: Gas Temp
325 °C
OF: Drying Gas
5.0 l/min
OF: Nebulizer
30 psig
OF: LC Stream Valve
Waste
OF: Ref. Mass Ions Found
Some Ions Found
Profile
MS
+ MS1 Profile at 1128.70 min.
Area: 1854164
m/z: 922.0148
FWHM: 0.0380
Height: 389984
Resolution: 24245
Time: 54008.2416
922.0148
923.0167
371.3176
400K
300K
200K
100K
0
500
1000
1500
2000
2500
3000
m/z (amu)
Click on Apply after making any changes ➔
Apply
Reset

5) Put the instrument into “On” mode.



6) Turn on the Reference Mass ions, using the Q-TOF and Ref Mass tabs.



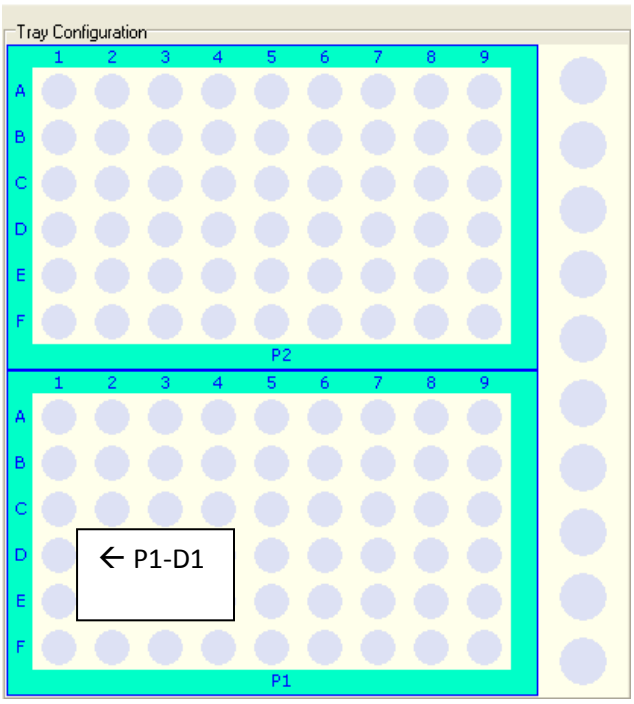
7) After the column has conditioned for 5 minutes, change the gradient to 95% water with 0.1% formic acid, and 5% acetonitrile with 0.1% formic acid.

8) Sample Preparation:
The protein sample should be desalted, and dissolved in water with 0.1% formic acid. The concentration should be at least 0.1 mg/ml. The minimum volume would be 10 ul if using a conical bottom insert in the vial.

Important! The sample must be filtered to remove any particulate matter.

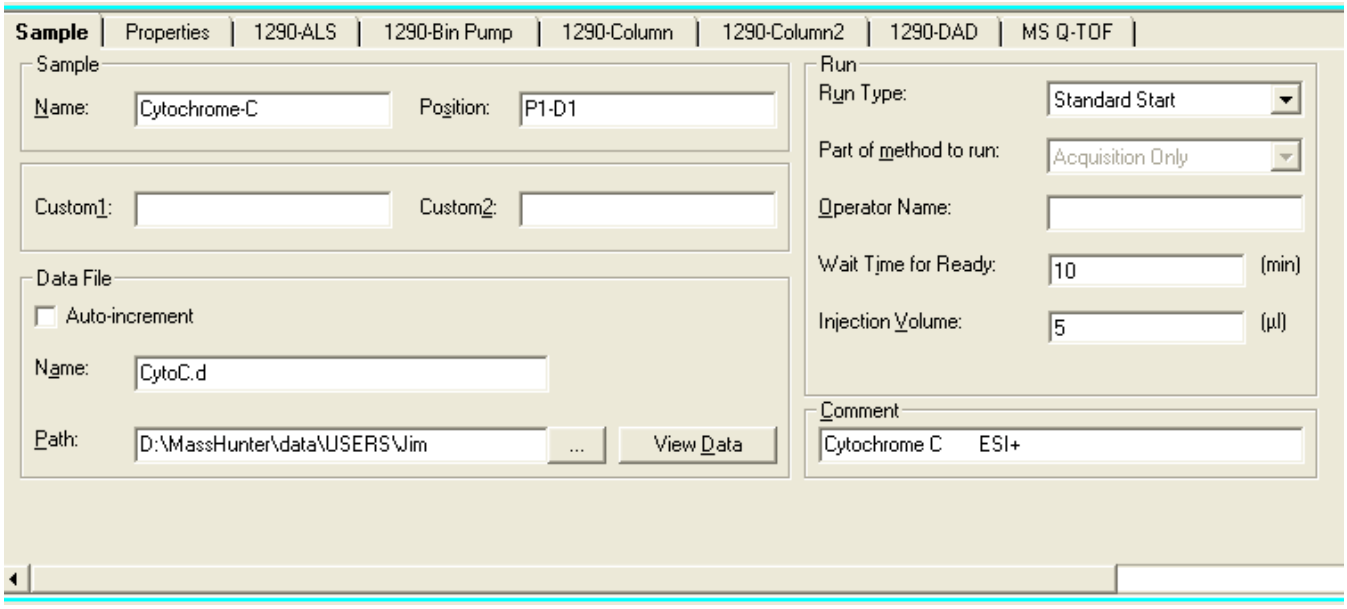
Typical 0.2 ml vial insert.

9) Place the sample in the Autosampler compartment, and note its position. It's position is designated by the plate number, followed by a dash, and then the well letter and number. For example, P1-D1 would be plate 1, row D and column 1.

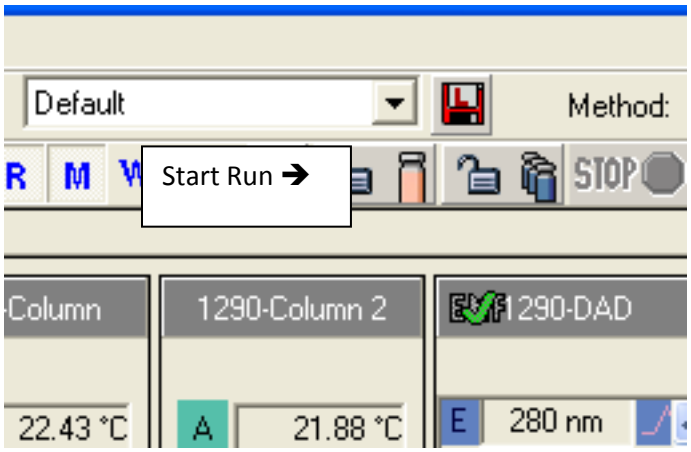


Click on the “Sample” tab in the method area near the bottom of the page, to set up the data acquisition.

The required parameters to set are the sample position in the autosampler tray, a data file name, comment, and injection volume.



10) Click on the Start Run icon.

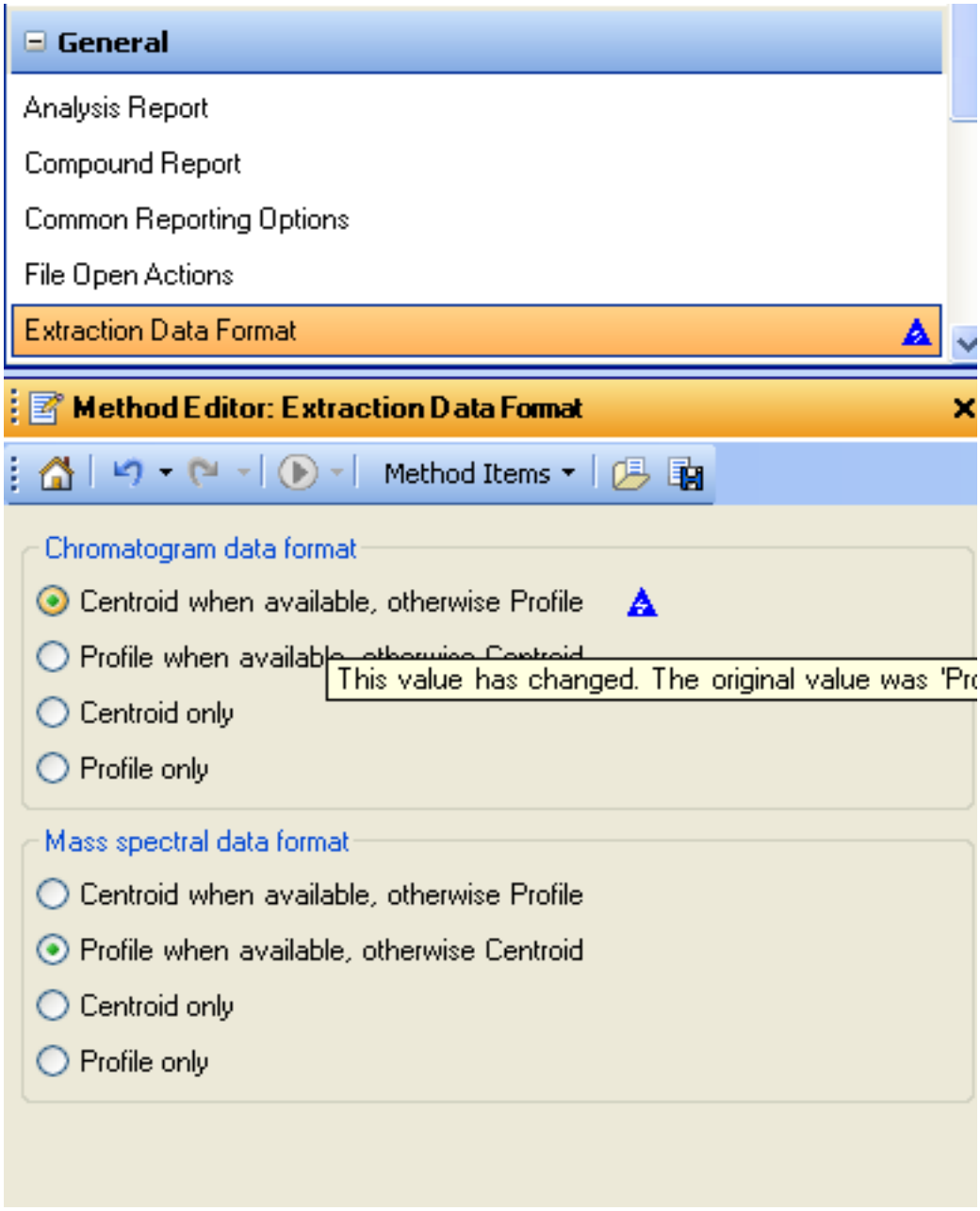


Data Workup After the Run Has Completed

1) Launch Agilent Qualitative Analysis Program.

