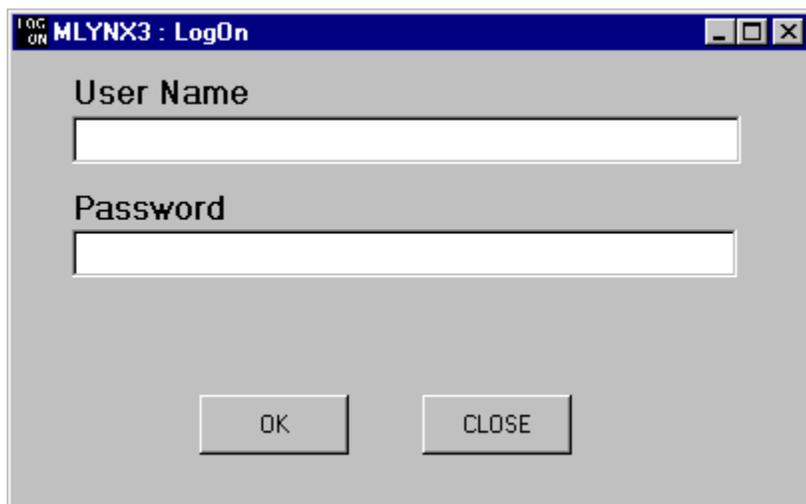
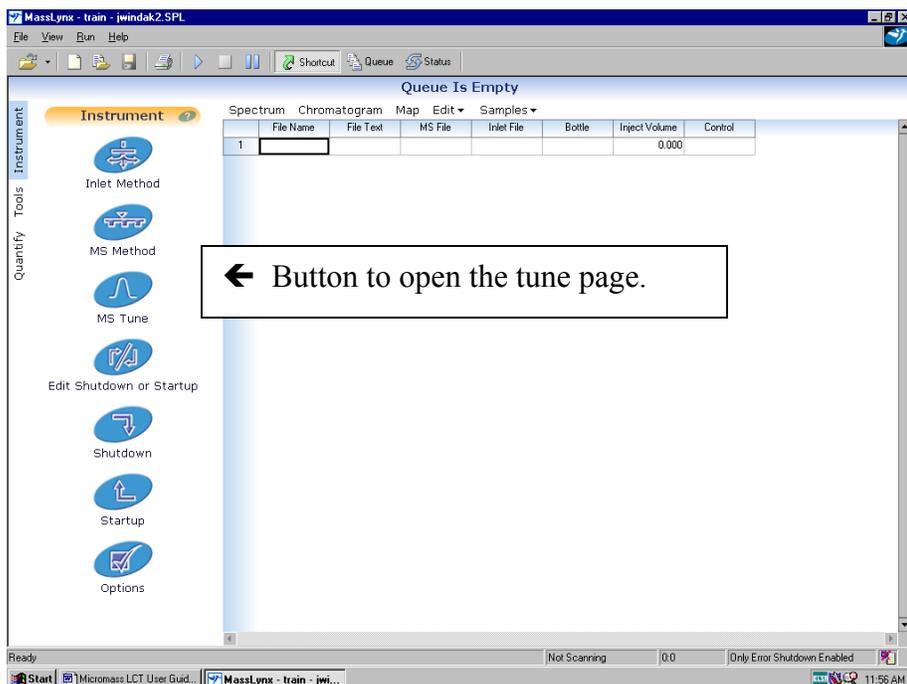


