AxION 2 TOF MS

User's Guide

PerkinElmer®

AxION 2 TOF MS
User's Guide

Release History

Part Number	Release	Publication Date
09931007	D	September 2013

Any comments about the documentation for this product should be addressed to:

User Assistance
PerkinElmer, Inc.
710 Bridgeport Avenue
Shelton, CT 06484-4794
U.S.A.

Or emailed to: info@perkinelmer.com

Notices
The information contained in this document is subject to change without notice.
Except as specifically set forth in its terms and conditions of sale, PerkinElmer makes no warranty of any kind with regard to this document, including, but not limited to, the implied warranties of merchantability and fitness for a particular purpose.
PerkinElmer shall not be liable for errors contained herein for incidental consequential damages in connection with furnishing, performance or use of this material.

Trademarks
Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are protected by law.

PerkinElmer is a registered trademark of PerkinElmer, Inc.

Contents

Introduction

The **AxION 2 TOF MS** combined with **Chromera** provides fast and reliable analysis of chemical samples by liquid chromatography mass spectrometry. A system consists of an AxION 2 TOF MS, HPLC Pump and Autosampler and allows the option of adding a column oven and UV/VIS or Fluorescence detector, if desired. These components are connected to a computer using the Windows 7 SP1, 32 bit operating system and running the TOF Driver V6.2 and Chromera 3.4.1 software that controls the data acquisition procedures and evaluation of results.

This guide is intended to provide an overview of the workflow to run an AxION 2 TOF MS analysis using Chromera. Before beginning, the AxION 2 TOF MS should be installed and connected to the LC instruments.

Chromera is a powerfully-easy data system for liquid chromatography. Any laboratory instrumentation is only as good as the software behind it. For maximum productivity and long-term return on investment (ROI), a Chromatography Data System (CDS) needs to be intuitive, application-focused and scalable. And when chromatography is being used in combination with mass spectrometry, the software also needs to provide complete control of both techniques and to allow the smooth integration of data from the two systems.

PerkinElmer's Chromera® CDS was specifically developed for chromatographers, but built to provide full mass spectrometer control and spectral data handling. This unparalleled integration enables the software to smoothly transition from one analytical technique to the other and to seamlessly merge data from the two instrument types. Chromera allows users to build and continually adapt a LC/MS system to suit their specific needs. By using unique, patented Instrument Device Descriptors, users can quickly and easily create custom configurations on the fly. It provides highly configurable and responsive LC instrument control for multi-detector systems, combined with an elegantly simple user interface for interactive processing, and flexible, multi-channel quantitation and reporting. Chromera is designed to display all of the necessary information on the screen to give you complete control of your system.

Starting

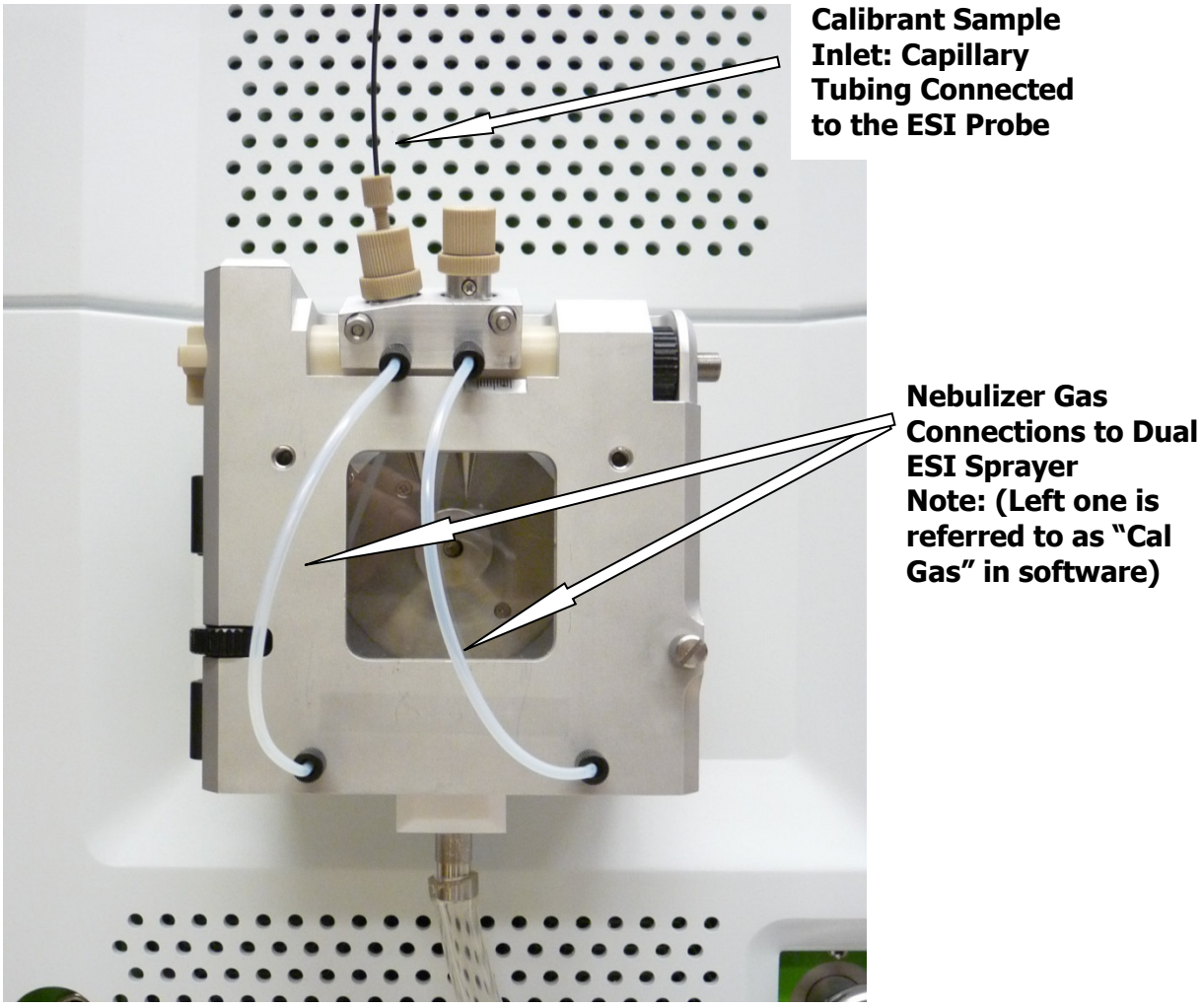
Overview

This document provides basic operating instructions for the AxION 2 TOF MS instrument including start-up, introduction of samples, data acquisition, data analysis, and shutdown.

The start-up instructions provided in this chapter assume that Chromera, the TOF MS Driver software, and the AxION 2 TOF MS instrument have been correctly installed by a representative of PerkinElmer.

NOTE: *When planning analyses, bear in mind that the instrument needs a minimum of 12 hours from initial installation power-on to establish the required vacuum. However, after venting for routine maintenance, allow 2 hours after pump down and HV activation to allow equilibration of all electronics prior to performing analyses.*

Capillary tubing delivers sample to the ESI, DSA, or APCI probe. Sample can be delivered from an LC system or from the syringe pump.



Starting the AxION 2 TOF MS Detector

WARNING! High voltage is present within the source during an experiment.

CAUTION! Do not move the instrument with the power on as this may damage the vacuum pumps.

To start the AxION 2 TOF MS system:

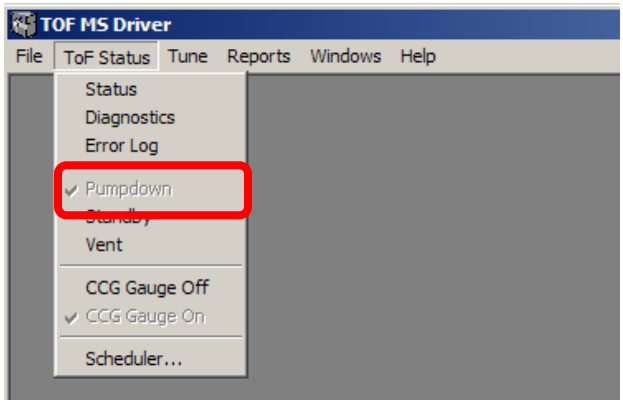
1. Switch the **Power ON/OFF** switch to position **On** (located on the right-side panel). Make sure the power switch on the roughing pump is on so that the Vacuum System will start when **Pumpdown** is selected in the AxION 2 TOF MS driver.

NOTE: *Do not switch the electronics on at this stage.*

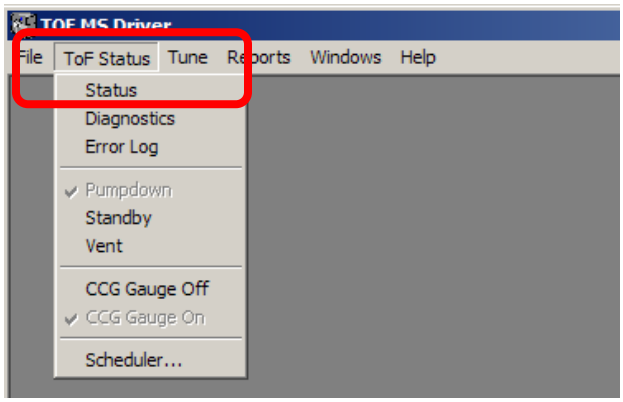
2. Check that the fans are operating. A cooling air flow should exit at the bottom of the instrument.

3. Double-click on the **TOF MS driver** icon on the desktop.

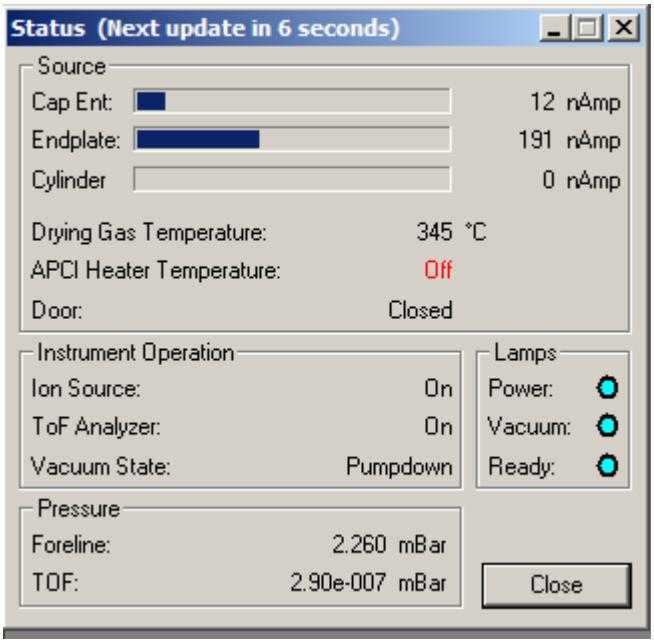
4. Select **Pumpdown** from the **TOF Status** menu.



5. Open the **Status** screen by selecting **Status** from the **Instrument** menu to display vacuum status during pumpdown.



6. Check the vacuum status displayed in the **Status** screen.



When ready, the **Lamps (Power, Vacuum, and Ready)** should all be green.

The **Vacuum** lamp changes from flashing to steady light at this stage.

NOTE: *The vacuum state light will take several hours to become ready and the system will not be able to load a Tune file.*

Checking the Position of the ESI Probe

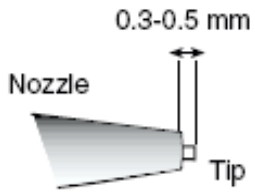
1. Observe the ESI probe through the inspection window.

2. Align it to the center of the Capillary by loosening the lock ring and moving the manifold by turning the position adjustment screw. Tighten the lock ring.

3. The marks on the probe assembly can be used for quick positioning. Observe the needle through the inspection window and adjust the tip if necessary.

4. Loosen the lock ring.

5. Turn the needle assembly adjustment screw until the needle tip protrudes about 0.5 mm from the nozzle.



6. Tighten the lock ring.

Starting Chromera

Configuring Chromera

The LC/MS system is configured in Chromera through the **Chromera Manager**; this acts as a control panel for the system. Closing Chromera Manager will not affect data acquisition or processing on a running instance of the Chromera.

If Chromera Manager has already been configured, you can skip this section.

Creating a System Database

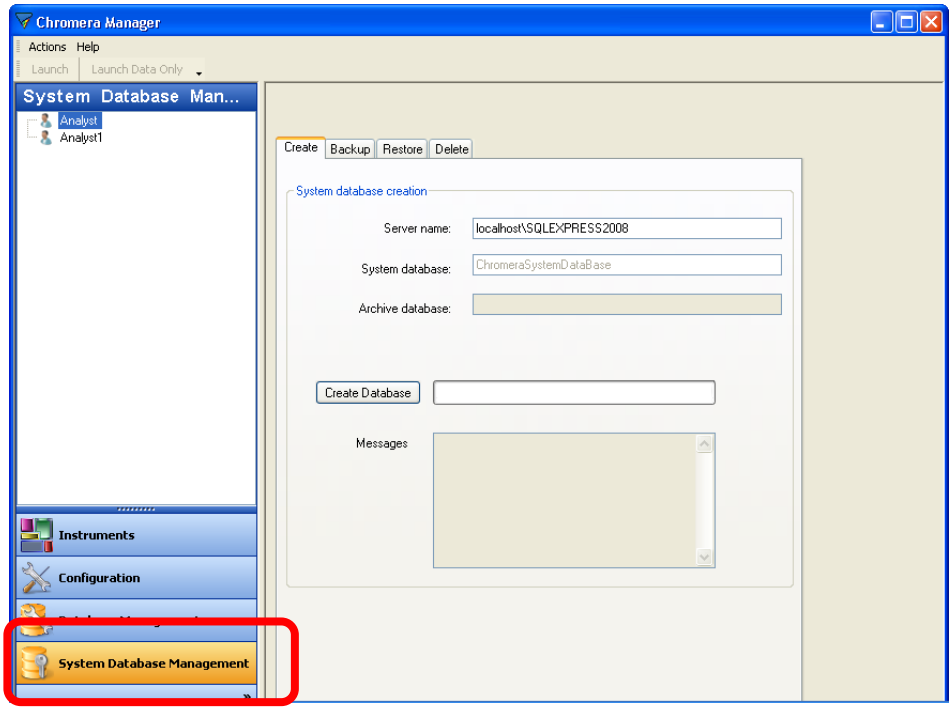
The first time you install Chromera you must create a System Database.

To create a System Database:

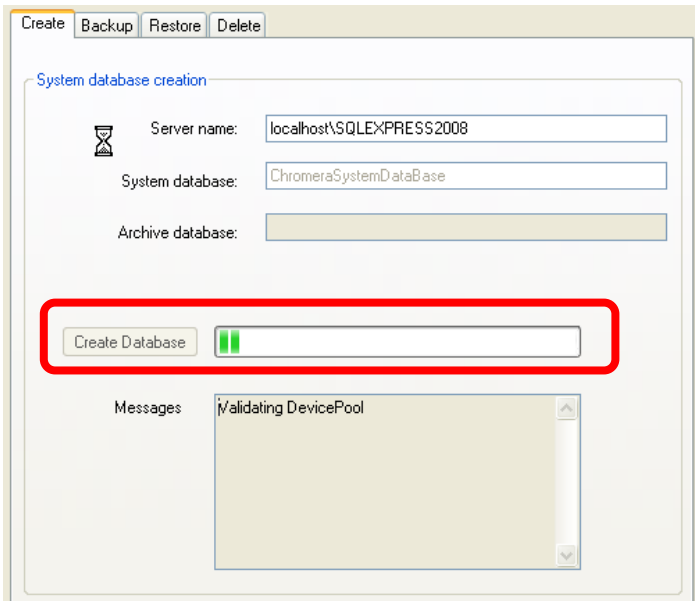
1. Create a **Chromera Manager** shortcut on your desktop.
   Click the Windows **Start** button, then click **All Programs**, locate then right-click on **Chromera Manager,** then select **Send To > Desktop** (create shortcut).

2. Start **Chromera Manager** by double-clicking on it.

3. Click the **System Database Management** button.
   The system database functions display in the **Create** tab.



4. Click the **Create Database** button and observe the progress bar as the system database is created.



5. Upon successful completion, the next step is to configure "an instrument" for the system.

   In Chromera, an instrument is defined as a collection of devices. For example, individual **Devices** such as Flexar or Series 200 autosamplers, pumps and detectors are combined to create an instrument. In addition, a **Port Name** (for communication to each device) must also be defined in the Instrument configuration. Next create an **LCMS** instrument to use with the AxION 2 TOF MS in a system.

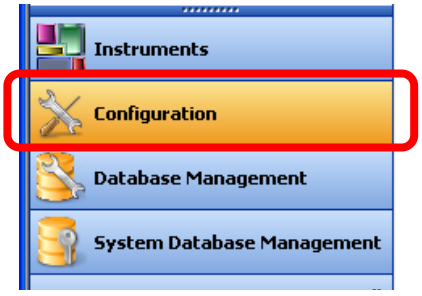
Creating an LCMS Instrument

NOTE: *Prior to creating an Instrument Configuration, make sure all cables are connected between all devices and the Edgeport box except for the AxION 2 TOF MS Detector since this device requires an Ethernet cable connection).*

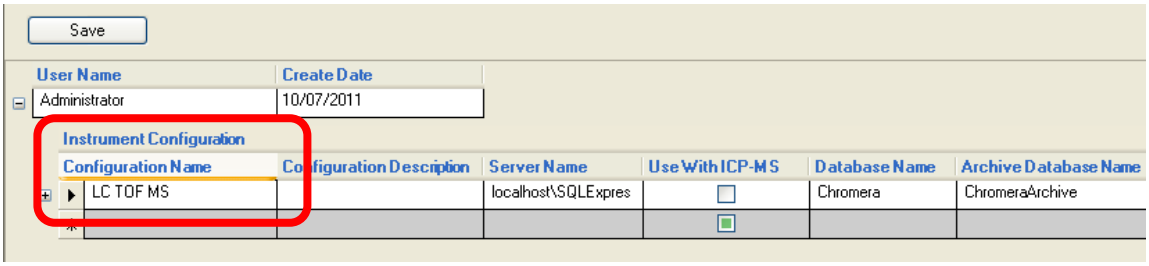
To create an LC instrument:

1. To create a new **Instrument Configuration** click on the **Configuration** button 

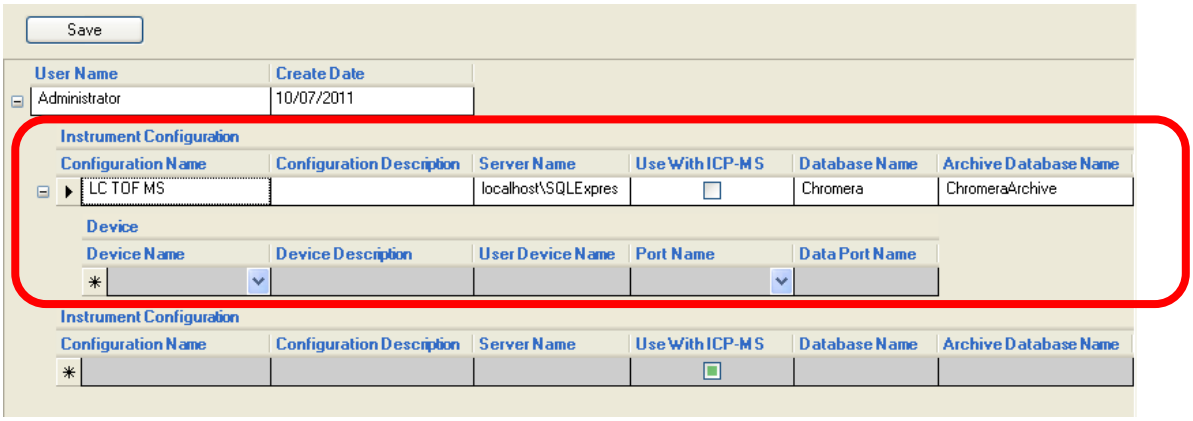
to display the initial **Configuration** screen.



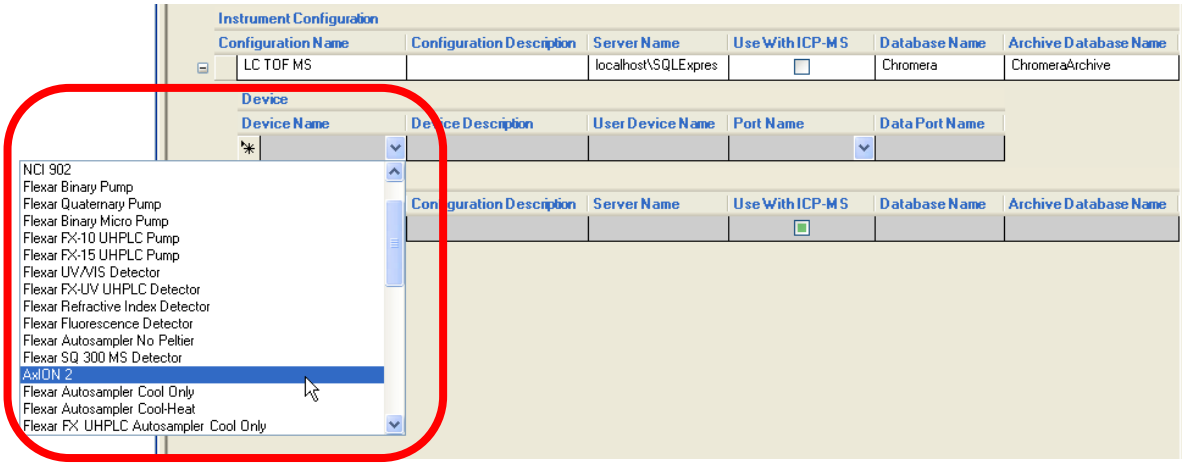
2. Under the **Instrument Configuration** row, click in the box under **Configuration Name** and type an instrument name (this example shows that **LC TOF MS** was typed), then press the **Enter** key.
   A ⊞ next to the row with the name displays.



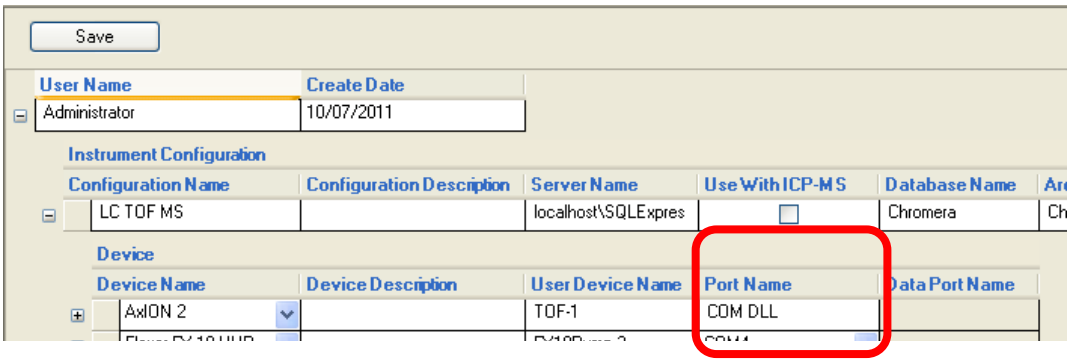
3. Click on the ⊞ and the **Device** row displays.



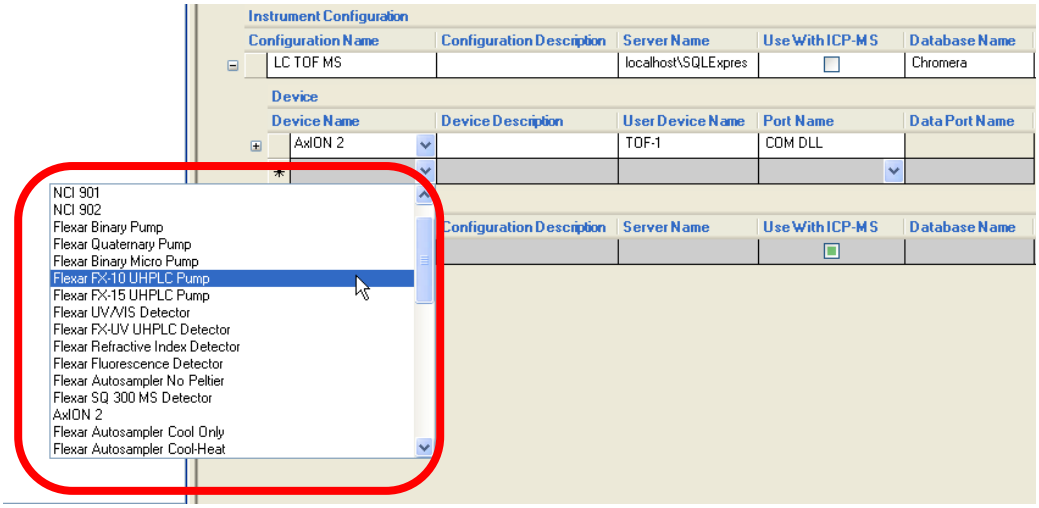
4. Click on the drop-down button in **Device Name** box, and device choices appear. Select the appropriate devices (modules) for the **Instrument** you are creating.
   In this example, select **AxION 2** from the drop-down list of devices.



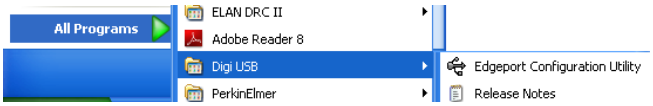
   The **AxION 2** detector automatically fills in the **Port Name** field with **COM DLL**.



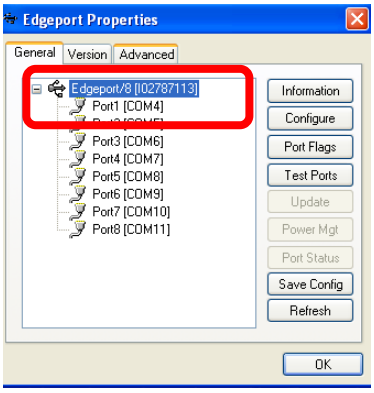
5. Select your LC Pump from the **Device Name** drop-down list.
   In this example, the **Flexar FX-10 UHPLC Pump**.



6. To determine a correct **Port Name** for the pump, open the **Edgeport Configuration Utility** from the **Start** button > **All Programs** > **Digi USB** > **Edgeport Configuration Utility**.



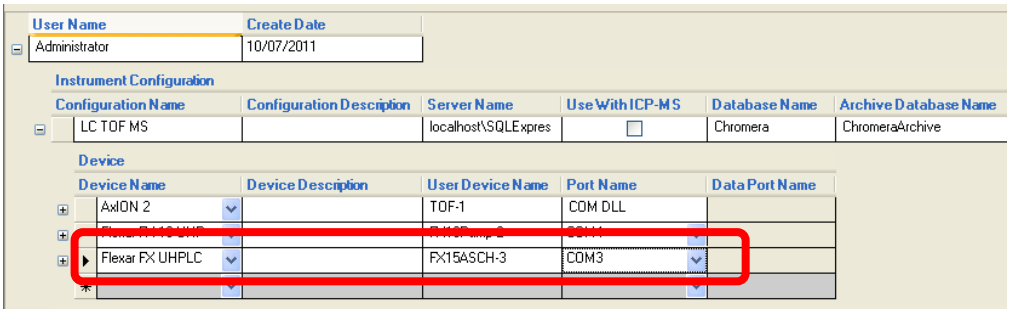
7. The **Edgeport Properties** dialog displays. Click the plus sign to display a list of the physical Ports (1 – 8) on the Edgeport with the corresponding COM port numbers.



8. In the **Device Name** pump row (in this example, Series 275 HRes Binary Micro Pump), click on the drop-down button in the **Port Name** field.

9. Select **COM4** from the **Port Name** drop-down list.

   If your LC Pump is plugged into Port 1 on the Edgeport the corresponding COM port is COM4 as shown in the Edgeport Properties dialog above.



10. Select your LC Autosampler from the **Device Name** drop-down list.

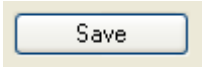
11. **Select the Port Name for the autosampler.**

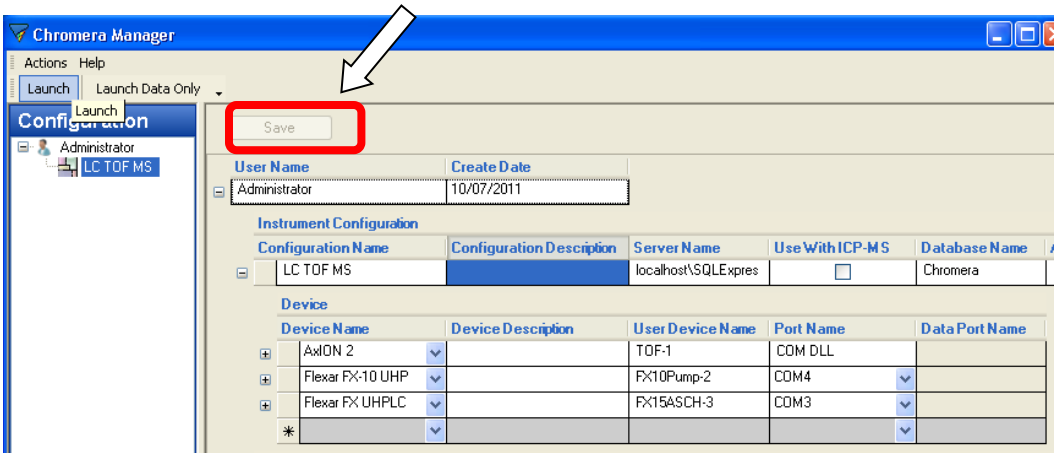
    If your autosampler is plugged into Port 2 on the Edgeport the corresponding COM port is COM5.

12. Observe the **Database Name** fields. This is where you define the database names.

The field **Database Name** is the name of the active database. The default database is **Chromera**. The field **Archive Database Name** is the name of the archived database when an archive is created. The default archive database is **Chromera Archive**. You can change the default names by typing new names into these fields. If the names are changed, the you must click the **Save** button.

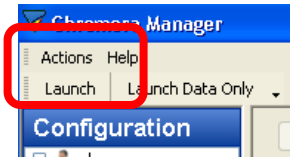
13. When all instrument components have been defined, click the **Save** button located at the top of the screen.



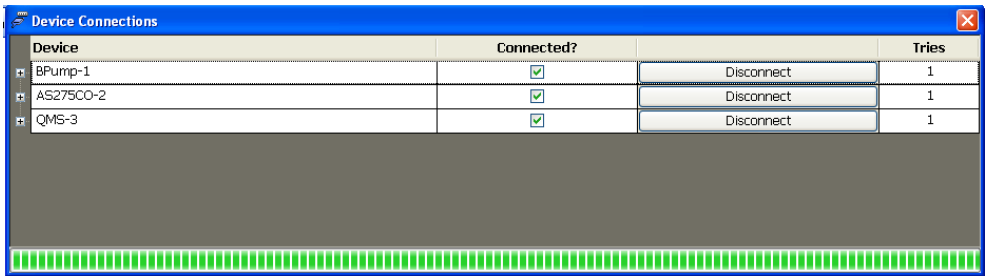


Once you save, the **Configuration Name** (in this example **LC TOF MS**) displays in the **Configuration** pane.

14. Click **Launch** to launch Chromera.



Chromera starts and displays **Device Connections** as it connects to the devices.



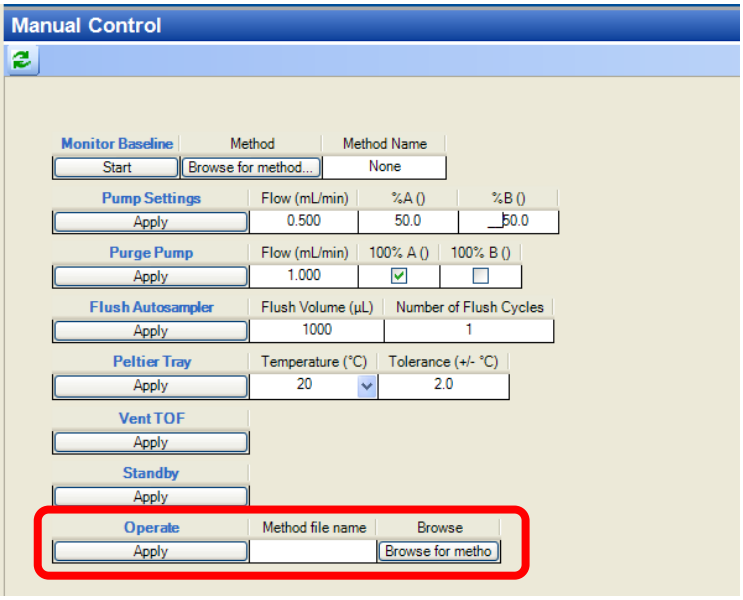
Upon successful connection, the **Run Time** screen displays.

Setting the Operate Method

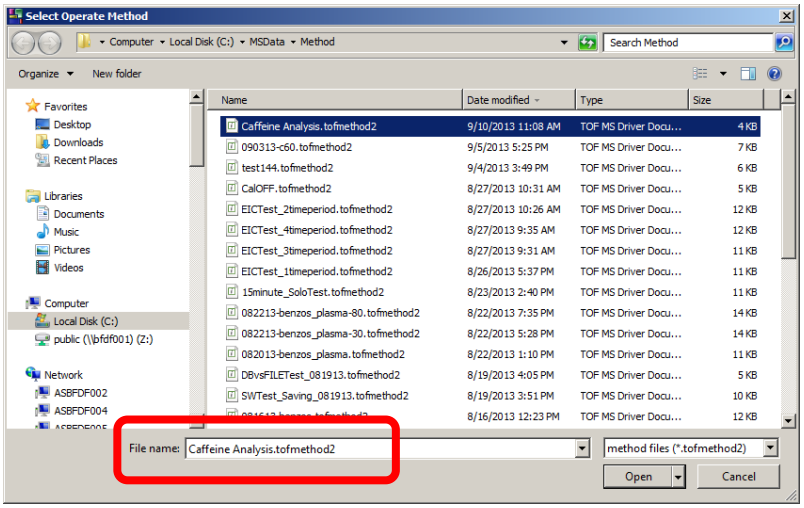
At this time you must assign an **Operate** method. The Standby method not selectable; all you have to do is apply it when necessary.

NOTE: *When you close Chromera you lose the **Operate** method settings. You must reassign it every time you Launch Chromera.*

1. In the **Operate** row, click **Browse for method**.

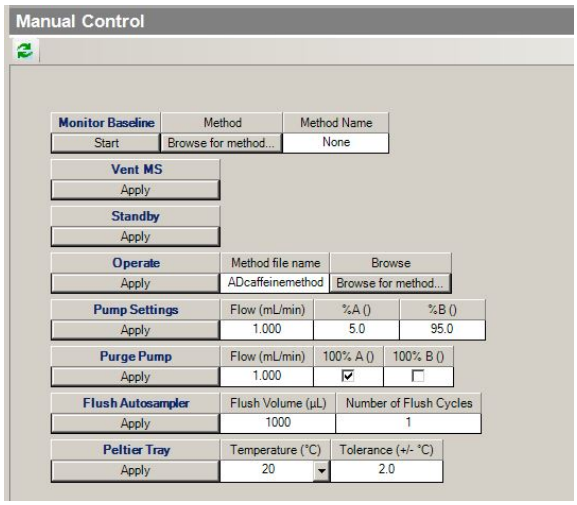


The **Select Operate Method** dialog displays.

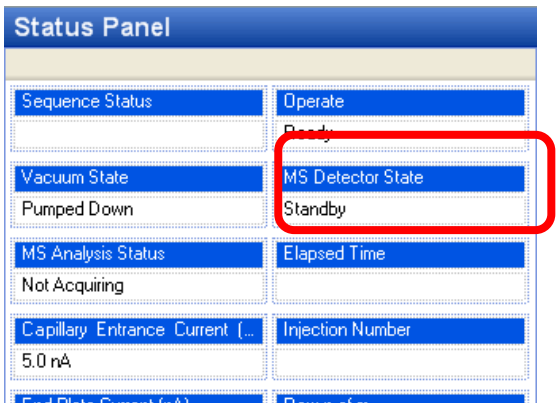


2. Select the method you created in the TOF MS driver, then click **Open**.
   This example shows **Caffeine Analysis.tofmethod2**

3. Look at the **Manual Control** section of the **Run Time** screen.



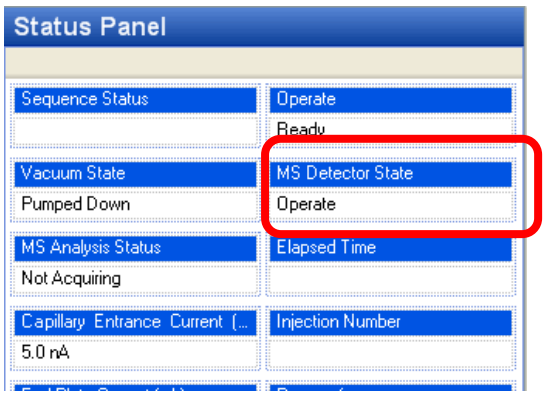
4. To verify the methods work with Chromera, in the **Standby** row click **Apply.**
   Observe that in the **Status Panel**, the **MS Detector State** displays **Standby**.