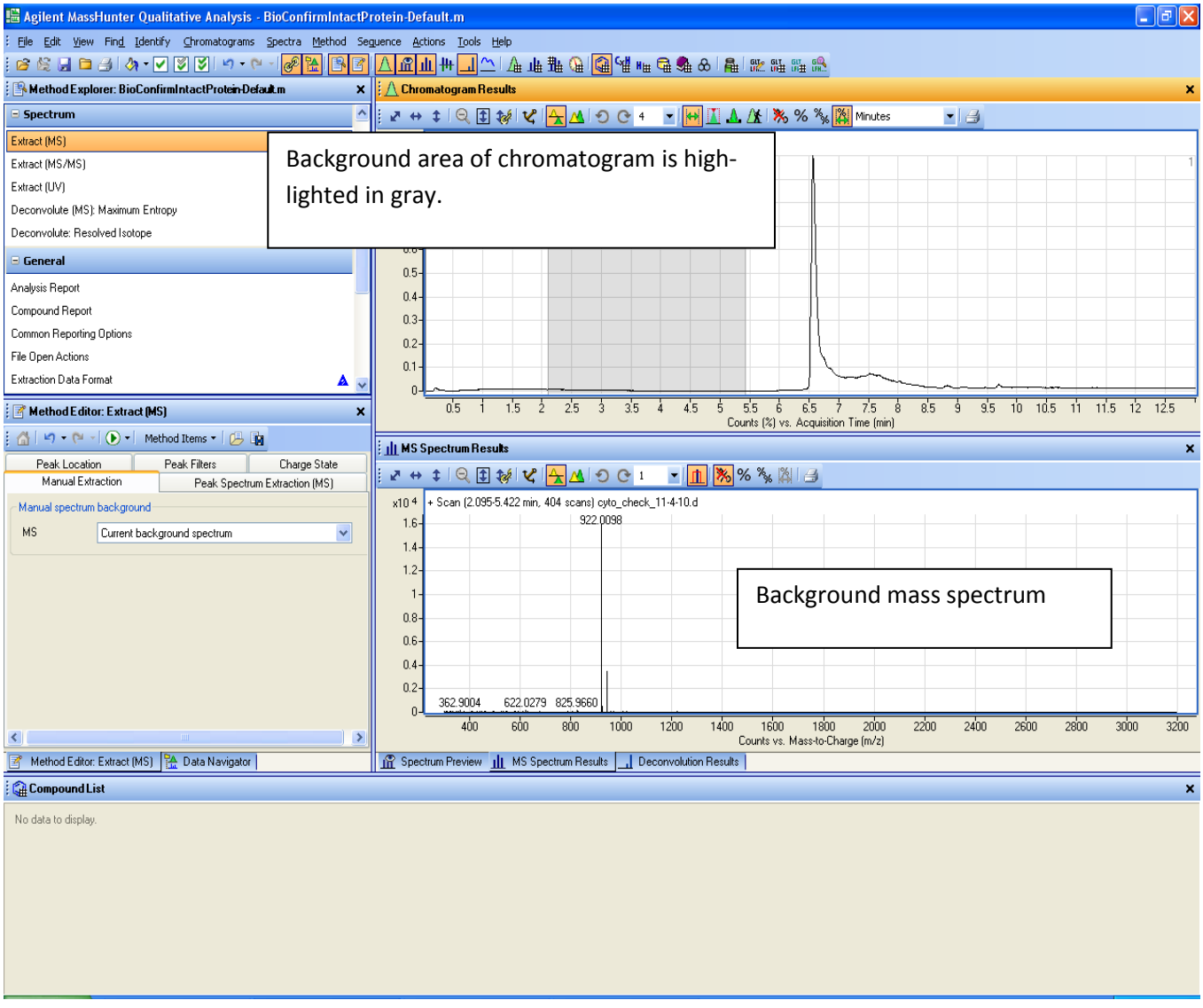
2) After the program comes up, go to the Tools menu, and Configure Workflow for “Bioconfirm”. Click OK and click on “No” to saving method changes.

3) On the left hand side, expand the “General” heading, and click on “Extraction Data Format”. For Chromatogram data format, click on “Centroid when available”, and for Mass spectral data format, click on “Profile, when available”

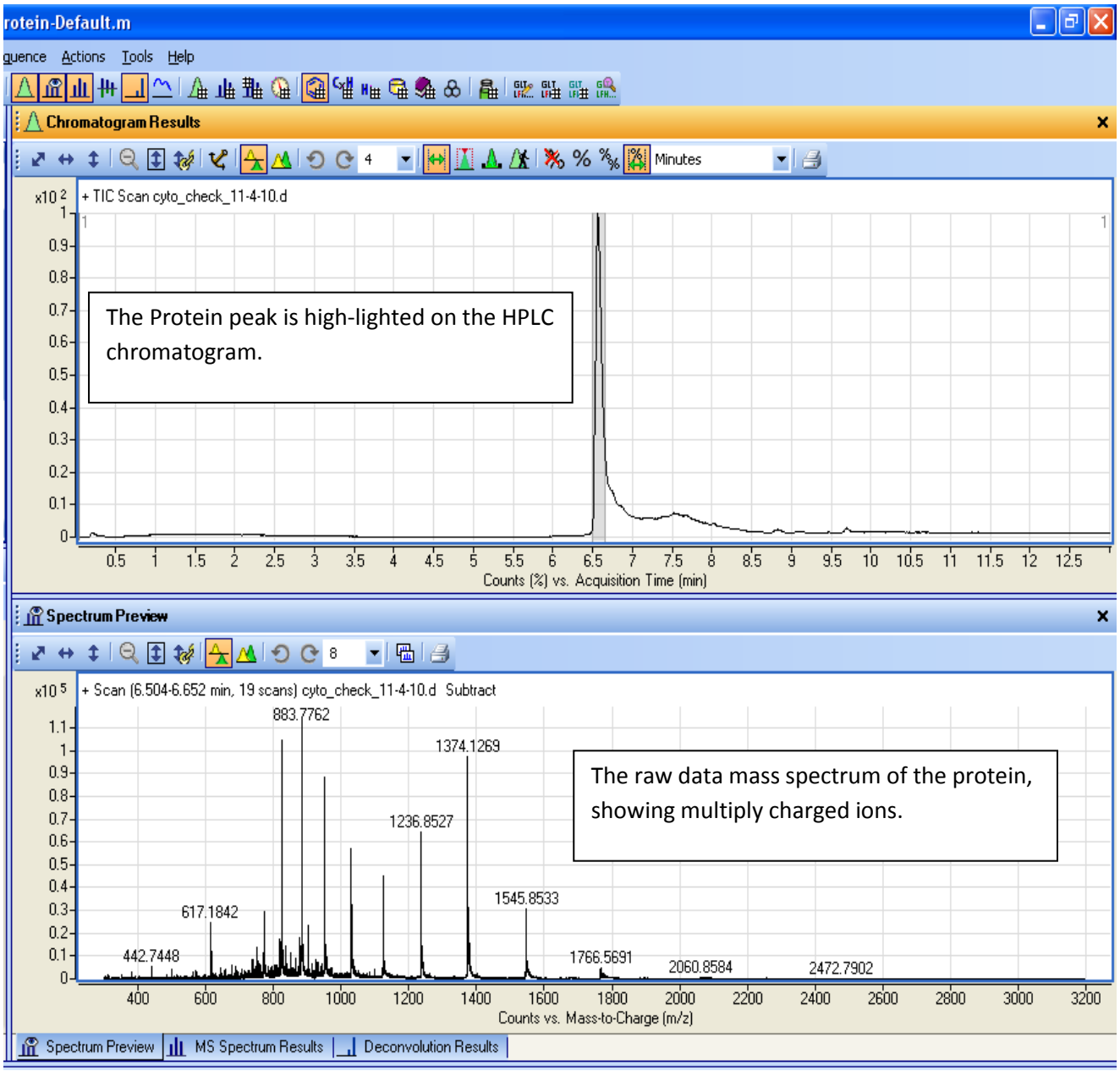


4) Next, open your data file, using File and Open.

5) On the HPLC chromatogram, left-click and drag across an area of background baseline with no peaks, in order to highlight that area. Then right-click the mouse to obtain a menu, and left-click "Extract MS Spectrum to Background". You should see a background spectrum in the MS Spectrum window, showing mostly m/z 922, which is a reference ion used for mass correction.

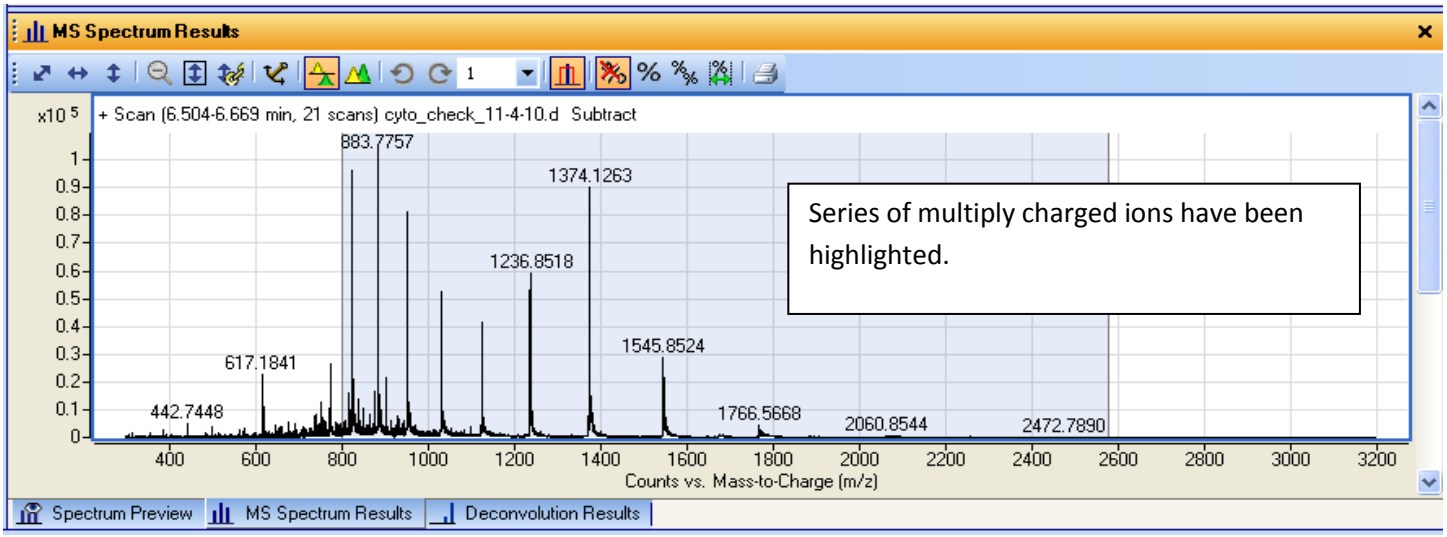


6) On the HPLC chromatogram, left-click and drag across the protein peak of interest to highlight it. Then right-click the mouse to obtain a menu, and left-click "Extract MS Spectrum". In the MS Spectrum window, you should see the mass spectrum of the protein, showing a series of multiply-charged ions.

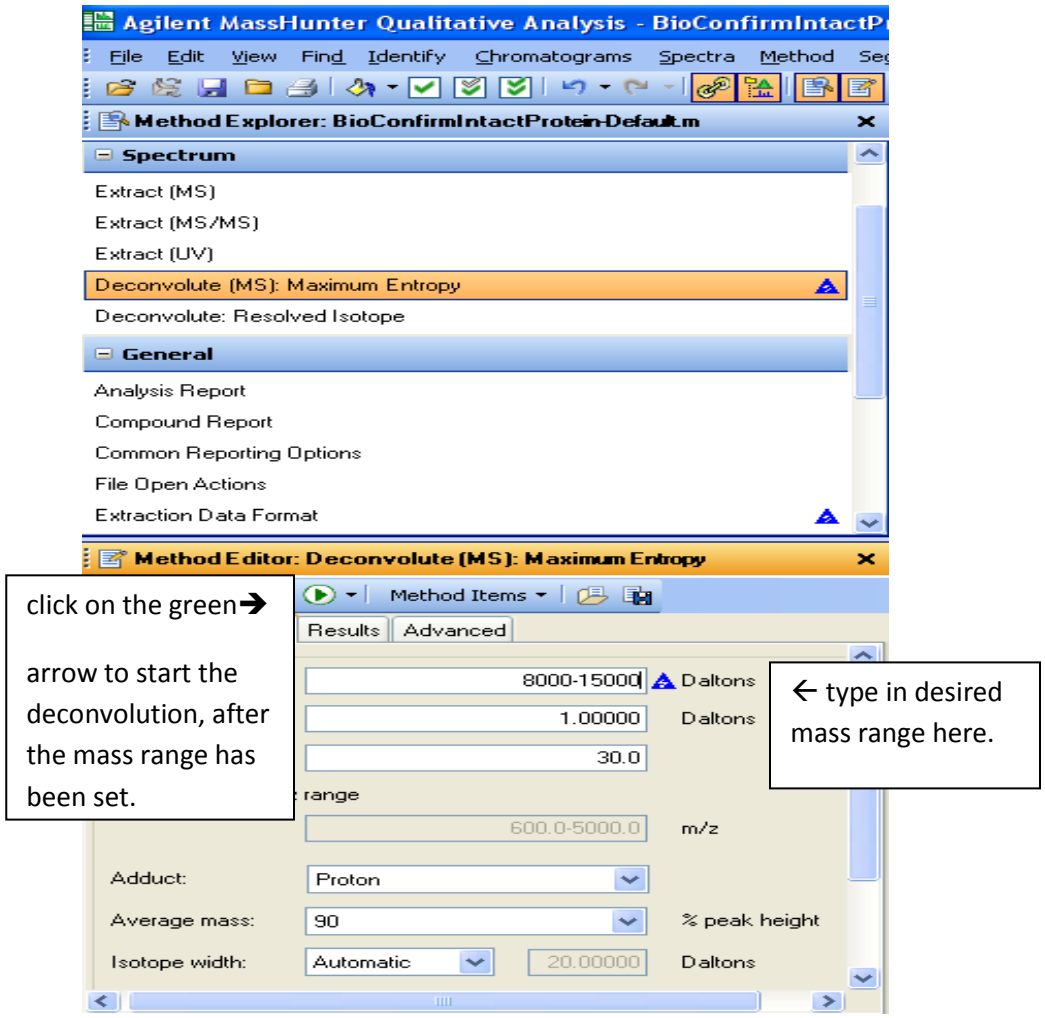


7) The mass spectrum needs to be copied from the Spectrum Preview window to the Spectrum Results window, in order to process and deconvolute it. To do this, right-click on the spectrum to obtain a menu, and left-click on "Copy to User Spectra".