Micromass LCT User's Guide

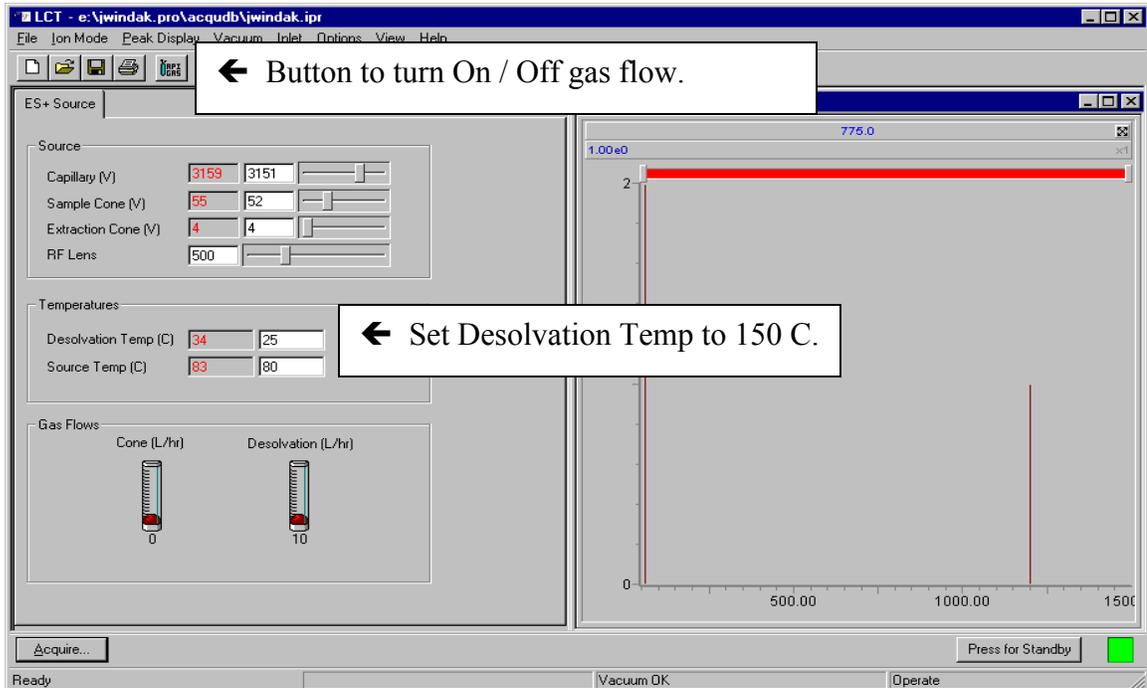
- 1) Log on to MassLynx with your username & password.



- 2) After you have logged in, the MassLynx software will automatically run.
- 3) After MassLynx has come up, open your project by clicking on the File menu, and then "Open Project". Select your project and click OK.
- 4) Click on the "MS Tune" icon in order to open the tune page.



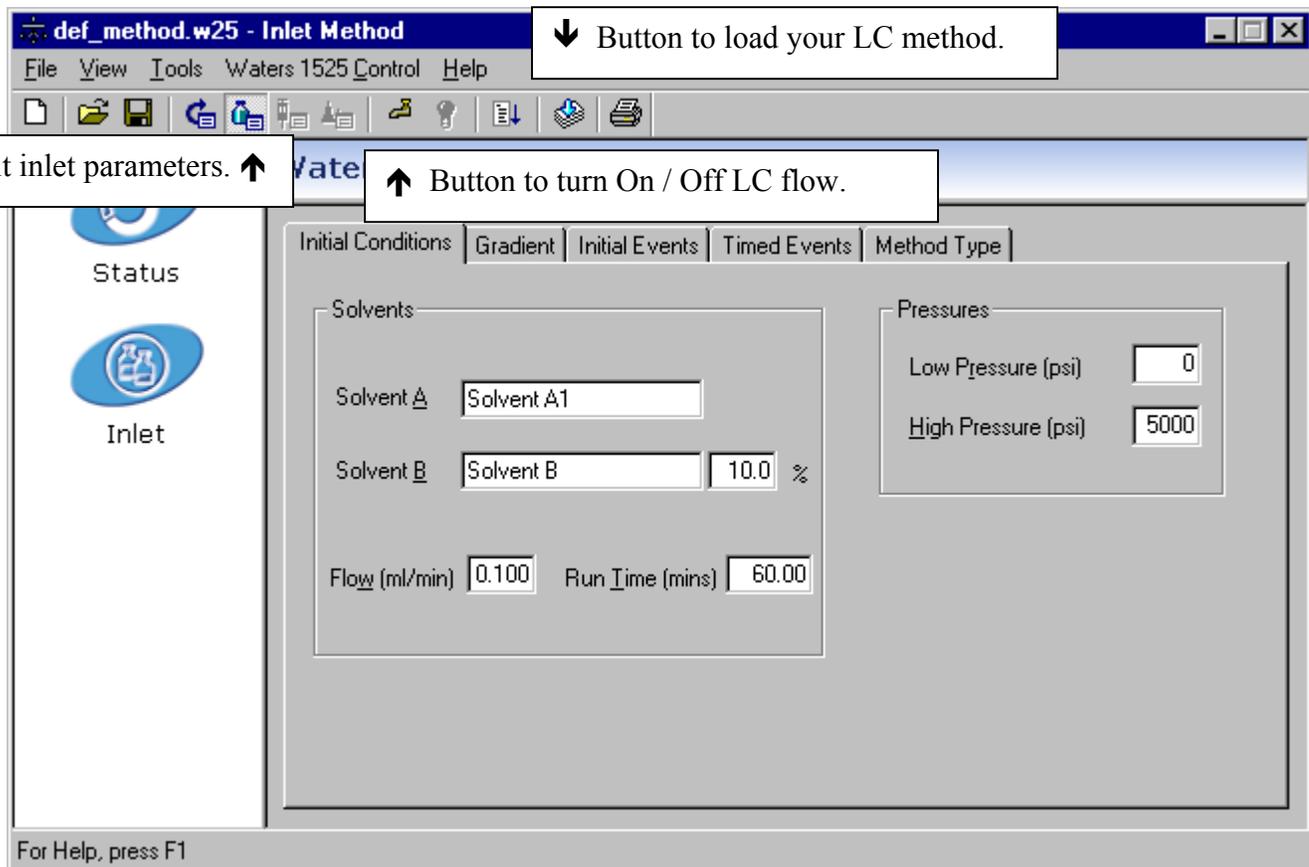
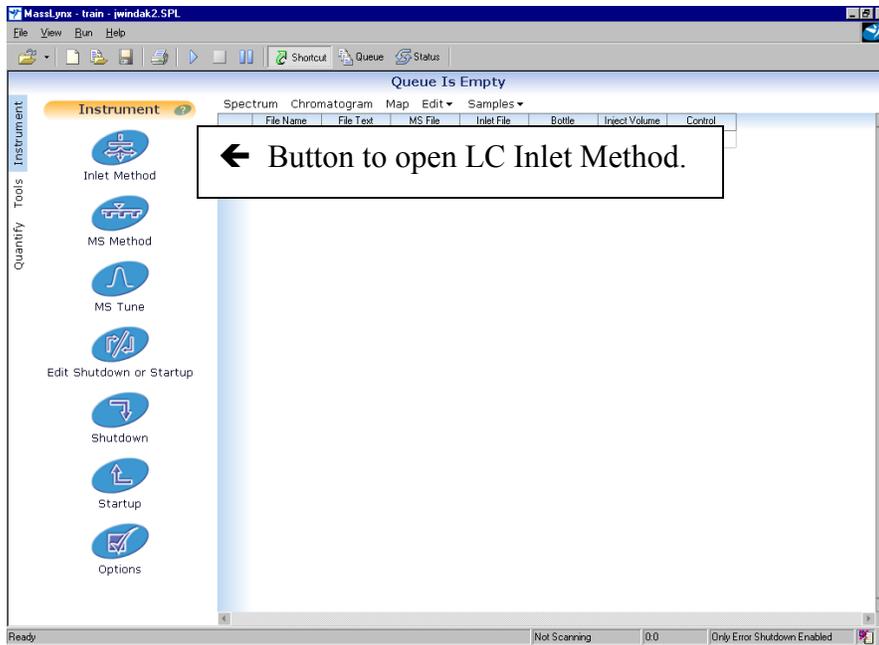
- 5) On the Tune Page, Click on the button on the toolbar that says “API Gas” to turn on the nitrogen gas flow. (If the button appears pressed in, the gas flow is on. If it appears out, the gas flow is off).