NOTE: *The default "Method" folder displayed in the Select Operate Method dialog is the ONLY location from where TOF MS Driver method can be selected. The Full path is C:\ProgramData\PerkinElmer\AxION\Method*

If a method is saved elsewhere, it must be moved to this folder to be applied properly. If a method is applied outside of the designated folder, Chromera will need to be restarted to proceed.

5. Next, in the **Operate row** click **Apply.**



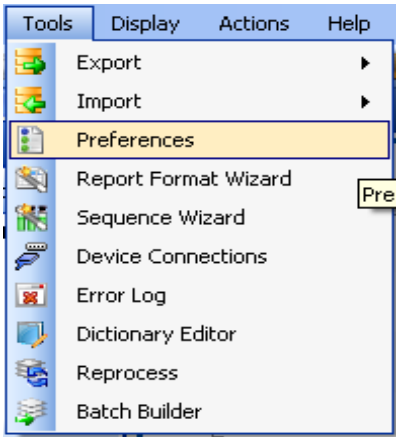
Observe that in the **Status Panel**, the **MS Detector State** displays **Operate**.

6. Leave the AxION 2 TOF MS Detector in the **Operate** mode.

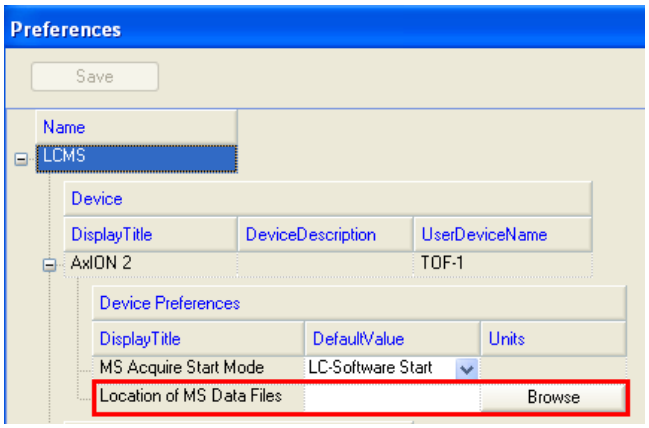
Setting the TOF Data Path

When you are in Chromera, follow this procedure to set the appropriate folder to deposit the TOF data files. Failure to do so may fill the local C:\ drive.

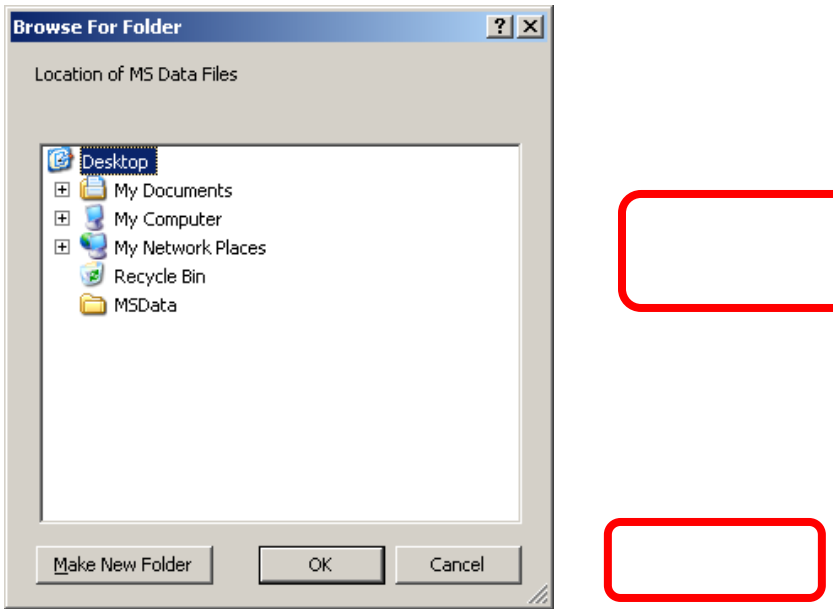
1. In Chromera, select **Preferences** from the **Tools** menu.



2. When the **Preferences** window opens look for **Location of MS Data Files** under **Device Preferences** as shown below.

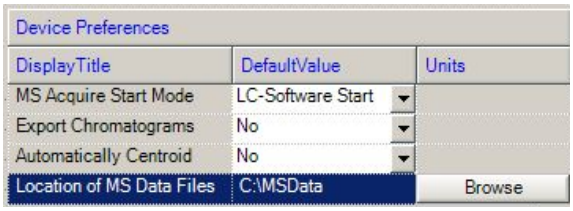


3. Click the **Browse** button.
   The **Browse For Folder** dialog displays.

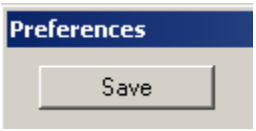


4. Expand **My Computer** and select **System (C:)**

5. Click the **Make New Folder** button. The **New Folder** displays.
6. Right-click on **New Folder** and rename it **MSData.**
7. Click **OK.**
   The location of MS data files are now set to **C:\MSData**.



8. The **Save** button in Preferences should be highlighted; click it to save changes.



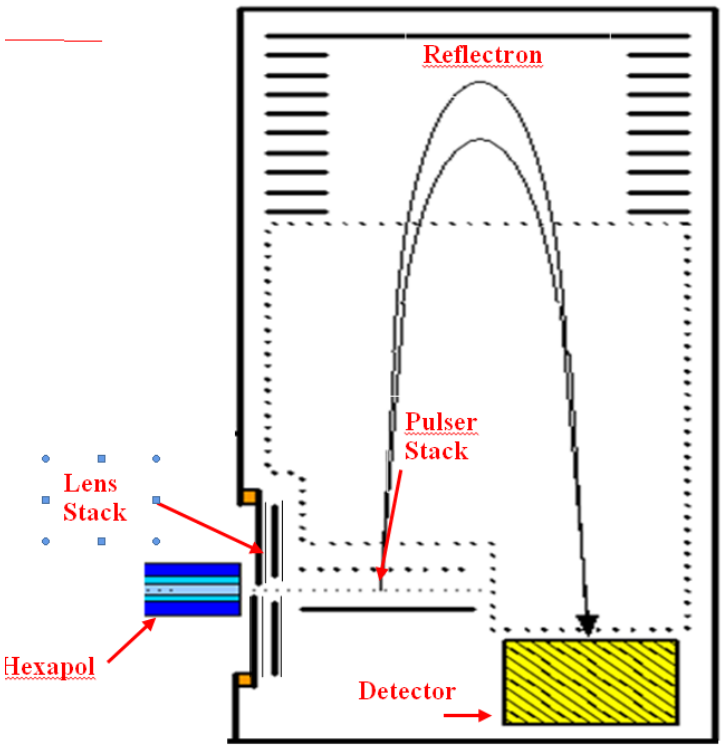
The TOF data will now be written to **C:\MSData** as defined in the preferences.

AutoTune the AxION 2 TOF MS Detector

Tuning the AxION 2 TOF sets ion source and ion lenses parameters to obtain optimal resolution and sensitivity, and involves adjusting the probes. Even though the instrument is tuned at the factory, it may be necessary to re-tune during installation. Tunings are recommended after running many applications, or when performance is not fulfilled.

Overview of the Tuning Procedure

The AxION 2 AutoTune is a script base program that tunes the time of fight mass spectrometer to optimize for signal intensity, resolution and peak symmetry. AutoTune works by ramping essential parameters in the ion optics and the flight tube optics The important tuning parameters are the hexapole DC offset, which controls signal intensity and to some degree resolution, the reflectron and PL1 Bias in the flight tube optics controls peak intensity, peak shape and resolution, lens1 (L1) and lens 3 (L3) and Lens 2 and Lens 4 (L4) are ramped in pairs these parameters control peak intensity and resolution and to a lesser degree peak symmetry. The schematic below shows an illustration of the tuning optics. Ions travel from the ion optics region where they pass through the hexapole and then into the lens stack where they then enter the pusher puller or pulser stack. From the pulser stack ions are ejected into the flight tube where they are accelerated up the tube to the reflectron and are then accelerated again down the flight tube until they finally reach the detector.



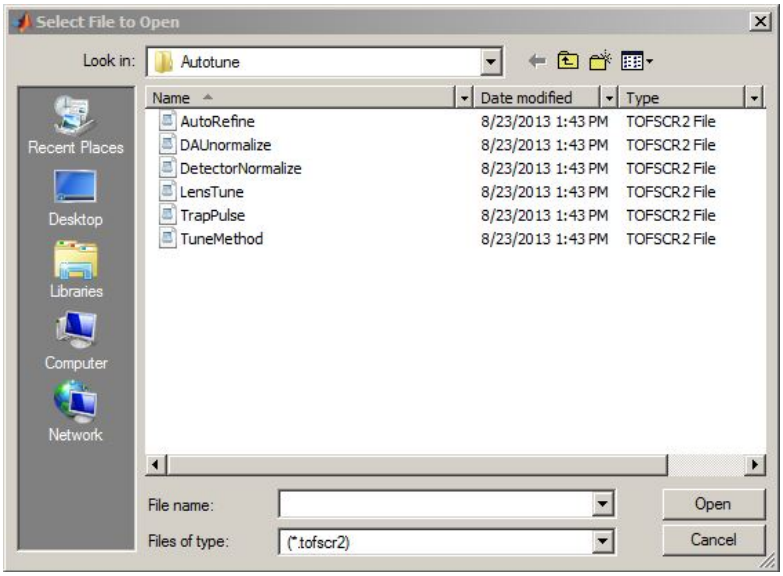
AutoTune also normalizes the Data Acquisition Unit (DAU) which provides a proper baseline that sets the cut off threshold for electronic noise. The detector is also normalized to set the proper detector voltage, to analyze ion response statistics and to set the proper isotope ratios. The detector normalization is also important for optimizing the dynamic range of the instrument.

About the AutoTune Algorithm

AutoTune can be performed using different starting tune settings (stored in Tune files) at different flight tube voltages in both positive and negative ion modes. There are positive and negative ion mode Tunes available at three different flight tube voltages: 5 Kilovolts (kV), 8 kV and 11kV. The 8kV range can be used for most work. For low mass work (i.e., *m/z* <600) the 5 KV flight tube voltage is optimal, whereas for high mass work the 11 KV flight tube voltage is optimal.

The AxION 2 TOF can be operated in 2 modes: pulse mode and trap pulse mode. Trap pulse mode allows the user to increase the signal to noise (S/N) over a narrow *m/z* window. This is accomplished by setting the timing of two parameters in the Trap Enhancement Tab. In trap pulse mode the ions get "trapped" between the hexapole and the lens stack by controlling the value of the D7 voltage gate. Setting D7 to longer times will allow trapping of more ions as they buildup in this region, while setting D7 to shorter times will result in trapping fewer ions. The second parameter is the value of D8 which ultimately controls which *m/z* region that will get enhanced. Shorter values of D8 will trap the lower mass region while longer values of D8 will trap the higher mass regions. For example: setting D7 to a value of 8 µsec and setting D8 to 22 µsec will trap in the region near *m/z* 130. Setting D7 to 22 µsec and D8 to about 45 µsec will enhance the signal to noise of ions in the range of *m/z* 600. D8 sets the timing for when the pusher puller stack will eject ions into the flight tube. Therefore, the desired mass region to be enhanced can be selected by setting values for D7 and D8 properly.

How the AutoTune Algorithm Runs



An AutoTune file, **TuneMethod.tofscr2**, runs the following four individual scripts in the order listed below:

a. DAUnormalize.tofscr2
b. LensTune.tofscr2
c. DetectorNormalize.tofscr2
d. TrapPulse.tofscr2

Below is the list of the individual parameters that are optimized as per the scripts in the following order:

Order of run	*.tofscr2 run	Parameter Set
1	DAUnormalize.tofscr2	DAU offset
2	LensTune.tofscr2	Tradeoff factor for resolution increase
		PL1 bias
		Reflectron
		Offset Voltage
		Lens 1 and 3
		Lens 2 and 4
		PL1 bias
		Reflectron
3	DetectorNormalize.tofscr2	Detector Voltage
4	TrapPulse.tofscr2	D7
		D8

AutoTune results are stored in two Tune files that are created – one for the pulse mode and another for trap pulse mode. Additionally a log file (*.txt) is created that gives the detailed information of the parameters optimized.

About the Samples

Tune Mix (PerkinElmer Part Number ZG2421A)

Negative Mode Ions: *m/z* 112.98558, 431.98233, 601.97897, 1033.98811, 1633.94978, 2233.91146, 2833.87314

Positive Mode Ions: *m/z* 118.08625, 322.04812, 622.02896, 922.00979, 1521.97147, 2121.93315, 2721.89483

Calibrants

The ESI Tune Mix (PerkinElmer Part Number ZG2421A) TOF mass calibration solution is suitable for both positive and negative ion tuning and mass calibration. It is used at 100:1 dilution for positive ion mode and 1000:1 for negative ion mode. The dilution is made in 95/5 LC-MS grade acetonitrile/water.

It is also recommended that caffeine (PerkinElmer Part Number MZ301236) is added to the mix for an additional lower mass peak in a 1:5 dilution in the ESI Tune Mix.

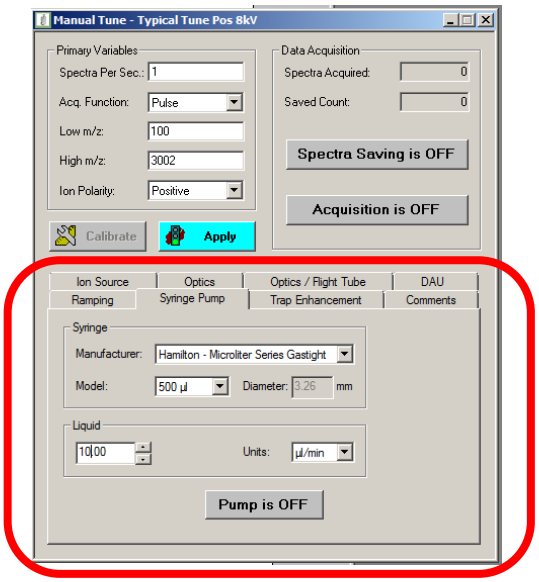
- 7.6 mL of LC-MS grade Acetonitrile
- 0.4 mL of LC-MS grade Water
- 2 mL of ESI Tune mix
- 40 µL of caffeine (2 mg/mL stock solution in water)

Calibrant Delivery by Syringe Pump

Calibrant can be introduced into the mass spectrometer by using one of the Calibrant Vials or with the syringe pump. Using a Calibrant Vial is detailed below. If, instead, using the syringe pump is desired, it can be set as shown in the following example:

***NOTE**: When using the 500 µL Hamilton syringe, set the inner diameter to 3.26 mm and the flow to 10 µl/min.*

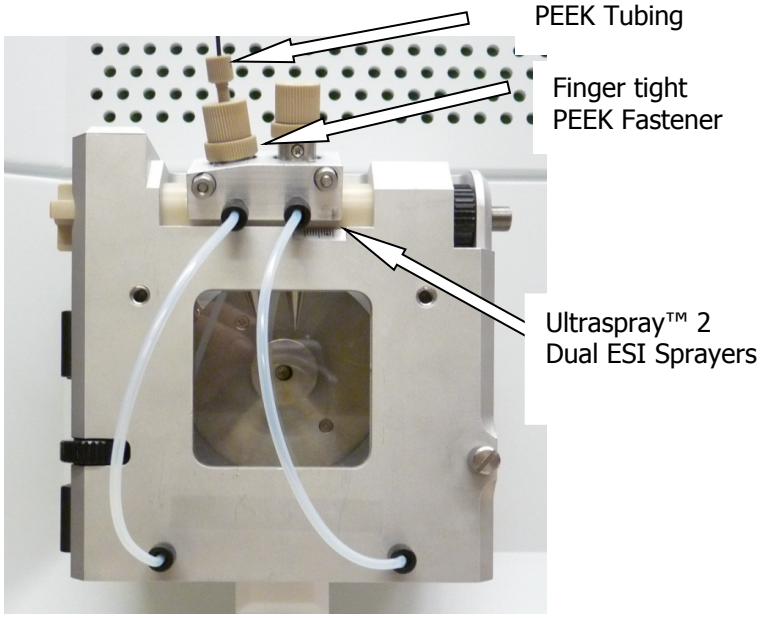
1. Select the **Syringe Pump** tab on the **Manual Tune** dialog.
2. Select **Hamilton** from the **Manufacturer** drop-down list.
3. Select **500 µL** from the **Model** drop-down list.
4. Select **3.26** from the **Diameter** drop-down list.
5. Select a **10** from the **Liquid** spin box.
6. Select **µL/min** from the **Units** drop-down list.



Preparing for AutoTune

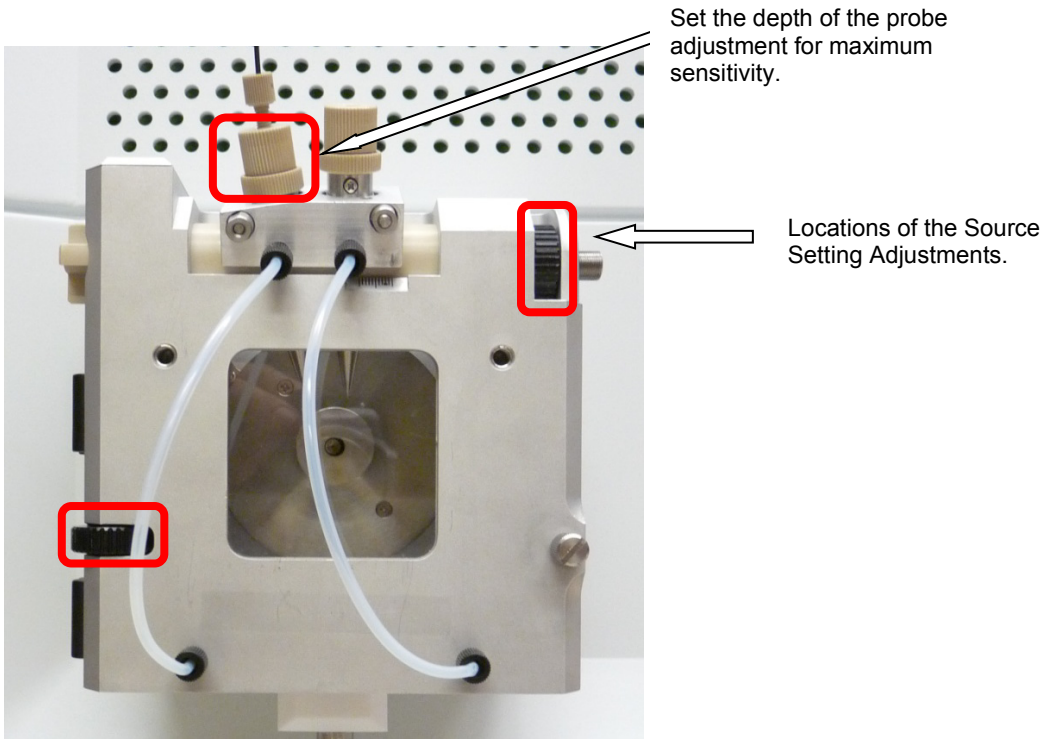
Typically use **the Positive Ion Tune mix**.

1. Fill the syringe with the Tune Mix (PerkinElmer Part Number ZG2421A) and mount the syringe in the syringe pump.
2. Connect PEEK™ tubing from the end of the syringe to the inlet of the ESI sprayer and use a PEEK finger tight fitting to secure the tubing as shown below to the left ESI sprayer.

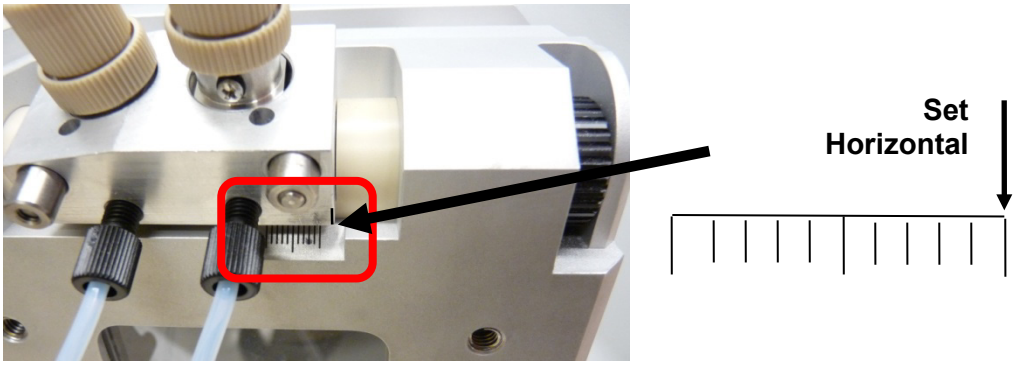


Running AutoTune

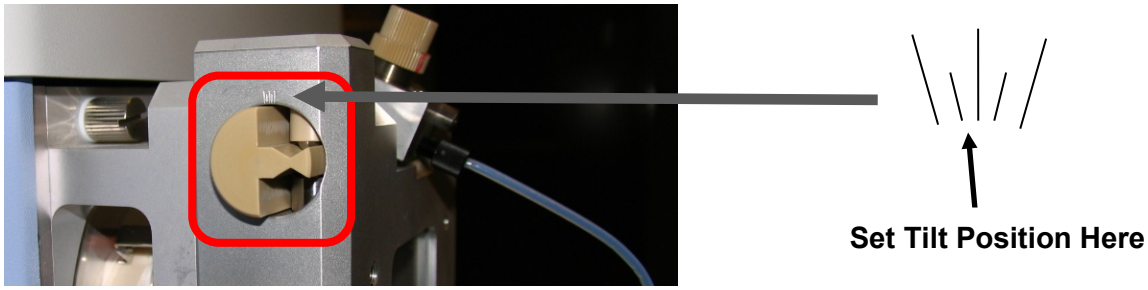
Set you source positions before you start the AutoTune procedure.



3. Set the **source horizontal position** so that the horizontal probe marking is positioned 10 spaces to the right side of the scale as shown below:

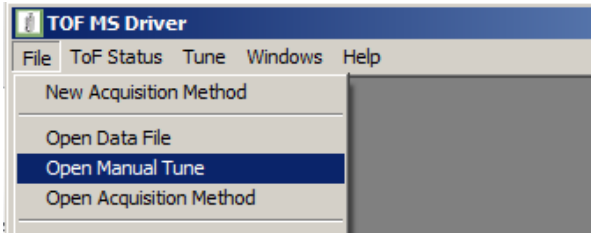


Set the **Probe Tilt** position as shown below:

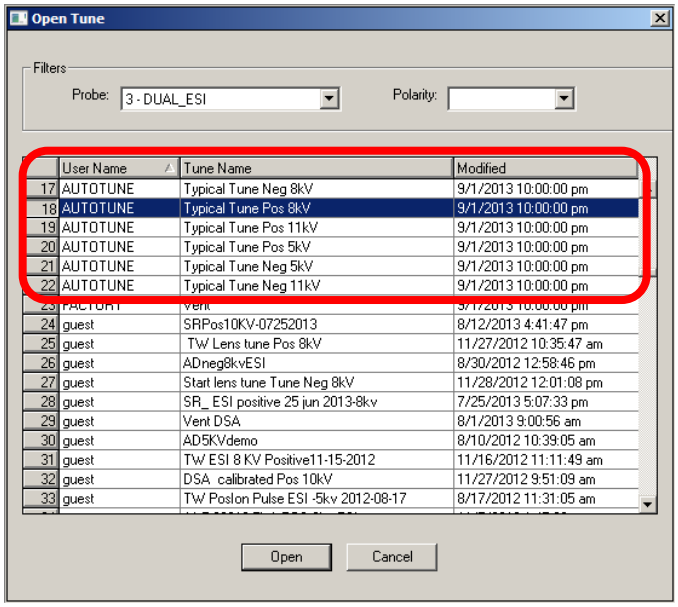


4. Double-click on the **TOF MS Driver** icon on the desktop.

5. Select **Open Manual Tune** from the **File** menu.



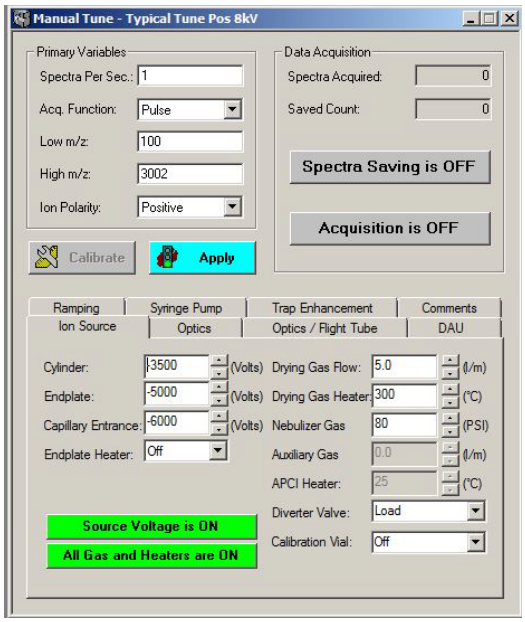
The **Open Tune** dialog displays.



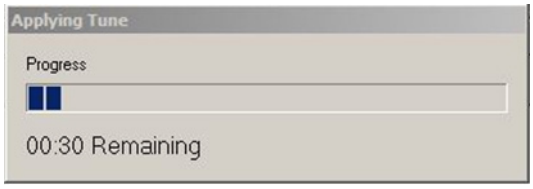
There are six **AUTOTUNE** files displayed.

6. Select **Typical Tune Pos 8kV.**
   The above example shows selecting **Typical Tune Pos 8kV** as the Tune file.

7. Click **Open.**
   The **Manual Tune - Typical Tune Pos 8kV** dialog opens.



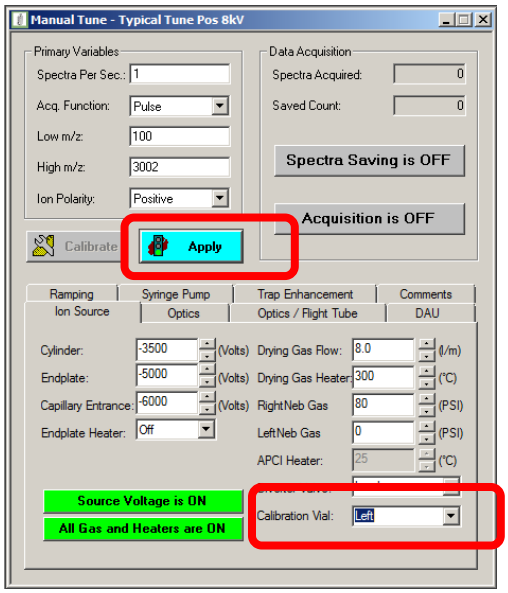
8. Click **Apply** to load the Tune. Once loaded, the **Manual Tune** dialog is active.



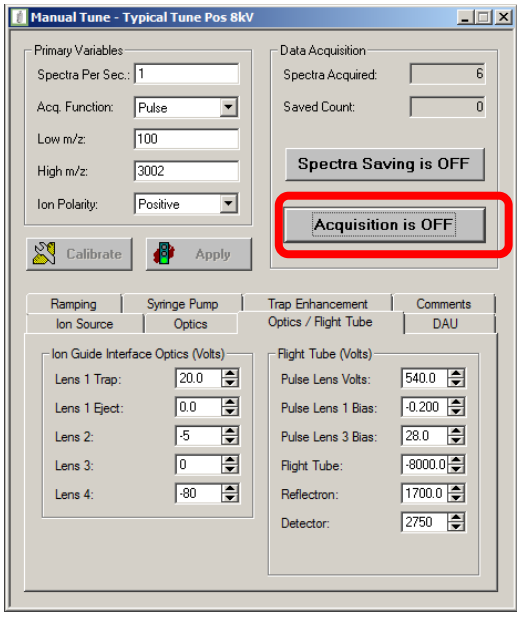
9. Click the **Ion Source** tab and set the **RightNeb Gas** or **LeftNeb Gas** to 80psi, depending on which probe is used for calibrant delivery.

NOTE: *The gas must be on when running dual sprayers.*

10. Select the **Calibration Vial** containing the Tune Mix and click the **Apply** button to activate the vial *or* turn on the syringe pump by selecting the **Pump is OFF** button in the **Syringe Pump** tab.



11. Click the **Optics/Flight Tube** tab to review the settings for **Typical Tune Pos 8kV**. Then click the **Acquisition is OFF** button to turn on Acquisition.



NOTE: *If the instrument had been in positive ion mode for at least two hours, let the instrument pulse for one hour to equilibrate the electronics and hardware. If the polarity was just changed, open the Tune and allow the instrument to pulse for at least 2 hours before running AutoTune in the current polarity.*

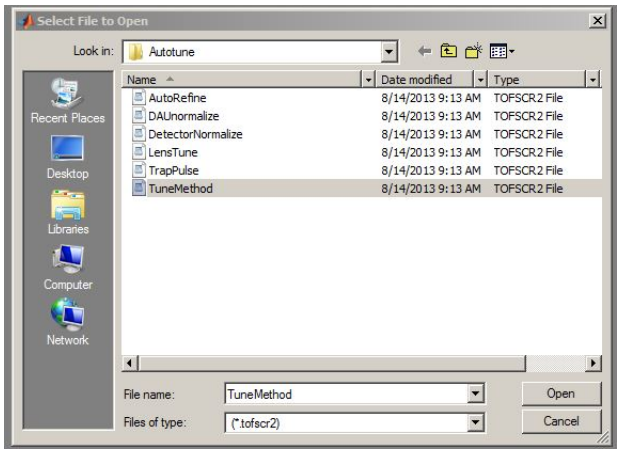
12. While the instrument is pulsing, infuse the tune mix at 10µl/min into the mass spectrometer and make sure all seven of the calibration ions are observed while the instrument is acquiring; then stop the infusion.

13. After the instrument has been given appropriate time to thermally stabilize (see **Note** above), close the TOF MS Driver program.

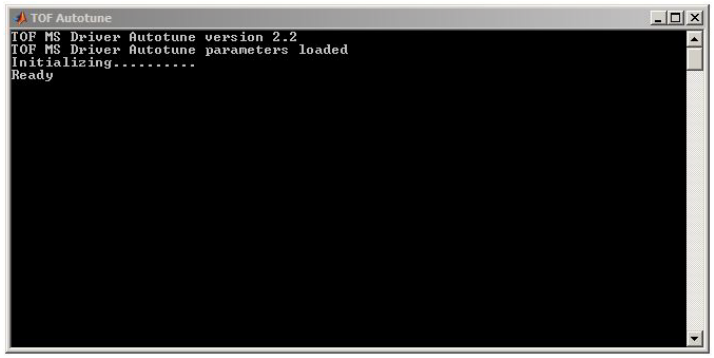
14. Before going to the next step, restart the infusion of the tune mix from either the calibrant vial or syringe pump at 10µl/min and let it run for a few minutes before starting AutoTune to insure a stable calibrant flow.

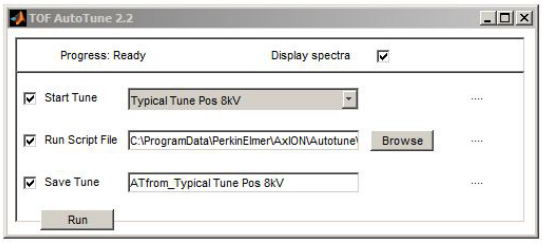
15. On your desktop, double-click on **AutoTune.exe**.

The AutoTune application starts and displays the **Select File to Open** dialog.

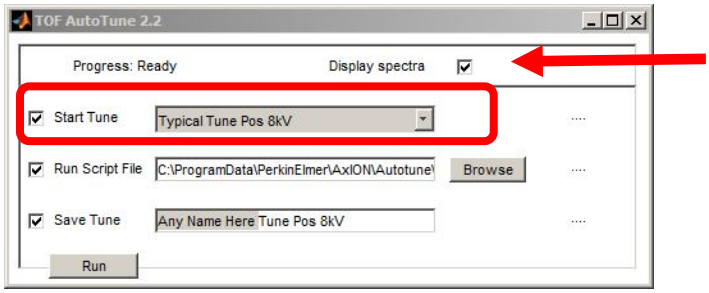


16. Select **TuneMethod** then click **Open**.
    The following AutoTune screens appear.

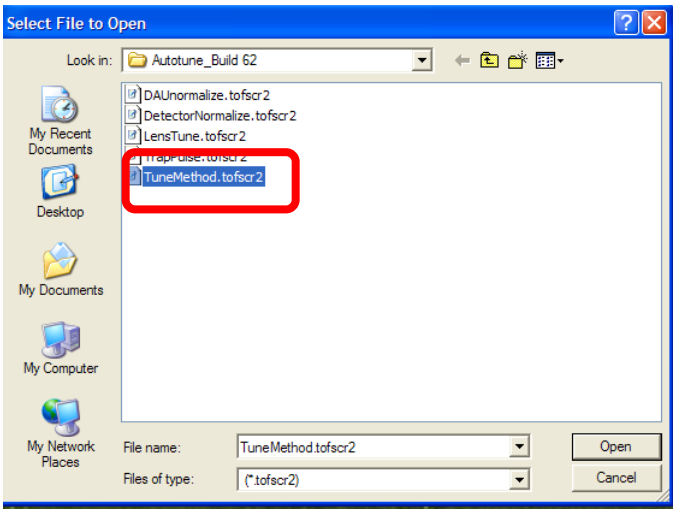




17. If necessary, in the **Start Tune** line, click the drop-down arrow and select **Typical Tune Pos 8kV** from the drop-down list. Also click in the check box to **Display spectra**.

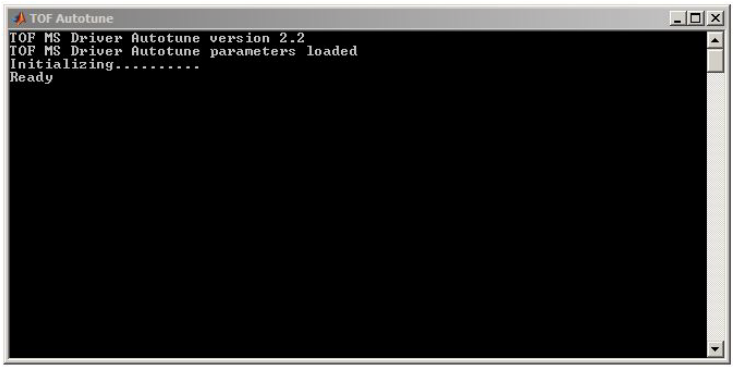


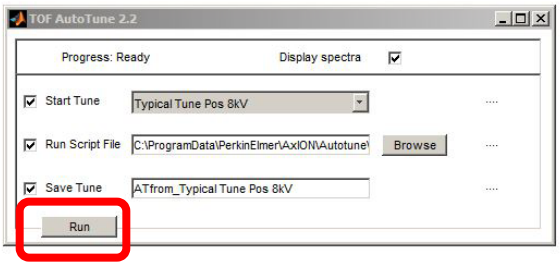
18. In the **Run Script File** line click **Browse**.
    The **Select File to Open** dialog appears.



19. Select **TuneMethod** then click **Open**.

    The following screens display.