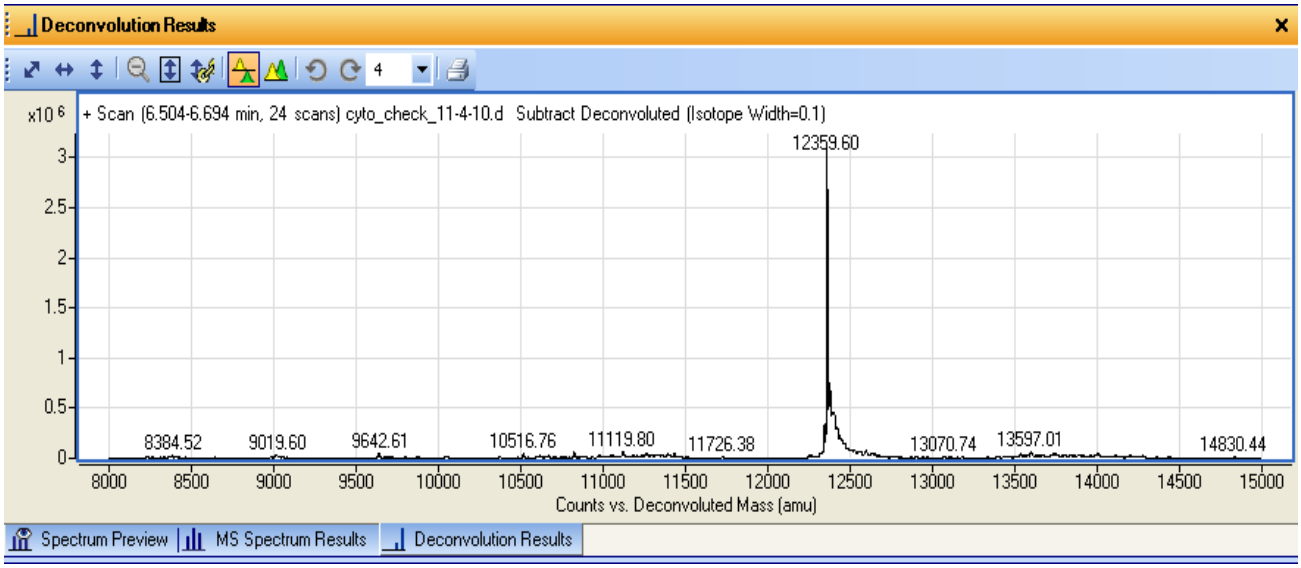
8) Left-click and drag across the spectrum to highlight the series of multiply-charged ions.



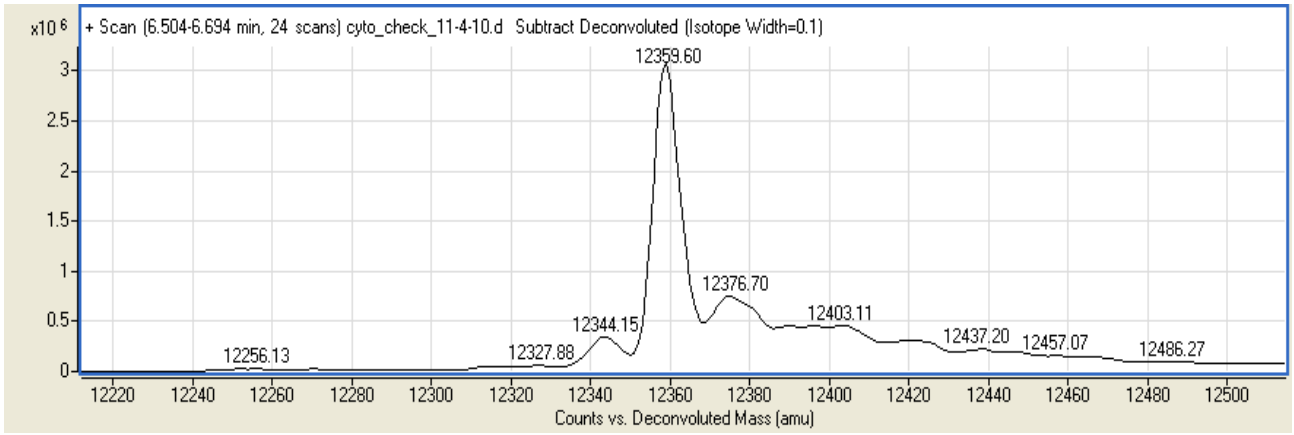
9) On the left hand side, under the Spectrum heading, click on Deconvolute MS: Maximum Entropy. Enter a reasonable mass range. (The wider you make the mass range, the longer it takes to complete the calculations). Click on the Green arrow to start the process.



10) After the calculation is complete, click on the Deconvolution Results tab to view the spectrum.

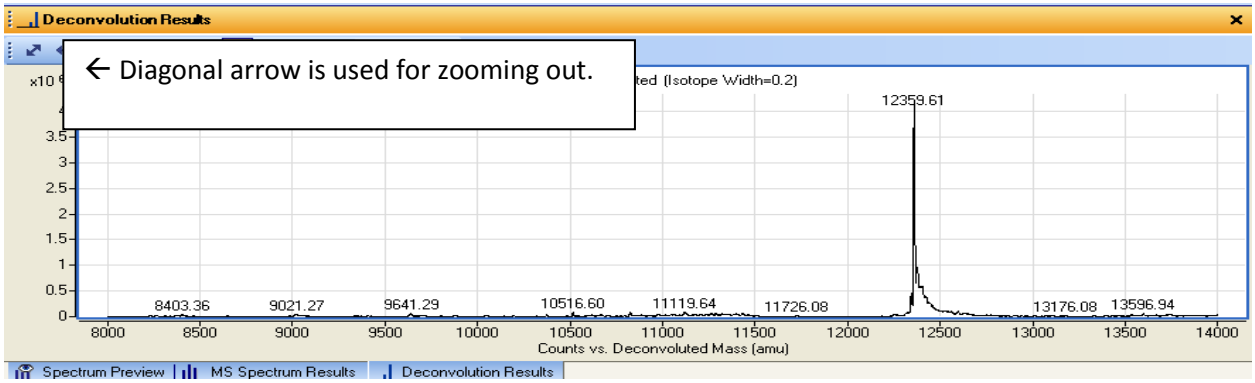


You can zoom in on a particular region, by left-clicking and dragging.



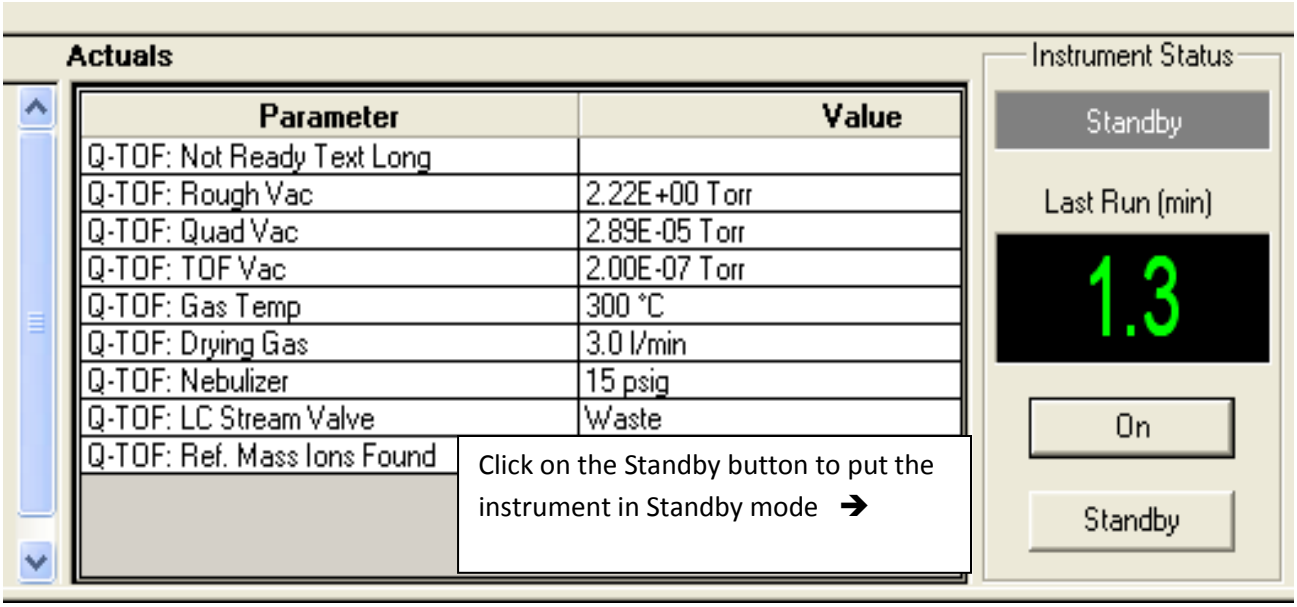
Spectra can be copied and pasted into a Word or Powerpoint document.

If you need to zoom out to the full spectrum, click on the diagonal arrow on the spectrum toolbar.



How to Shut Down When You Are Finished

1) Put the Instrument in Standby, by clicking on the Standby button.



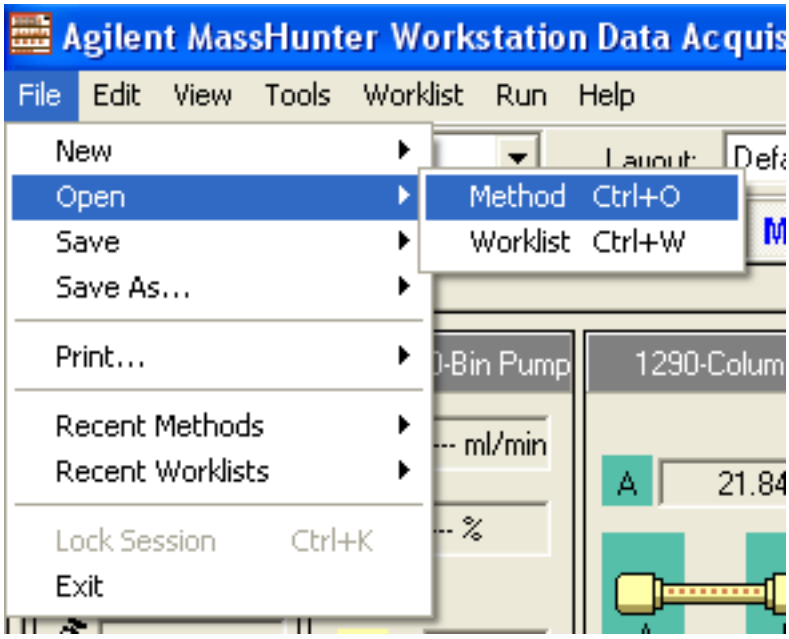
2) Exit from the Data Acquisition program. Exiting from the Data Acquisition program is what logs you off, so that no more time is billed to your account.

3) Remove the Poroshell HPLC column.