- 6) On the Tune Page, Set the Desolvation Temperature to 150 C and press Enter.

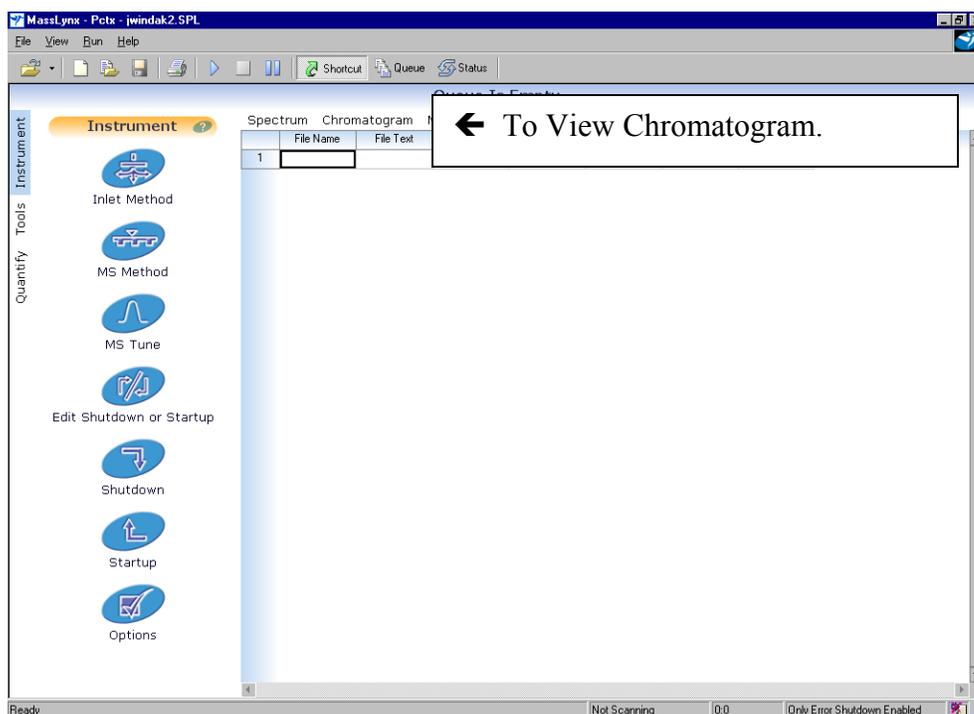
Please note that the API gas must be on before you increase the Desolvation temperature. Otherwise, the probe will overheat and be damaged.