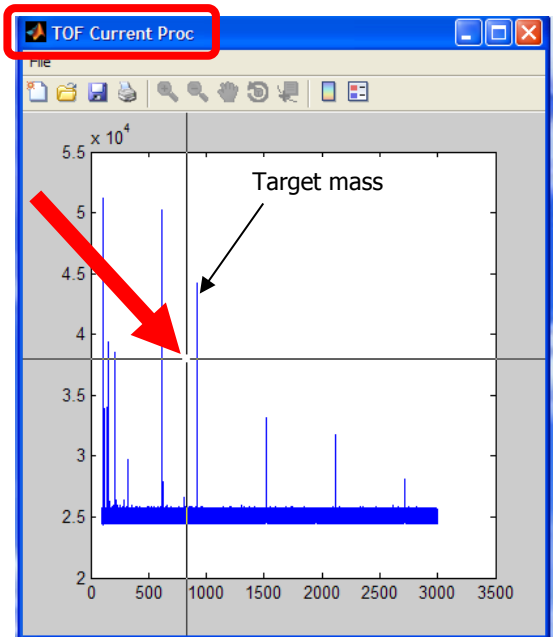




Regardless if **Save Tune** is checked or unchecked, AutoTune automatically saves 2 Tunes; one for pulse mode and one for trap pulse mode with the prefix **ATfrom_**with the ending either Trap or Pulse.

If Save Tune is checked, AutoTune still automatically saves a Trap Tune with the prefix **ATfrom_** followed by the name you gave it, and ending with Trap.

20. Click **Run** to start the AutoTune.

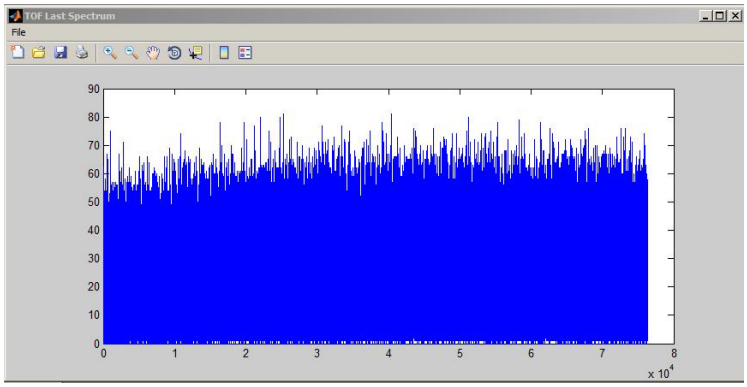


21. In the **TOF Current Proc** screen, as you move the mouse, the pointer turns to a cross hair.

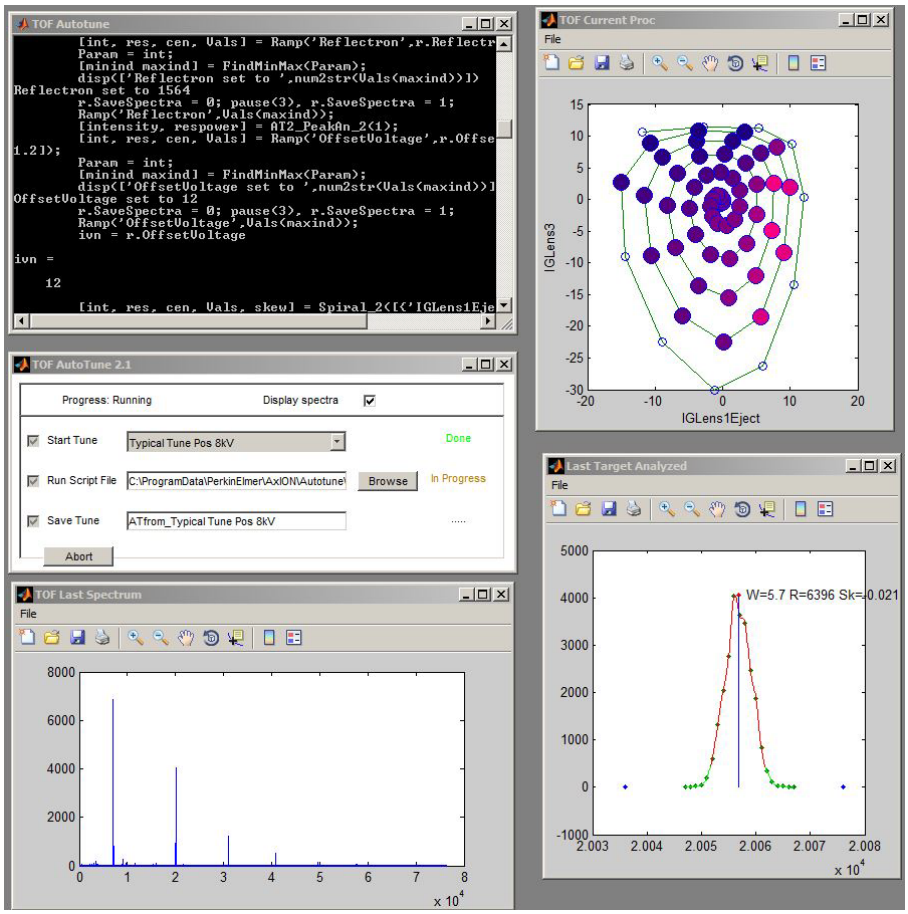
22. First click on the left side of the target mass you want to tune on. Typically in positive ion mode select *m/z* 922 and in negative ion mode select *m/z* 1033.

    If you are only concerned with **Trap** mode, AutoTune on the mass that is closest to your target mass. If your target mass lies between two of the calibration peaks, choose one and later you can open the trap Tune and adjust the D7 and D8 values to optimize your trap enhancement while infusing your target compound.

23. Position the cross hair to the right of the target mass. When you click the mouse, AutoTune immediately starts to run.



As AutoTune runs though the tuning procedure, many windows appear and disappear from the monitor. For example, as Lens 1 and Lens 3 are tuned, the following windows appear…



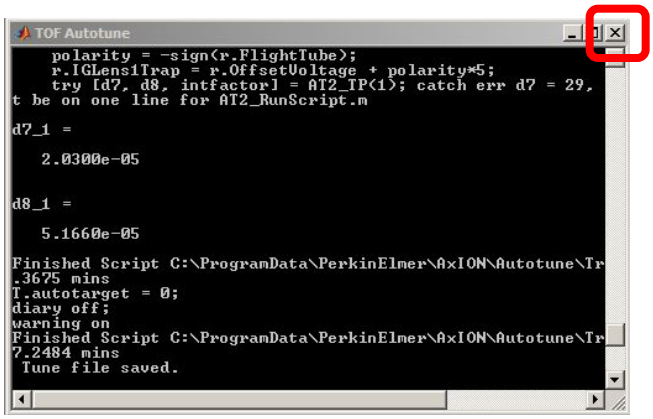
If you see the following information box dialog display.....

.... the instrument tunes on the next isotope. No action is required.

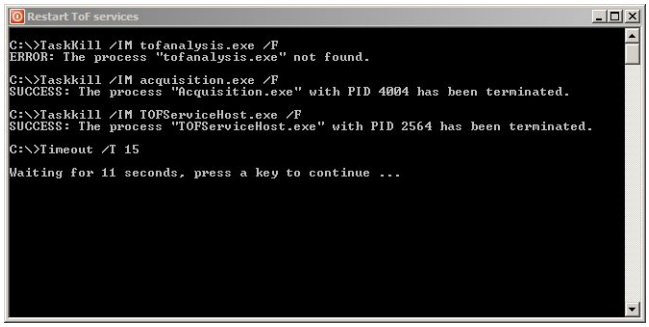
If you see an error display, this means that the Trap script did not complete. To overcome this, open the pulse Tune that AutoTune just created as shown on the next page and give it a different name. Then go back and run AutoTune again using the TrapPulse.tofscr2 script with this newly named Tune.

When AutoTune is complete it will save a Tune with the prefix **ATfrom** with your name followed by Trap at the end of the Tune name.

24. After AutoTune is complete close the AutoTune windows by clicking on the **X** in the upper-right of the TOF AutoTune screen as shown below and all of the other windows will close.

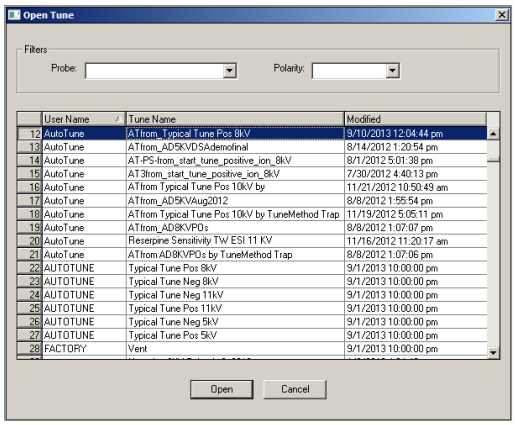


25. Click the red button to restart TOF services



26. Open the **TOF MS Driver.**

27. Open the pulse Tune that AutoTune just created.



28. Open the Tune in pulse mode while infusing the tune mix.

    Right click on the apex of the *m/z* 922 ion (in positive ion mode) and check the resolution calculated. The AxION 2 TOF has a resoluton specification of >12,000 at *m/z* 922 **at 11kV** flight tube voltage (not at 8kV, that the example above used). Always remember that there is a trade off between resolution and sensitivity. To achive the ultimate resolution possible requires sacrificing sensitivity! (**Note:** In negative ion mode, the *m/z* 1033 ion can be used to check the calculated resoluton.)

    - AutoTune was designed to automatically achieve very good resolution, but also to balance the need for sensitivity. Consequently, it may not deliver resolution that meets or exceeds the AxION 2 instrument specification of >12,000 at *m/z* ~1000 (using 11kV flight tube voltage).

    - If the goal is proving that the instrument performance specification for resolution can be met, then typically some minor manual tuning will be required after an AutoTune to demonstrate the resolution specification.

    - If AutoTune provides the desired performance required by the user, then proceed to the section on how to perform a default calibration (associated with the specific Tune).

    - If the user requires even better performance than that which was delivered using Auto Tune, contact a PerkinElmer Product Specialist.

Calibration

Calibration is accomplished using the AxION 2 TOF MS Driver. If Tune Mix (PerkinElmer Part Number ZG2421A) is to be used for the default calibration in positive ion mode, the solution should be diluted 100:1 with 95/5 LCMS grade acetonitrile/water. In negative ion mode, the dilution should be 1000:1 with the same solvent.

To calibrate the instrument, start by opening the desired Tune in the MS TOF Driver, click the acquire tab and let the instrument start pulsing. If the instrument has not been acquiring data (pulsing) for some time, let the instrument pulse for 30-60 minutes to allow the electronics to thermally stabilize. When switching polarity prior to a calibration, it is best to let the instrument pulse for 30-120 minutes prior to doing the calibration.

The masses for the calibration mix in negative and positive ion modes are given below.

About Samples

Tune Mix *(PerkinElmer Part Number ZG2421A)*

Negative Mode Ions: *m/z* 112.98558, 431.98233, 601.97897, 1033.98811, 1633.94978, 2233.91146, 2833.87314

Positive Mode Ions: *m/z* 118.08625, 322.04812, 622.02896, 922.00979, 1521.97147, 2121.93315, 2721.89483

Calibrants

The Tune Mix (PerkinElmer Part Number ZG2421A) TOF mass calibration mix is suitable for positive ion and negative ion tuning and mass calibration. It is used at 100:1 dilution for positive ion mode and 1000:1 for negative ion mode.

It is also recommended that caffeine (PerkinElmer Part Number MZ301236) is added to the mix for an additional lower mass peak in a 1:5 dilution in the ESI Tune Mix.

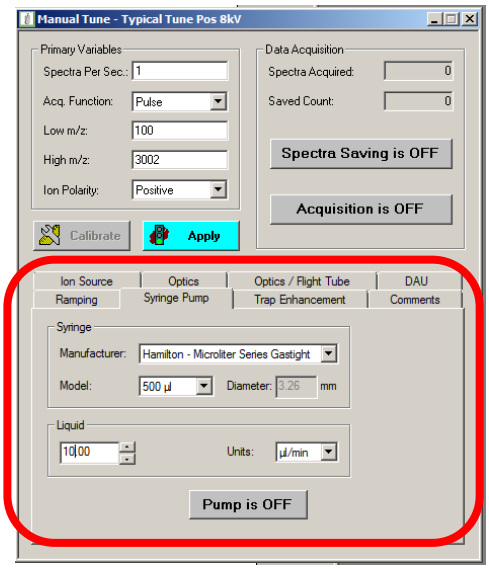
- 7.6 mL of LC-MS grade Acetonitrile
- 0.4 mL of LC-MS grade Water
- 2 mL of ESI Tune mix
- 40 µL of caffeine (2 mg/mL stock solution in water)

Calibrant Delivery by Syringe Pump

Calibrant can be introduced into the mass spectrometer by using one of the Calibrant Vials or with the syringe pump. Using a Calibrant Vial is detailed below. If, instead, using the syringe pump is desired, it can be set as shown in the following example:

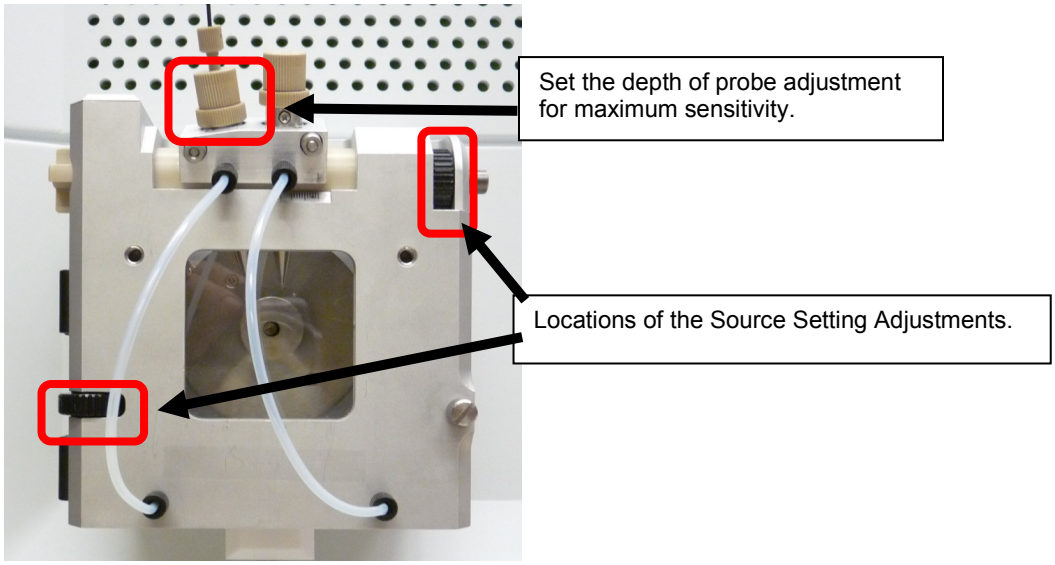
NOTE: *Using a 500 µL Hamilton syringe, set the inner diameter to 3.26 mm with a flow rate of 5 to 10 µL/min.*

1. Select the **Syringe Pump** tab on the **Manual Tune** dialog.
2. Select **Hamilton** from the **Manufacturer** drop-down list.
3. Select **500 µL** from the **Model** drop-down list.
4. Select **3.26** from the **Diameter** drop-down list.
5. Select a value between **5 and 10** from the **Liquid** spin box.
6. Select **µL/min** from the **Units** drop-down list.

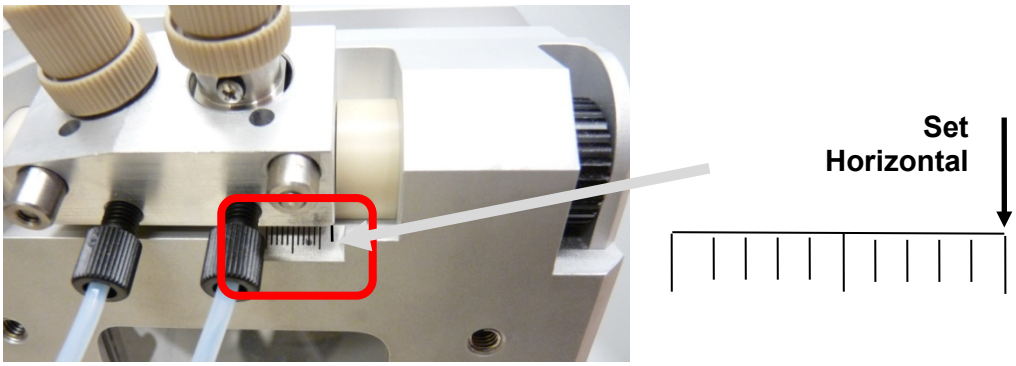


After the instrument has stabilized, while acquiring, infuse the tune mix. Examine the spectra to make sure that all of the target masses are present. Adjust the probe needle depth, horizontal position and the tilt for the single probe sprayer as shown below. If you are using a dual probe sprayer make sure that both probes are inserted into the source.

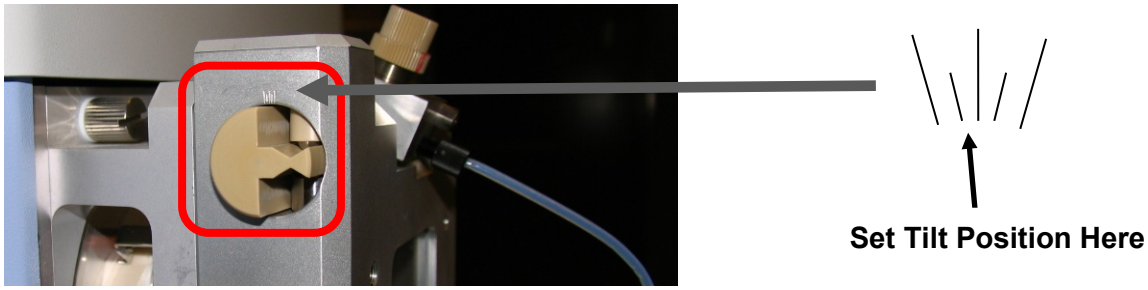
For the Dual ESI source, connect the infusion line to the left hand side of the dual sprayer. (The left sprayer is recommended since it must be used when the lockmass calibrant is infused simultaneously with the LC effluent.) The probe needle depth and the tilt positions should be the same as for the single probe sprayer but the horizontal position may have to be adjusted to optimize the sensitivity of the calibration mixture.



1. Set the **source horizontal position** so that the horizontal probe marking is positioned 10 spaces to the right side of the scale as shown below:

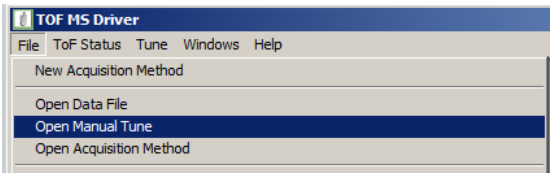


Set the **Probe Tilt** position as shown below:

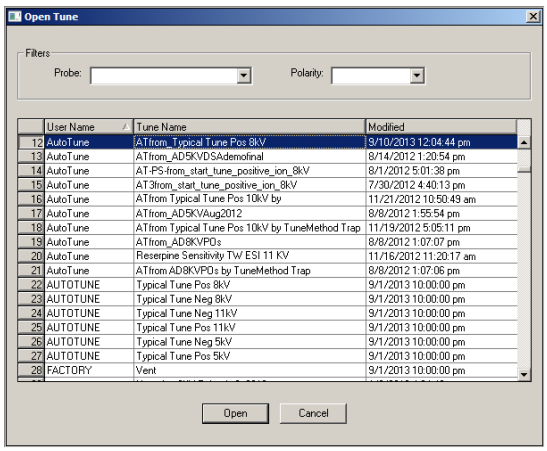


2. To start the calibration, double-click on the **TOF MS Driver** icon.

3. From the **File** menu, open **Manual Tune**.

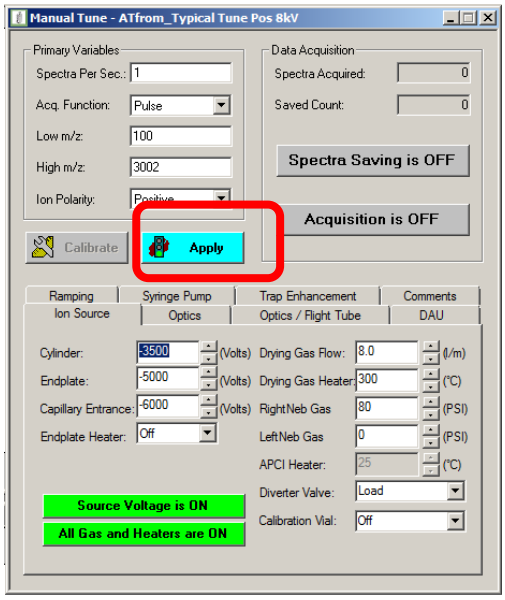


4. Select the Tune you want to calibrate by scrolling to the Tune then click on it to open it.
   In this example, we have selected the Tune: **ATfrom_Typical Tune Pos 8kV**

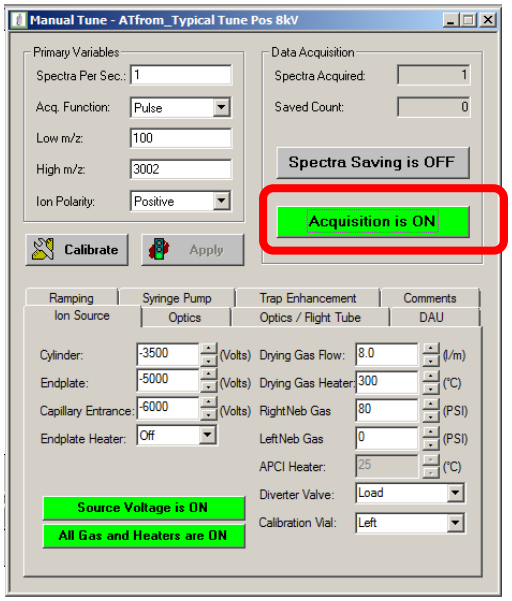


5. Click **Open**.
   The **Manual Tune - ATfrom_Typical Tune Pos 8kV** dialog displays.

6. If using a calibrant Vial to introduce Tune mix, update the drop-down to choose the appropriate Calibrant Vial. Then click **Apply** to apply the Tune and turn on the pump for the appropriate Calibrant Vial. If introducing Tune Mix using the syringe instead of the calibrant vial, **Apply** the tune and then select the Syringe Pump tab and select the **Pump is OFF** button.
   The instrument loads the Tune.

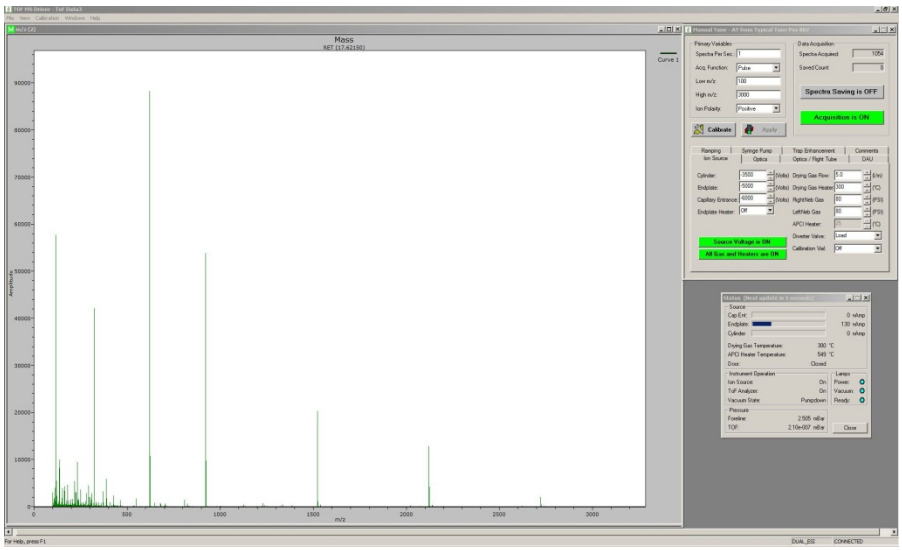


7. Click on **Acquisition is OFF** button to turn the acquisition on.

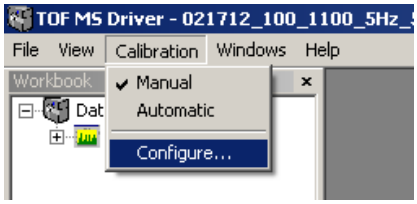


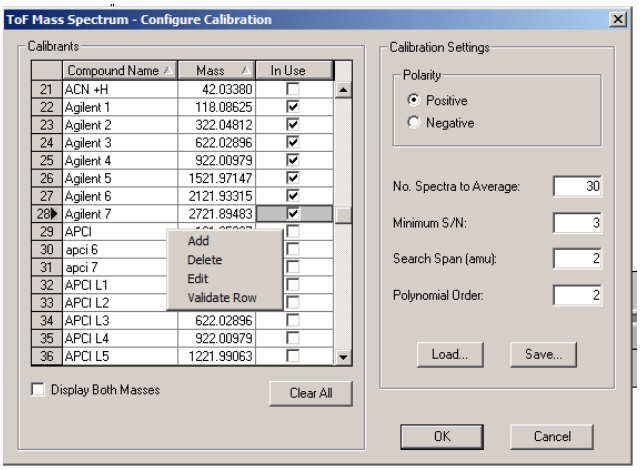
The TOF will start pulsing and the **Acquisition is OFF** button will light up green and change to **Acquisition is ON**. With the acquisition on, allow the electronics to thermally stabilize as describe at the beginning of this Section.

Make sure the **Drying Gas Flow** is set between 6-8 (l/m), the **Drying Gas Heater** to 300 C, the **Endplate Heater** is OFF and the **Nebulizer Gas** for the probe introducing the tune mix is set to 80 PSI.



8. From the **Calibration** menu select **Configure** to open the **TOF Mass Spectrum - Configuration Calibration** window.





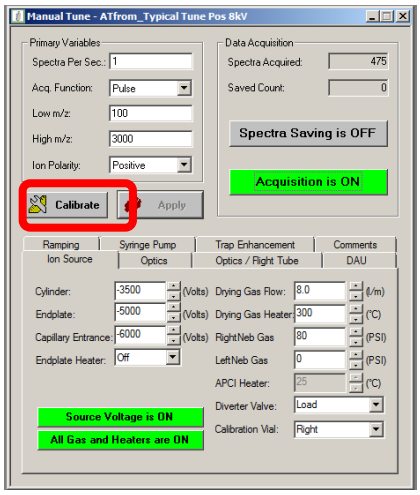
9. Select the ion **Polarity** (Positive or Negative) and check the masses for the calibration series by left clicking in the boxes under **In Use** column.

   The example above shows that we are calibrating on seven masses. To enter, edit or delete a value in the calibrant mass list, right click on the list of compound/masses and choose the appropriate menu item. Validation checks the entry selected is within acceptable mass specifications.

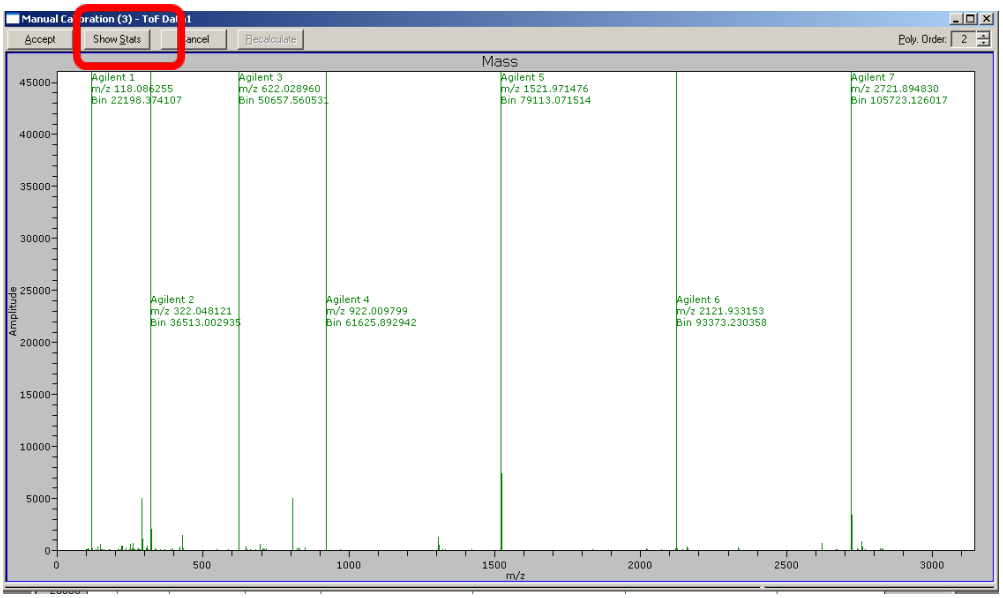
10. Use **30** for the **No. Spectra to Average** and use the value 3 for the **Minimum S/N**. The **Search Span** should be set to **2** amu; however if the masses are off more than **2** mass units you will have to open the span wider. The **Polynomial Order** should be **2** but if this does not work well you can set the order to 1 or 3 and rerun the calibration.

11. Click **OK** to set these values**.**

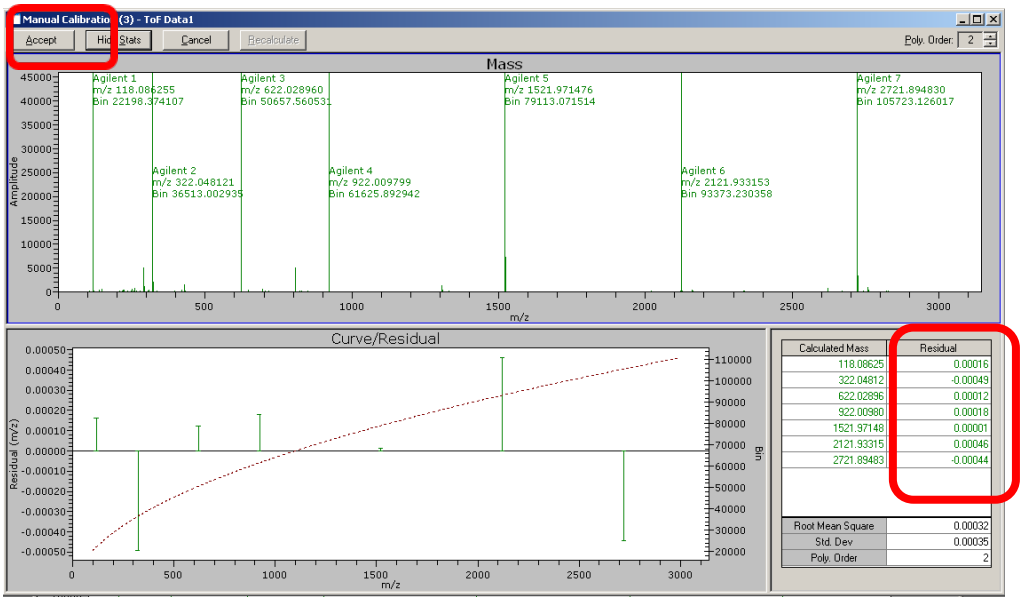
12. In the **Manual Tune** dialog, click on the **Calibrate** button and allow the calibration to complete.



When the calibration is finished the calibration window will appear.

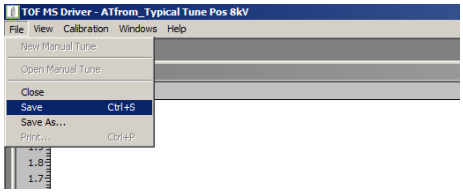


13. Click the **Show Stats** button.
    The information appears on the bottom half of the screen.

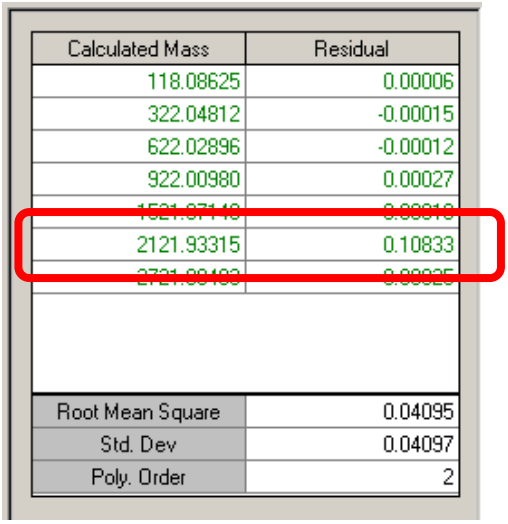


14. Make sure the Residuals are less than 0.000x as shown above (there should be three zeros for each of the Residuals). Click the **Accept** button.

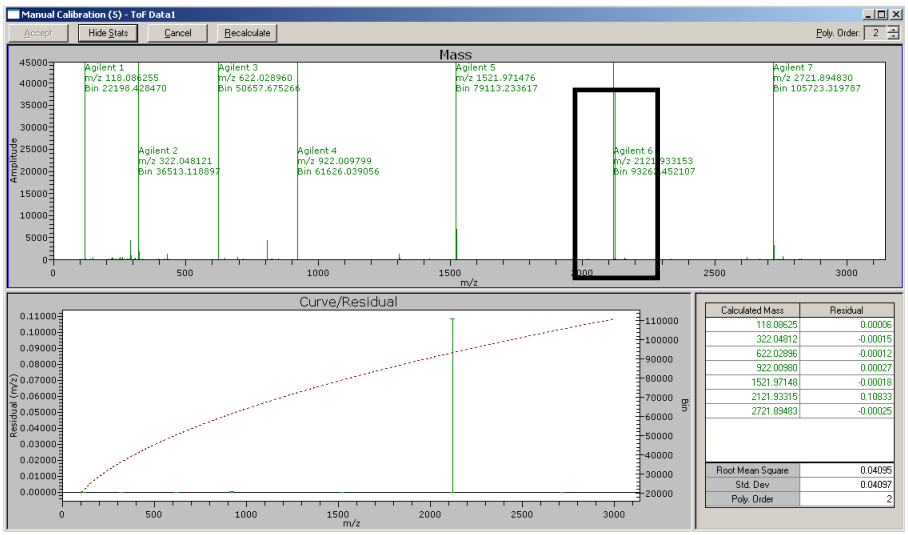
15. If all of the Residuals are less than 0.000x as shown above, select **Save** from the **File** menu. This ties the calibration information to this Tune file.



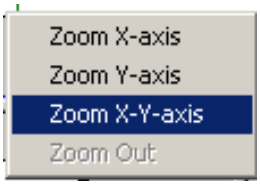
16. If any Residuals are too high, as shown in the following example, perform the following procedure to adjust the residuals to an acceptable level.



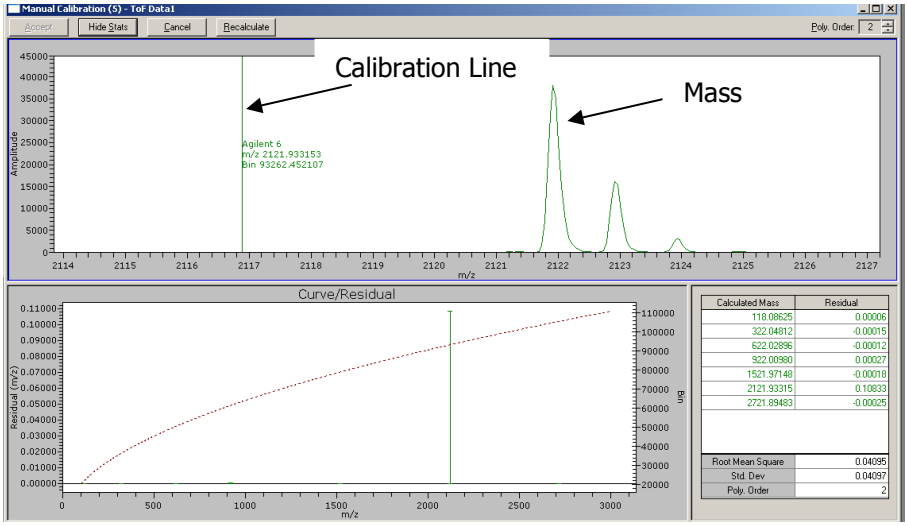
   a. Left-click and drag the mouse to draw a box around the mass and the calibration line. The following example shows the mass and calibration line close to each another.