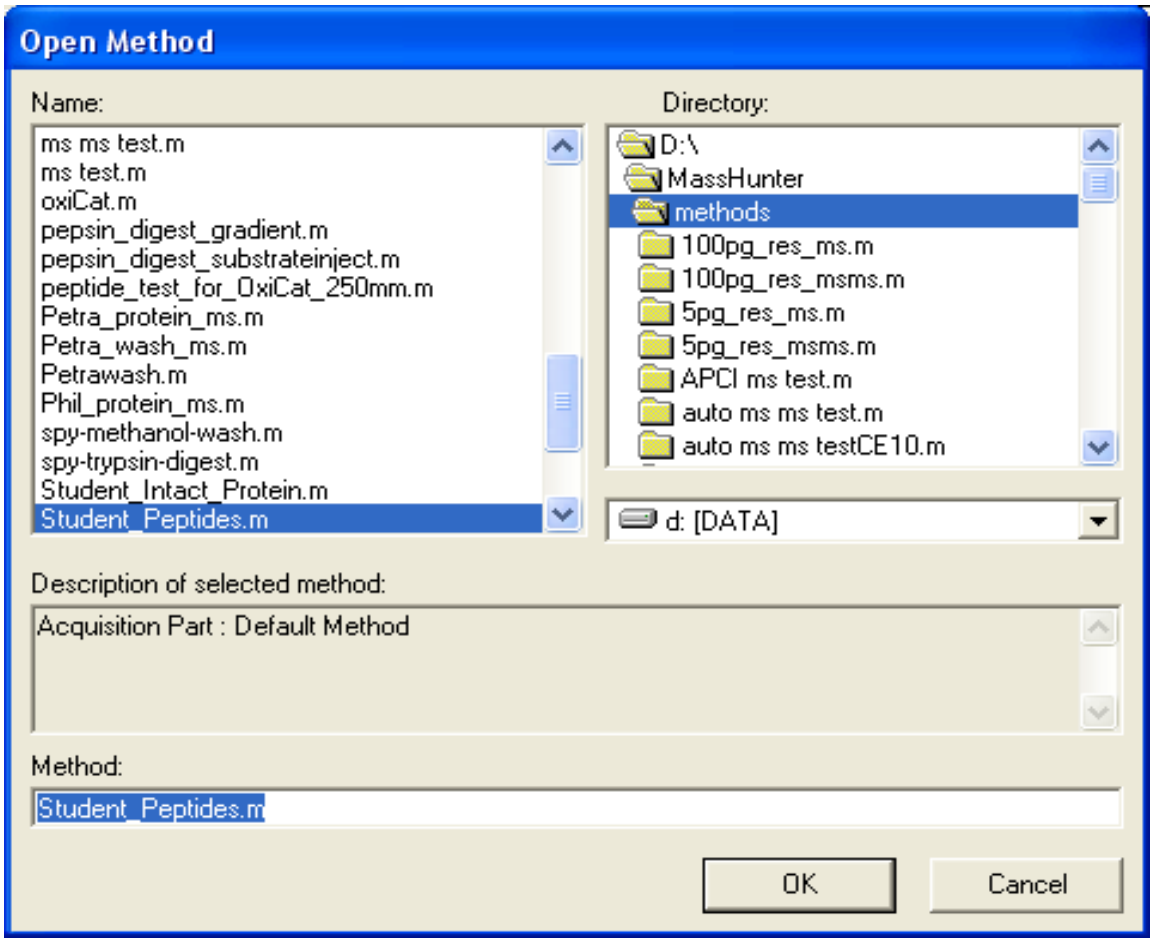
4) Install a union in place of the column, to prevent solvent from leaking out of the tubing.

Peptides

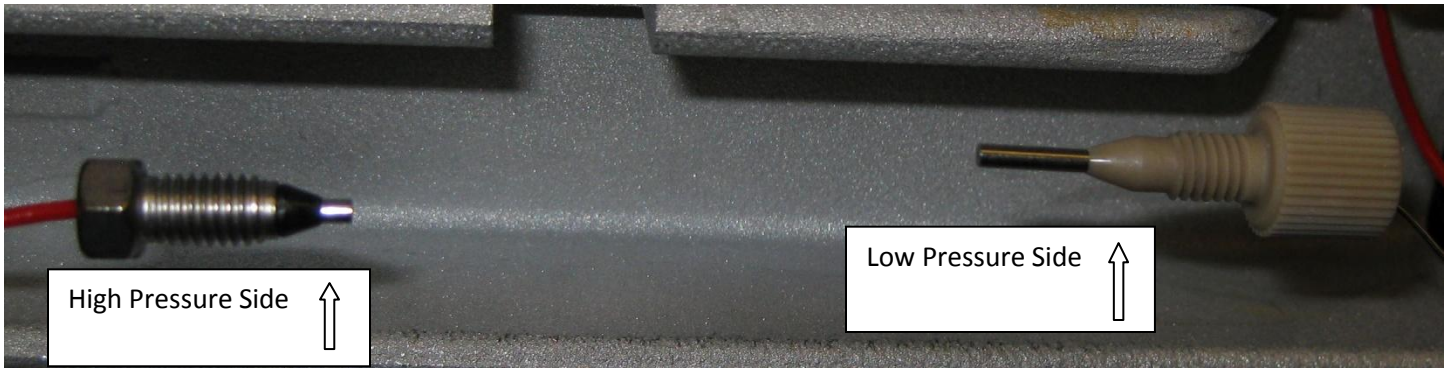
1) Go to the File menu, and click on Open Method….

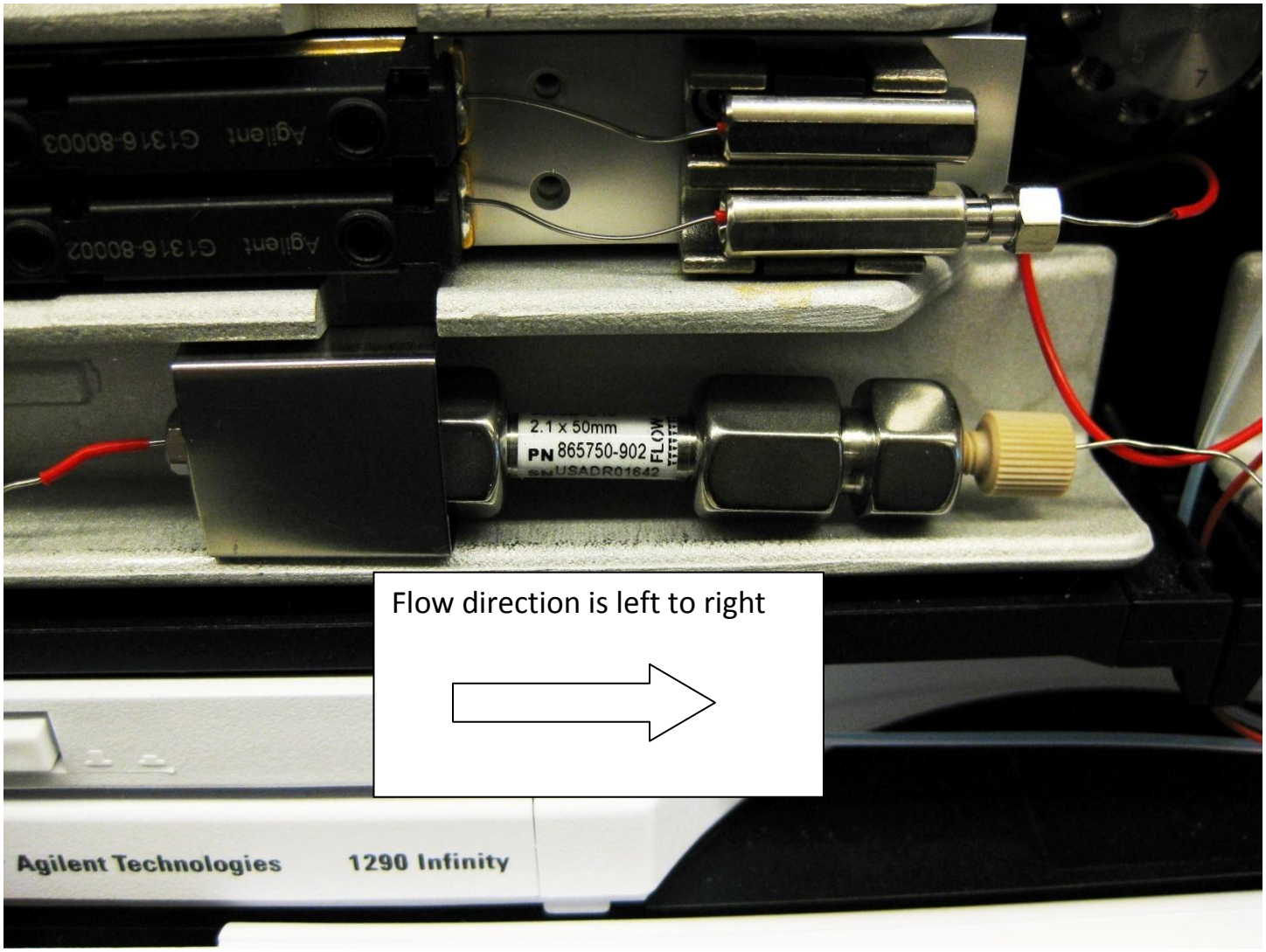


2) Choose the method called "Student_Peptides".