- 7) Go back to the MassLynx page, click on the “Inlet Method” icon. This will take you into the control panel for the Waters’ LC pump. Click on the inlet parameters icon on the toolbar. Check that the flow is 0.1 ml/min. Then click on the Load Method icon to load this method. Then click on the icon that looks like a water faucet. This will turn on the LC flow. If water looks like it’s flowing out of the faucet icon, then LC flow is on. Please note that communication with the LC is slow, so that it will take perhaps 15 seconds for the faucet button to respond after clicking it.

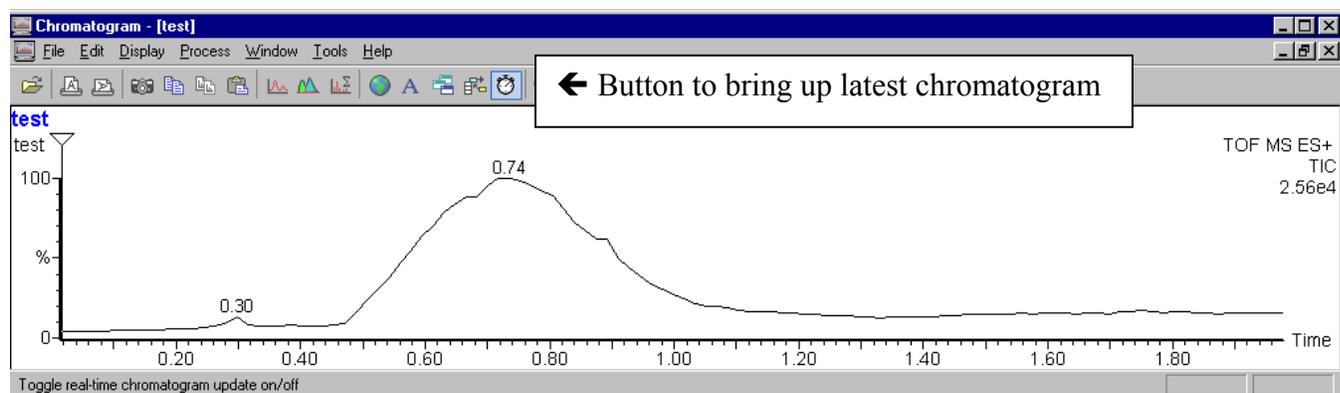


- 8) Verify that the LC is flowing properly, by clicking on the “Status” icon. You should see a flow of 0.100 ml /min, and Solvent A (methanol) should be around 90%, and solvent B (water) should be around 10%.

- 9) You can minimize the Inlet Method box, but do not close it. You will need it again when you shut off the pump when you are done.
- 10) Go back to the Tune Page, and click on the Acquire button. Enter a filename, and a text description of the sample. Choose “Centroid” for the Data Format. Click on Start to start the acquisition.
- 11) The sample solution should have been prepared according to the instructions given at the end of this booklet. The concentration should be approximately 1 ng /ul for a nominal mass spectrum. Move the injector lever to the “Load” position. Rinse out the syringe with blank solvent. Fill your syringe with 20 ul of sample solution without air bubbles, and inject. (The sample loop has an actual volume of 5 ul). Move the injector lever to the “Inject Position”.
- 12) Rinse out the syringe
- 13) Within 30 seconds, you should see spectra from your compound in the tune page window.
- 14) After your compound has eluted, click on “Stop” to stop the acquisition. It will ask if you really want to stop the acquisition. Click Yes.
- 15) To display your data, go to the MassLynx page, and click on “Chromatogram”.



- 16) To bring up the last chromatogram acquired, or the one being acquired, click on the stopwatch icon.



- 17) To display a spectrum, right-click a scan or a range of scans on the chromatogram.
- 18) The spectra you have acquired have been mass measured against an external calibration, with no internal lock mass. They will be accurate to plus or minus a few tenths of a Dalton. You should have the peak annotation set to one decimal place to reflect the accuracy of the data. The peak annotation setting is found under the Display menu, under “Peak Annotation...”.
- 19) To obtain a listing of the spectrum, you can click on “List Spectrum” under the Display menu. You can control the threshold for peak listing by clicking on “View...”, under the Display menu. Then under “Data Threshold” you can enter a percentage above which peaks will be listed. To go back to the spectral view, click off “List Spectrum” under the Display menu.
- 20) To run another sample, go back to the Tune page and click on the Acquire button to set up your next data acquisition. Otherwise, if you are done, go to the Shutdown Procedure.