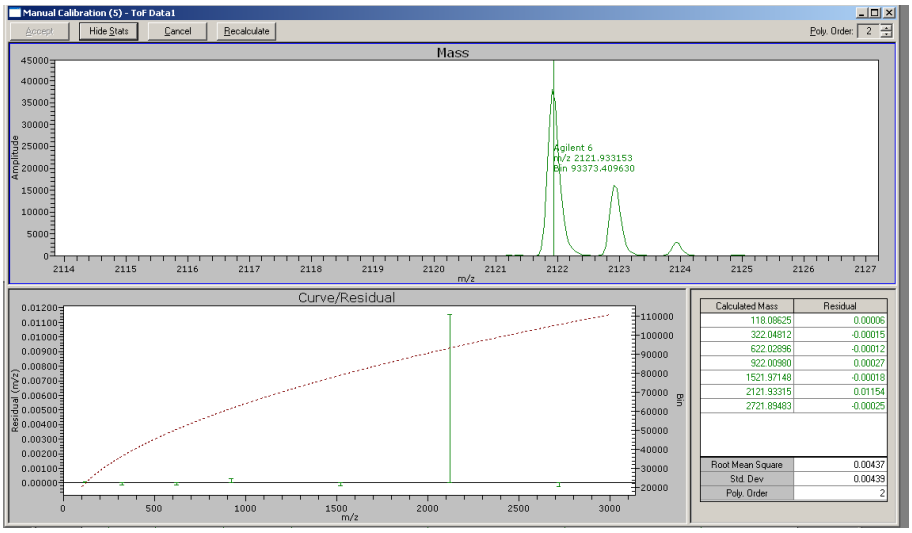
After drawing the box, release the mouse button and the Zoom pop-up box appears.



b. Select **Zoom X-axis** or **Zoom X-Y axis**. Continue to Zoom in by redrawing a box until the mass and the calibration line are visible as shown below.

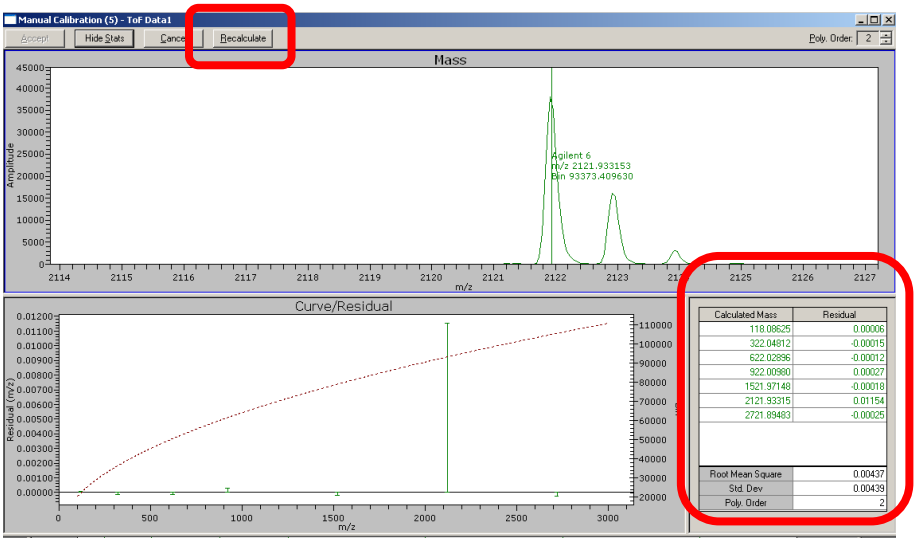


c. Left-click on the calibration line and drag it to the center of the mass peak as shown below.



Repeat this procedure for all masses where the Residual is greater than 0.000x.

d. Click the **Recalculate** button and observe the Residuals to make sure they are within specification.



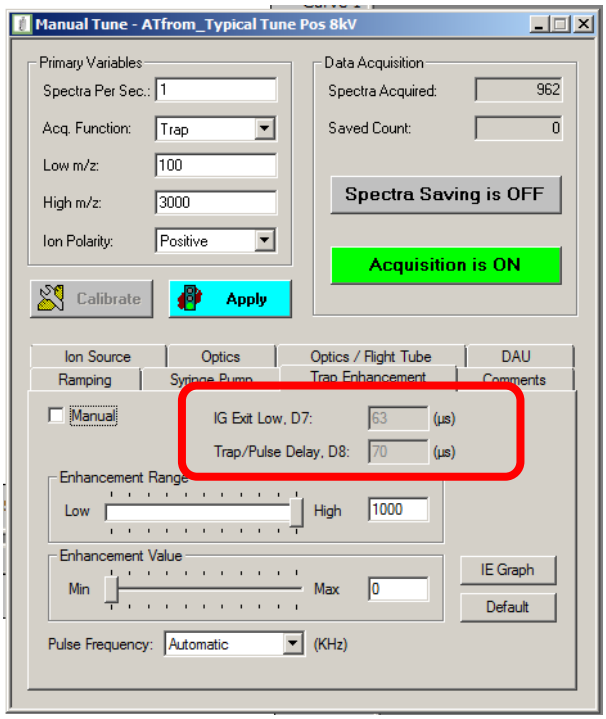
e. To complete the calibration procedure, click the **Accept** button. Then from the TOF MS Driver window, select **Save** from the **File** menu.

17. If this procedure fails, increase the concentration of your calibration mix.
    - For the Tune Mix in positive ion mode, change it from 100:1 to 50:1.
    - In the negative ion mode, change concentration from 1000:1 to 100:1
    - Then rerun the calibration procedure.

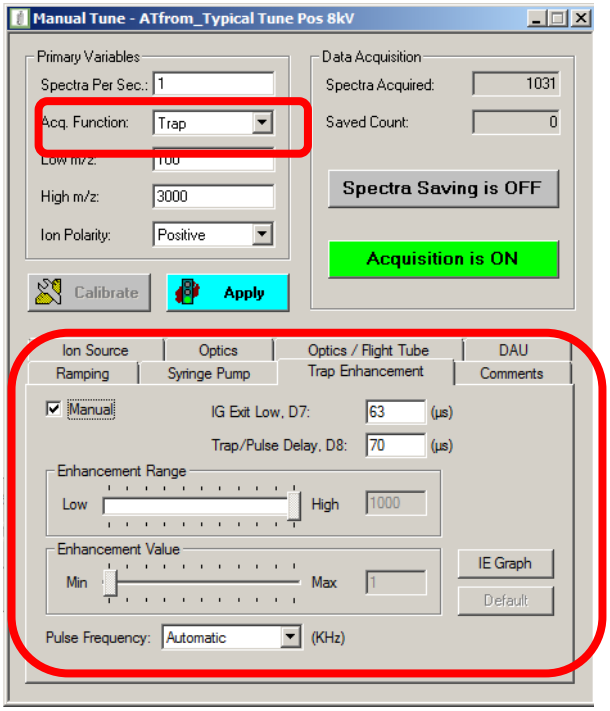
    When the calibration is complete, apply the **Pulse Tune** calibration to the Trap Tune that AutoTune created.

18. Open the Trap Tune.

19. On the **Manual Tune** dialog, click the **Trap Enhancement** tab and write down the values for D7 and D8.



20. Open the calibrated Pulse Tune and select **Trap** as the **Acq. Function.** Then click the **Trap Enhancement** tab, click the **Manual** check box, type the values for **D7** and **D8** that you wrote down in the previous step.



21. Then from the TOF MS Driver window, select **Save as** from the **File** menu and save the file as the Trap Tune. Now the calibration is applied to the Trap Tune.

Ramping the AxION 2 TOF MS Capillary Exit

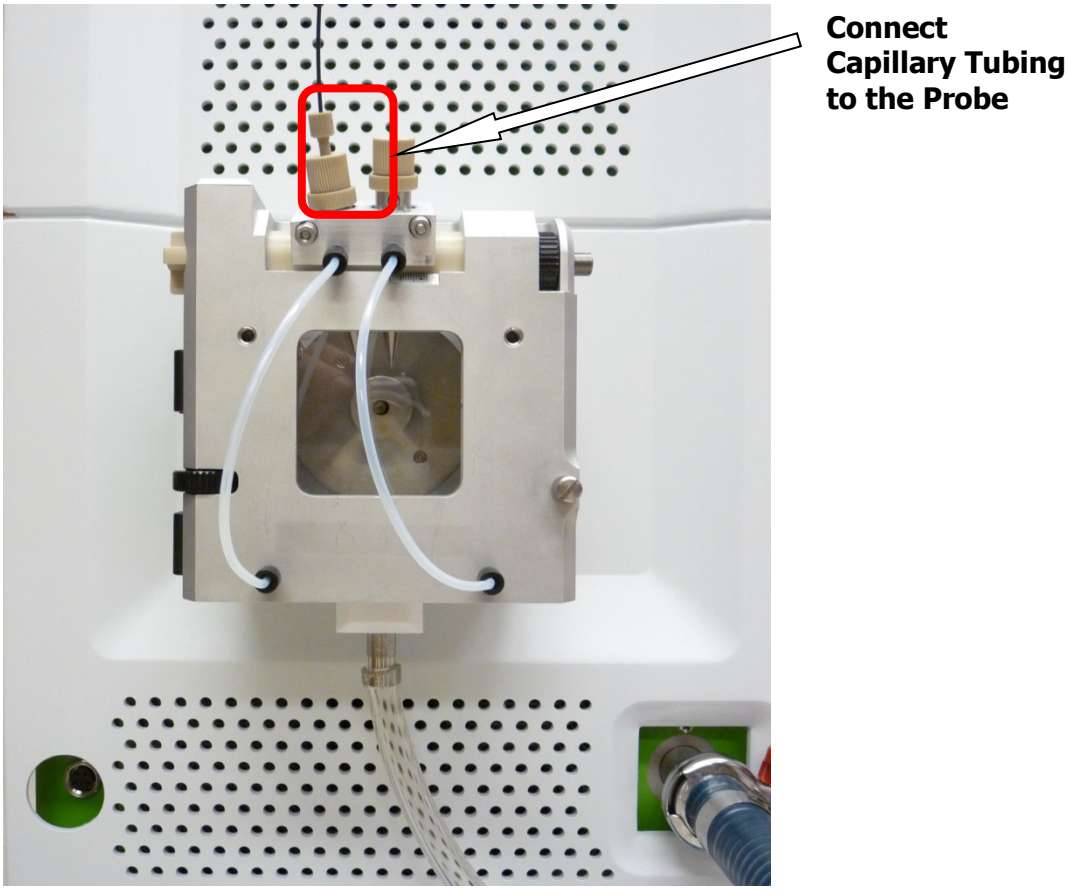
Setting up a Sample Infusion

To optimize a specific variable, it is typically necessary to infuse a continuous amount of sample into the ion source. There are 2 ways to accomplish this, infuse the standard at a low flow rate directly into the MS, or infuse it into the LC stream running at a typical flow rate in order to also optimize "flow dependent" MS parameters such as temperature and drying gas. The following example demonstrates how to infuse a reserpine standard directly into the MS using a syringe. Reserpine was chosen because it is a widely used standard in the LCMS community and it is readily available from a variety of commercial sources.

1. Fill a syringe with a ~50 pg/µl reserpine solution in LCMS grade methanol and water in a 75:25 ratio in 5 mM ammonium formate

2. Since this example demonstrates a dual-probe system, connect a Peek transfer line from the syringe needle to the left ESI sprayer. (Either sprayer may be used if infusing a solution.)

3. With the syringe prepared for infusion, the next part of the process covered in the next section involves ramping (to optimize) the capillary exit voltage.



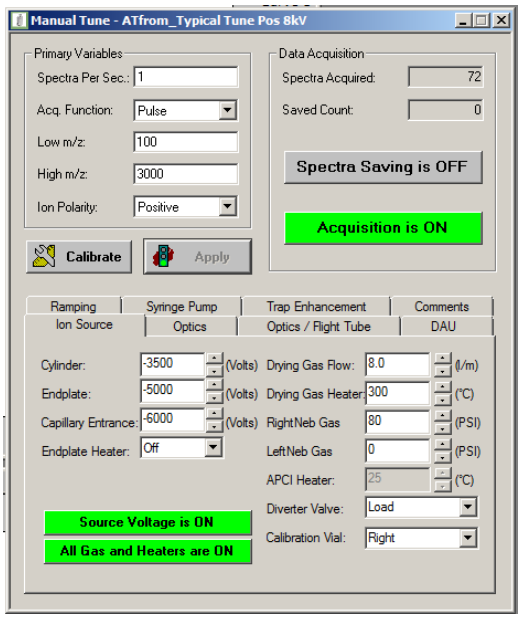
Ramping Parameters - Optimizing the Capillary Exit Voltage

The AxION 2 TOF MS driver allows you to ramp certain Tune parameters to determine the best settings for those parameters. However, the AutoTune routine has eliminated the need to do this except for the one parameter that is "compound dependent", the Capillary Exit voltage. This is the one parameter that should be checked in order to obtain the best sensitivity for quantitative analyses, or minimize or maximize molecular fragmentation information for qualitative analyses, or both.

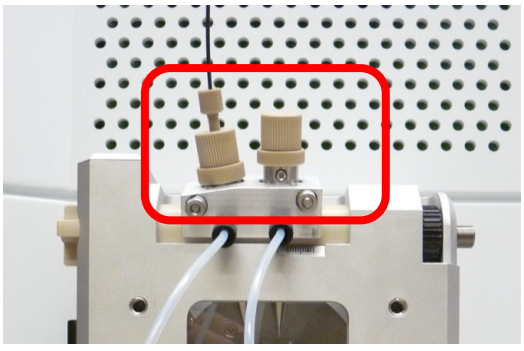
To Ramp the Capillary Exit voltage:

1. Open a **Manual Tune**.

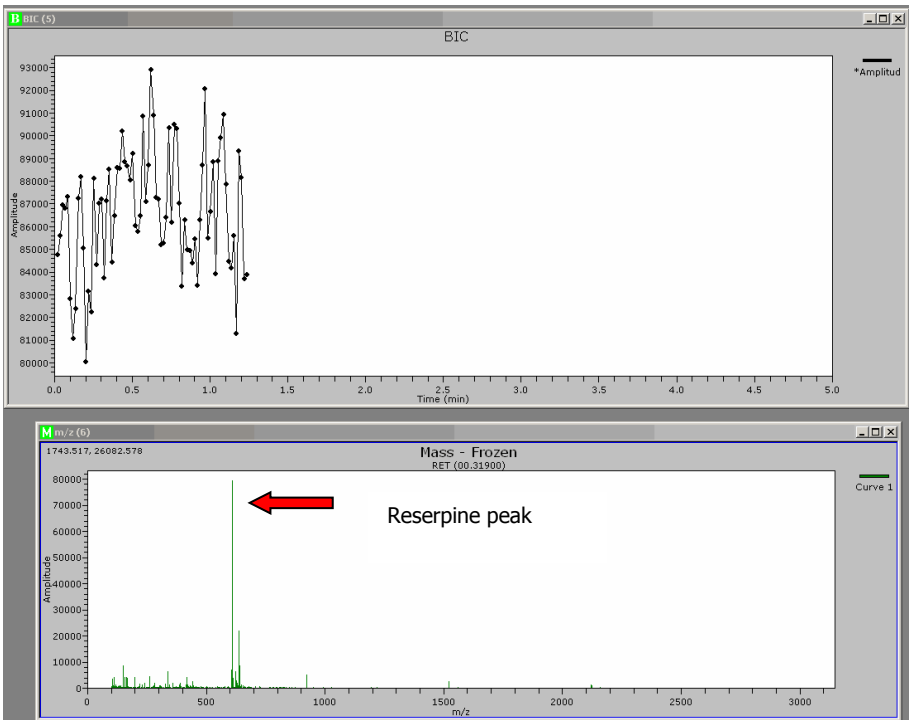
2. When using a dual sprayer, click on the **Ion Source** tab and turn on the appropriate Calibrant Vial as previously described.



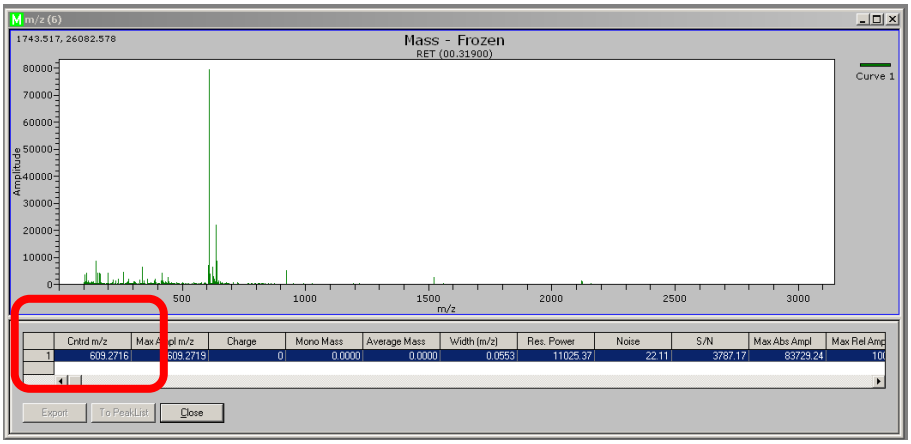
3. Start infusing your target compound (in this example it is reserpine) into the probe on the left.



Observe your target mass (in this example reserpine) in the TOF Driver window.



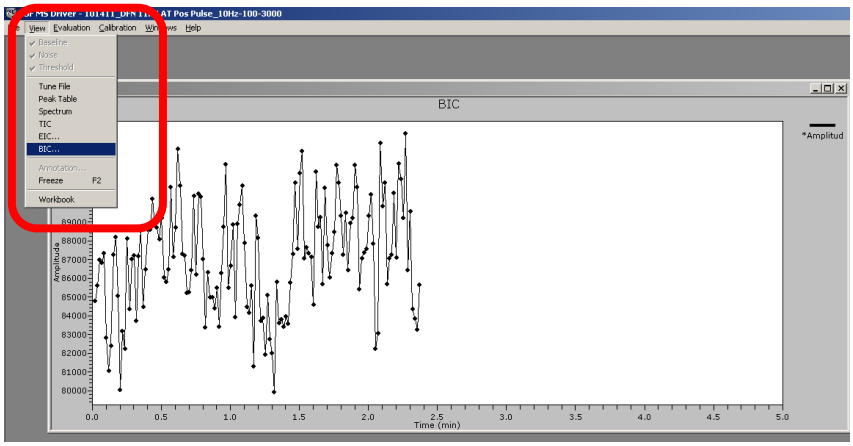
4. In the ***m/z*** screen, right-click on the apex of the target mass and write down the observed values.



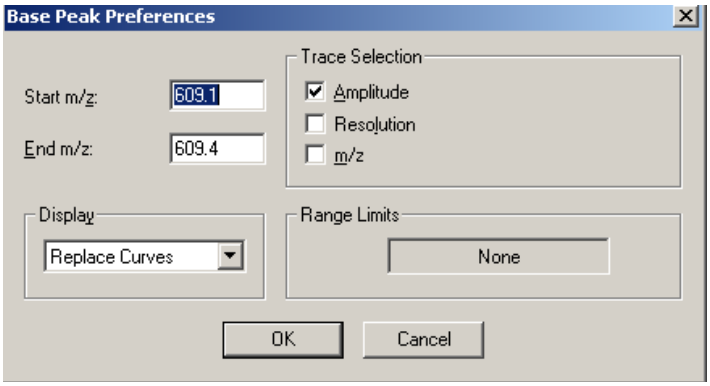
NOTE: *The above window shows the observed value of m/z 609.2716.*

5. Click **Close**.

6. In the TOF MS Driver screen, select **BIC** from the **View** menu.



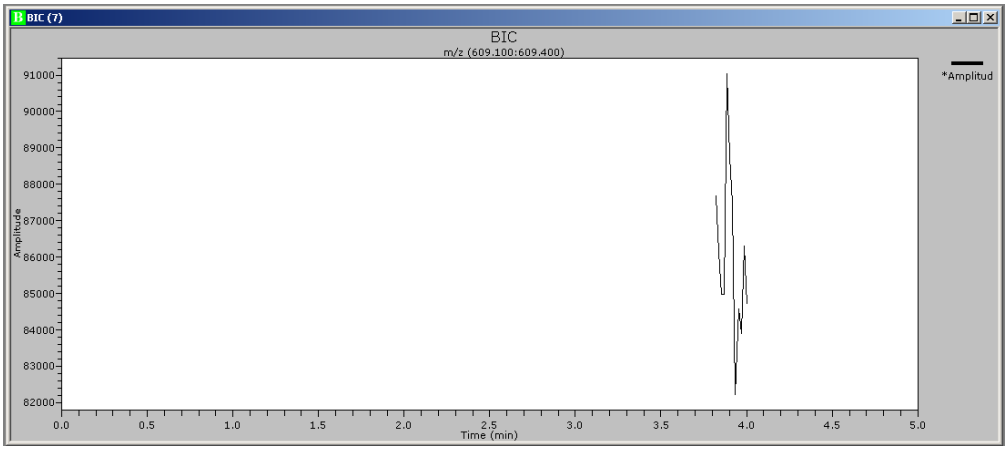
The **Base Peak Preferences** dialog displays.



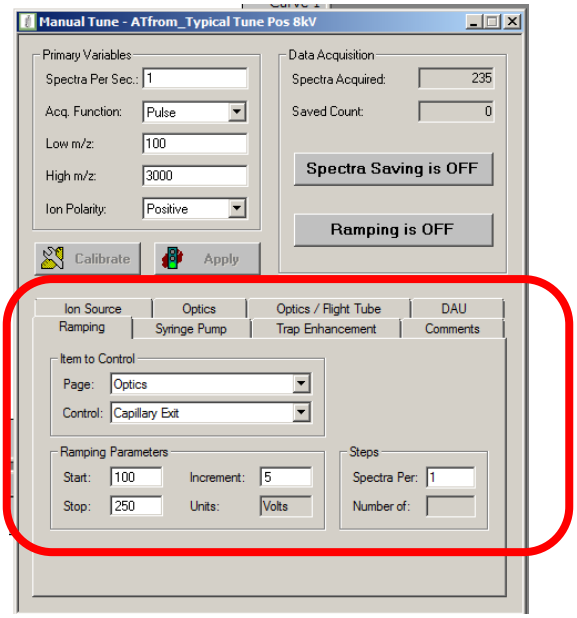
7. Type a **Start** ***m/z*** value to the left of your target peak and an **End** ***m/z*** value to the right of your target peak. Select **Amplitude** for the **Trace Selection** and **Replace Curves** for the **Display**.

8. Click **OK**.

   The BIC screen displays.



9. Stop acquiring by clicking the green **Acquisition is ON** button. The button changes to **Acquisition is OFF**.
10. Select the **Ramping** tab. The **Acquisition is OFF** button changes to **Ramping is OFF**.

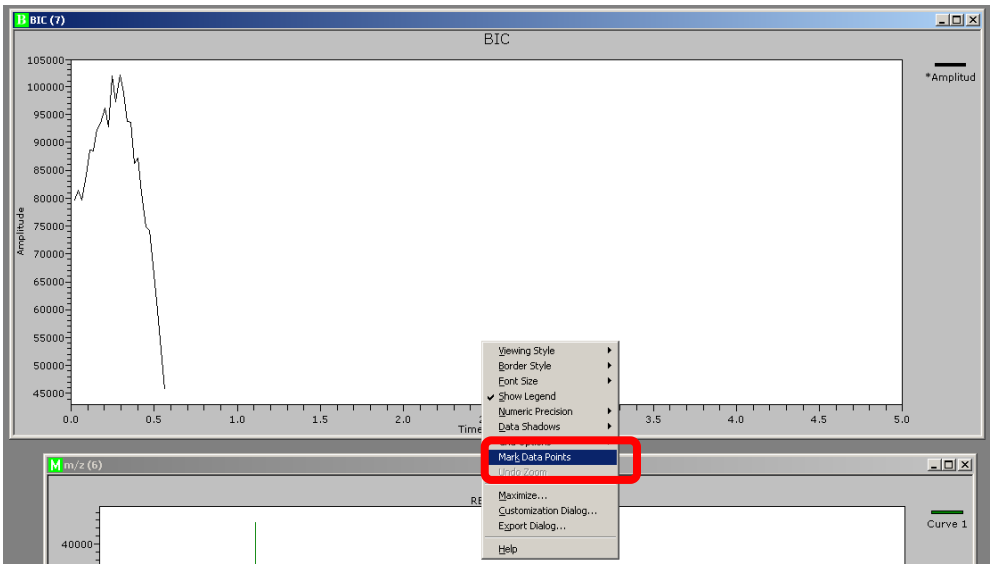


11. Select or enter values for the following: **Item to Control**, **Ramping Parameters**, and **Steps**.
    In this example we entered the following:

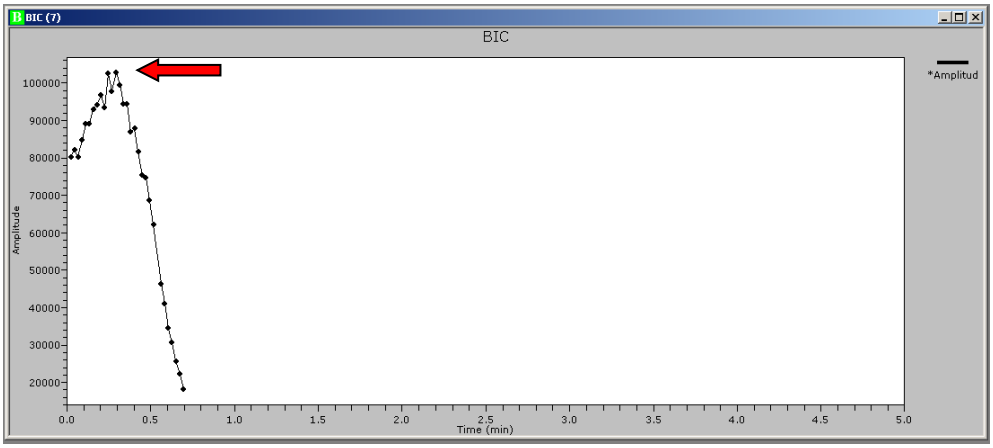
Function	**Parameters**	**Values/Settings**
Item to Control	Page	Optics
	Control	Capillary Exit
Ramping Parameters	Start	100
	Stop	250
	Increment	5
	Units	Volts
Steps	Spectra Per	1

12. To start the ramp, click on the **Ramping is OFF** button.
    It turns green and changes to **Ramping is ON** 

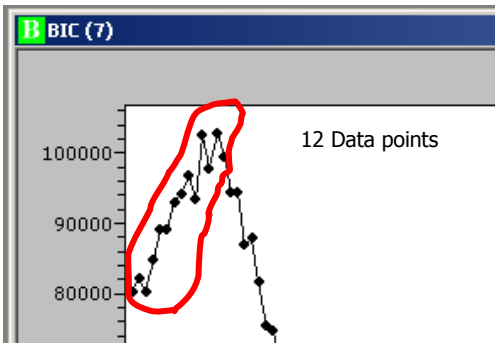
13. When the ramp completes, right-click in the **BIC** screen and select **Mark Data Points** from the pop-up menu.



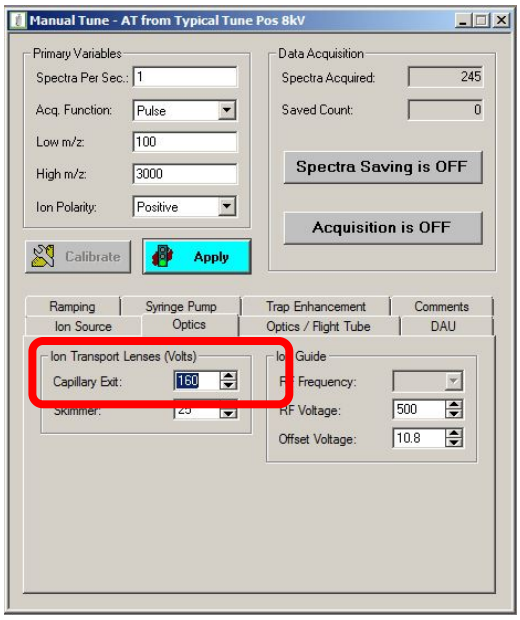
The Ramp marks the data points as it runs to completion.



14. Start counting the data points from the left side of the BIC screen to the apex of the displayed peak. Each data point is 5 volts (V); this is the value you entered as an **Increment** in the Ramping parameters. In this example we counted 12 data points.



15. Multiply the counted number of data points (12) by the voltage per data point (5V) 12x5=60. Add this 60 value to the **Ramping Parameters Start Mass** value, in this example the Start Mass value is 100. 100+60=160. This value is now your optimum **Capillary Exit** voltage for reserpine.
16. Click on the **Optics** tab. Enter the above calculated value of 160 as the **Capillary Exit** value.



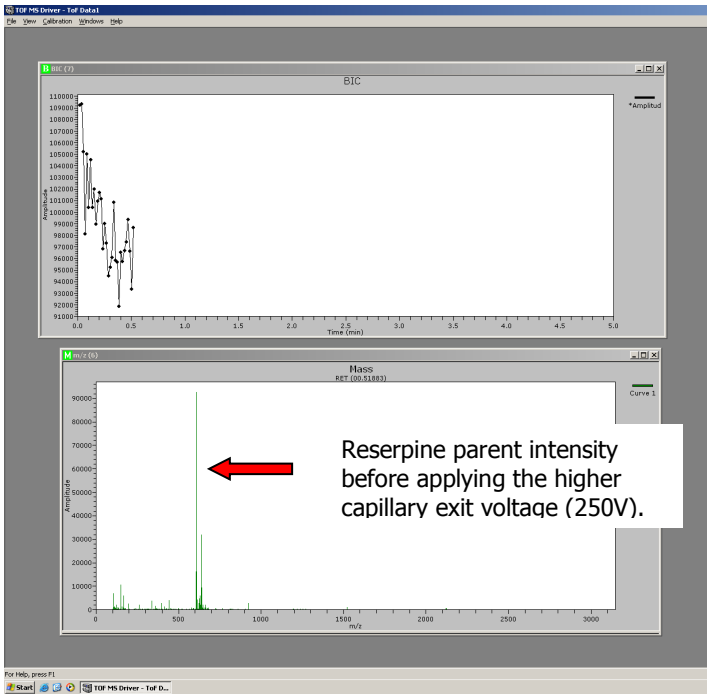
17. Select **Save Tune** from the **TOF MS Driver File** menu.

18. Turn the acquisition on and view you spectrum in the Mass screen.

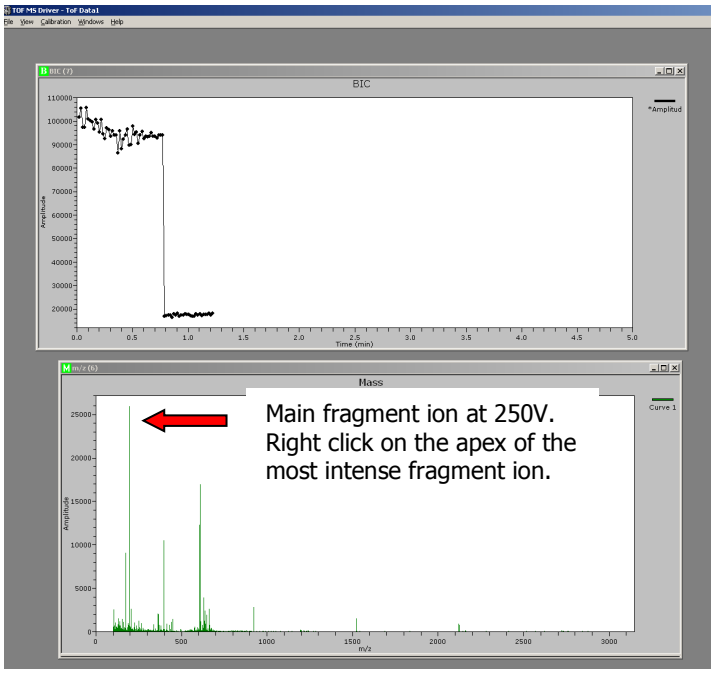
Ramp Again to Optimize the Signal Intensity of a Fragment Ion

Obtaining fragmentation information on a compound can be very valuable for a number of reasons. It gives insight into the structure of the compound, which is typically applicable to other compounds with the same base structure (e.g., drugs and their metabolites). It also provides additional ions associated with the analyte that can be measured instead of, or in addition to, the protonated molecular ion. For example, in quantitative analyses, there is occasionally an issue with a contaminant or mobile phase ion at the same nominal mass as the analyte to be measured. This may have a significant effect on the detection limits of the analyte due to background noise. To overcome this, fragments of the analyte can be monitored instead of, or in addition to, the protonated molecular ion. The probability of the interfering ion having a fragment at the same *m/z value* as a fragment from the analyte is extremely low (but still must be verified!)

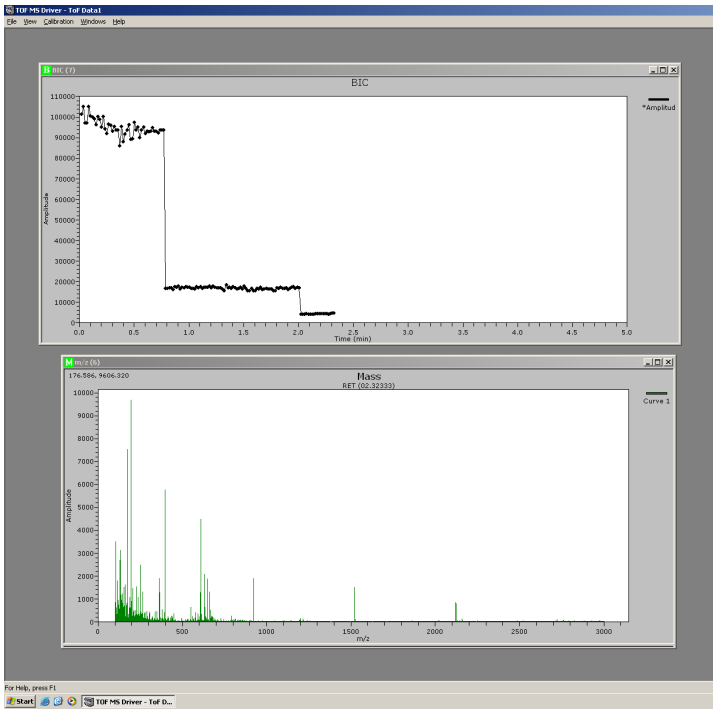
The following screen shows reserpine optimized at 160V.



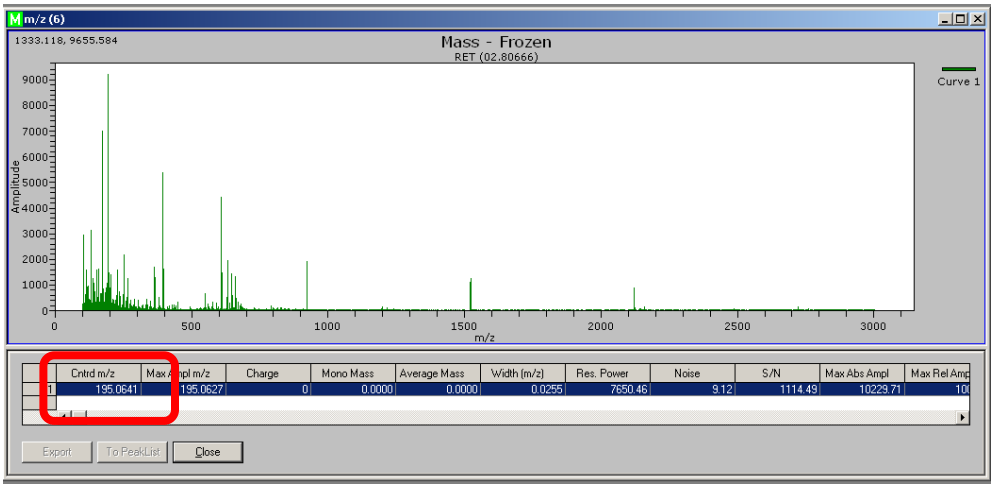
1. To view the fragment, increase the **Capillary Exit** value (in this example we set it to 250V) and click **Apply**.



2. Increase the **Capillary Exit** value (in this example we set it to 300V) and click **Apply**.

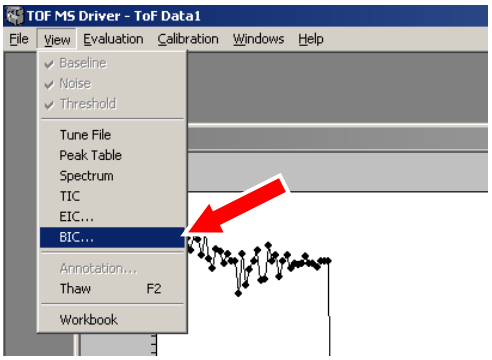


3. In the ***m/z*** screen, right-click on the apex of the most intense fragment ion(s) observed and write down the observed values.