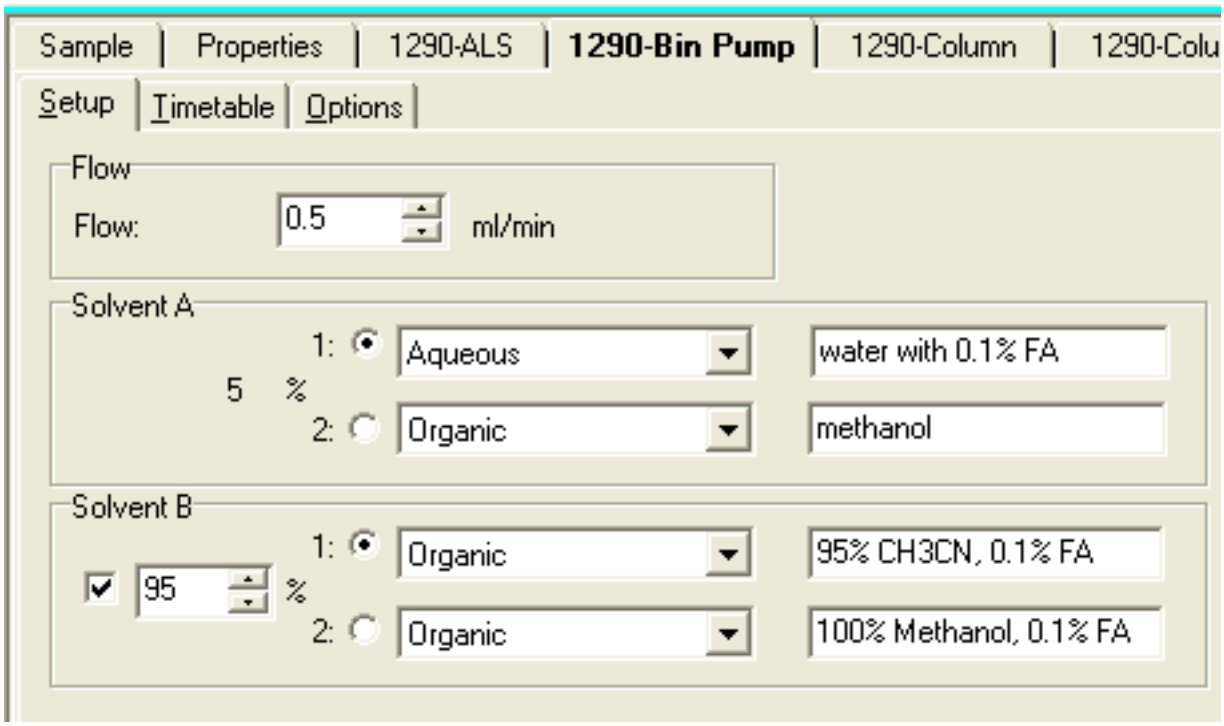


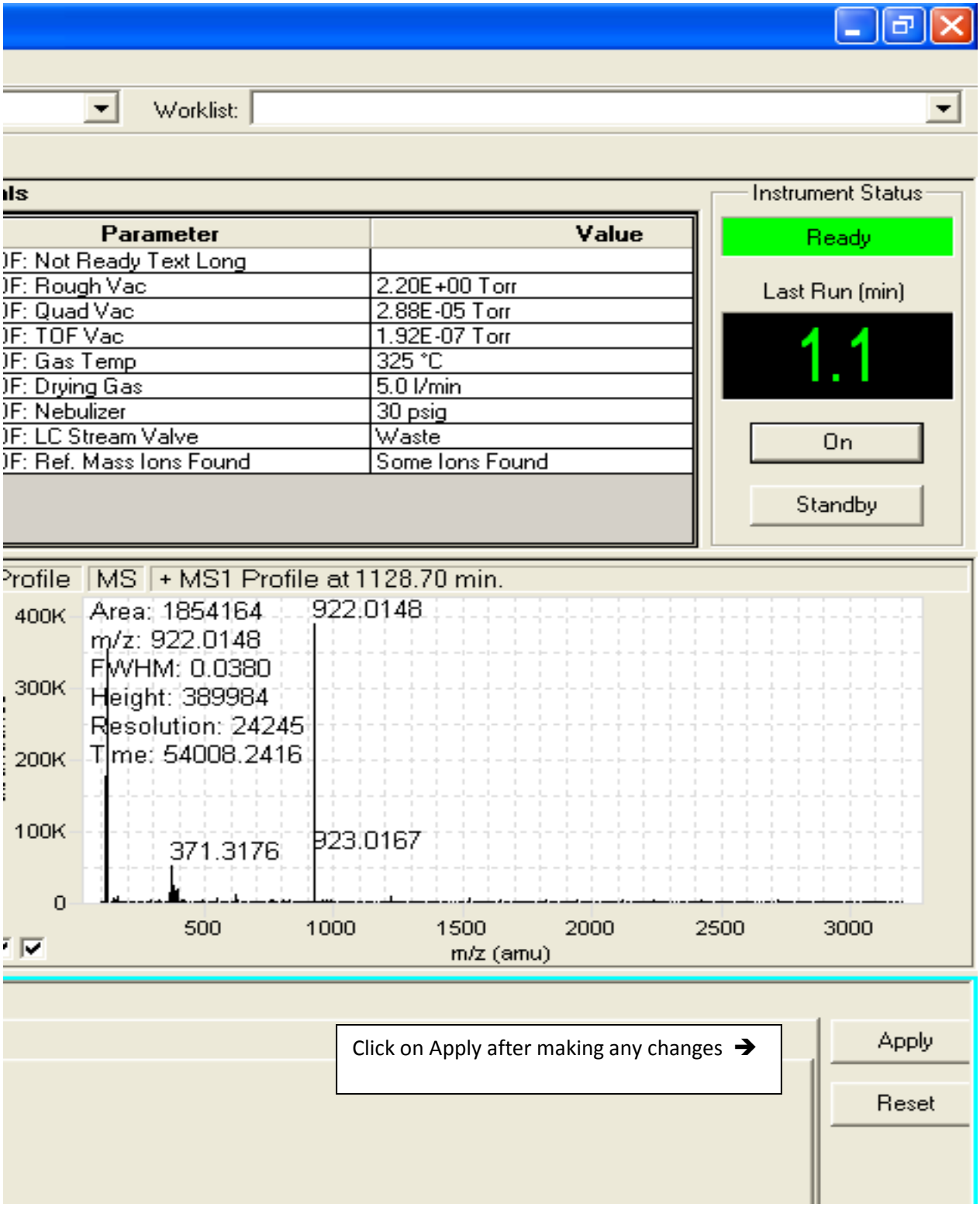
3) Install the ZORBAX 300SB-C18 column. Note that the flow direction is left to right. The left side is the high pressure side, and requires a nut and ferrule. The low pressure side on the right only requires a one-piece PEEK fitting.



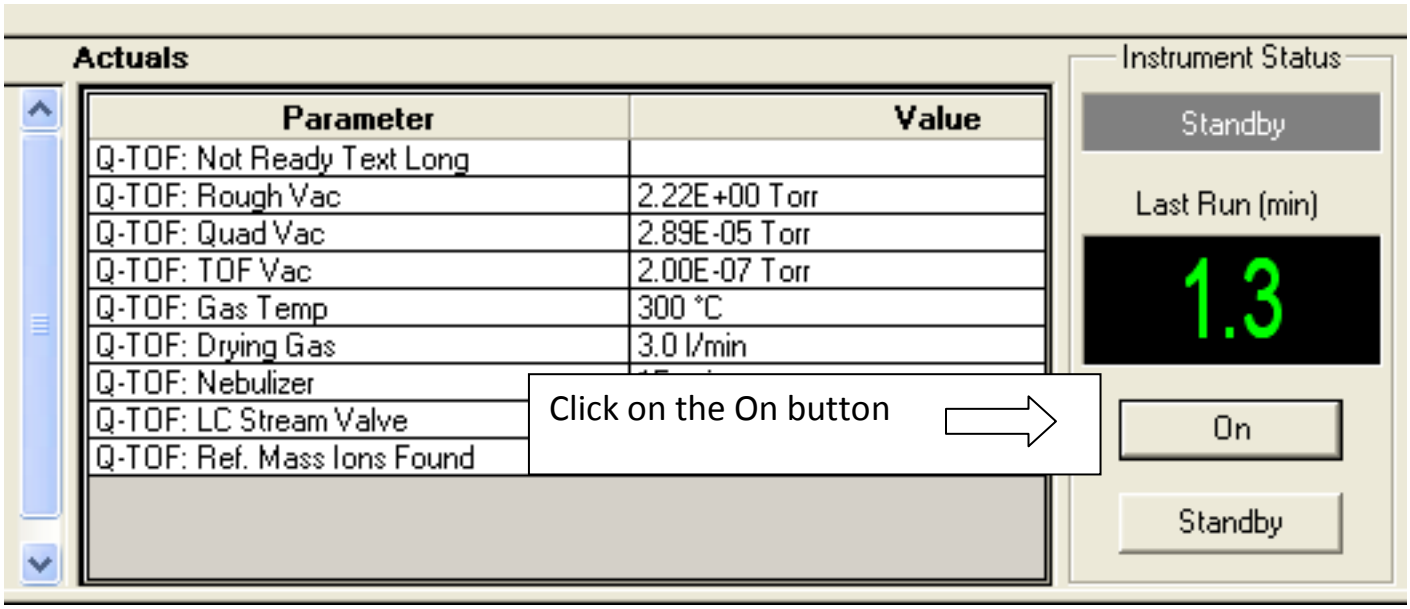


4) Set the initial solvent flow to 95% acetonitrile with 0.1% formic acid, and 5% water with 0.1% formic acid, at a flow rate of 0.5 ml/min, using the “1290-Bin Pump” tab on the method editor. Click on the “Apply” button.

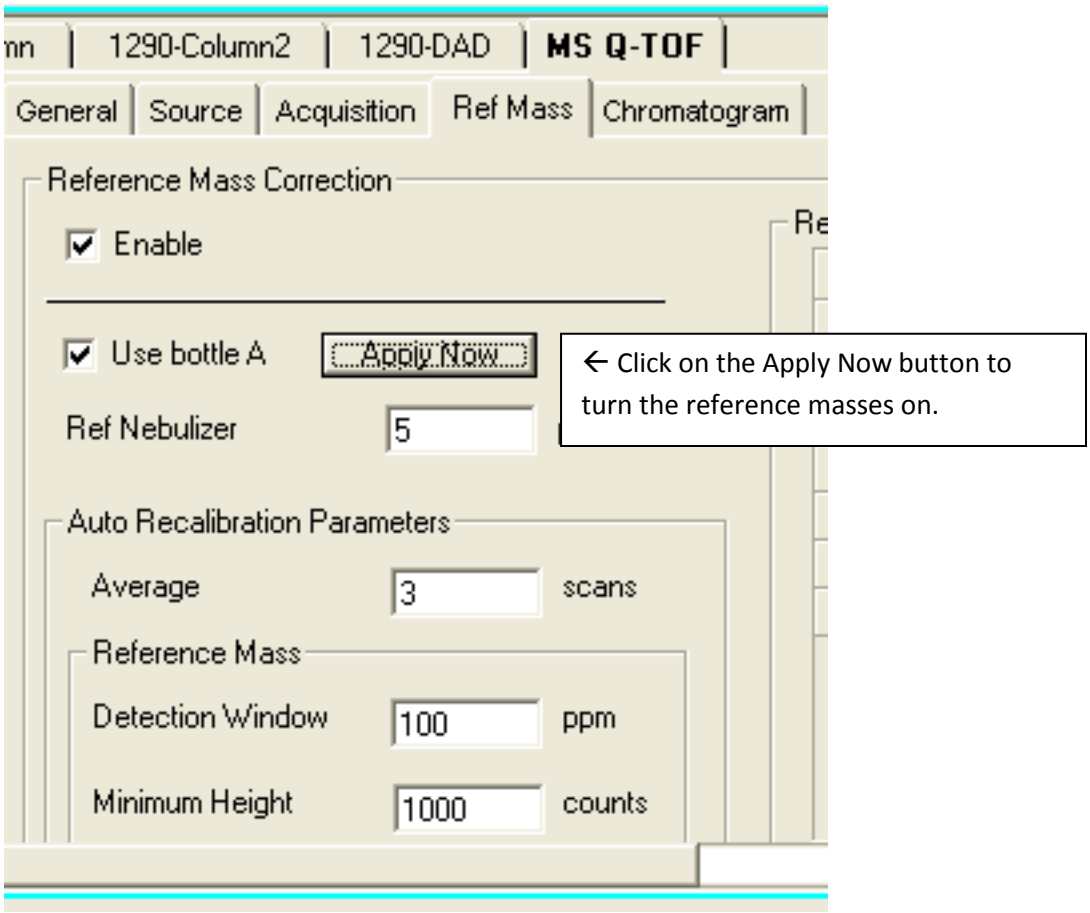



Worklist:
Instrument Status
Ready
Last Run (min)
1.1
On
Standby
Parameter
Value
OF: Not Ready Text Long
OF: Rough Vac
2.20E+00 Torr
OF: Quad Vac
2.88E-05 Torr
OF: TOF Vac
1.92E-07 Torr
OF: Gas Temp
325 °C
OF: Drying Gas
5.0 l/min
OF: Nebulizer
30 psig
OF: LC Stream Valve
Waste
OF: Ref. Mass Ions Found
Some Ions Found
Profile
MS
+ MS1 Profile at 1128.70 min.
Area: 1854164
m/z: 922.0148
FWHM: 0.0380
Height: 389984
Resolution: 24245
Time: 54008.2416
922.0148
923.0167
371.3176
400K
300K
200K
100K
0
500
1000
1500
2000
2500
3000
m/z (amu)
Click on Apply after making any changes ➔
Apply
Reset

5) Put the instrument into “On” mode.



6) Turn on the Reference Mass ions, using the Q-TOF and Ref Mass tabs.



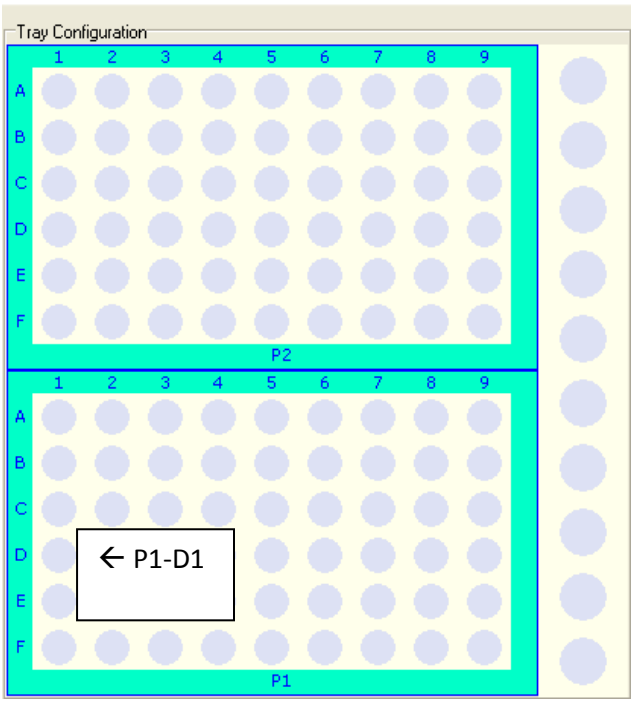
7) After the column has conditioned for 5 minutes, change the gradient to 95% water with 0.1% formic acid, and 5% acetonitrile with 0.1% formic acid.

8) Sample Preparation:
The protein sample should be desalted, and dissolved in water with 0.1% formic acid. The concentration should be at least 0.1 mg/ml. The minimum volume would be 10 ul if using a conical bottom insert in the vial.

Important! The sample must be filtered to remove any particulate matter.

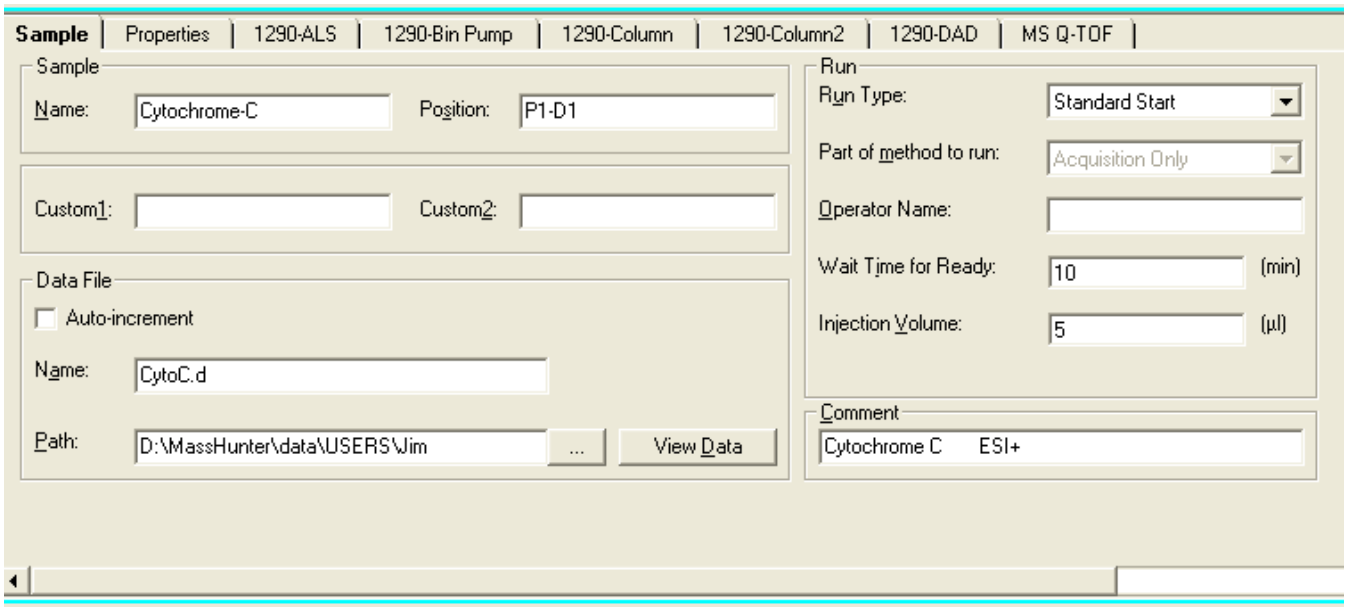
Typical 0.2 ml vial insert.