Shutdown Procedure

- 1) On the MassLynx page, click on the “Inlet Method” button. Turn off the LC flow, by clicking on the faucet icon. (Communication with the LC is slow). Verify that the LC flow status is off, by clicking on “Status”.
- 2) On the Tune Page, set the Desolvation Temperature to 20 C, and press Enter. Wait until the actual temperature falls to 100 C or less.
- 3) On the Tune Page, click off the API gas button to turn off the gas flow.

Please note that the Desolvation heater must be off before turning off the API gas. Otherwise the probe will overheat and be damaged.

- 4) Close the Tune Page. If you are asked whether to save changes to the parameter file, choose **Yes**.
- 5) Close any Spectrum and Chromatogram windows, and exit from MassLynx.
- 6) Exiting from MassLynx will automatically log you off. Be certain to remember to do this. Otherwise you will keep on accumulating time that you will be billed for.

Recipe for Preparing Organic Molecule Samples, With Molecular Weights Between 300 to 1500 daltons For Electrospray-MS on the Micromass LCT:

1) Dissolve 1 mg of sample in 1 ml of 80% MeOH / 20% Dichloromethane. The ratio of MeOH to Dichloromethane can be adjusted if the sample is not very soluble in methanol. You could use 50% MeOH / 50% Dichloromethane, for example.

This stock solution will have a concentration of 1 milligram per milliliter, which is the same as 1 microgram per microliter, which is also the same as 1000 nanograms per microliter.

2) Dilute this stock solution 1:1000, by taking 1 microliter of the stock solution, and adding 1 ml of a Dilution Solution. (See the following page for Dilution Solution Recipes).

This diluted solution will now have a concentration of 1 nanogram per microliter, which is the same as 1000 picograms per microliter.

This concentration is suitable for injecting into the 5 ul LC sampling loop, in order to obtain a strong intensity, nominal mass spectrum.

When doing exact mass measurements, this solution would have to be diluted further to 100 pg/ul.

Dilution Solution Recipes

Dilution Solution Recipe for Observing $[M+Na]^+$ Adducts:

45 ml of MeOH

5 ml of H₂O

Add 50 microliters of a 10 mg / ml NaCl solution in water, in order to obtain a Na⁺ concentration of approximately 0.2 milliMolar.

Dilution Solution Recipe for Observing $[M+H]^+$ Adducts:

45 ml of MeOH

5 ml of H₂O

Add 500 microliters of Glacial Acetic Acid

Use high purity solvents, and try to avoid using solvents that have been in plastic squeeze bottles. The solvents will pick up plasticizers (phthalate esters) that will show up be Electrospray.

Procedure for Doing Exact Mass Measurements

This following procedure is used to obtain exact mass measurements. The LCT is capable of mass measuring to within 5 ppm error for masses between 400 and 1000 daltons.

Two points are very important for obtaining accurate results. First, you must have a known “Lockmass” present. A known standard that produces a known molecular ion must be introduced simultaneously with your “unknown” sample.

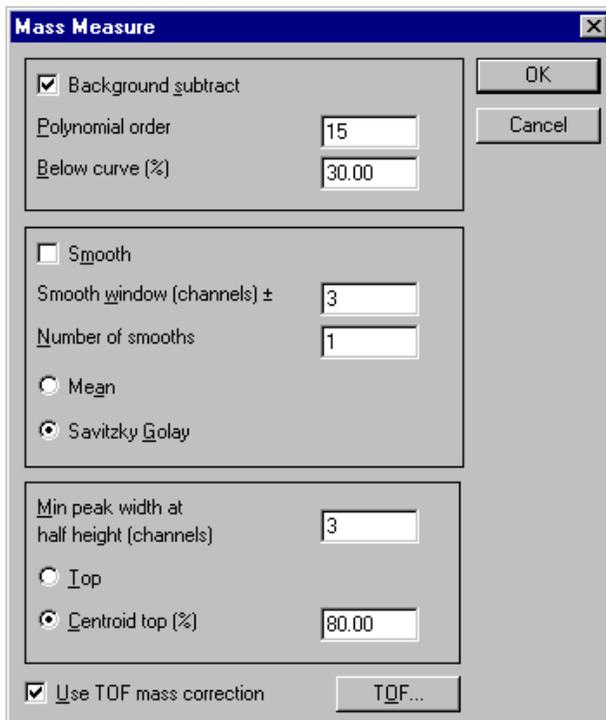
Second, both your standard lockmass, and your unknown masses must be below 200 counts in intensity when run in continuum mode. If either your sample or lockmass exceed these intensity values, the mass peak shape will become skewed, and your accuracy will not be good. This is caused by the phenomenon called “detector dead time”.