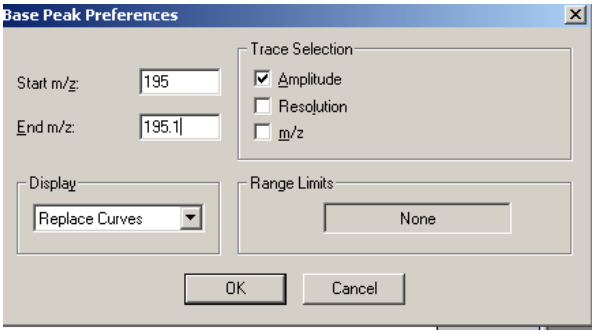


NOTE: The above window shows the observed value of *m/z* 195.0641.

4. Select **BIC** from the **TOF MS Driver View** menu.



The BIC **Base Peak Preferences** dialog displays.

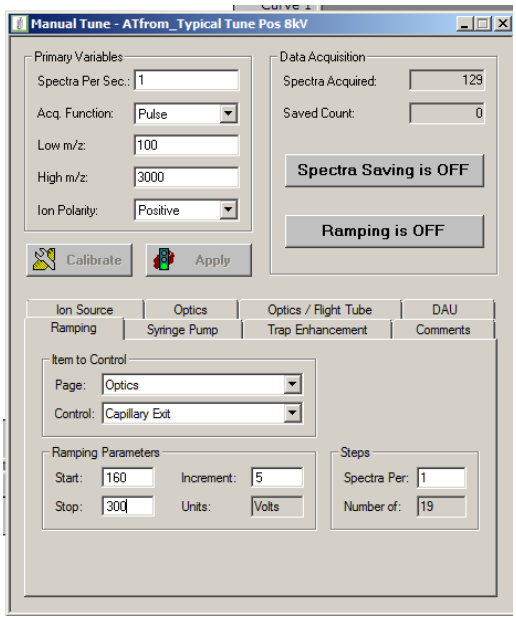


5. Type a **Start** ***m/z*** value to the left of your target peak and an **End** ***m/z*** value to the right your target peak. Select **Amplitude** for the **Trace Selection** and **Replace Curves** for the **Display**.

6. Click **OK**.

7. Stop acquiring by clicking the green **Acquisition is ON** button. The button changes to **Acquisition is OFF**.

8. In the **Manual Tune** dialog, select the **Ramping** tab.



9. Select or enter values for the following: **Item to Control**, **Ramping Parameters**, and **Steps**.
   In this example we used the following settings:

Function	Parameters	Values/Settings
Item to Control	Page	Optics
	Control	Capillary Exit
Ramping Parameters	Start	160
	Stop	300
	Increment	5
	Units	Volts
Steps	Spectra Per	1

10. To start the ramp, click on the **Ramping is OFF** button.

    It turns green and changes to **Ramping is ON** 

11. Right-click in the BIC screen and select **Mark Data Points** from the pop-up menu.

12. After the ramp is complete as shown in the previous example, start counting the data points from the left side of the BIC screen to the apex of the displayed peak.
    Each data point is 5V; this is the value you entered as an **Increment** in the Ramping parameters.

13. Multiply the counted number of data points by the voltage per data point (5V).

14. Click on the **Optics** tab. Enter the above calculated value as the **Capillary Exit** value.

15. Select **Save Tune** from the **TOF MS Driver File** menu.

16. Turn the acquisition back on and view you spectrum in the Mass screen.

Creating Methods and Sequences

Creating an MS Method

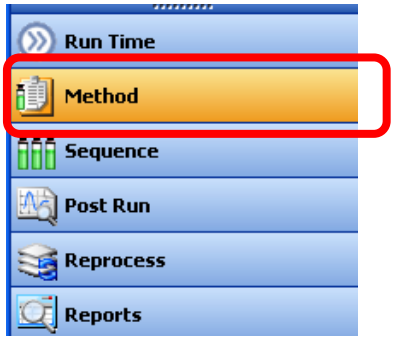
The foundation for an optimal MS Method is starting with a good mass calibrated Tune. Using the previous calibration instructions, ensure the tune is proven to be optimized, using Calibration, before proceeding.

The following example shows how to create an MS method that will acquire both a total ion chromatogram (TIC) and an extracted ion chromatogram (EIC), along with the spectra. While the TIC gives an indication of the sum of the ion intensities observer during a scan (written to disk), the EIC is used to selectively identify specific masses in real time to indicate when they are observed within a scan. This is extremely valuable for quantification purposes, since the protonated molecular ion (in positive ion analyses) of a molecule or fragment thereof, may be quantified within a batch of samples containing the appropriate calibration standards.

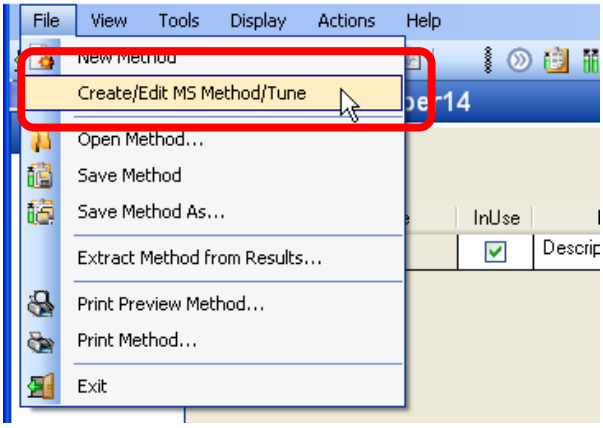
IMPORTANT: If quantification of a compound using Chromera is desired, then the EIC masses of the ions of interest must be predefined in the TOF MS Driver Method in order to be available to Chromera for processing. EICs added post-acquisition are invalid for reprocessing.

To create an MS method:

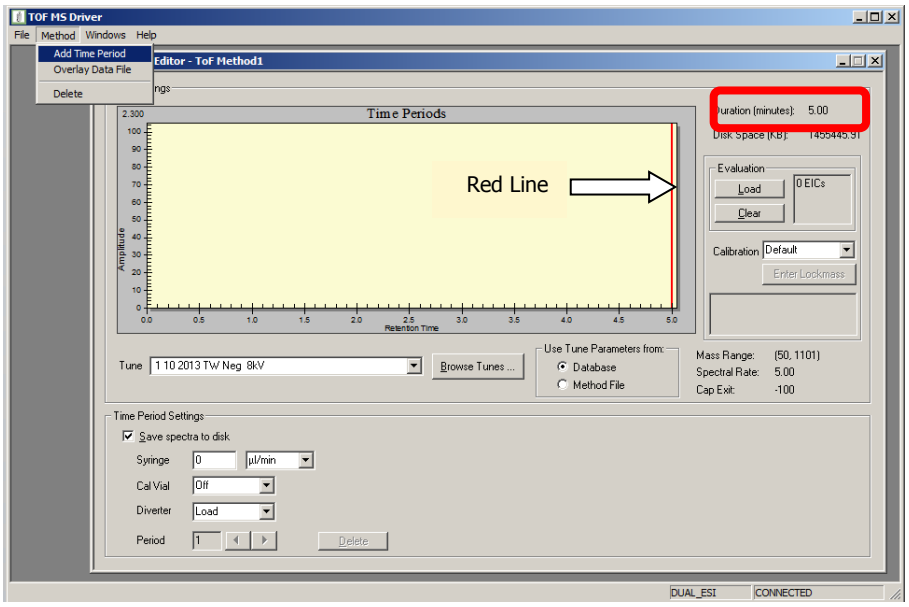
1. Click **Method** to open the **Chromera Method** screen.



2. Select **Create/Edit MS Method/Tune** from the **File** menu.



The **Method Editor** screen displays in the **TOF MS driver** screen.

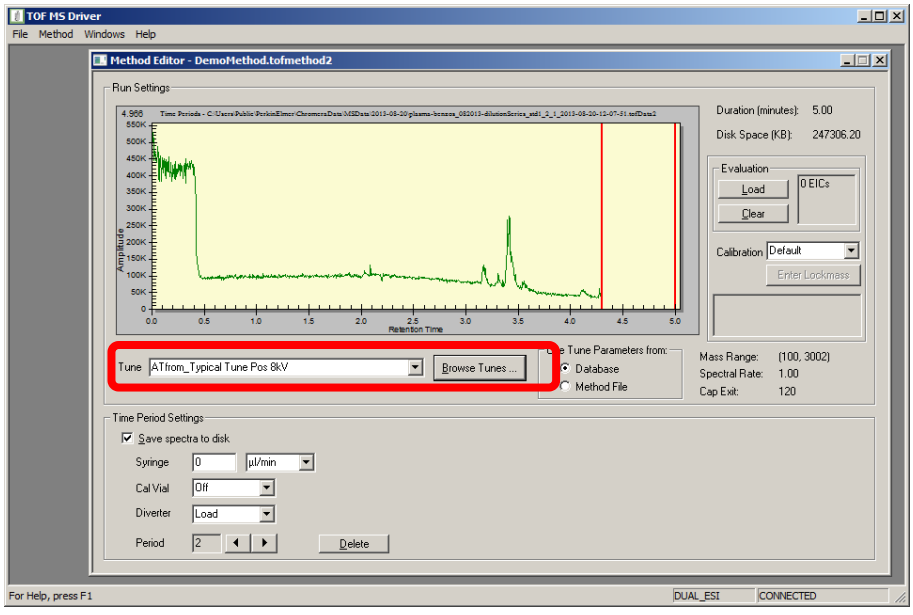


3. Click and drag the red bar in the graph to set the time of the experiment to the same time required for the previously developed LC method. Addition time periods can be added by selecting Add Time Period in the Method menu. Also, existing data (.tofdata2) can be loaded as an overlay to aid in assignment of multiple time periods.

   In the above example, it is set to 5 minutes.

4. Select a Tune from the drop down list or by selecting the **Browse Tunes...** button, then double-click on the Tune name. As soon as a tune is assigned to a method, the parameters for the tune are written into the method file (tofmethod2).

   This example shows **ATfrom_Typical Tune Pos 8kV** was selected. This example also shows the addition of a second time period and the overlay of a tofdata2 file.



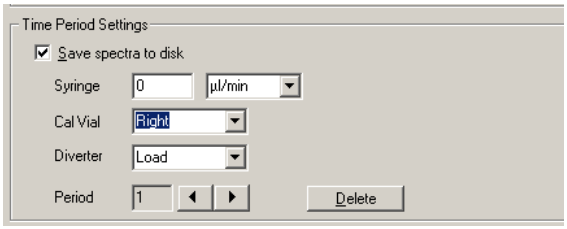
5. Select whether the tune parameters from the **Database** or the **Method File** will be used. The **Database** selection means that later updates to the parameters of that tune (through Manual Tune,

Autotune and/or Calibration) will automatically be used when the method is run. This occurs without re-associating the tune to the method. In contrast, the **Method File** selection keeps the tune parameters static. The **Database** option is recommended and the default selection for new methods and for methods created using earlier versions of the TOF MS Driver software.

NOTE: *If, during acquisition, a method is configured to use a tune stored in the database but that tune name is not found (renamed or deleted), the system will automatically use the tune parameters in the method file. Also, if a method file is set to read only then the functionality to pull the tune from the database is not functional.*

6. The peripheral device settings in the lower part of the screen are method *and* time period specific. These specific settings in the method will be used over the tune settings during acquisition and when the TOF Driver method is loaded into Chromera to get to Operate mode.

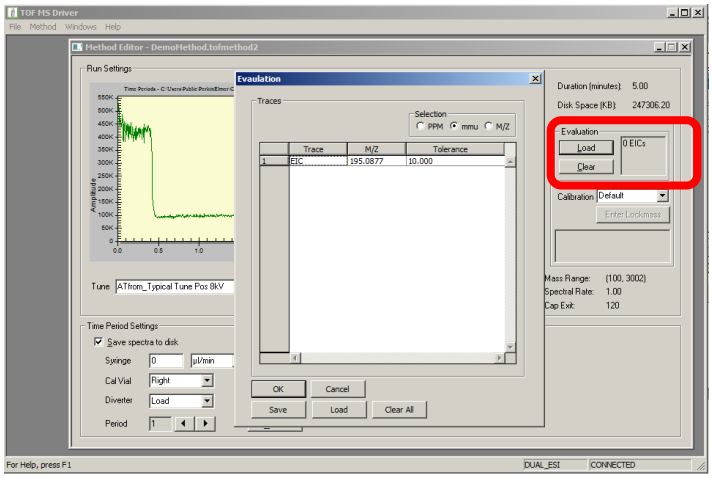
    a. Only those parameters shown below can be changed at the method level. Consequently, be very careful with the **Method File** option for fetching the Tune Parameters. With this selected, the peripheral values that cannot be set within the method are also static. For example, the syringe TYPE. If a method uses the **Method File** tune parameters and the syringe TYPE is changed in the tune, that change is not adopted during method-controlled acquisition. The **Database** option for fetching tune parameters will adopt such changes.



IMPORTANT: *For processing in conjunction with Chromera, ALWAYS have "Save spectra to disk" selected.*

7. Define EICs: In the **Evaluation** section of the Method Editor screen, click the **Load** button.

    The following dialog displays.



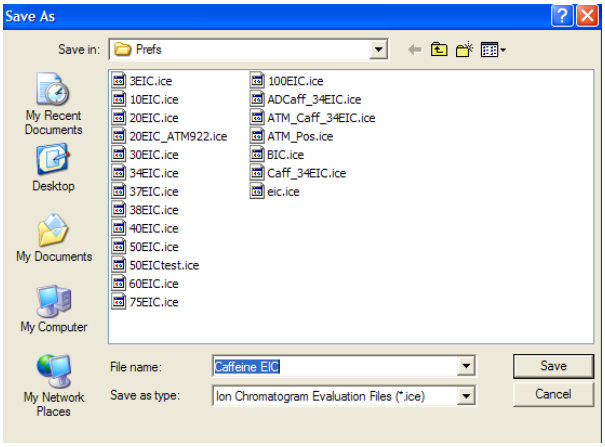
The EIC values entered here will be displayed in real time during acquisition in Chromera and will be available for quantitation in Post Run. Edits to the EIC list cannot be applied to acquired data.

8. Click the **Insert** key on your keyboard and enter the EIC value in *m/z*; with this example it is 195.0877 for protonated caffeine.

9. The Tolerance (+/-) of the EIC can be selected in parts per million (ppm), millimass units (mmu), or *m/z* as in the **Selection** section; here, **mmu** was used for the EIC tolerance with a value of 10 millimass units. Make sure to press the Enter key on the keyboard to update the method with this value.

NOTE: *The resolving power and exact mass measurement capabilities of the AxION 2 TOF provides a tremendous amount of selectivity that should be utilized to the user's advantage. Entering an EIC mass to 4 decimal place accuracy and specifying a tolerance of a few mmu (or ppm) will significantly increase the selectivity of detection for the specified analyte.*

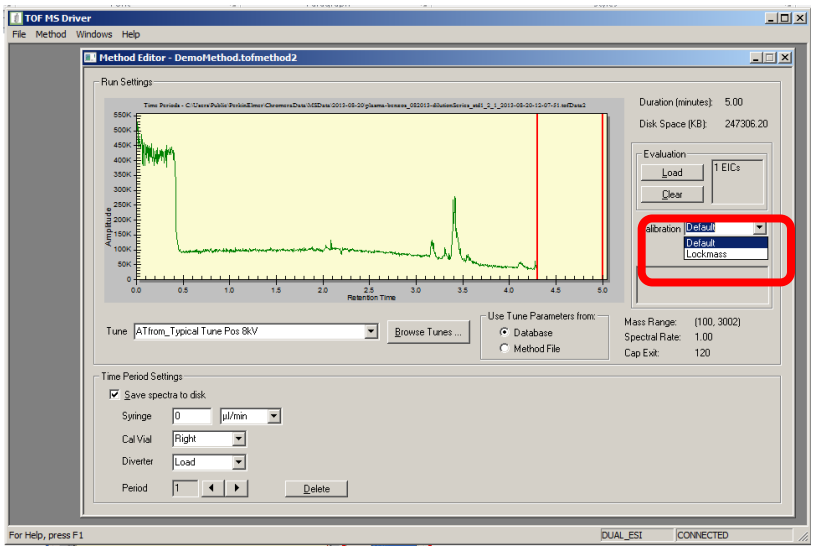
10. Click the **Save** button (to save the EIC for future use).
    The following **Save As** dialog displays.



11. Type a **File name**.
    In this example, the name is **Caffeine EIC**.

12. Click the **Save** button.

13. Apply Lockmass Parameters: In the Calibration section of the screen, select **Default** or **Lockmass** from the drop-down.

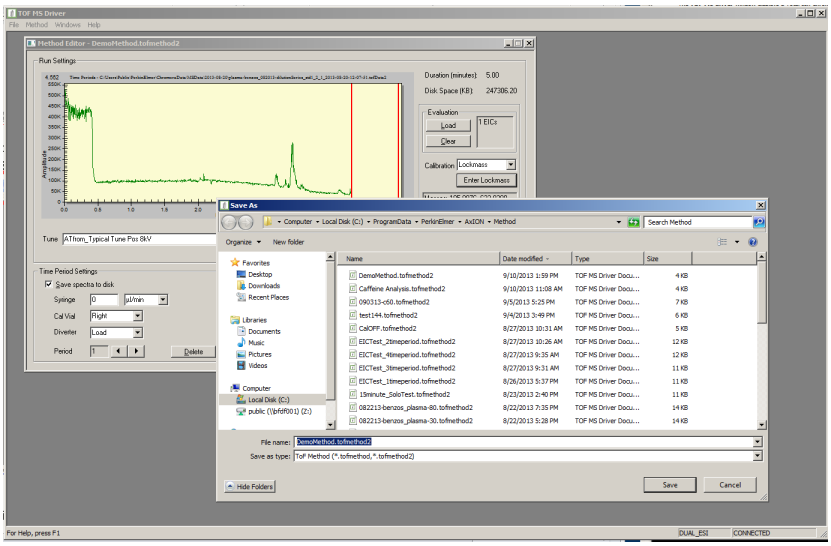


In this example we selected the **Default** Calibration setting which uses the calibration that was updated within the selected Tune (by utilizing the **Calibrate** button on the Tune page).

Alternatively, two Lockmass values can be assigned to be used for mass correction during acquisition. Parameter sets can be saved for future use. Refer to the next section for additional information.

14. To continue, click **Save** from the main **File** menu on the **TOF MS Driver** window.

    The **Save As** dialog appears:



15. Type a **File name**, then click **OK**. To use a method in Chromera it must be saved in C:\ProgramData\PerkinElmer\AxION\Method.

    In this example the name is **DemoMethod.tofmethod2**.

Using Lockmass in Real-Time

These instructions can be used to create LC-TOF methods using Lockmass calibration in real-time. For a complete guide on setting-up on-the-fly lockmass, refer to the last section of this manual.

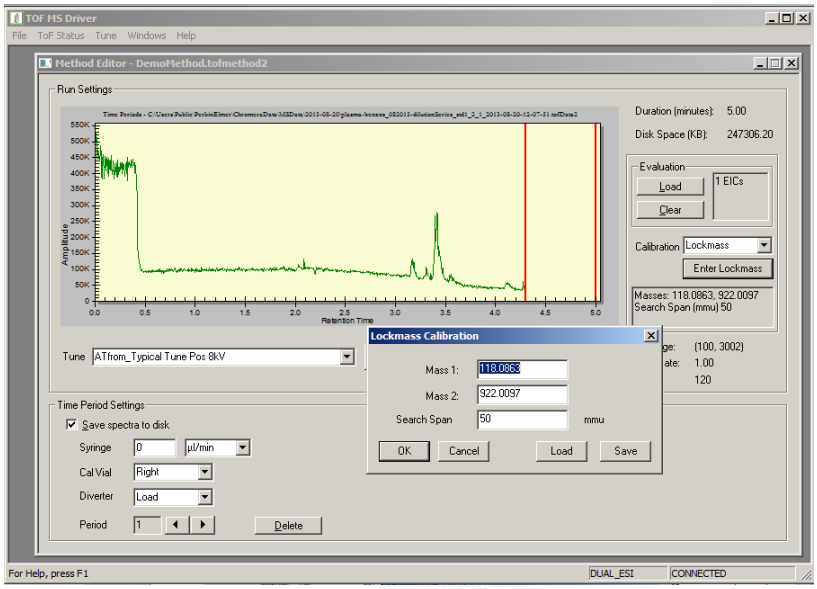
For Lockmass calibration runs, ideally it is better to infuse calibrant continuously in the left probe in the dual ESI source while the other ESI probe will be connected to effluent from the LC. Lockmass

calibration runs may be done with single probe ESI source also, but in this case, both the eluent from LC and the calibrant from a syringe pump must be connected to a tee before connecting it to the ESI sprayer.

The advantange of analytical runs with lockmass calibration done in real-time is that the mass measurement accuracy can be improved to 2 ppm (or sometimes better, depending on the mass measured and the flight tube voltage) even with small drifts in room temperature during the course of the analyses.

1. From the **File** menu on the TOF MS driver window, main menu, select **New Acquisition Method**.

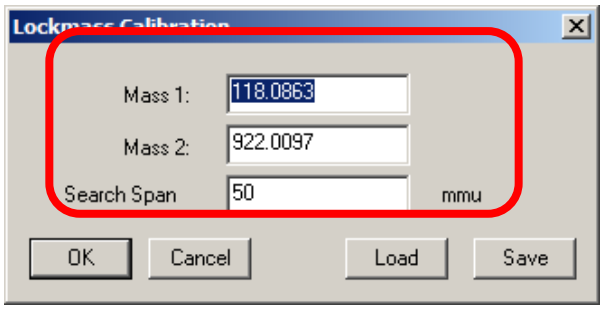
2. Select a TOF Tune with the desired scan range and rate that contains a recent calibration.
   The **Method Editor** window will look similar to the following:



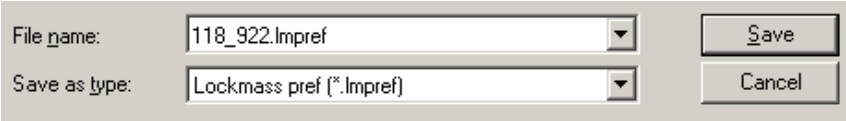
   Choose two **Lockmasses** from your calibration solution, which will typically be infused into the second (left) ESI sprayer of the Ultraspray 2 ion source. Ideally, the masses of the target compound sample should be between the two lock masses. In this case for reserpine (*m/z* 609.2) as the target compound, the two masses for calibrants entered would be 118.08625 and 922.00979 u.

3. In the **Calibration** section of the screen, select **Lockmass** from the drop-down, then click on the **Enter Lockmass** button.

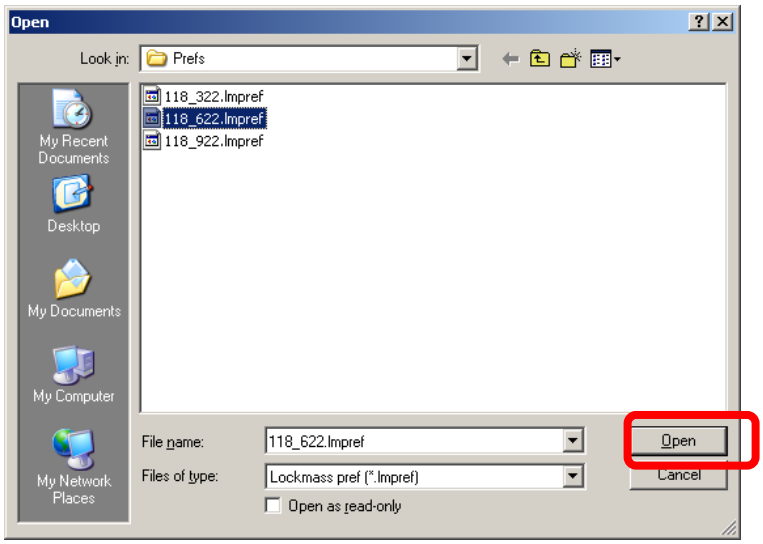
4. Type in the desired mass values in the **Lockmass Calibration** window; these can be saved for future reference by clicking the **Save** button. Selecting **OK** will associate the configuration (saved or not) to the method.

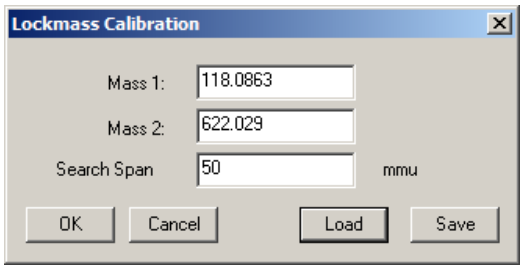


5. Choose a suitable name for the lock mass preferences and click **Save**:

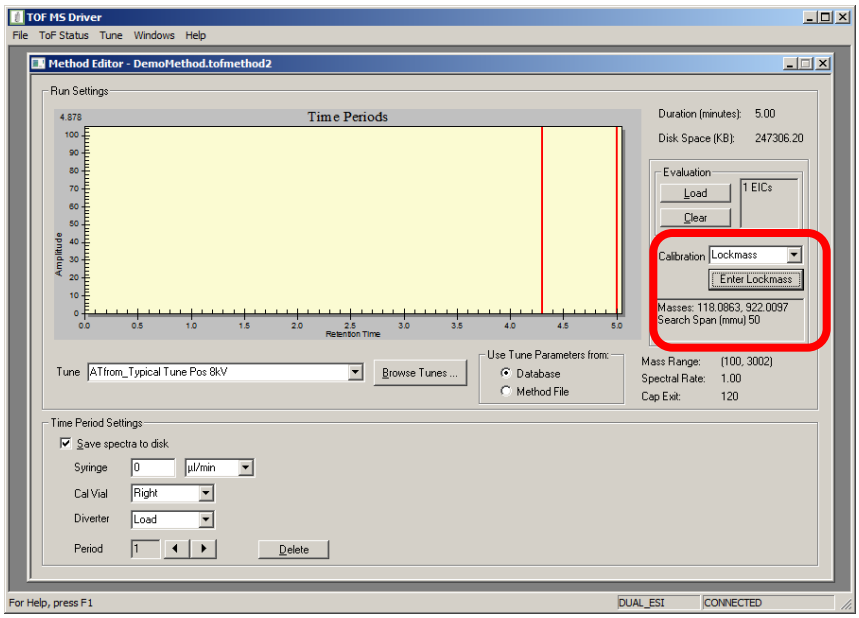


6. Saved lock mass values can be retrieved by clicking on **Load** and selecting the desired lock mass preference file and clicking **Open**:





7. Click **OK** to update the method.
   The screen displays with lock masses as 118.086 and 922.010.



8. Save the above method. This setup can be used for performing on-the-fly lockmass.

NOTE: *If a method is configured to apply lockmass calibration but the system does not find the peaks, the system will revert to using the default calibration in the tune. Batch Convert can be used to apply different lockmass parameters in the case, for example, the wrong mass was originally specified.*

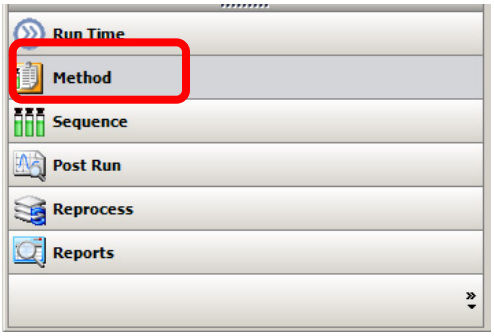
9. Close the **TOF MS Driver** window.

Creating a Chromera Method

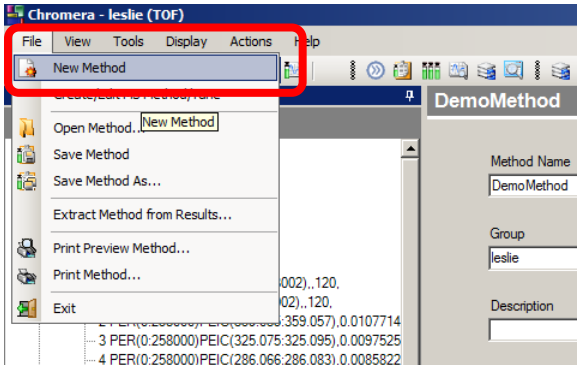
After creating the MS method in the TOF MS Driver application, open Chromera to create a Chromera method. The Chromera method will define all the operating requirements for all the other components in the Chromera configuration.

To create a Chromera method:

1. Click **Method** to open the Method screen.

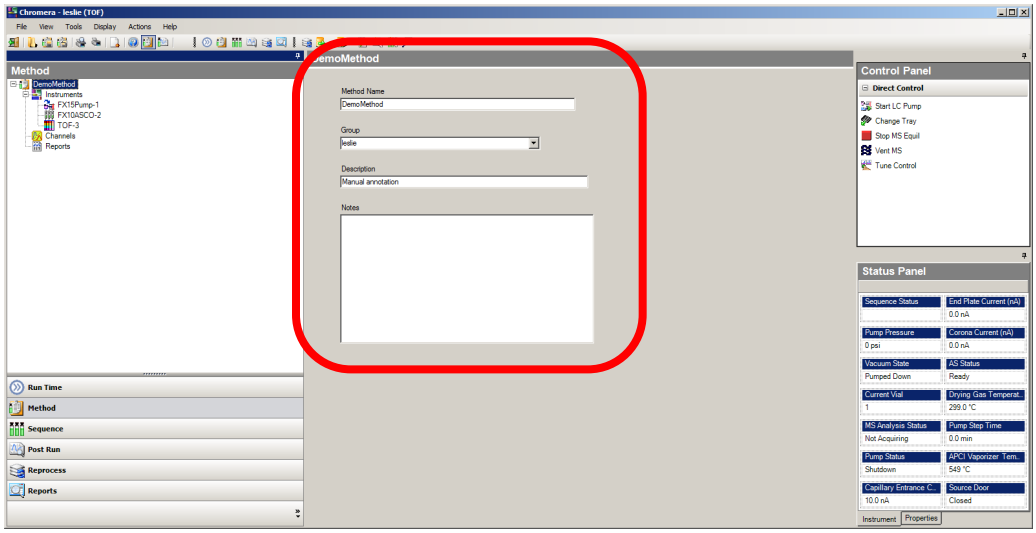


2. Select **New Method** from the **File** menu.



3. Type a **Method Name** and a **Group**.
   Optionally, you can also enter a **Description**.

This example shows a **Method Name** of **TOF Demo** and a **Group** of **TOF Group.**

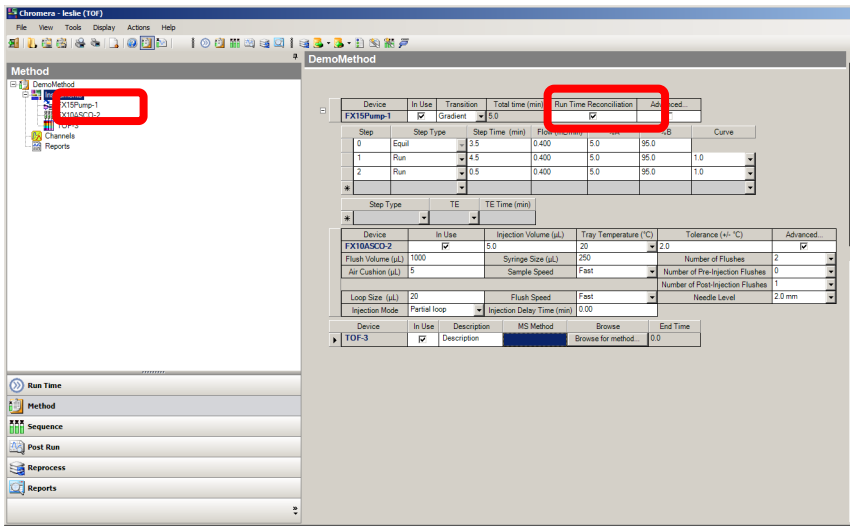


4. Select **Save Method** from the **File** menu.

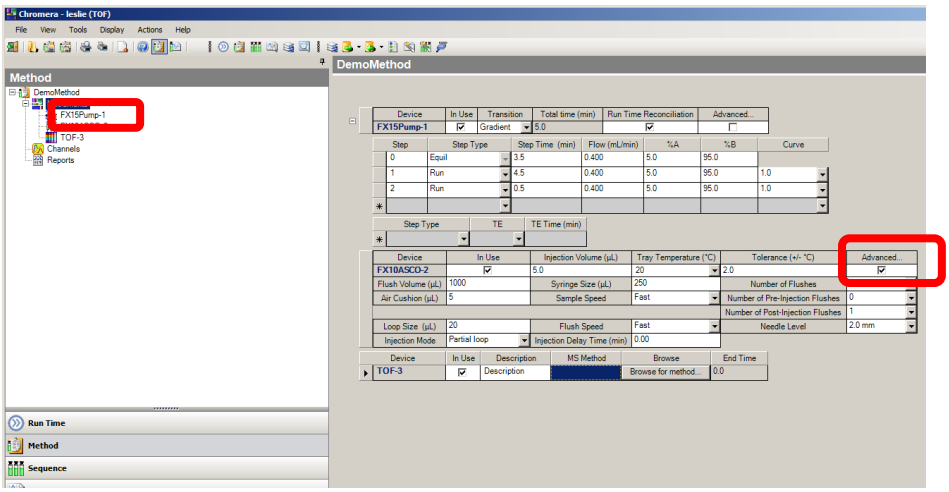
5. Enter your instrument parameters by clicking on each instrument.

   Click on **FX15Pump-1** and enter the pump parameters. Click **Gradient** to specify a step-wise buffer method or **Isocratic** and the **Advanced** checkbox, as appropriate for the chromatographic run conditions. The pump protocol will drive the totally processing time for the method.

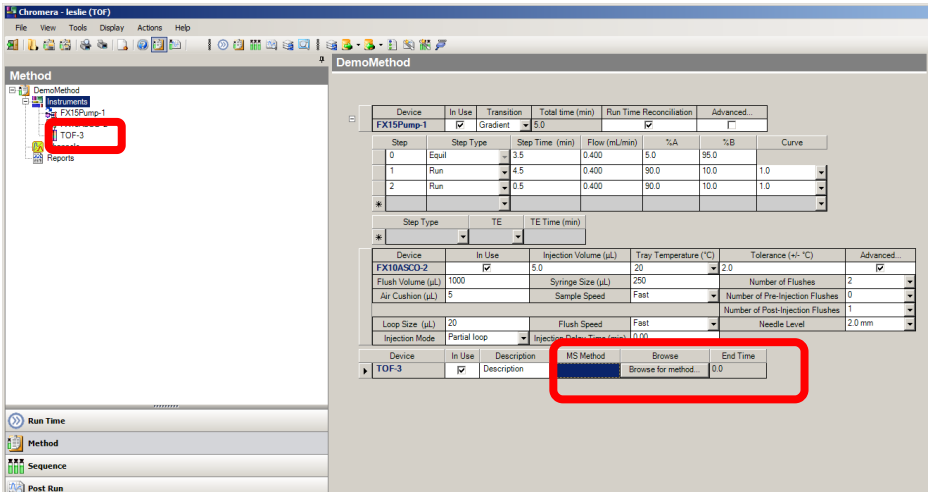
IMPORTANT: *Be sure* ***Run Time Reconciliation*** *is checked.*



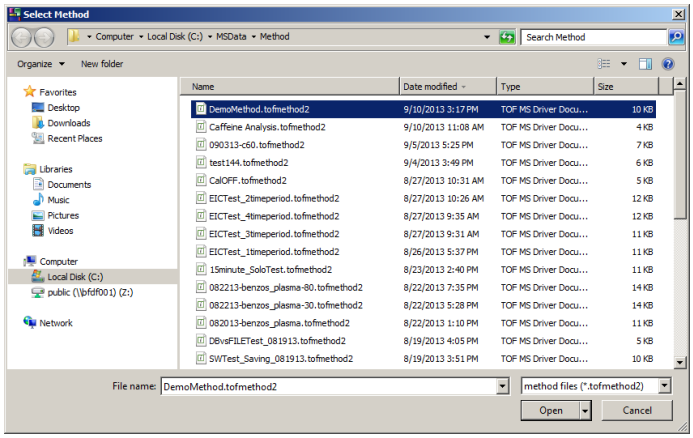
6. Navigate to the autosampler parameters section. Click **Advanced** to show additional autosampler parameters. Update those parameters as necessary to the instrument configuration. Injection volume specified here will be automatically applied when the Sequence of runs is defined.