9) Place the sample in the Autosampler compartment, and note its position. It's position is designated by the plate number, followed by a dash, and then the well letter and number. For example, P1-D1 would be plate 1, row D and column 1.

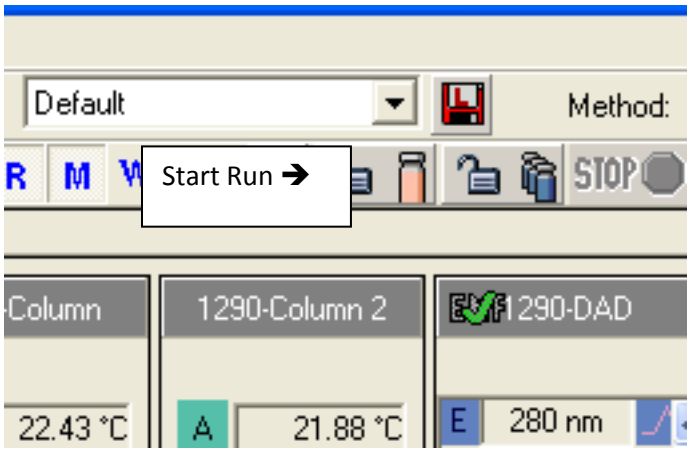


Click on the “Sample” tab in the method area near the bottom of the page, to set up the data acquisition.

The required parameters to set are the sample position in the autosampler tray, a data file name, comment, and injection volume.



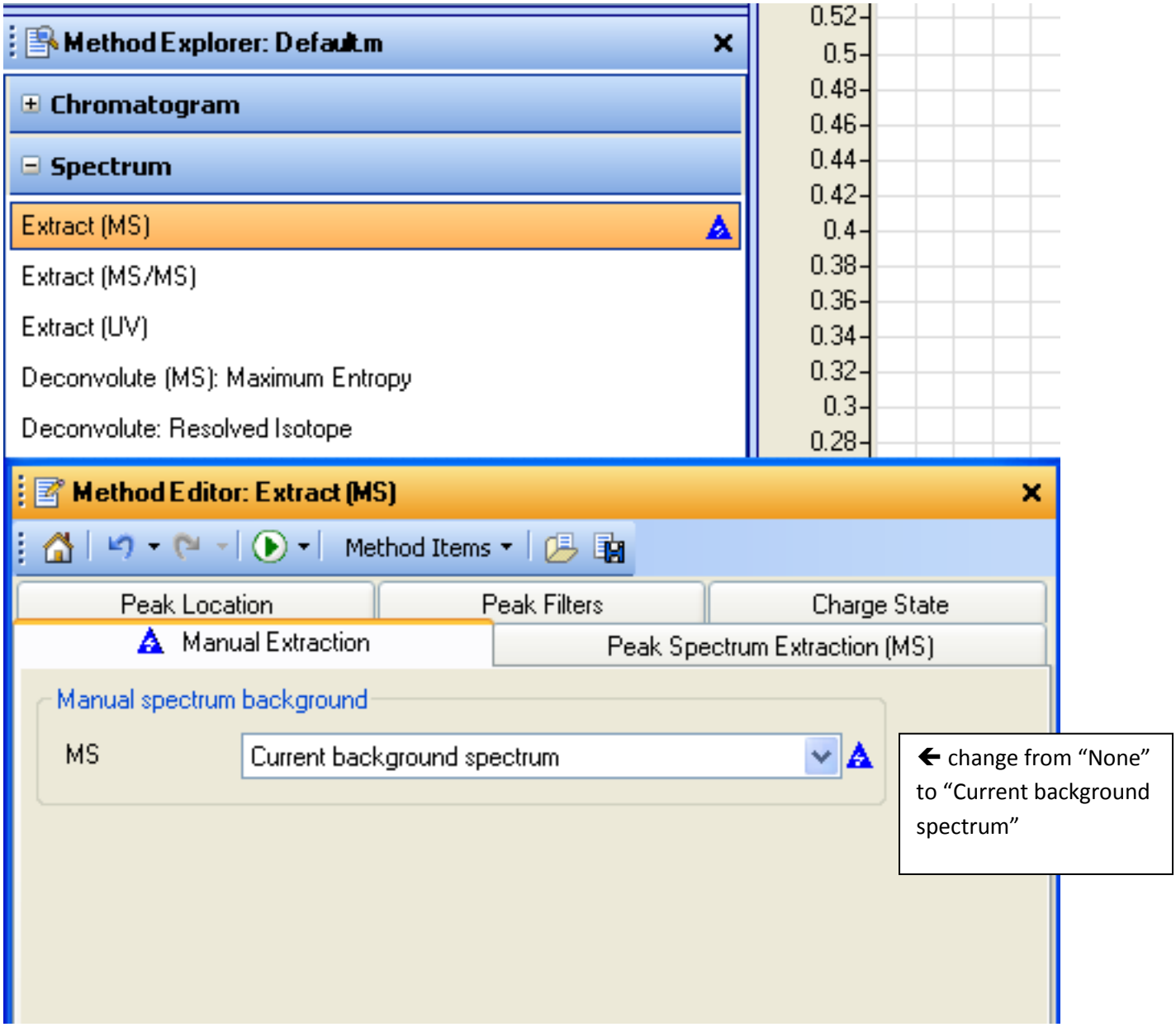
10) Click on the Start Run icon.



1) Launch Agilent Qualitative Analysis Program.

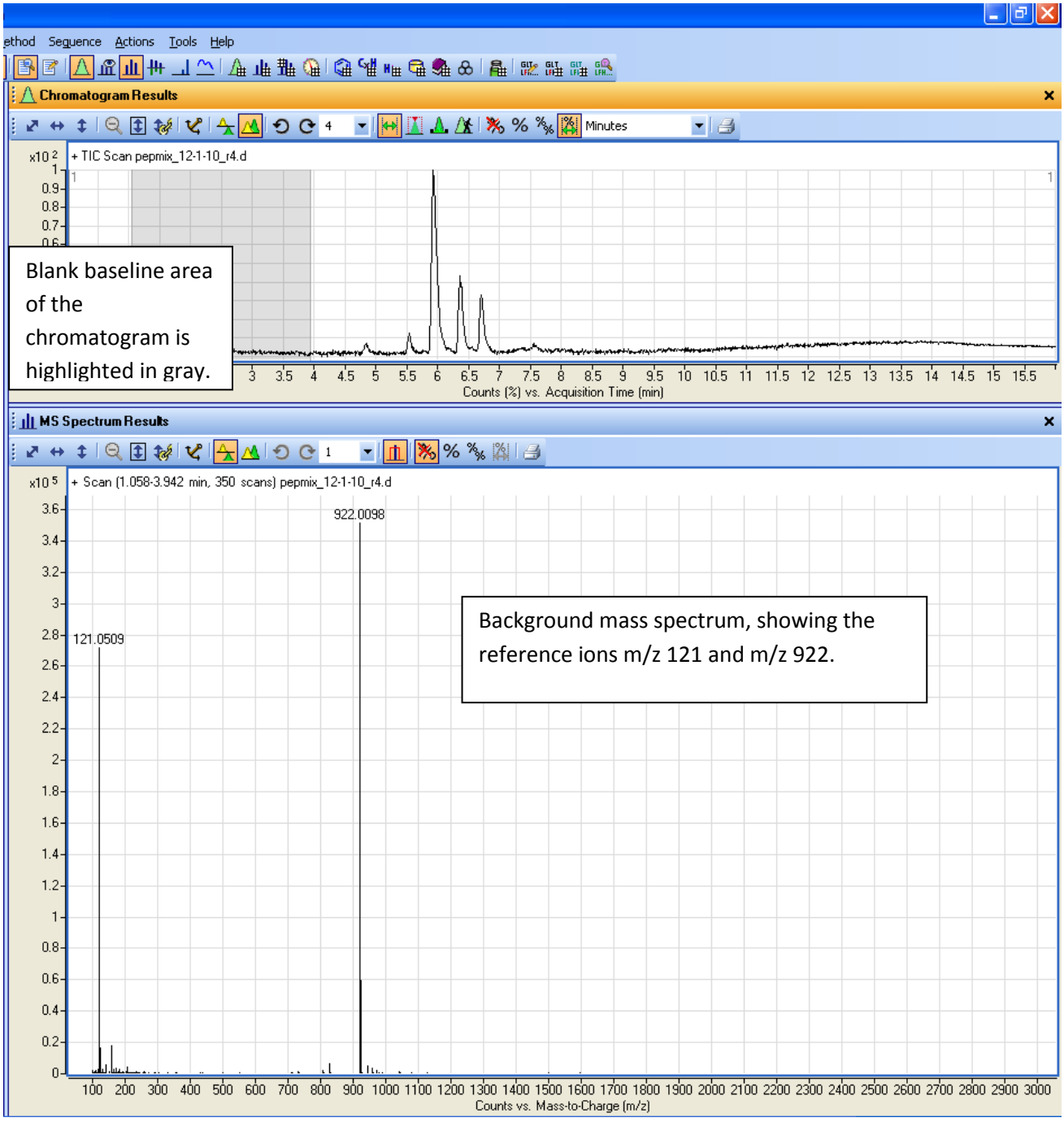
2) After the program comes up, go to the Tools menu, and Configure Workflow for “General”. Click OK and click on “No” to saving method changes.

3) On the left hand side, expand the “Spectrum” heading by clicking on the “+” sign in front of it. Then click on “Extract(MS)”. The method editor will come up, and you will need to change the drop down menu choice from “None” to “Current background spectrum”. This will set up automatic background subtraction.