A good lockmass standard to use is the compound Reserpine. It produces a strong $[M+H]^+$ ion at m/z 609.2812.

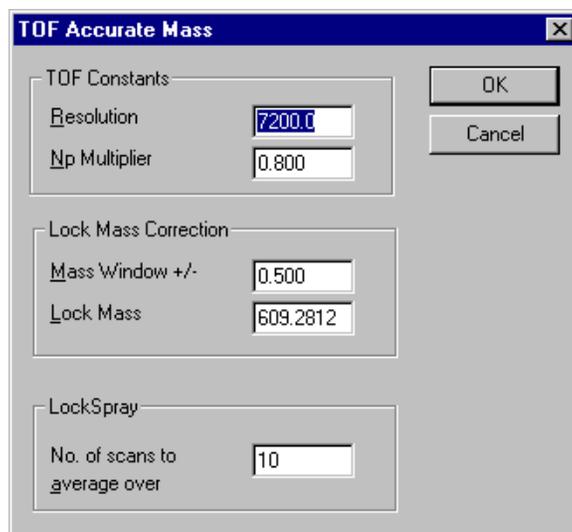
This procedure assumes that you are already logged in, and that MassLynx is already running, and that the gas flow is on, and the Desolvation Temperature is already at 150 C, and the LC pump is flowing at 0.100 ml/min.

- 1) Tee into the line from the LC pump with the line that goes to the syringe pump.
- 2) Fill the 500 ul syringe with the 1 ng/ul solution of Reserpine. Place it in the syringe pump, and put the fitting on the end of the syringe needle. Push some solution through the line to clear out whatever was previously in the line. Press the run/stop button on the syringe pump to turn it on. Set the flow rate to around 5-7 ul /min.
- 3) Monitor the intensity of the Reserpine peak at m/z 609 while set up for viewing the peaks in Continuum mode. Adjust the flow rate of the syringe pump, and also the cone voltage if necessary, in order to obtain an intensity of around 200 counts on m/z 609.
- 4) Click on the Acquire button to set up and start the acquisition. Collect the data in Continuum mode. Your sample should have a concentration of around 100 picograms per microliter. Inject your sample solution. After your sample peak has completely eluted, stop the acquisition.

- 5) View the chromatogram. Right click on the peak to display a spectrum. Observe the number of intensity counts on your m/z of interest. You want to use only those scans where the intensity of your unknown m/z is less than 200 counts. The intensity of the Reserpine m/z 609 should also be around 200 counts or less. Combine only those spectra that fit these conditions.
- 6) Set the peak annotation to 4 decimal places by clicking on the Spectrum Display menu, and clicking on “Peak Annotation...”. Set the decimal places value to 4, and click OK.
- 7) Click on the Process menu, and click on Mass Measure. If your mass peaks have a good shape, then it is not necessary to smooth. You should background subtract, and also center the spectrum. Set the center channel parameter to $\frac{1}{2}$ of the number of channels across the peak width at $\frac{1}{2}$ height. Click on the option to Use TOF mass correction.



Click on the TOF... button, and the following box will come up:

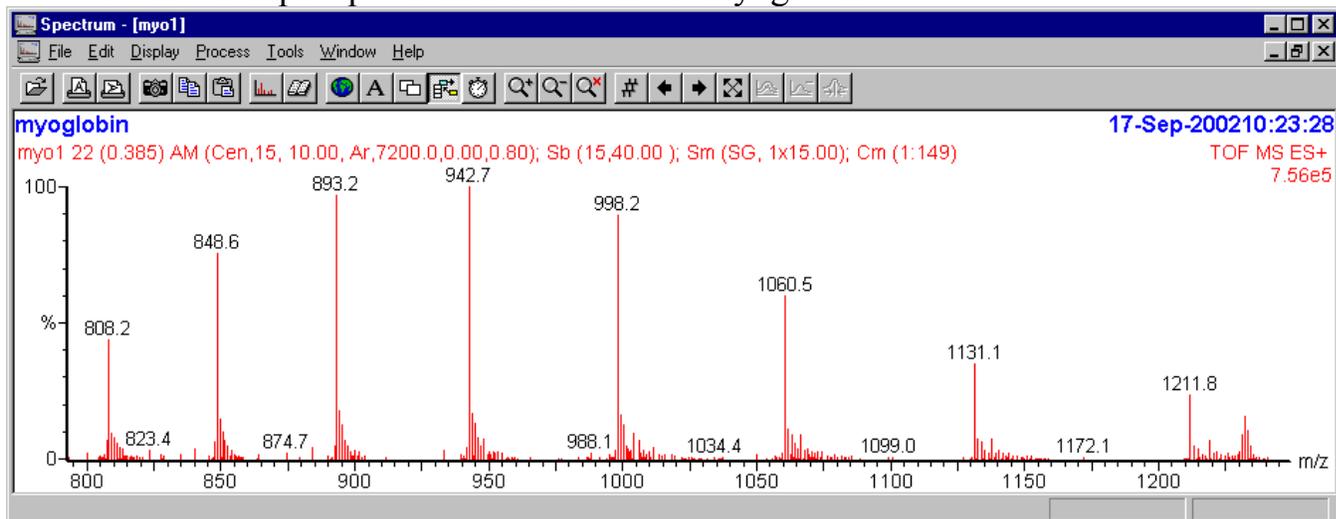


Set the Lock Mass to the $[M+H]^+$ mass of Reserpine, which is 609.2812. Click OK, and then click OK again. The spectrum will be processed. The mass of the unknown sample peak should now be correct to within 5 ppm.

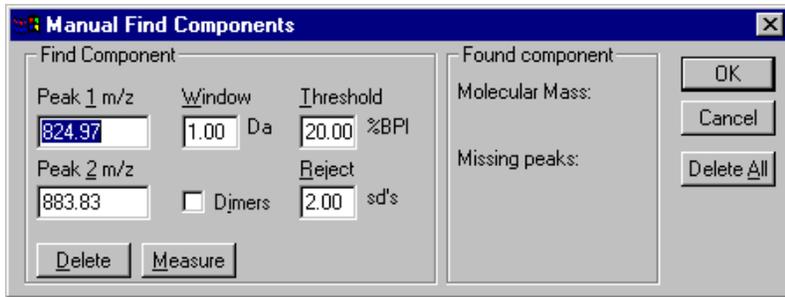
- 8) If you wish to run another sample, go back to step 4). Otherwise, set the peak annotation back to 1 decimal place. Set the data acquisition mode back to Centroid. Flush out the syringe pump line with methanol. Disconnect the syringe pump line from the tee, and put the blank plug back into the tee. Then do the normal shut down procedure.

Procedure for Running Protein Samples on the LCT

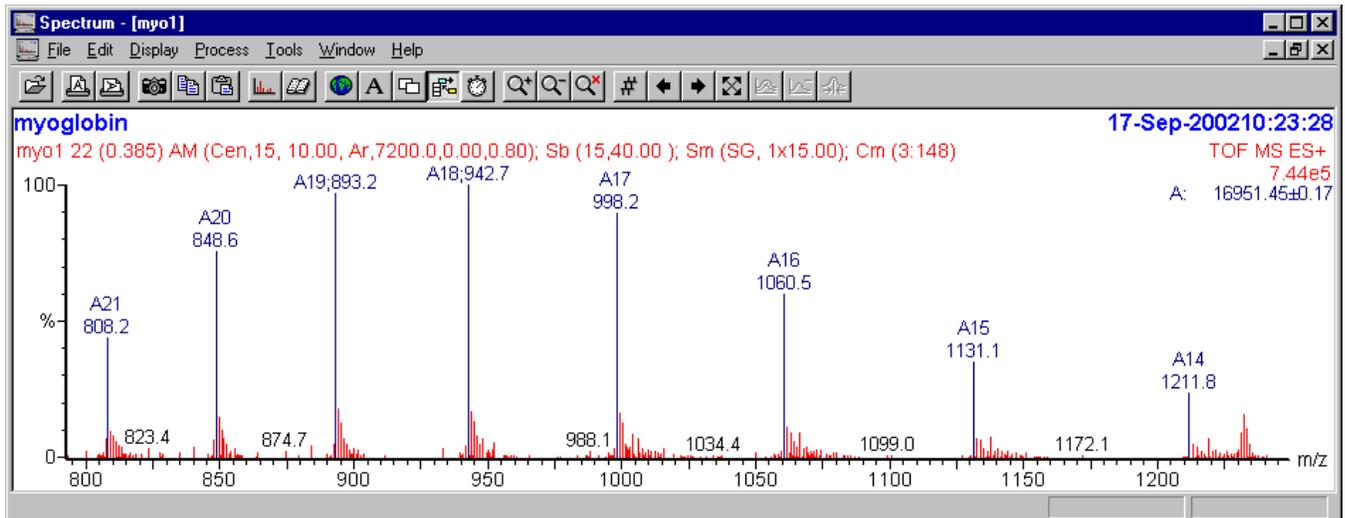
- 1) Disconnect the line from the LC pump. Connect the line from the syringe pump to directly infuse your sample solution.
- 2) Start up the LCT as normal, by logging in, and turning on the API gas, and setting the desolvation temperature to 150 C.
- 3) Infuse your protein solution into the LCT. Suggested concentration is 10 picomoles per microliter. The flow rate should be set to whatever gives good signal intensity, generally around 10-30 ul/min. Do not use TFA if possible, because this will suppress your signal. Adding 1% formic acid will help to enhance the signal intensity. You may need to increase the cone voltage to get good signal intensity.
- 4) Acquire data in Continuum mode. Average for at least several minutes.
- 5) View the chromatogram, and combine all of the scans into one spectrum.
- 6) Smooth, background subtract, and center the resulting spectrum. Shown below is an example spectrum for horse heart myoglobin.