7. Navigate to the AxION TOF section in order to link the MS Method previously defined in the TOF driver software to the Chromera method being defined.



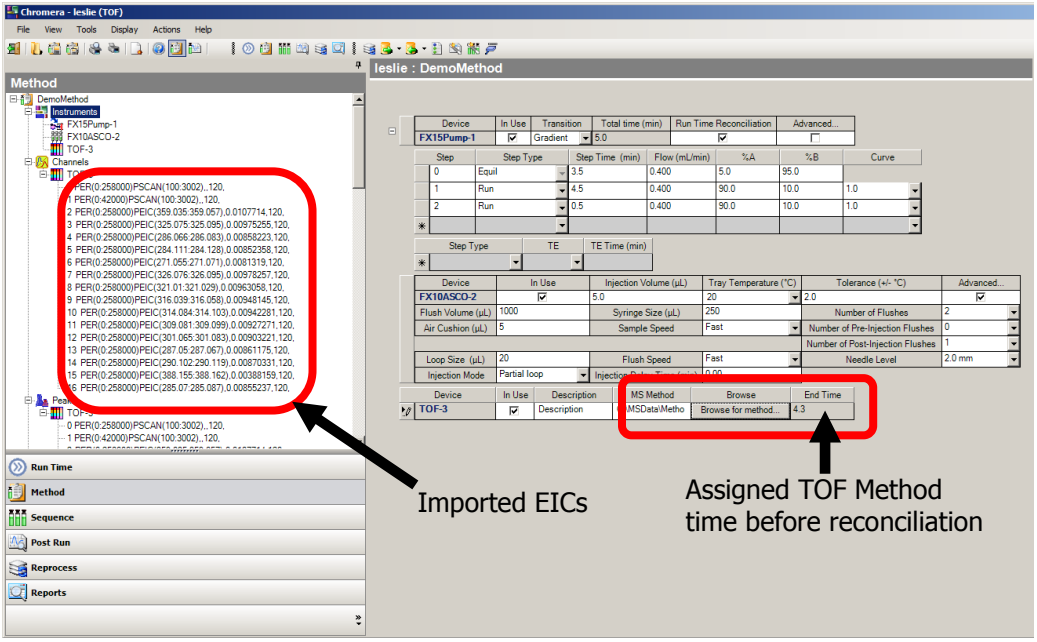
8. Click **Browse for Method**.
   The **Select Method** dialog displays.



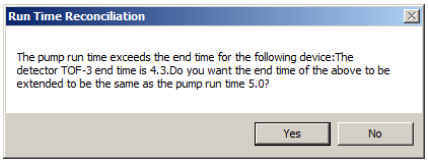
9. Select the method you created in the TOF MS driver in C:\ProgramData\PerkinElmer\AxION\Method, then click **Open**. The system will load the EIC information stored in the TOF method into the Chromera method.

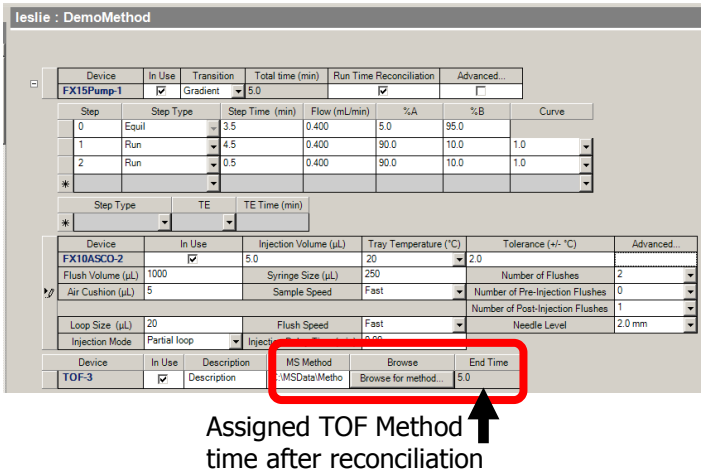
   This example, below, shows the association with **DemoMethod.tofmethod2**

NOTE: *There is a known issue, as of TOF Driver version 6.2, with the display of the total run time of the TOF acquisition method in Chromera. When multiple time periods are configured in the TOF method, the total run time displayed in Chromera only accounts for one time period. To resolve this issue, the run time reconciliation done when the Chromera method is saved will give the user the option to extend the TOF run to the total defined pump run.*



10. Select **Save Method** from the **File** menu. The Run Time Reconciliation will ask if the TOF method run time should be extended. Select the **Yes** button.



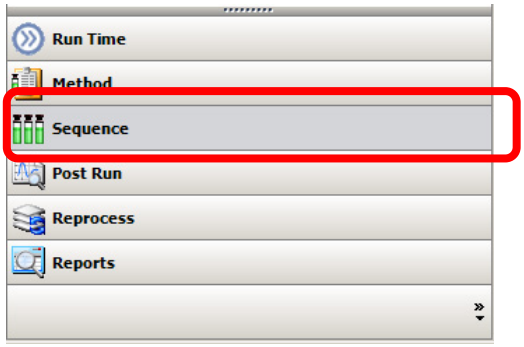


Creating a Chromera Sequence

After creating a Chromera method, create a simple Chromera Sequence to run the method.

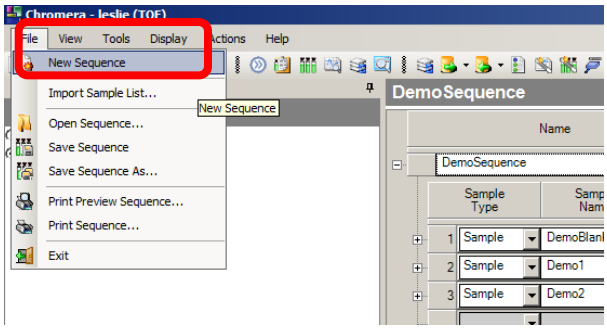
To create a Chromera sequence:

1. Click **Sequence** to open the sequence screen.



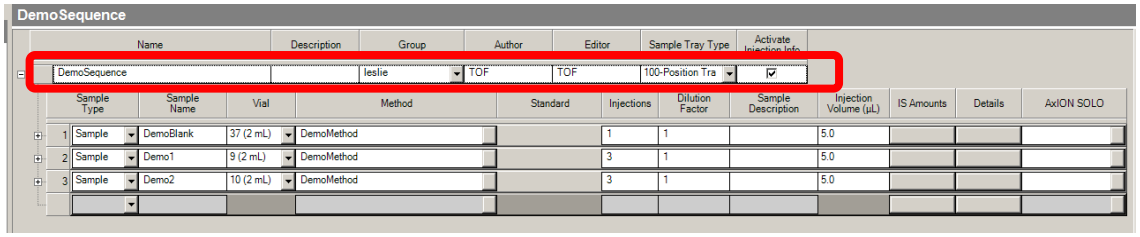
The Sequence screen opens with the last run sequence displayed.

2. Select **New Sequence** from the **File** menu.



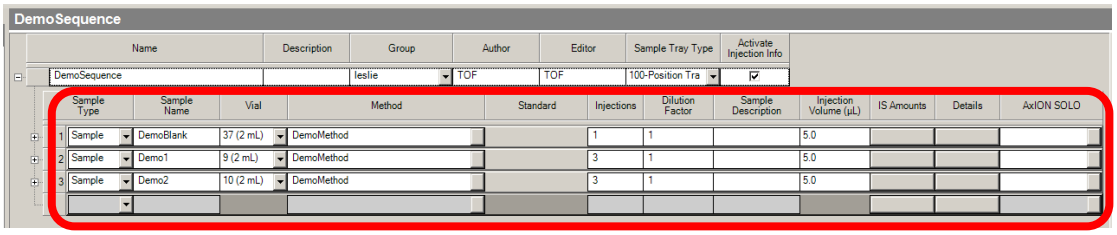
A blank sequence screen displays.

3. Set the sequence identifiers.
   - Click in the **Name** box and type a name for this sequence.
   - Select the **Group** from the drop-down list.
   - Select the **Sample Tray Type** of your autosampler. This example shows **100-Position Tray**.



4. Enter the Sequence Parameters.
   - Select the **Sample Type** from the drop-down list. This example shows **Sample**.
   - Type a **Sample Name**. This example shows **DemoBlank**.

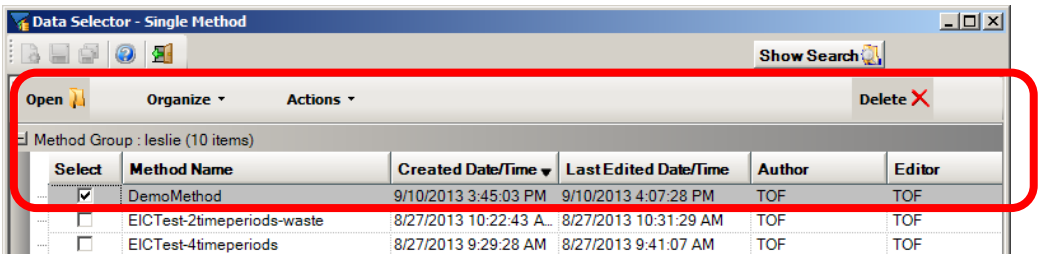
- Type the number of **Injections**. This example shows **1** injection.
- Type the **Injection Volume (µL)**. This example shows **5.0** µL.



5. Select the **Method** for this sequence by clicking the button 

in the **Method** field.

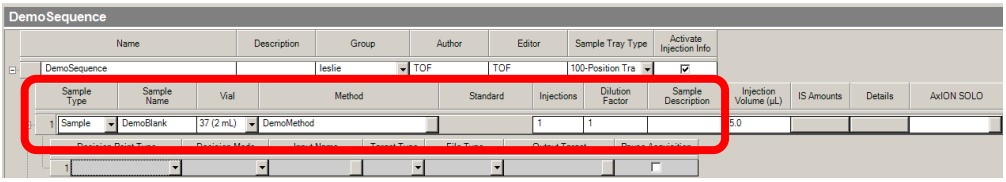
6. The **Data Selector – Single Method** dialog displays. Click the plus sign ⊞ to expand the appropriate **Method Group**



7. Click in the **Select** box to select the method. This example shows **DemoMethod** is selected.

8. Click **Open** to insert this method in the sequence.

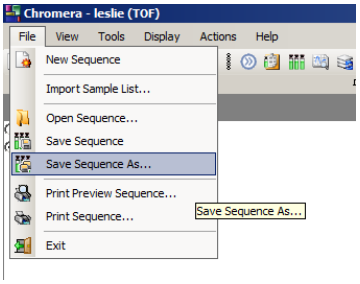
9. Click the plus sign ⊞ to display additional run parameters.



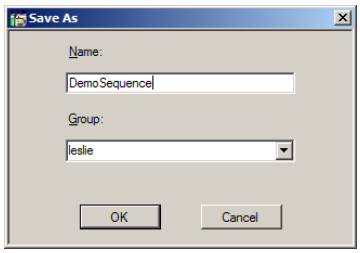
You can enter more parameters or save what you entered to this point.

10. Additional runs added to the Sequence will inherit the values of the run before it.

11. Select **Save Sequence As...** from the **File** menu.



12. If not auto-populated, type a sequence **Name** and select a sequence **Group** from the drop-down list.



13. Click **OK**.

Starting Data Acquisition

Preparing for an Analysis

Prepare the system with mobile phase, a column, and the sample listed below for the example analysis. The analysis conducted for the example shown on the following pages utilizes an isocratic HPLC method.

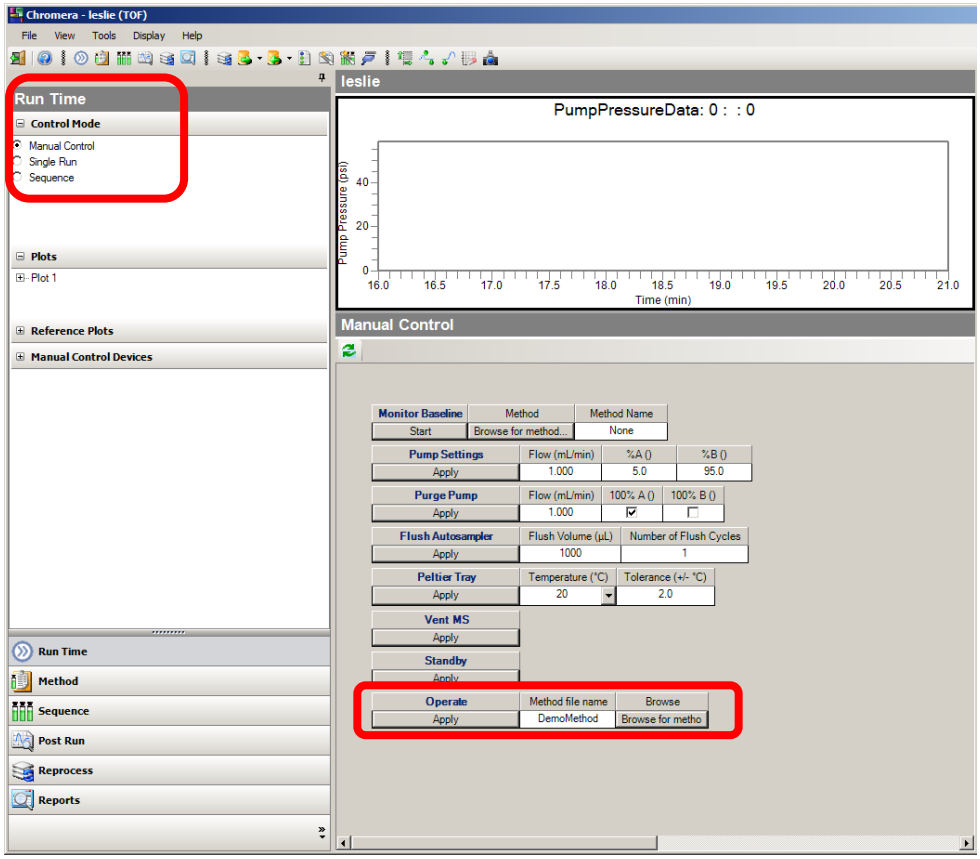
- Mobile Phase: A: Methanol; B: 50/50 Methanol/Water
- Sample: 50pg/µL caffeine
- Column: 3x3 CR C18 column and column holder

Equilibrate the System

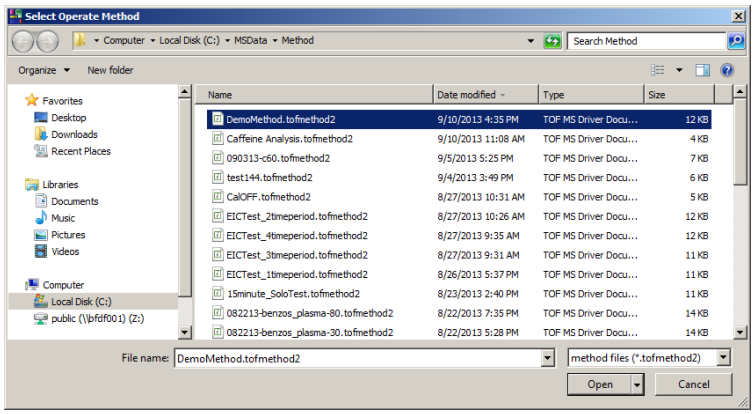
Before running an analysis, the LC system and the TOF must be equilibrated to achieve a stable chromatographic baseline and to properly condition the LC column.

To equilibrate the system:

1. In Chromera click **Run Time** then click **Manual Control** for the **Control Mode**.



2. In the **Operate** row, click **Browse** for a TOF Method.
   The **Select Operate Method** dialog displays.



3. Select the method (in this example **DemoMethod.tofmethod2**) and click **Open**.
   The selected method displays in the **Method file name** field.

4. In the **Operate** row click **Apply**. The settings for the initial time period of the TOF method will be loaded.

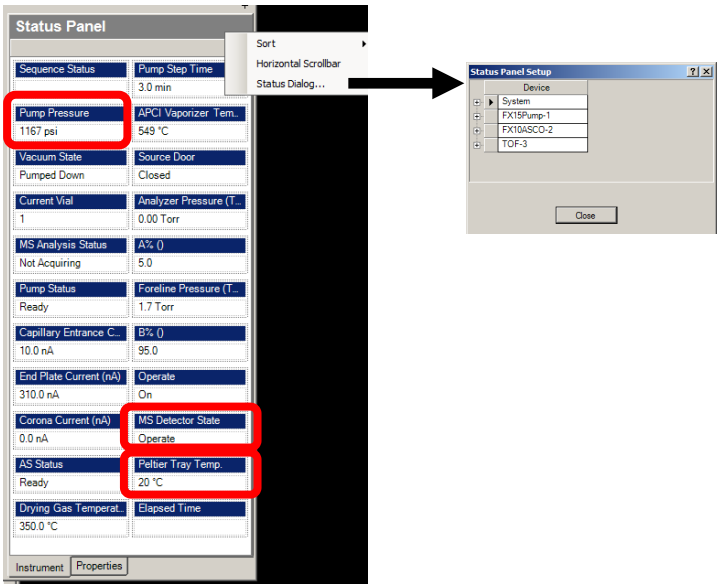
NOTE: *The mass accuracy of a TOF MS (running <u>without</u> lockmass) is very much dependent on the thermal stability (i.e., temperature equilibrium) of the system. If ion polarity has not been switched, the TOF should be allowed to pulse over the desired mass range for at least 30 minutes prior to calibration (the best results are obtained with 60-120 minute equilibration time). After calibration, the analyses to be performed should be run as soon as possible to avoid "cooling off" of the flight tube and electronics. If the samples cannot be run immediately after a calibration, the TOF should be left pulsing to keep it thermally stable. If the analyzer's polarity is switched, the instrument should pulse for 2 hours before running a calibration.*

5. Make sure the chromatographic tubing is connected between the LC system and the AxION 2 TOF MS detector.

6. Enter **Pump Settings** and click **Apply** to start the pump.
   In this example, enter a 1.0 mL Flow, 5% A, and 95% B.

7. Set the autosampler **Peltier Tray** temperature, if applicable. Enter the target temperature and tolerance and click **Apply** to ramp to target.
   In this example, enter 20°C Target with a ± 2.0 °C Tolerance.

8. Monitor the parameters in the **Status Panel**. Right clicking on the Status Panel Header will bring up a menu. Status Dialog can be selected and additional parameters added or removed from the panel.

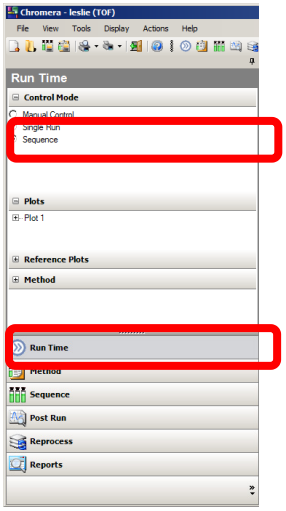


Running a Sequence

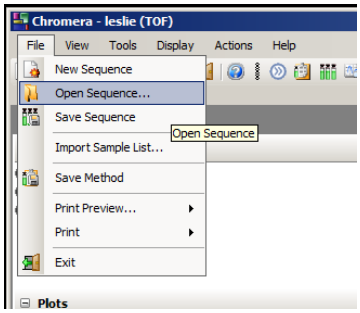
Once the system has reached equilibration, you can load and run the sequence. This example shows a simple sequence.

To run a sequence:

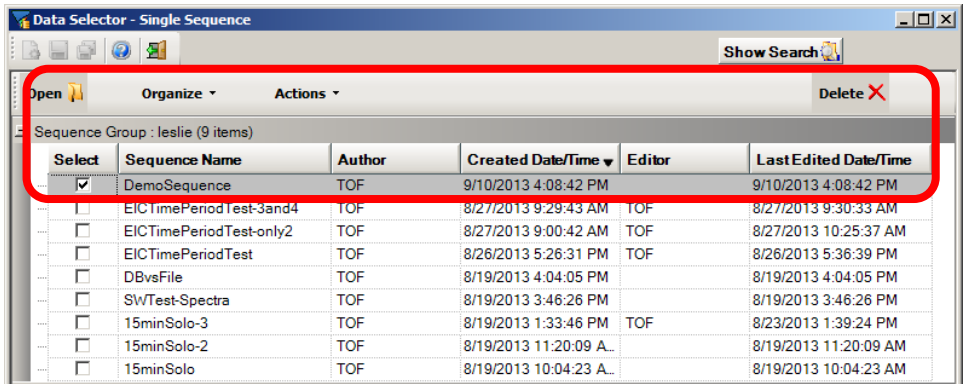
1. Select the **Run Time** Group and the **Sequence** radio button



2. Select **Open Sequence** from the **File** menu.



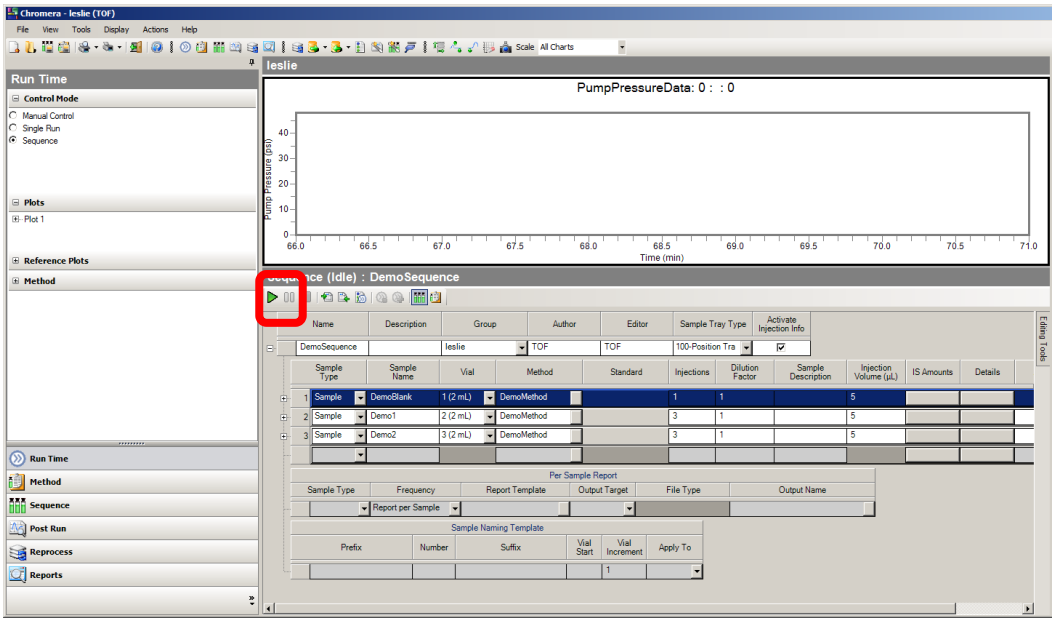
The **Data Selector – Single Sequence** screen displays.



3. Click the plus sign  to expand the appropriate **Sequence Group**. This displays all sequences saved in this group.

4. Click in the **Select** box to select the sequence. This example shows the Sequence named **DemoSequence** is selected. Then click **Open** to open this sequence.

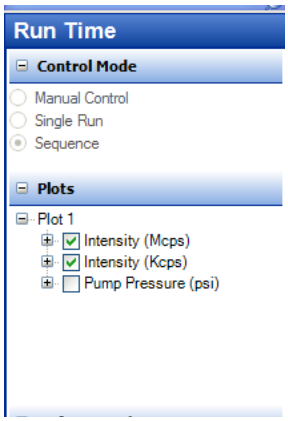
   The sequence displays and is ready to run, indicated by the green **Start** button.



5. Click on the green **Start** ▶ button. The sequence starts to run.

   The running sequence is displayed as a green line. The Total Ion Chromatogram or TIC, which is the sum of intensities for all ions observed in each scan is is displayed as a black line, and the EIC of *m/z* 195.13 is displayed as a blue line.

6. Observe the **Plots** pane on the left side. Click the plus signs to expand the plots.



   When the run completes the display clears. You can review the results in **Post Run**.

Running a Chromera Internal Calibration

An internal standard is a chemical substance that is added in a constant amount to samples, the blank and calibration standards in a chemical analysis. This substance can then be used for calibration by plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentration of the standards. This is done to correct for the loss of analyte during sample preparation or sample inlet. The internal standard is a compound that is very similar, but not identical to the chemical species of interest in the samples, as the effects of sample preparation should, relative to the amount of each species, be the same for the signal from the internal standard as for the signal(s) from the species of interest in the ideal case. This ratio for the samples is then used to obtain their analyte concentrations from a calibration curve. The internal standard used needs to provide a signal that is similar to the analyte signal in most ways but sufficiently different so that the two signals are readily distinguishable by the instrument.

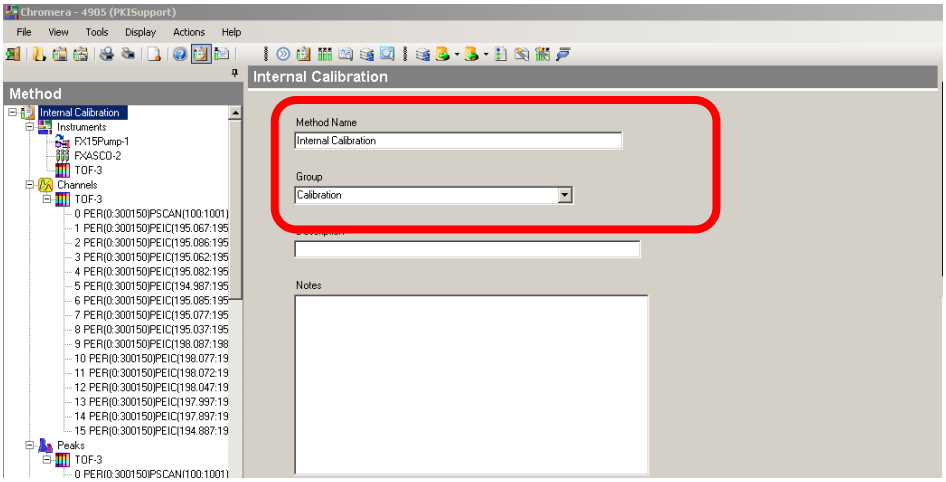
The Calibration View is used for viewing and interpreting calibration curves generated from the measurement of your standard solutions. The Calibration View allows you to evaluate the quality of the calibration by viewing both the graphic plot of the calibration points and by reviewing statistical information on the curve fit. You can also evaluate the effect of eliminating individual calibration points or replicates and changing the origin treatment.

In the calibration graphs the results from each replicate injection is plotted and not the average. This also means that the ability to exclude a calibration point will mean exclusion of a single replicate and not an entire level. You are able to select whether each replicate is included in the calibration curve using the **In Use** checkbox displayed for each replicate.

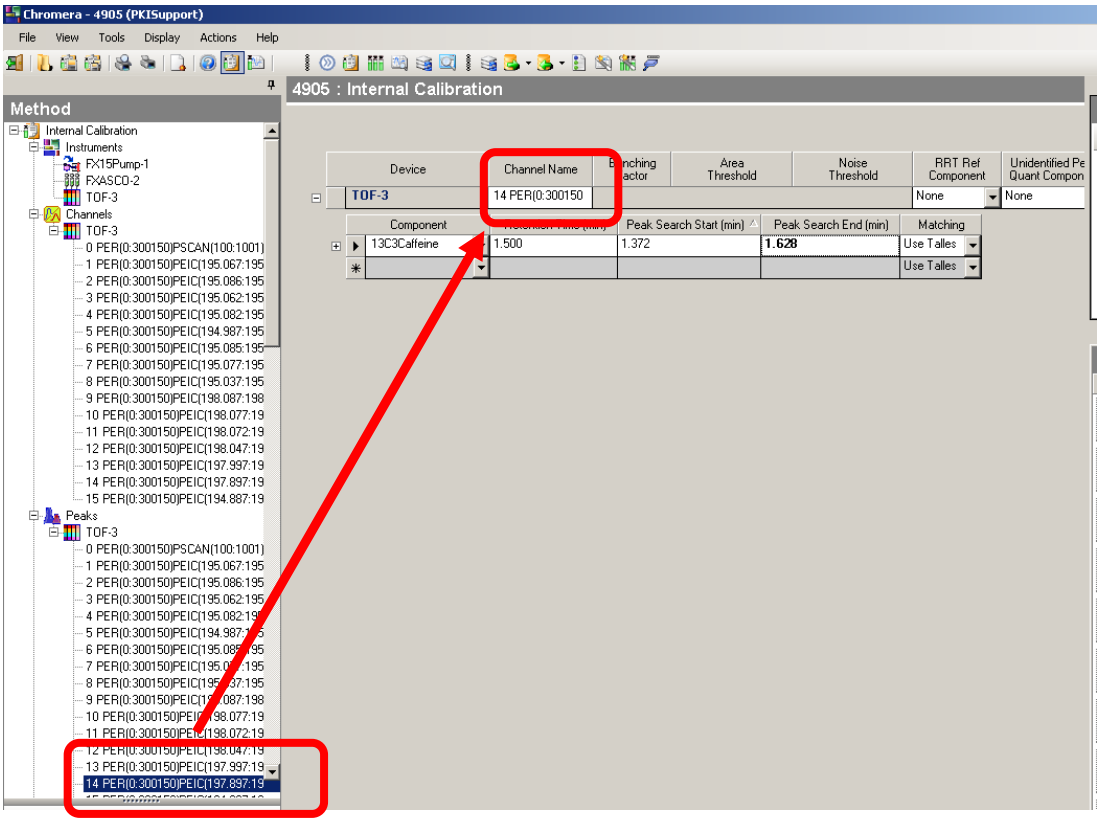
The Calibration section contains three tabs (Summary, Detail, and Setup Standards). The **Summary** tab shows thumbnails displays of the curves for all components/species; for all detector devices or a single device, depending on the method tree selection. The **Detail** tab shows details of the calibration for a selected component. Double-clicking on a curve on the Summary tab will cause the Detail tab to be displayed with that component selected. The **Set Up Standards** tab provides an easy approach to creating standards and entering standard amounts for all components.

The following example shows how to create and run an internal calibration in Chromera:

1. Start by creating a Chromera method.
   In this example, the method is named **Internal Standard** and the Group is named **Calibration**.

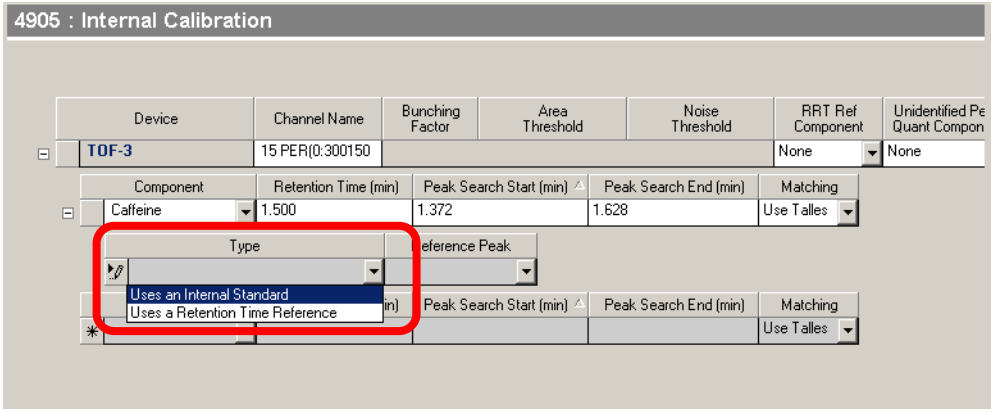


2. In the **Peaks** tree select a channel for your internal standard.
   In this example, we selected the channel **14 PER(0:300150)PEIC(197:897...** and appears in the **Channel Name** field.



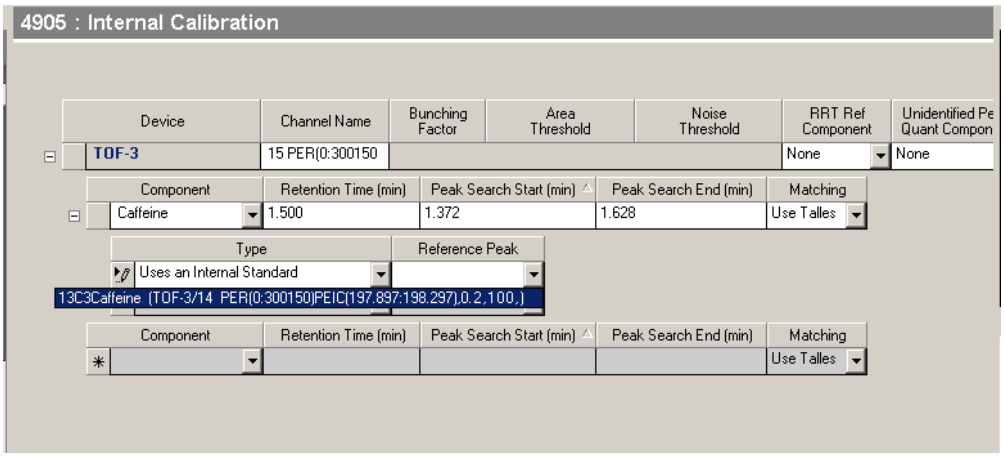
3. Type a **Component** name.
   In this example, **13C3Caffeine**.

4. Click the plus sign to the left of Caffeine to display additional fields.
   The **Type** and **Reference Peak** fields display.

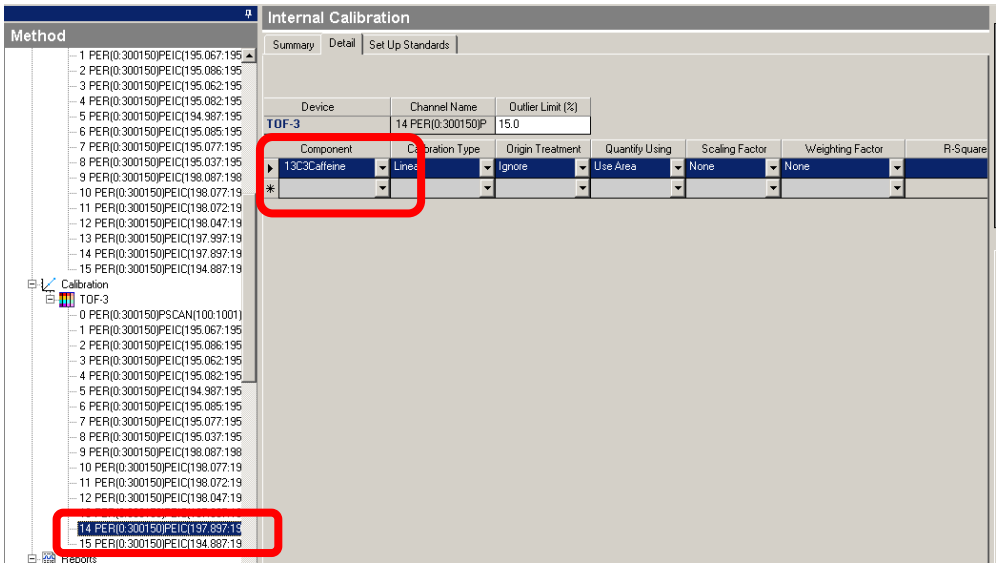


5. Select **Uses an Internal Standard** from the **Type** drop-down list.

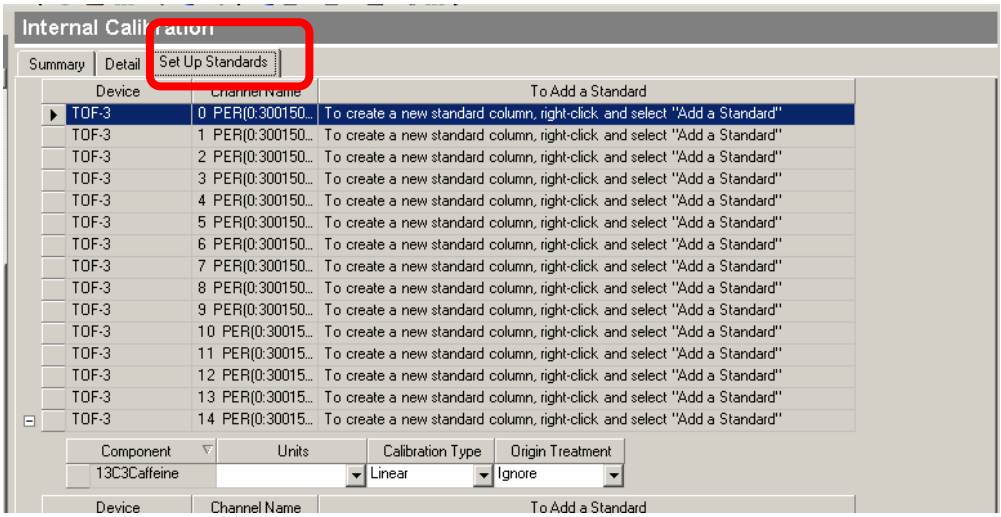
6. Select a reference peak from the Reference Peak drop-down list.
   In this example, **13CECaffeine (TOF-3/14...)** was selected.