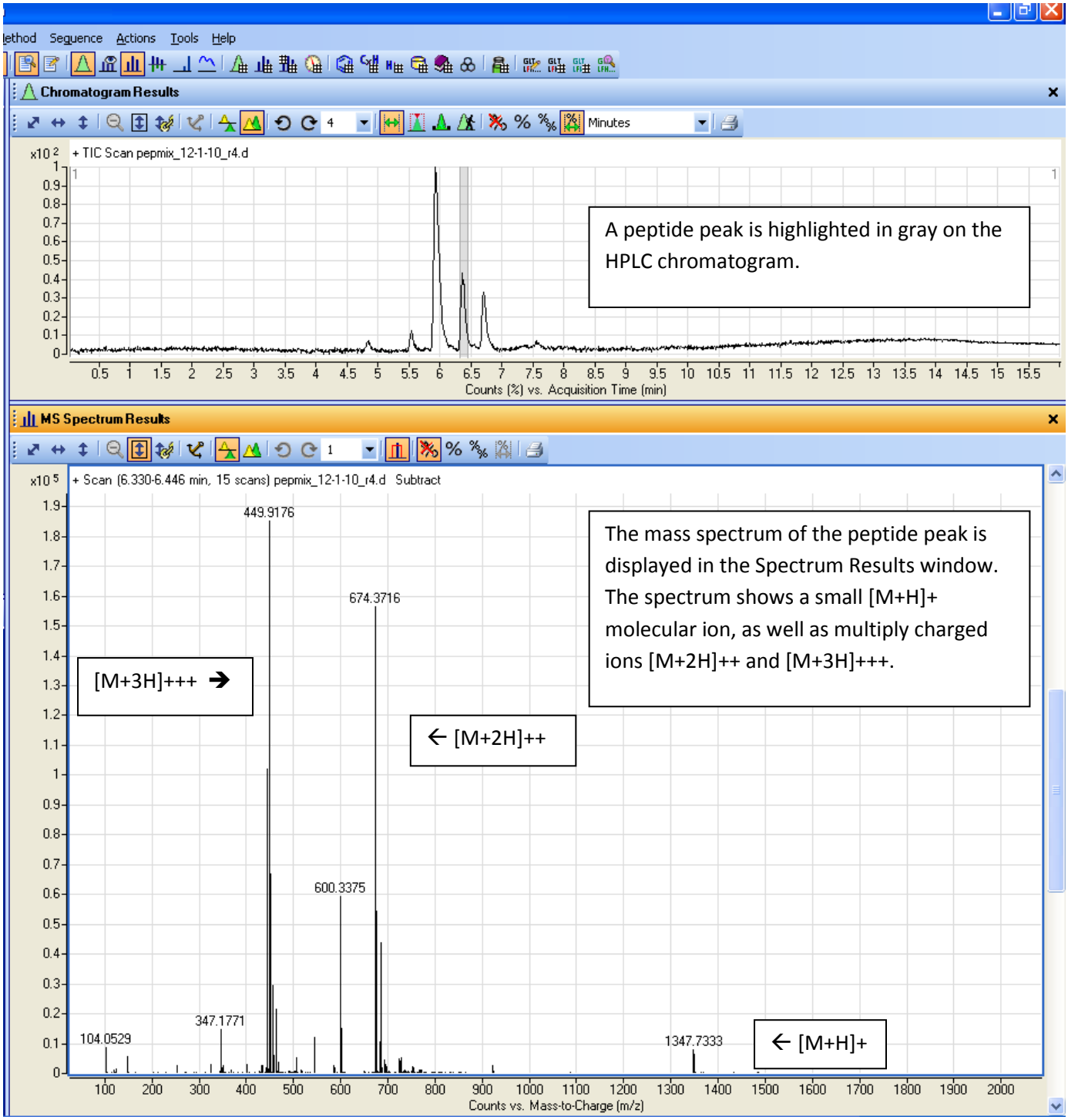


4) Next, open your data file, using File and Open.

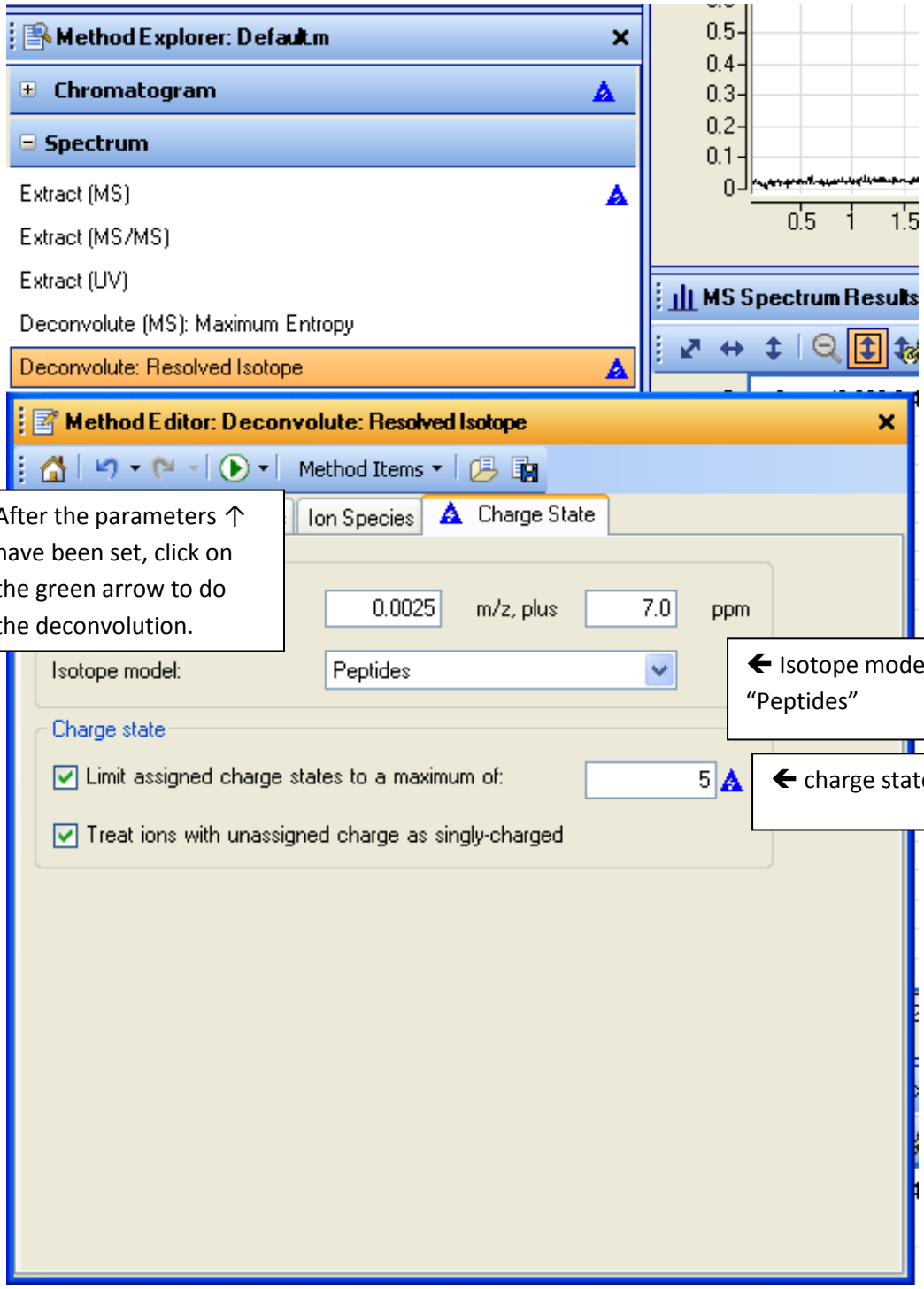
5) On the HPLC chromatogram, left-click and drag across an area of background baseline with no peaks, in order to highlight that area. Then right-click the mouse to obtain a menu, and left-click "Extract MS Spectrum to Background". You should see a background spectrum in the MS Spectrum window, showing mostly m/z 121 and m/z 922, which are reference mass ions used for mass correction.



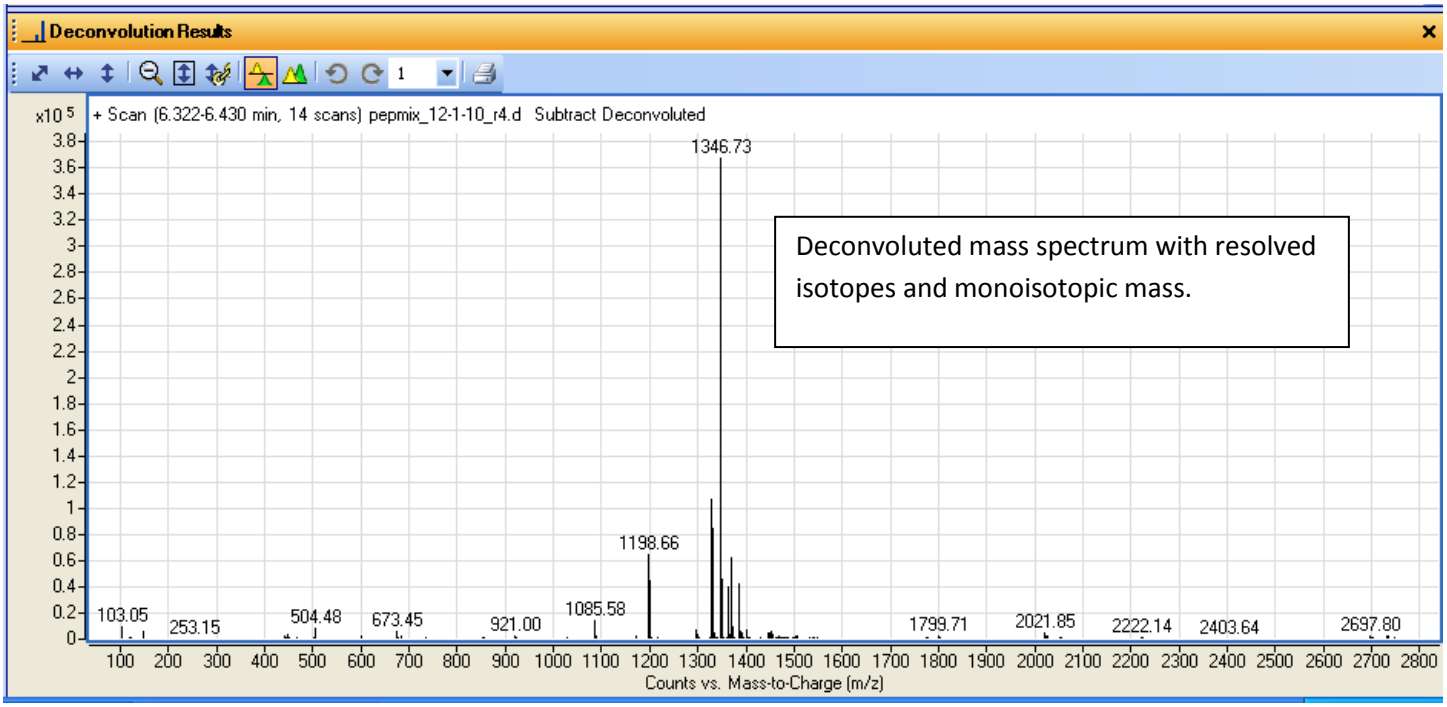
6) On the HPLC chromatogram, left-click and drag across a peptide peak of interest to highlight it. Then right-click the mouse to obtain a menu, and left-click "Extract MS Spectrum". In the MS Spectrum window, you should see the mass spectrum of the peptide. The background spectrum is automatically subtracted from this spectrum, so there should be little or no presence of m/z 121 and m/z 922.



7) To deconvolute the spectrum, first check the parameters in the deconvolution method. To do this, under the "Spectrum" heading on the left-hand side, click on "Deconvolute: Resolved Isotope". A dialog box will come up. Under the "Charge State" tab, make sure that the Isotope Model is set to "Peptides", and the charge state maximum is set to 5. Click on the green arrow to do the deconvolution.



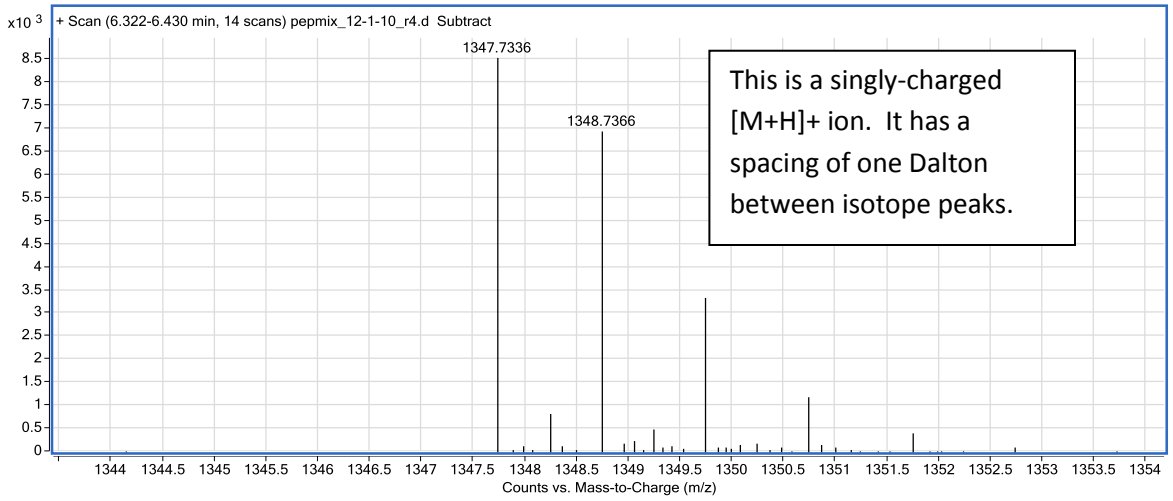
After the deconvolution has been done, you will see the deconvoluted spectrum in the Deconvolution Results window. This spectrum will show the monoisotopic mass of the peptide without additional protons or charges.