Click on the Process menu, and click on Component, and Find Manual...
This box will appear:



Highlight the Peak 1 m/z, and then right click a peak in the spectrum series. Highlight the Peak 2 m/z box, and then right click and adjacent peak in the spectrum. Then click on the Measure button, and all of the peaks in the series will be identified by the number of charges, and the approximate mass of the neutral protein will appear on the right of the spectrum.



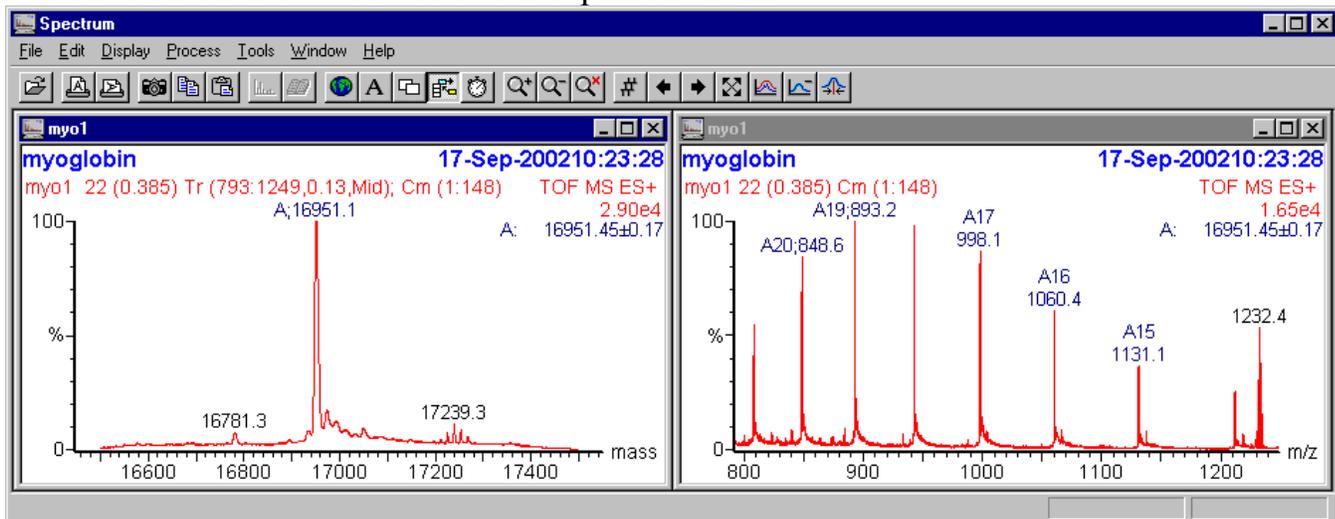
To obtain a transformed spectrum, do the following procedure:

Go back to the original continuum data spectrum, that has not been smoothed or centered. Display the best region of the spectrum that shows only reliable multiply charged peaks in the series. Then click on the Process menu, and Transform... The following box will appear:



Enter reasonable values for the Min mol mass and Max mol mass, that span a small region around the estimated molecular weight. Then click on OK. After around 30 seconds, the transformed spectrum will appear alongside the original spectrum. The transformed spectrum represents the average molecular weight of the original neutral protein without any extra charges. This transformed spectrum can then be smoothed,

background subtracted, and centered to give the most accurate value. The observed mass should be within 0.01% of the expected value.



When finished, please do the following steps:

- 1) Rinse out the syringe pump line with clean methanol.
- 2) Disconnect the syringe pump line, and reconnect the LC pump line to the Electrospray probe.
- 3) Set the data acquisition mode back to Centroid.
- 4) Set the desolvation temperature to 0 C and press enter. When the temperature falls below 100 C, turn off the API gas.
- 5) Exit from the Tune page, and say Yes to save changes.
- 6) Exit from MassLynx.