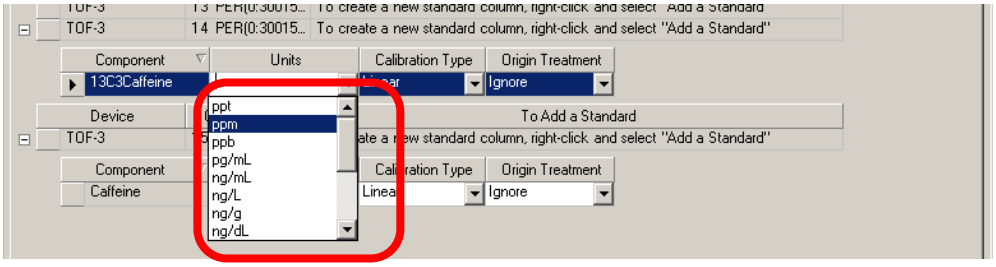
7. In the **Calibration** tree select a channel for your calibration data.
   In this example, we selected the channel **14 PER(0:300150)PEIC(197:897…**



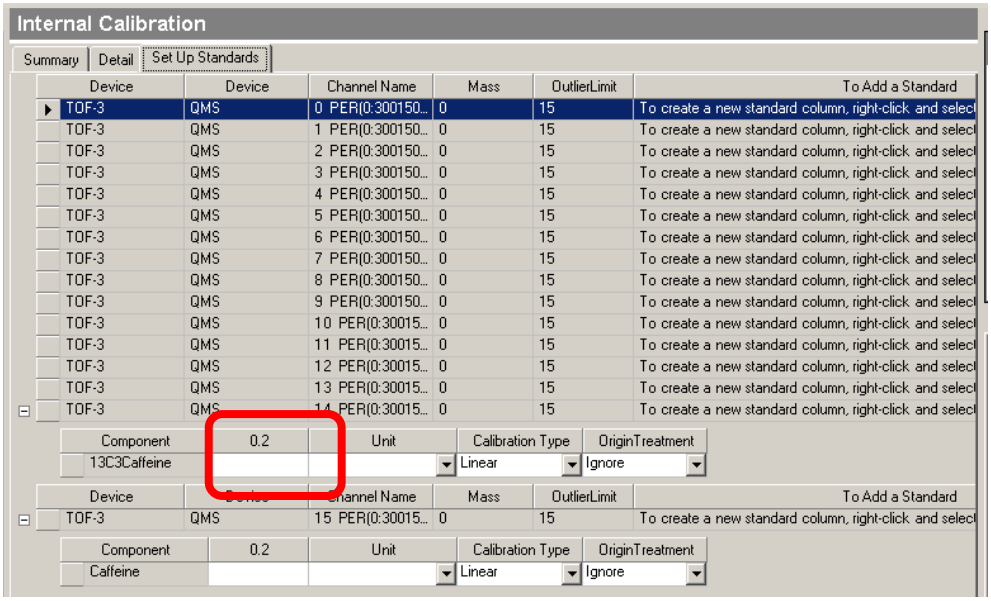
8. Click on **Set Up Standards** tab.



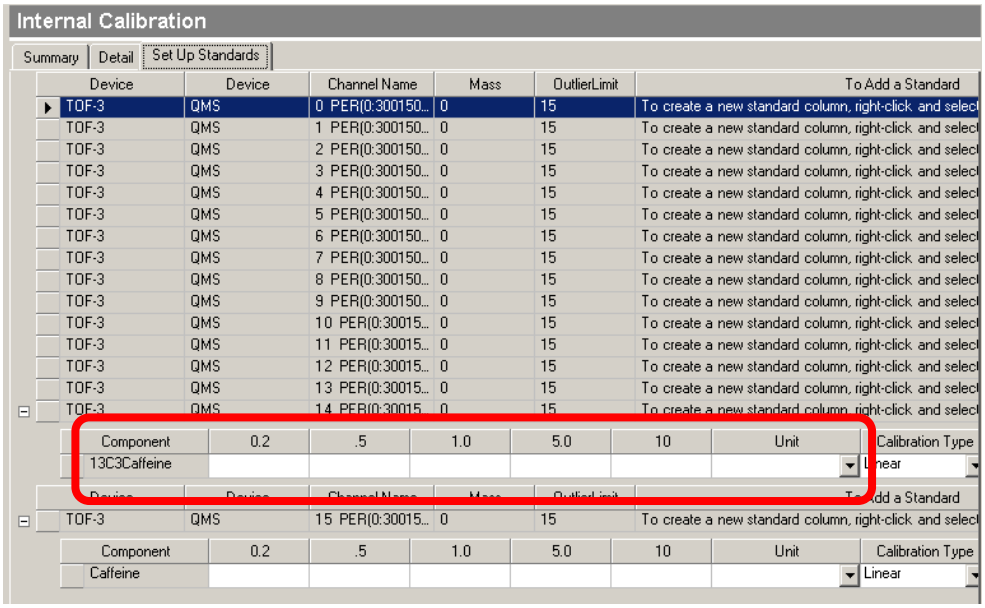
9. Click in the **Units** field and select **ppm** from the drop-down list.



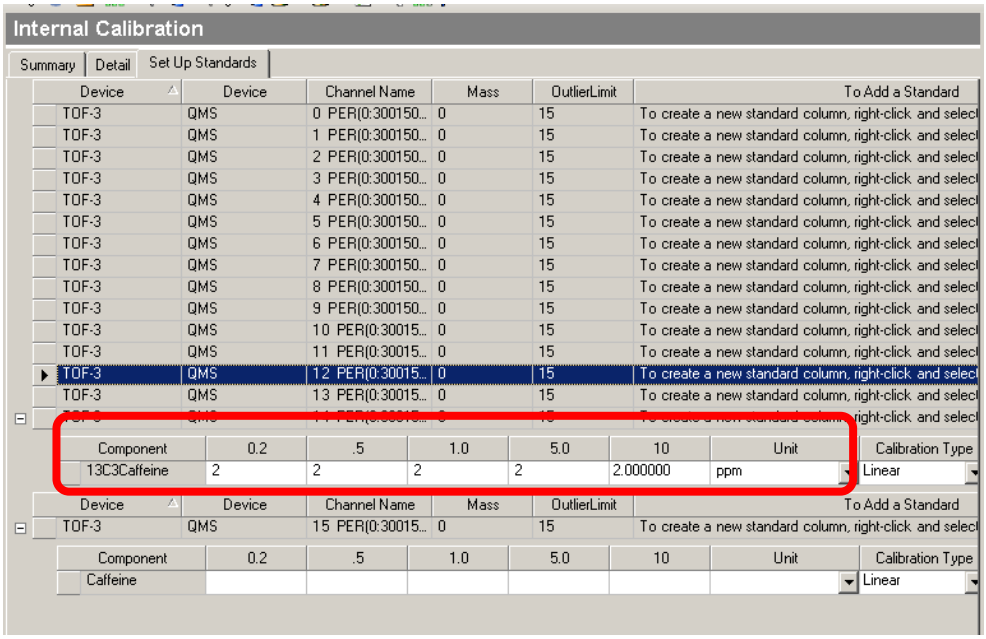
10. Add a new standard column by right clicking and selecting **Add a Standard**.



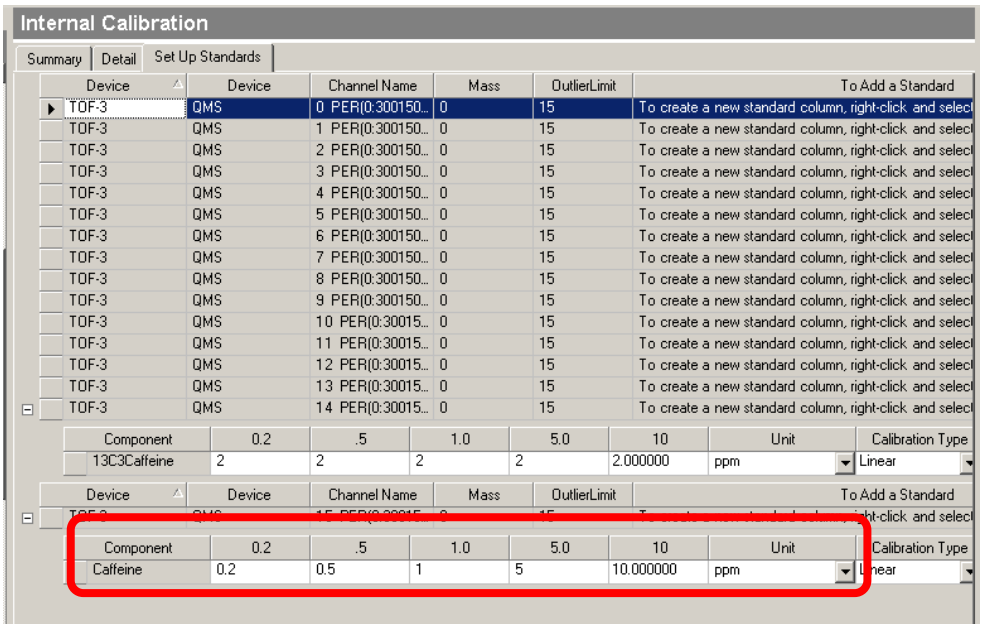
11. Type a standard name.
    In this example, **0.2**. Do this for each of the standards (0.5, 1.0, 5.0., and 10).



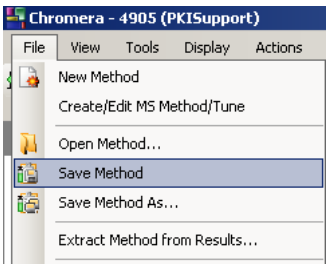
12. Enter your internal standard of 2 in each field.



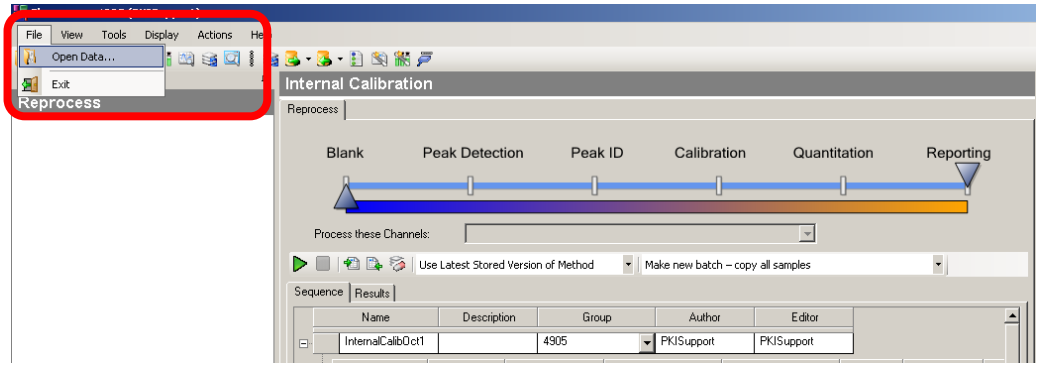
13. Now for the Caffeine component enter 0.5, 1.0, 5.0., and 10 into each column.



14. Select **Save Method** from the **File** menu.

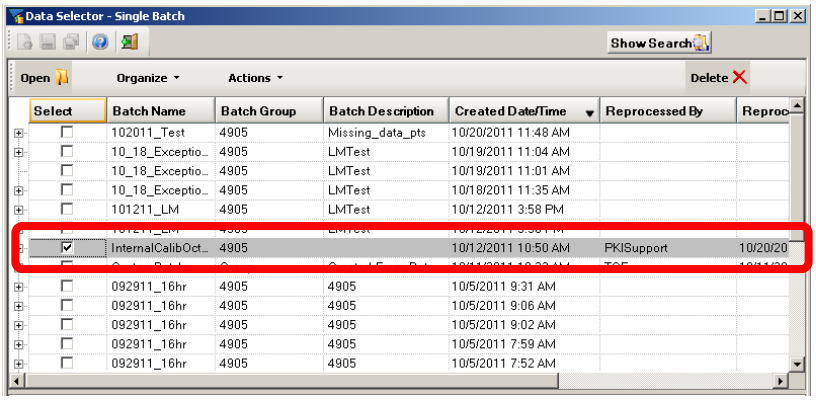


15. Click on the **Reprocess** button in the lower-left pane; then select **Open Data** from the **File** menu.

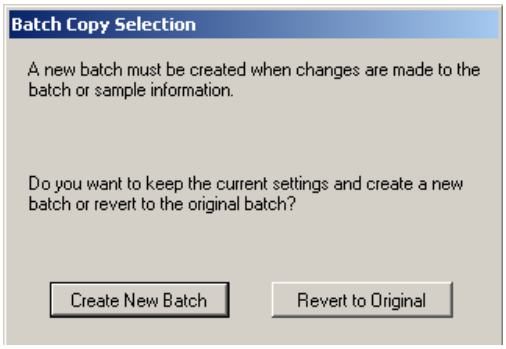


The **Data Selector** displays.

16. Select the batch that you want to analyze.
In this example we selected **Internal Calibration**.

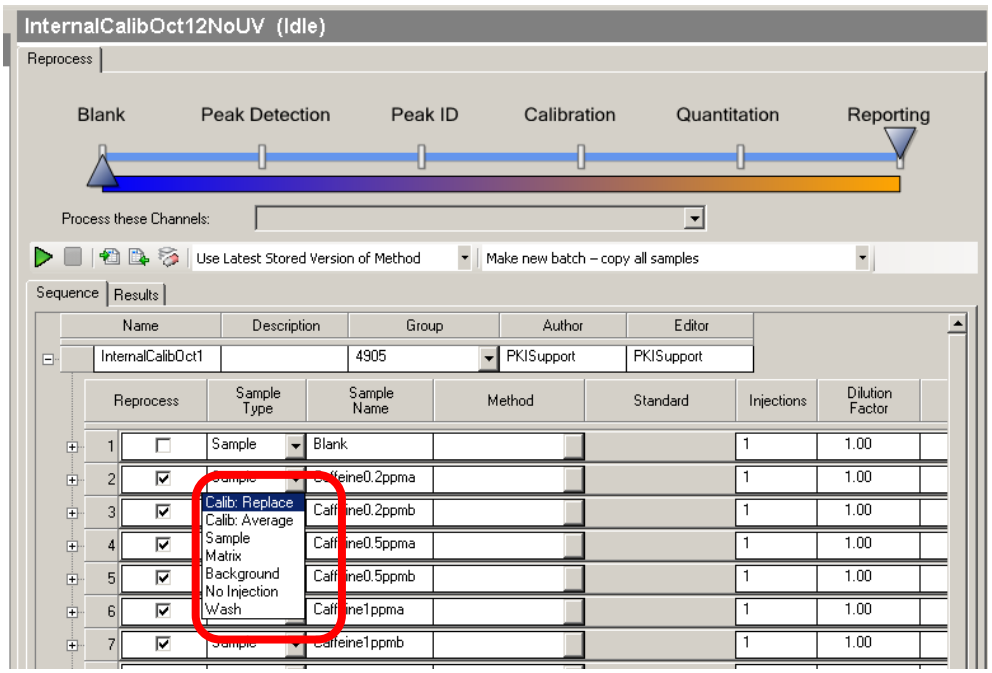


17. Click the **Open** button.
The **Batch Copy Selection** box appears requesting if you want to create a new batch.

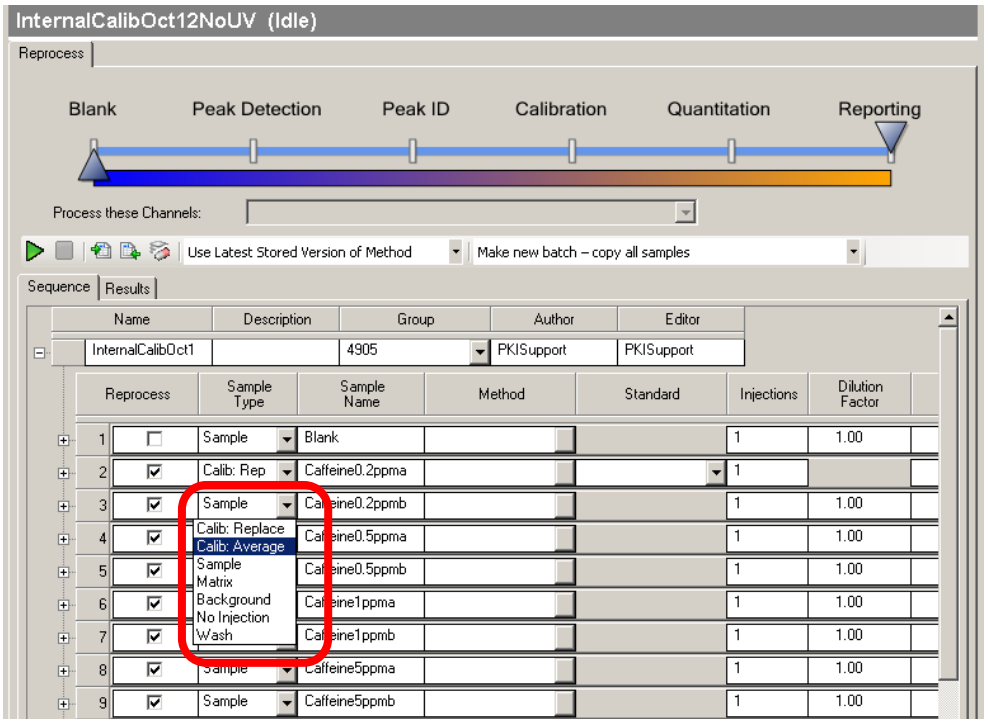


18. In this example we clicked **Create a New Batch**.
The new batch displays.

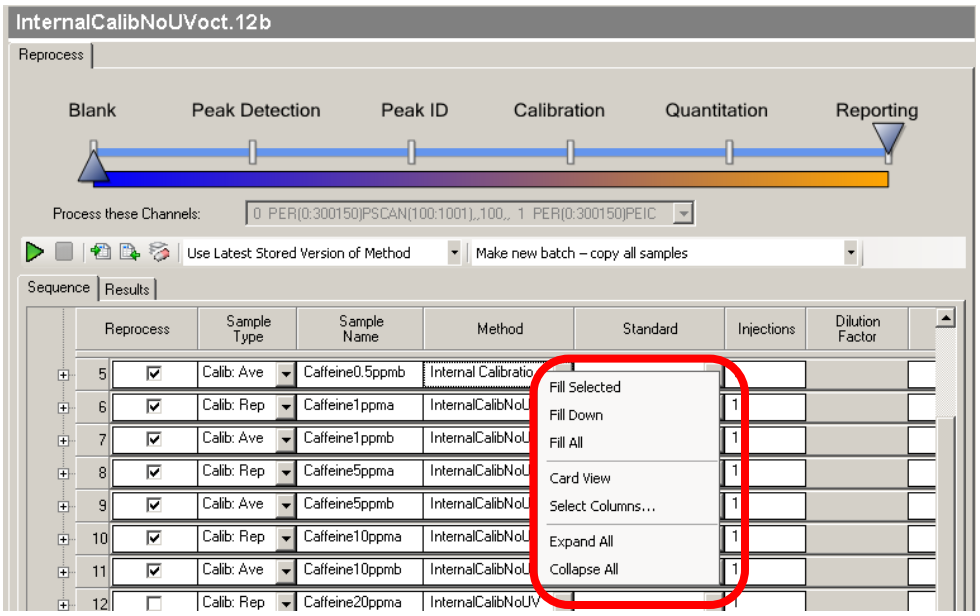
19. Click in **Sample Type** field and select **Calib. Replace**.
You are replacing to reset your calibration curve.



20. Select **Calib. Ave**.
    One run is a replace and the other run is an average.

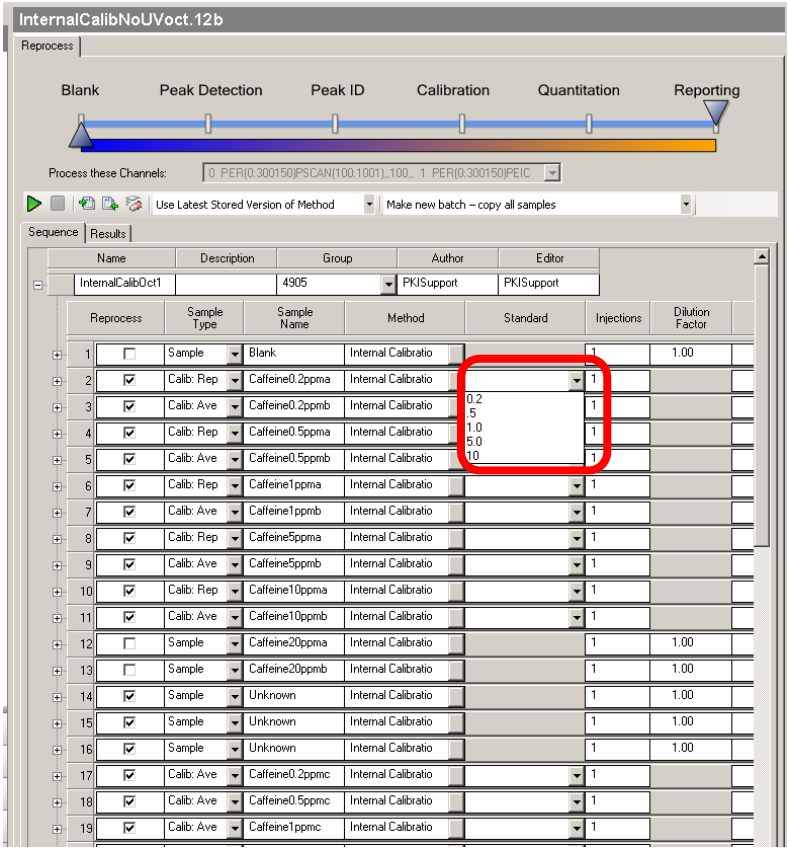


21. Click in the **Method** column, then right-click and select **Fill Down**.

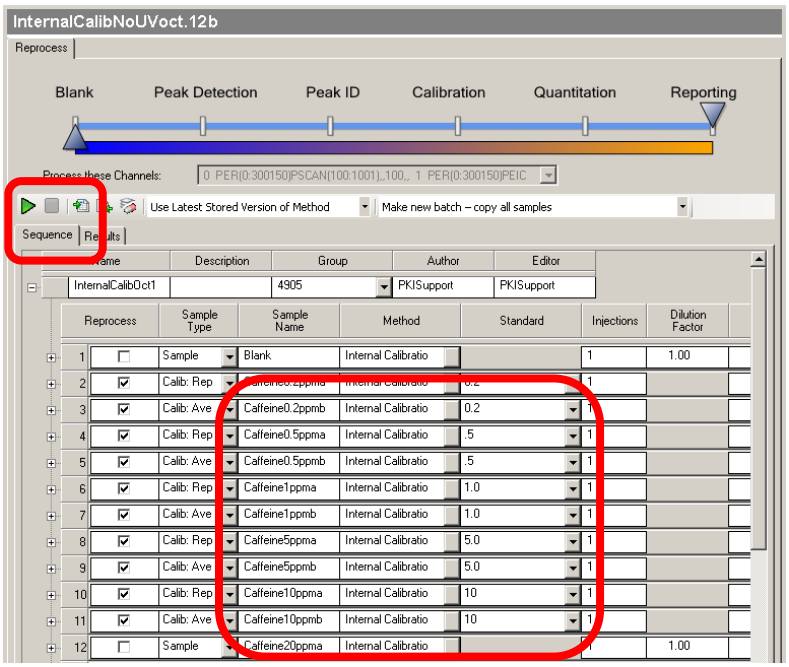


22. For the **Caffeine0.2ppma** sample, click in the **Standards** column, click on the drop-down, and select **0.2** from the list.

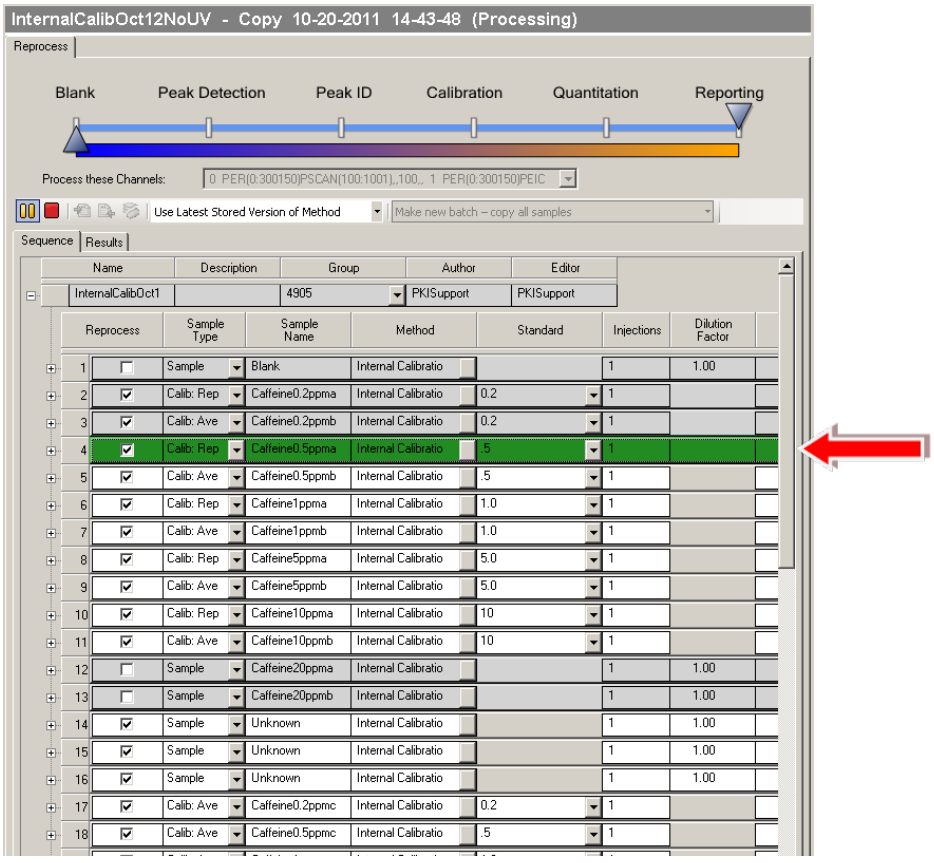
    This drop-down contains a list of the standard you defined earlier in the Set Up Standards screen.



23. Repeat this process by selecting the standard that corresponds to each sample name.

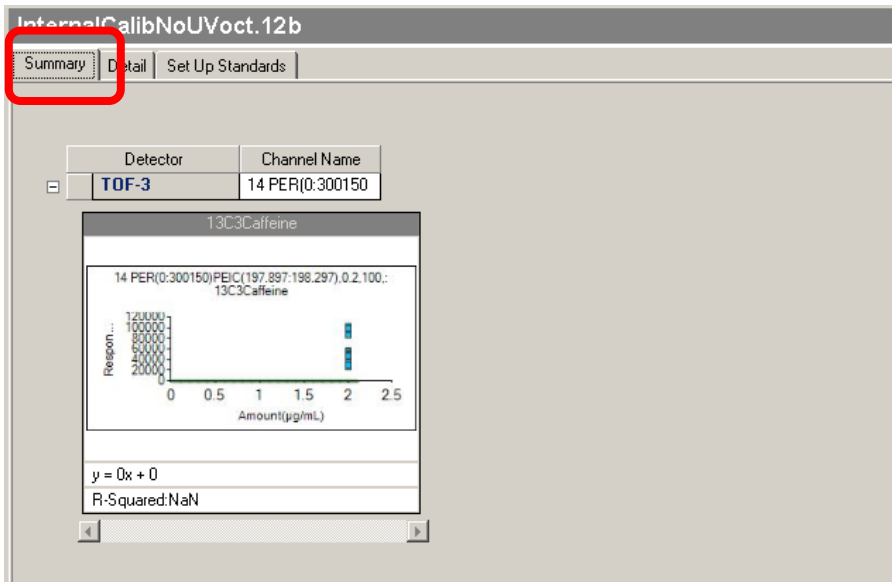


24. Click on the green **Start** ▶ button to start reprocessing.

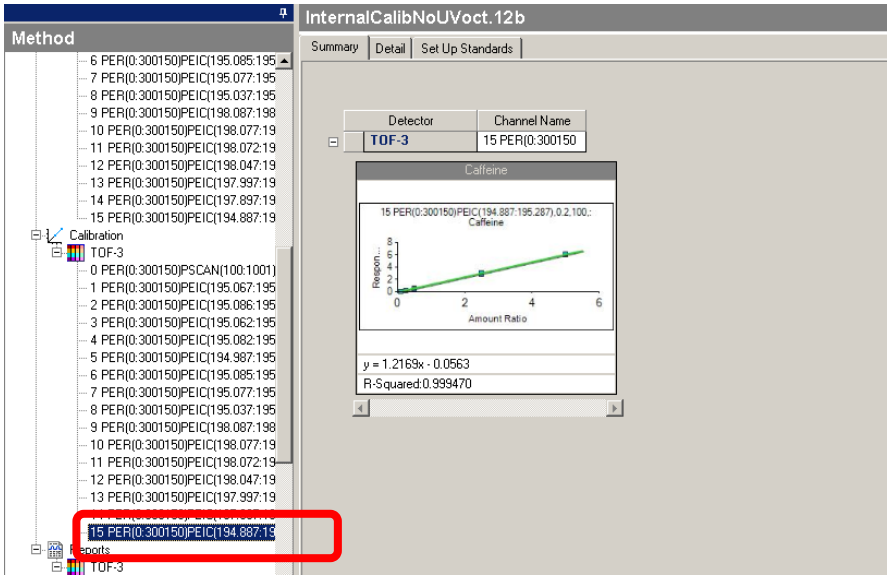


As reprocess runs the row being reprocessed is shown in green.

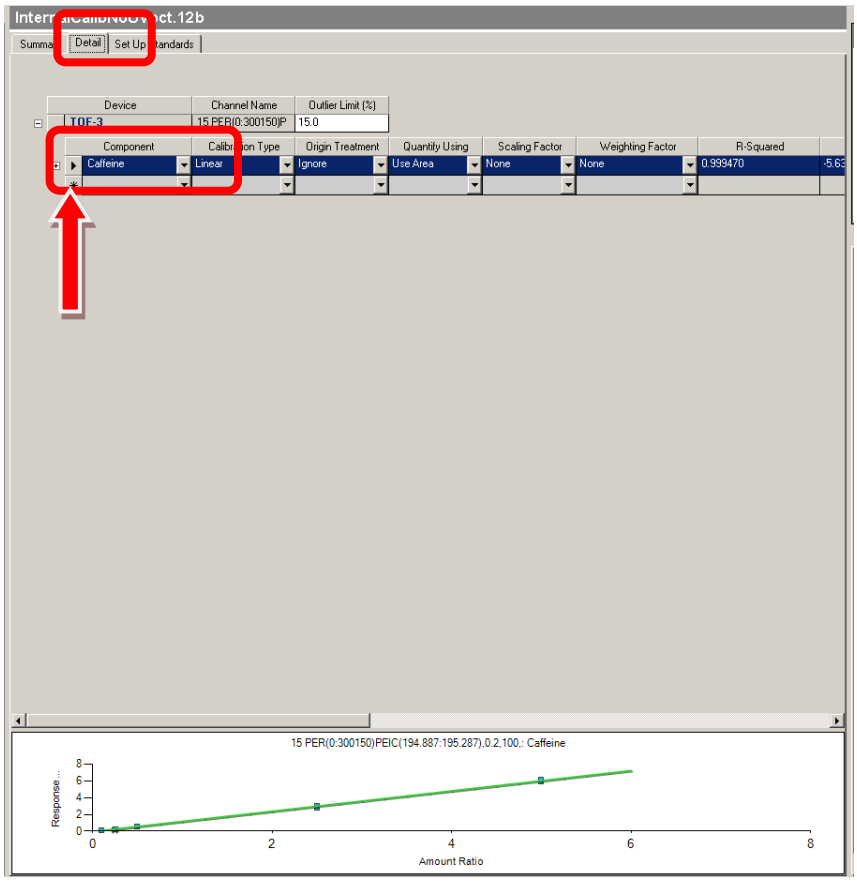
25. When complete, click the **Method** button in the navigation pane then click the **Summary** tab.



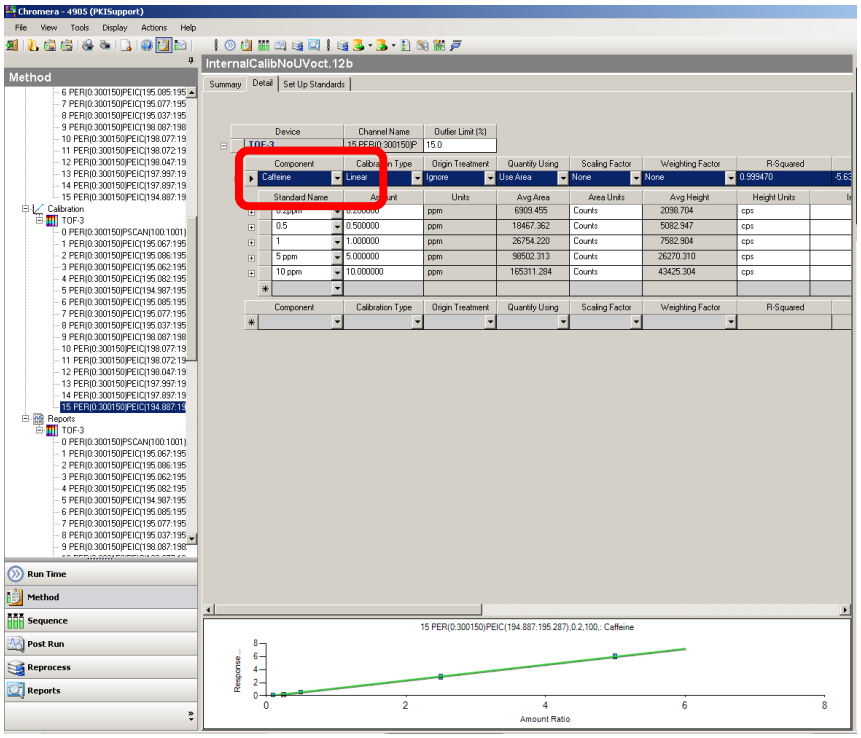
26. In the **Calibration** tree, select **15 PER(0:300150)PEIC(194:887...**



27. Click the **Detail** tab to display the calibration curve on the bottom of the screen.



28. For more detail click the plus sign to the left of the **Component Caffeine**.



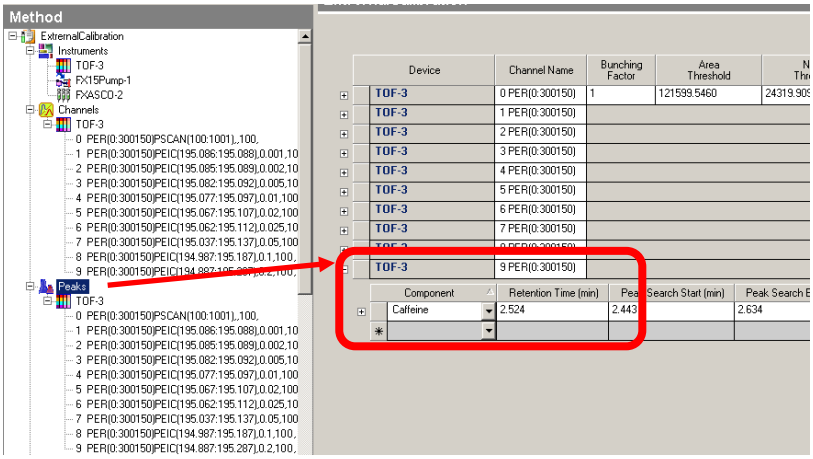
Running a Chromera External Calibration

The Calibration View is used for viewing and interpreting calibration curves generated from the measurement of your standard solutions. The Calibration View allows you to evaluate the quality of the calibration by viewing both the graphic plot of the calibration points and by reviewing statistical information on the curve fit. You can also evaluate the effect of eliminating individual calibration points or replicates and changing the origin treatment.