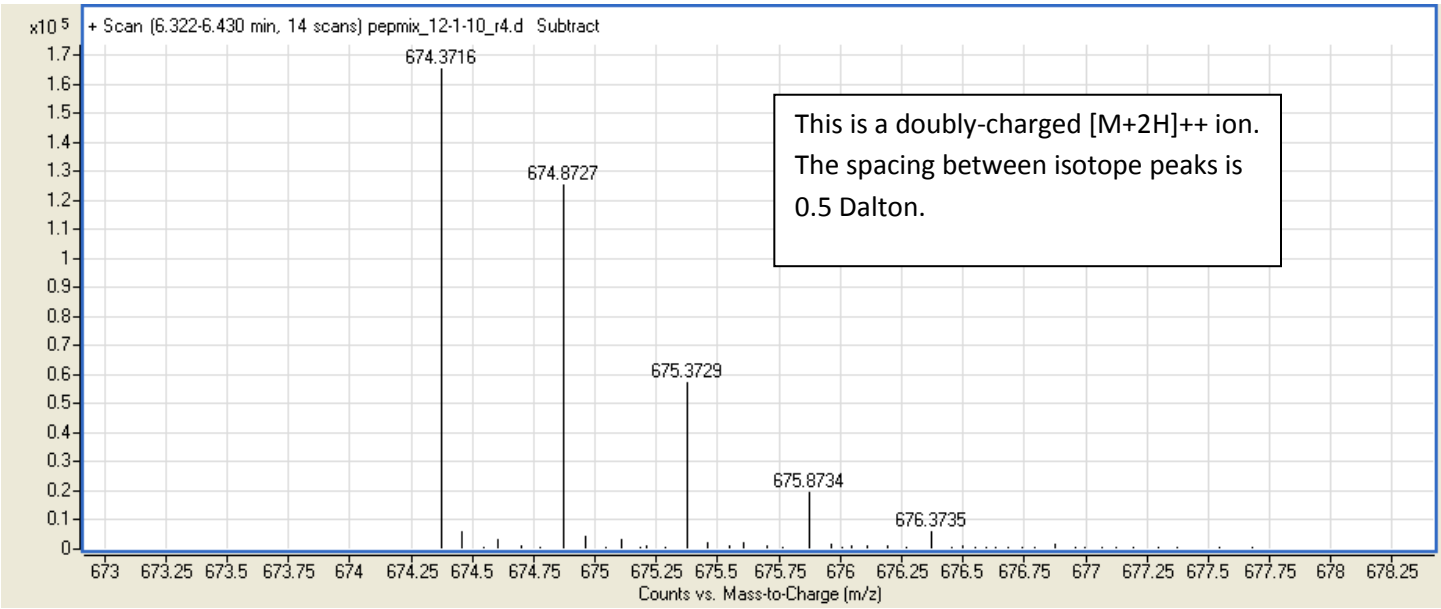
Note: Once your parameters have been set, from then on you can obtain a deconvoluted spectrum simply by right-clicking the mass spectrum to get a menu, and then left-click on "Deconvolute (Resolved Isotope)".

8) Other peptide peaks can be worked up in the same way. Here are some useful things to know about peptide spectra:

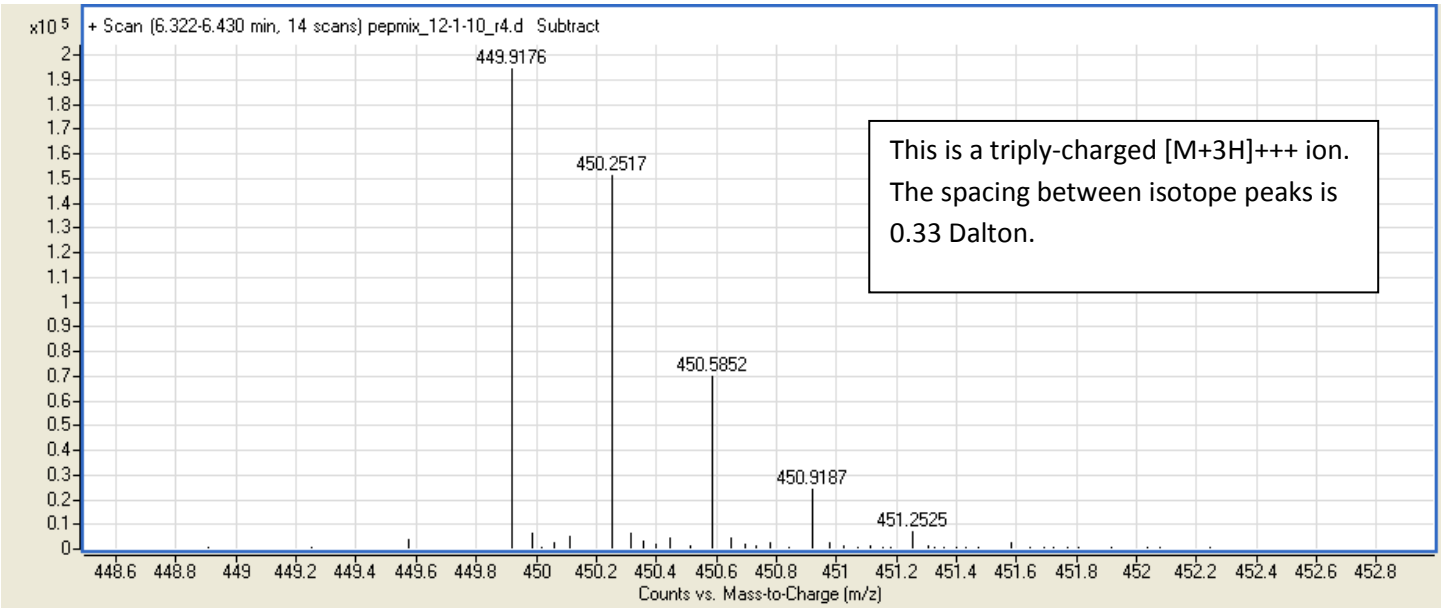
If an ion is singly-charged, then the spacing between adjacent isotope peaks will be one Dalton.



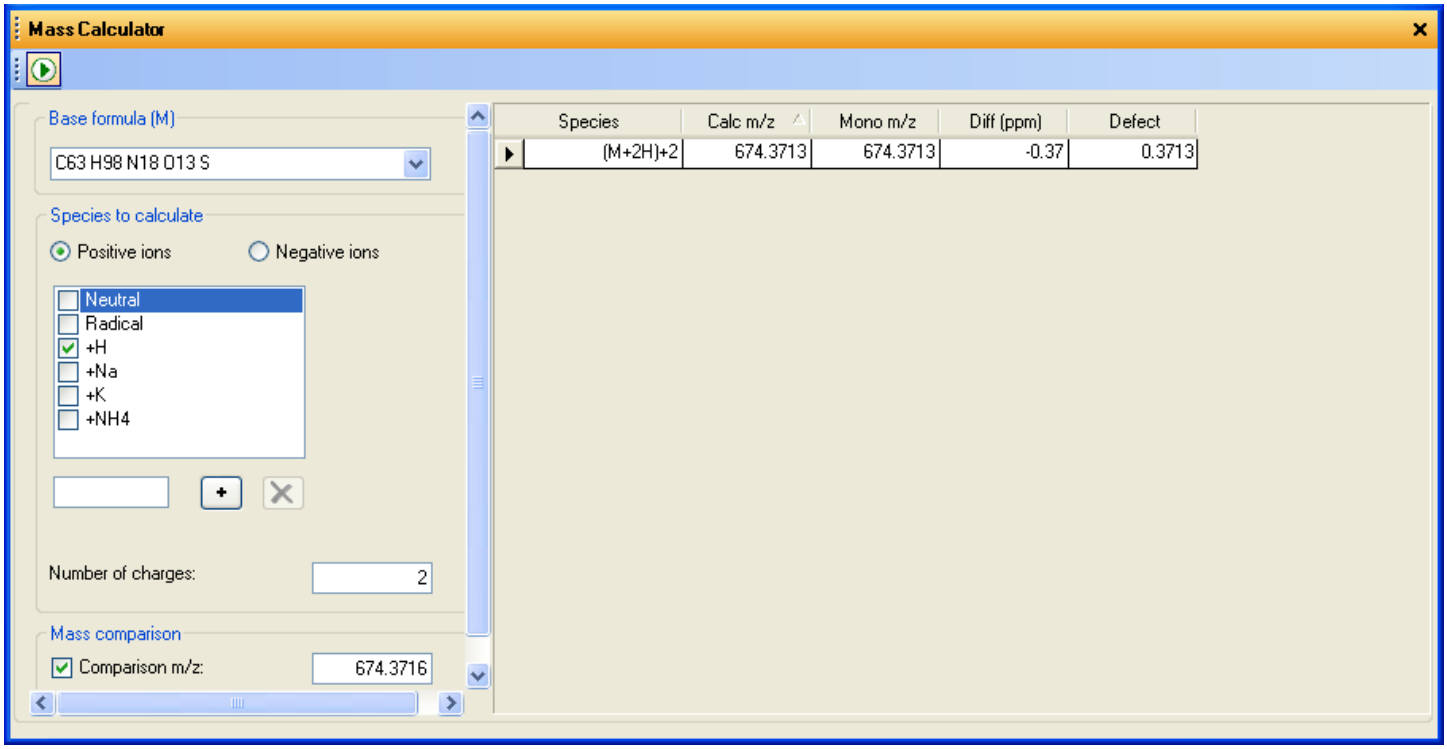
If an ion is doubly-charged, then the spacing between adjacent isotope peaks will be 0.5 Dalton.



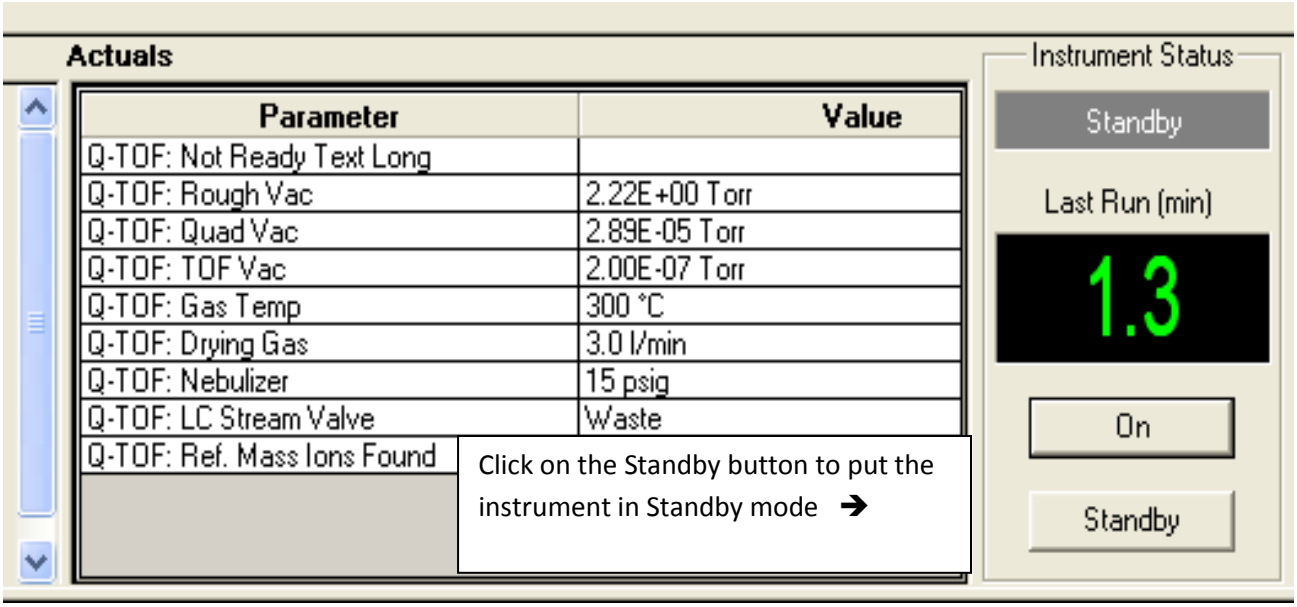
If an ion is triply-charged, then the spacing between adjacent isotope peaks will be 0.33 Dalton.



You can calculate the theoretical monoisotopic masses of the various ions by clicking on the "Tools" menu, and clicking on "Show Mass Calculator". Type in the molecular formula of the neutral molecule in the "Base Formula(M) field. Select "Positive ions" and click on the "+H" option. Set the number of charges. If you wish, you can type in the observed value in the "Comparison m/z" field, and it will calculate the difference between the observed and theoretical values. Click on the green arrow to do the calculation.



5) Put the Instrument in Standby, by clicking on the Standby button.



6) Exit from the Data Acquisition program. Exiting from the Data Acquisition program is what logs you off, so that no more time is billed to your account.

7) Remove the ZORBAX 300SB-C18 HPLC column.

8) Install a union in place of the column, to prevent solvent from leaking out of the tubing.