The following example shows how to create and run an external calibration in Chromera:

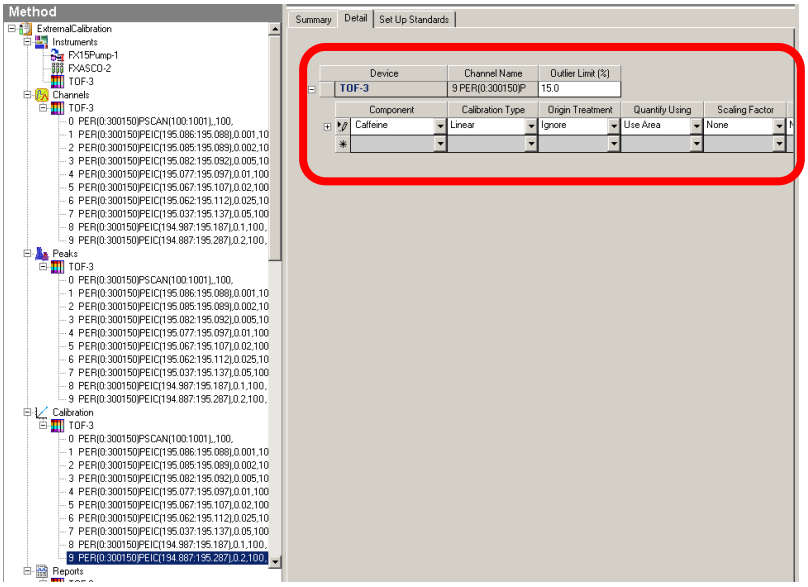
1. Start by creating a Chromera method.
   In this example, the method is named **External Calibration** and the Group is named **Calibration**.

2. Click on **Peaks** and select a channel to define your **Component** and **Retention Time**.

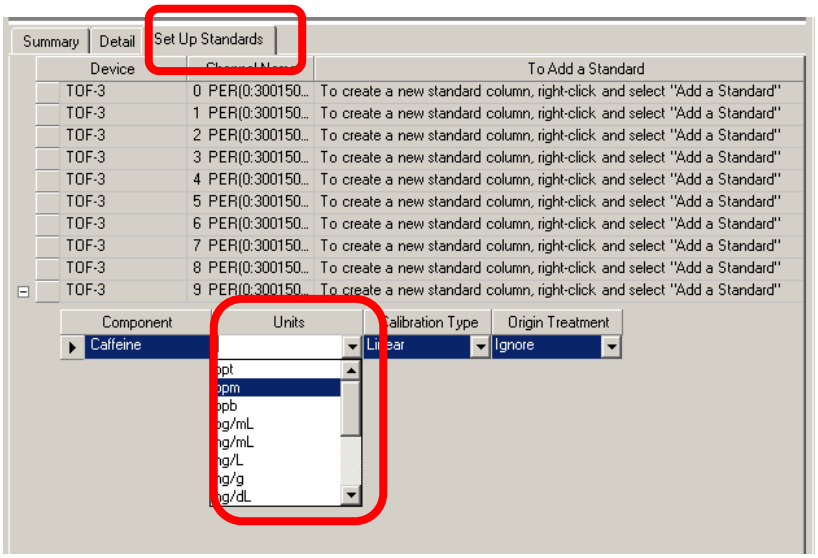


   In this example we defined the component as **Caffeine** and a retention time of **2.524** min.

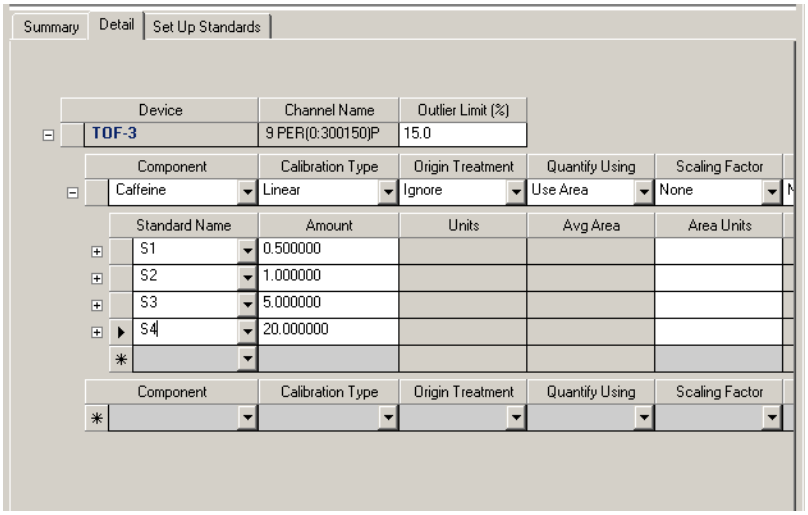
3. In the **Calibration** tree, select a channel, then click the **Detail** tab.
   In this example we selected, **9 PER(0:300150)PEIC(194:887:195:287),0.2,100...**



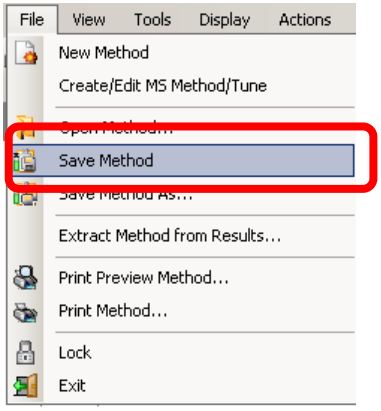
4. Click on **Set Up Standards** tab then click in the **Units** field, and select **ppm** from the drop-down list.



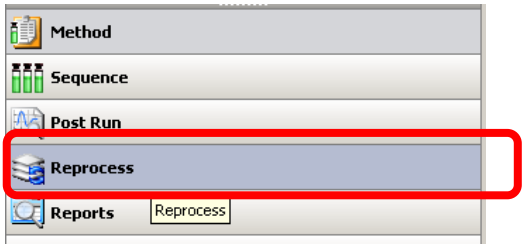
5. Click the **Detail** tab and add the **Standard Names** corresponding to the **Amount**.
In this example we added **S1** (0.500000), **S2** (1.000000), **S3** (5.000000), and **S4** (20.000000)



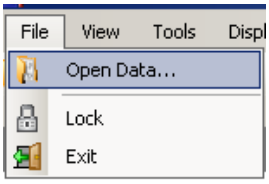
6. Save the method by selecting **Save Method** from the **File** menu.



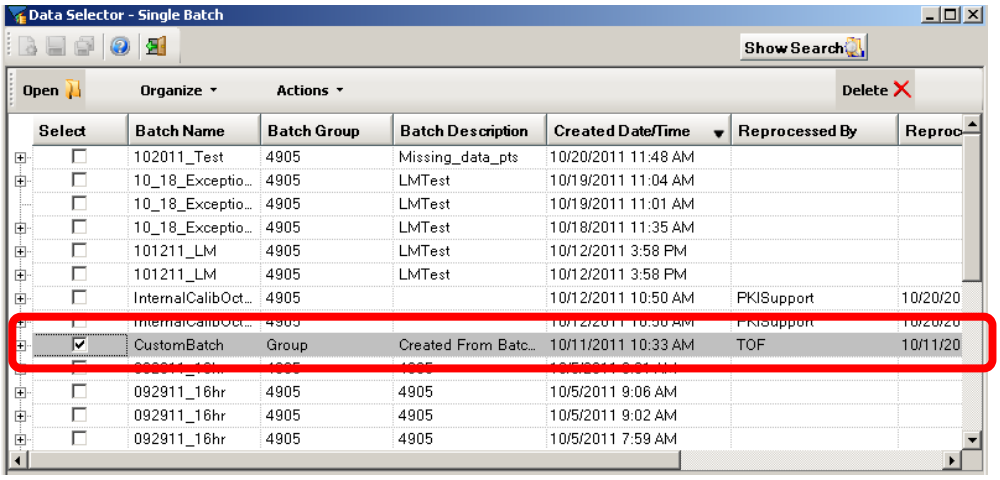
7. Now that your method is created and saved, we will reprocess the data using this method. Click the **Reprocess** button in the lower-left pane.



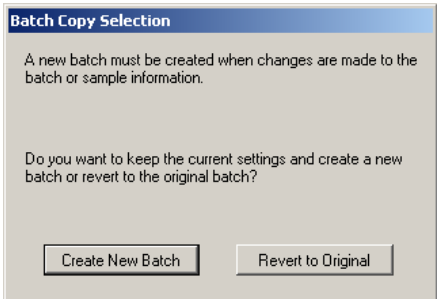
8. Select **Open Data** from the **File** menu.



The **Data Selector** displays.

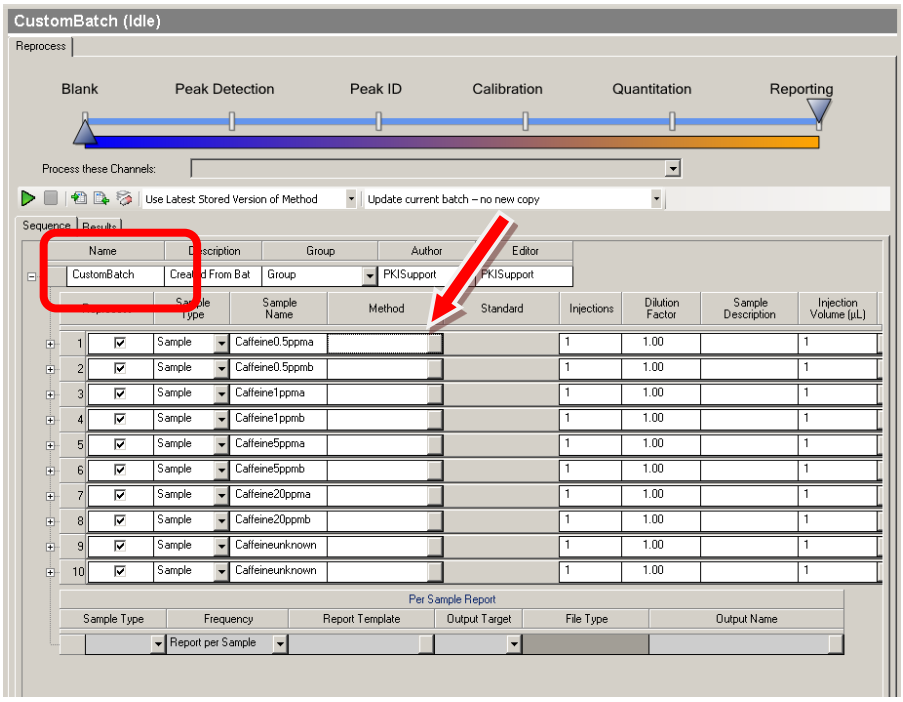


9. Select you data from the list and click **Open**.
   In this example we selected **CustomBatch.**
   The **Batch Copy Selection** box appears requesting if you want to create a new batch.

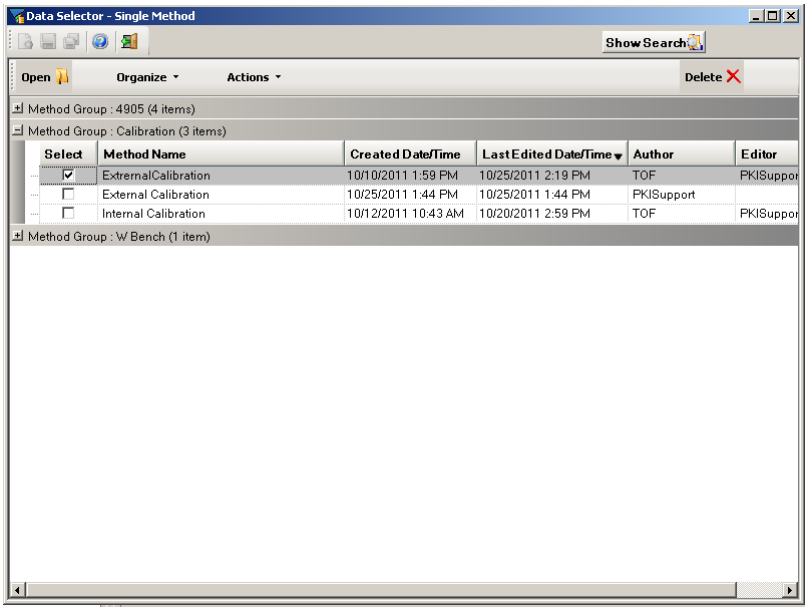


10. In this example we clicked **Create a New Batch**.
    The new batch displays. In this example it is named **CustomBatch**.

11. Click in the **Method** field.

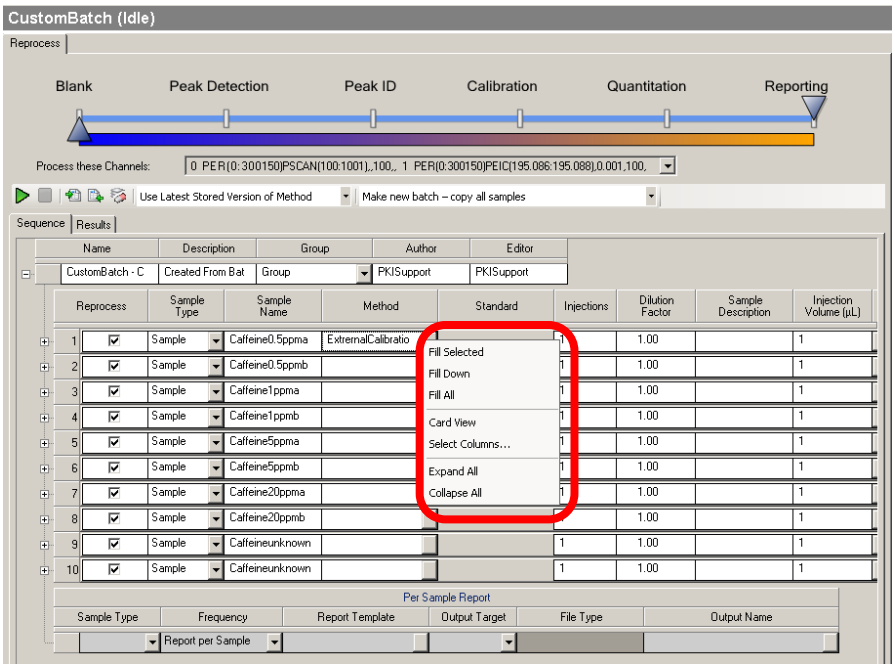


The **Data Selector** opens.



12. Select the method from the list displayed in the Method Group.
    In this example it is **External Calibration**.

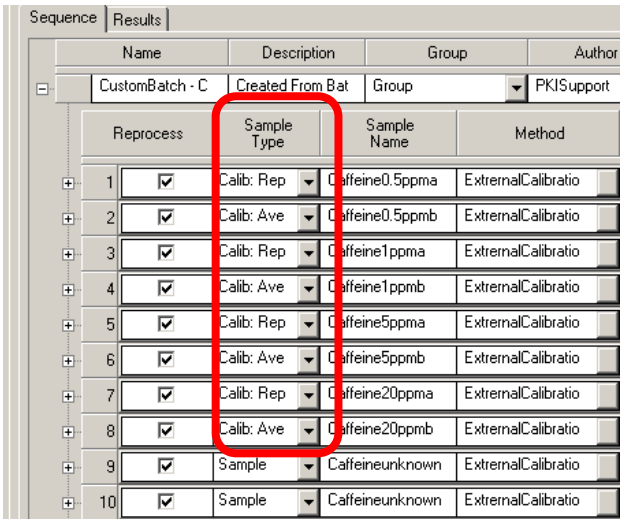
13. Right-click on the method and select **Fill Down** from the pop up box.



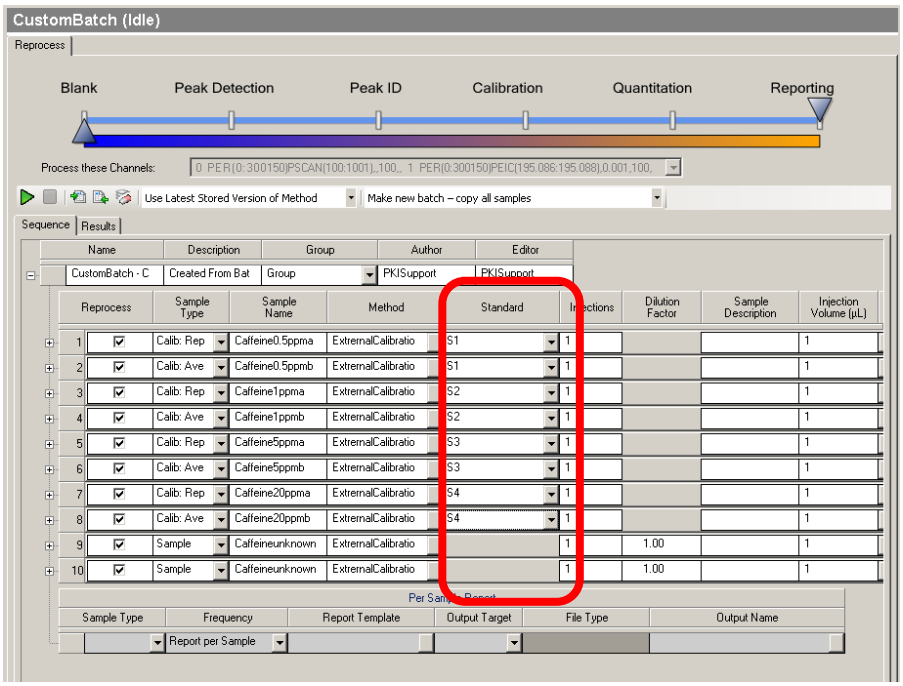
The method is associated with all rows to reprocess.

14. Click in **Sample Type** field and select **Calib. Replace** from the drop-down list.
    You are replacing to reset your calibration curve.

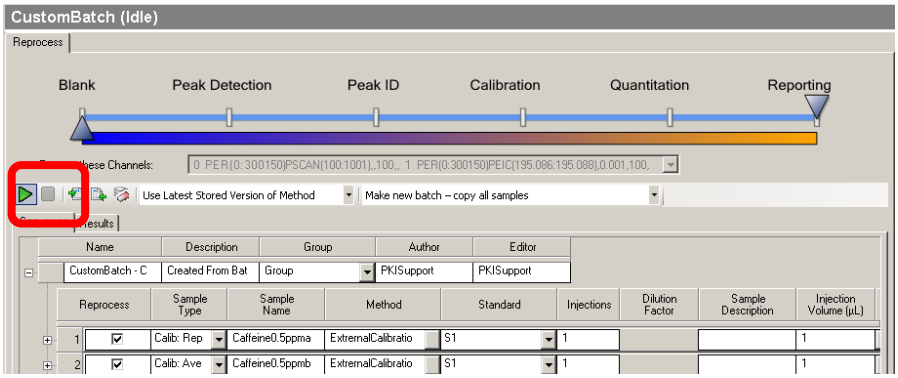
15. In the next row select **Calib. Ave** from the drop-down list.
    In this example, one run is a replace and the other run is an average.



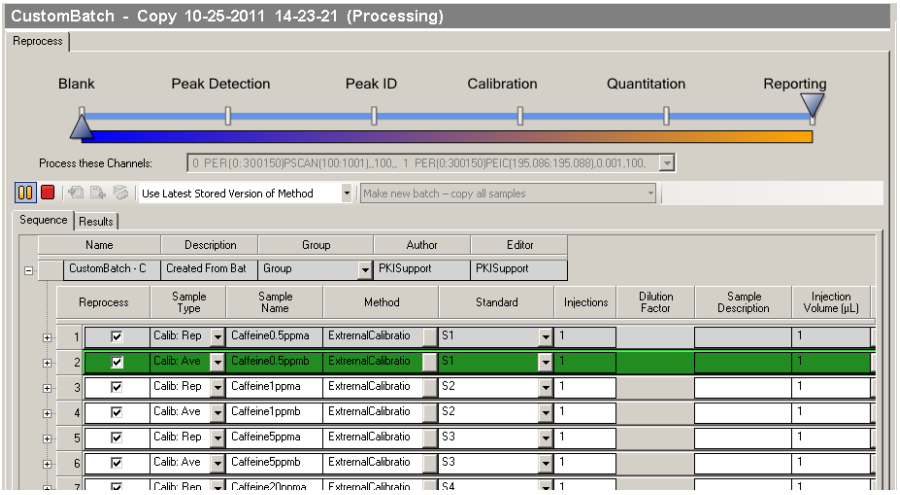
16. Enter your **Standard** for each sample row you will reprocess.
    In this example we did this for S1, S2, S3, and S4.



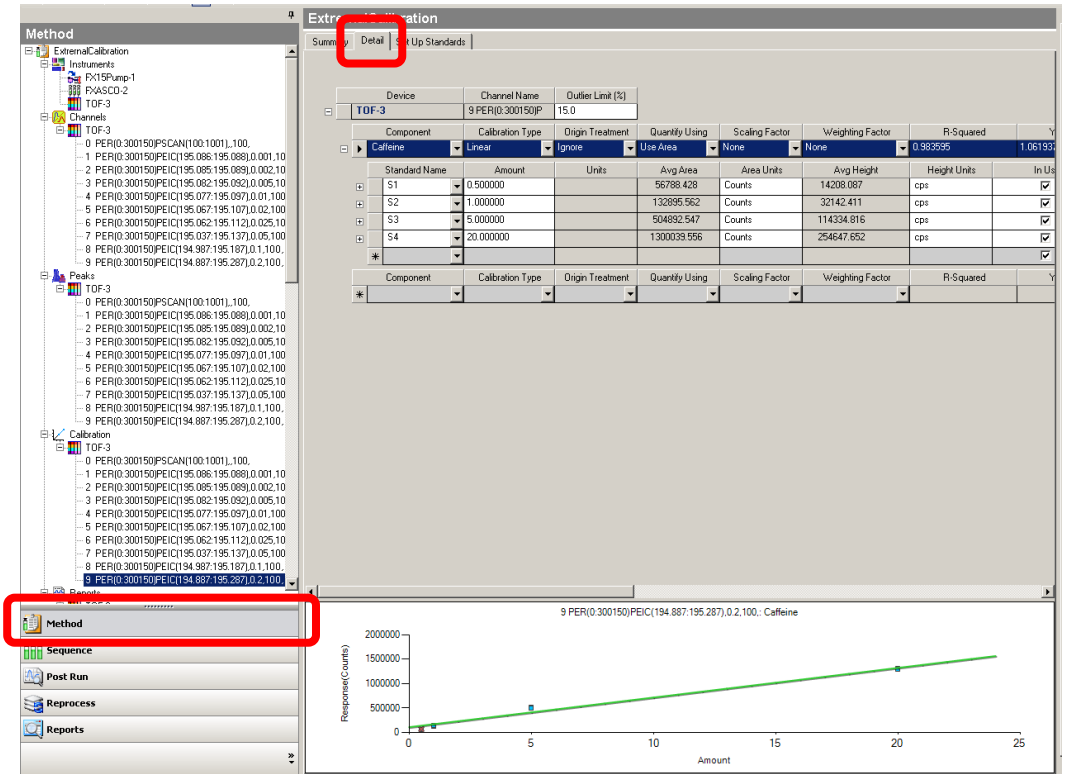
17. Click on the green **Start** button to start reprocessing.



As reprocess runs the row being reprocessed is shown in green.



18. When complete, click the **Method** button in the navigation pane then click the **Detail** tab.



Analyze Results in Post Run

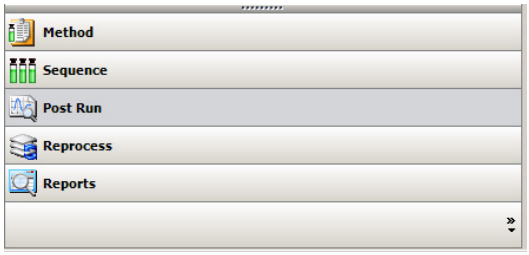
Viewing the Results in Post Run

Whether reprocessing existing data or acquiring new data, the completed samples will be displayed in the **Post Run** environment, and can be inspected by navigating through the Sample tree and interacting graphically with the chromatographic display.

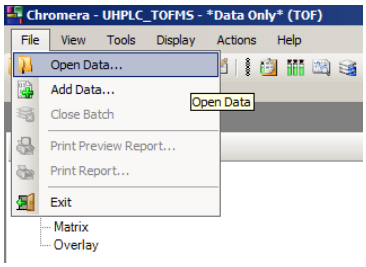
- Data can be treated as view only from the standard **Post Run** display**.**
- Individual results can be optimized graphically.
- The current version of the method can be graphically modified (**GME**, Graphic Method Editing) using the selected sample data.
- Data can be viewed in **Single Plot** mode, **Stacked Plot** mode, **Matrix** mode for multiple channels and replicate injections, or in **Overlay** and **3D** mode (**3D** mode is only available for PDA data at present).

To view results in Post Run:

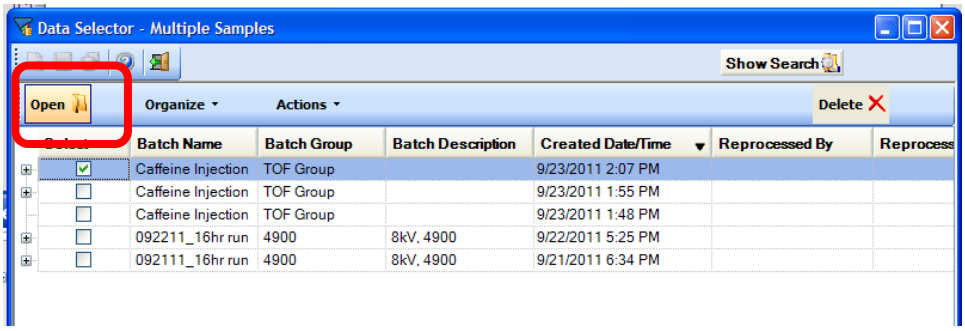
1. Click the **Post Run** button in the navigation pane.



Last run results are displayed or previously stored data can be loaded by selecting **Open Data** from the **File** menu. Note that in the screenshot below, Chromera is being run in Data Only mode. This functionality works the same way when the instrument system is connected.

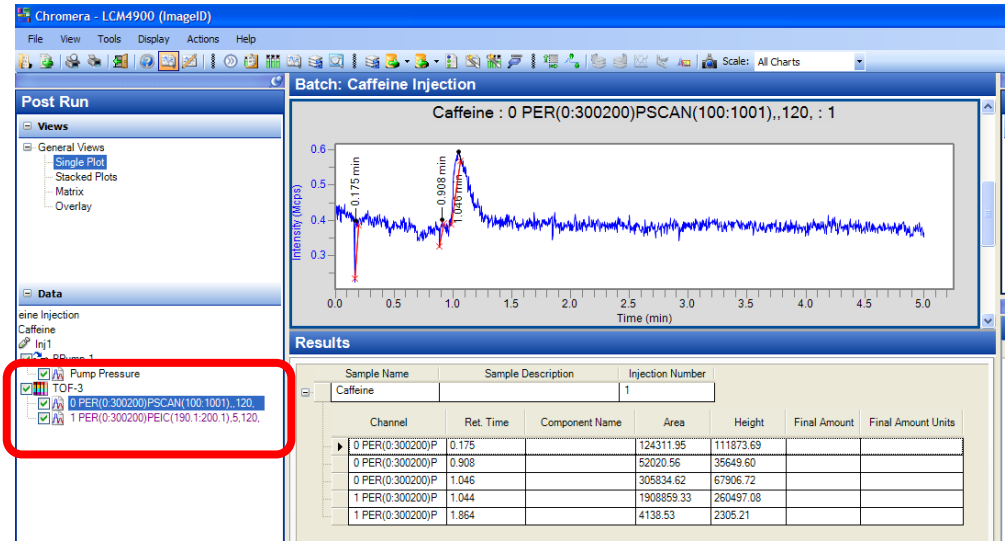


This displays the **Data Selector**. Search and select the data you want to analyze then click **Open**.



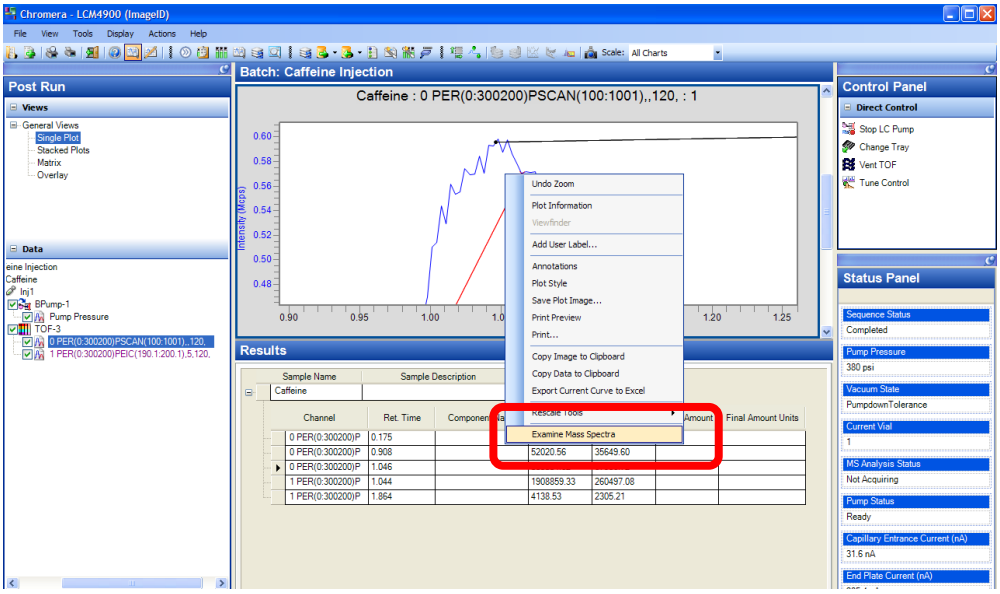
2. Click on **TIC** or **EIC** in the Data tree.

In this example, TIC data are displayed in the **Results** pane.



3. Click on **Scan.**

   The TIC (Total Ion Chromatogram) is displayed in the top plot window and the TIC chromatographic data are displayed in the **Results** pane.

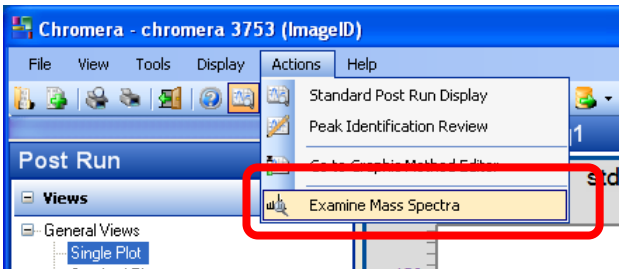


4. Move the mouse pointer to the apex of the peak (it turns to a hand) at retention time ~1.0 min. and then right-click.

5. Select **Examine Mass Spectra** from the menu.

   The spectrum from the selected retention time opens in the lower portion of the TOF MS driver window, and a copy of the TIC is displayed in the top portion of the window.

   Another way to enter the mass spectral processing domain (demonstrated on a different data file) is to select **Examine Spectra** from the Chromera **Actions** menu.



The spectra open in the **TOF MS driver** window.

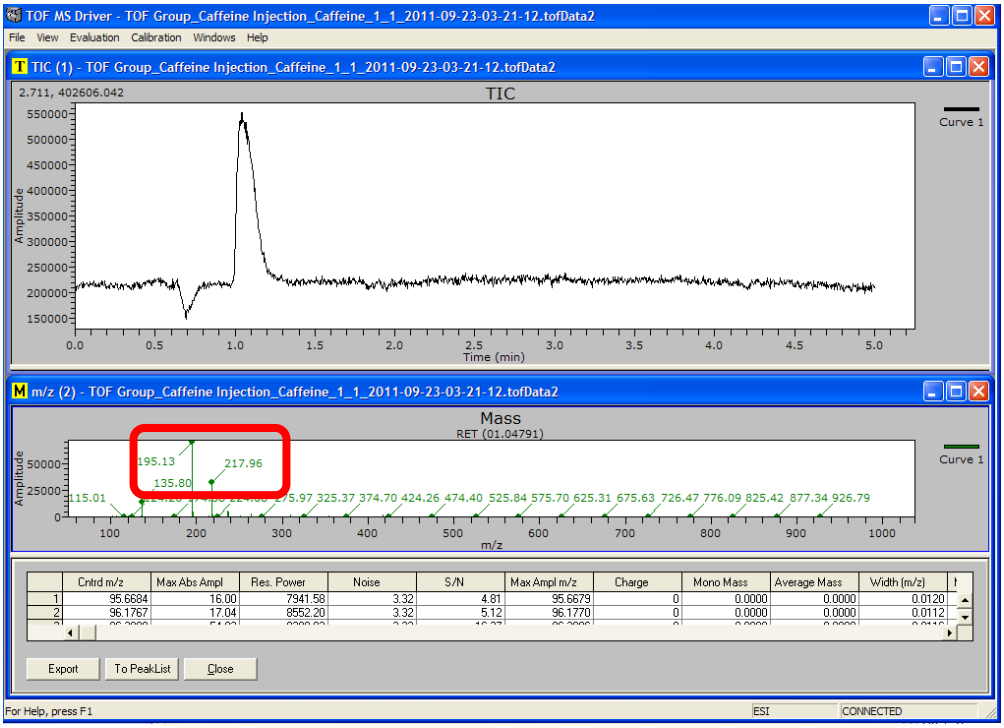
The **TOF MS driver** window displays a Total Ion Chromatogram **(TIC)** in the upper half of the window. If the mouse was right-clicked in the Chromera chromatogram (as in the first example above), the spectrum from that retention time will be displayed. If no point in the Chromera chromatogram is selected, then the first spectrum from the acquisition will be displayed.

The TIC is a chromatogram where each data point represents the sum of intensities of all the ions detected for a scan. Consequently, each data point in a TIC has a scan associated with it. The TIC mirrors a typical chromatogram displayed in an LC analysis.

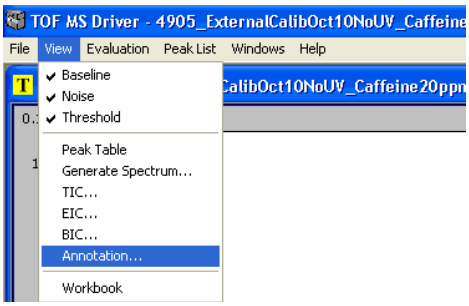
However, "all ions" are only those that were within the sampled mass range, which was determined by the Method used for the data acquisition and the Tune contained within that Method.

6. Move the mouse pointer to the apex of the *m/z* **195.13** peak (it turns to a hand) then right-click to display the peak table on the bottom of the window.

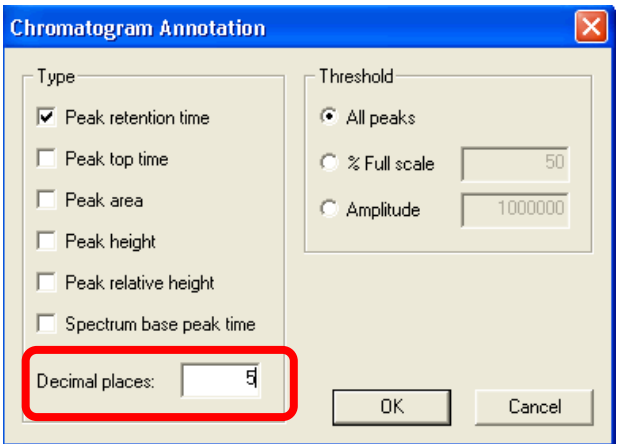
   The peak table provides some statistical data on the identified peak including absolute intensity, the peak width, etc.



7. If you choose more decimal places, in the TOF MS driver window, select **Annotation** from the **View** menu.



The **Chromatogram Annotation** dialog displays.

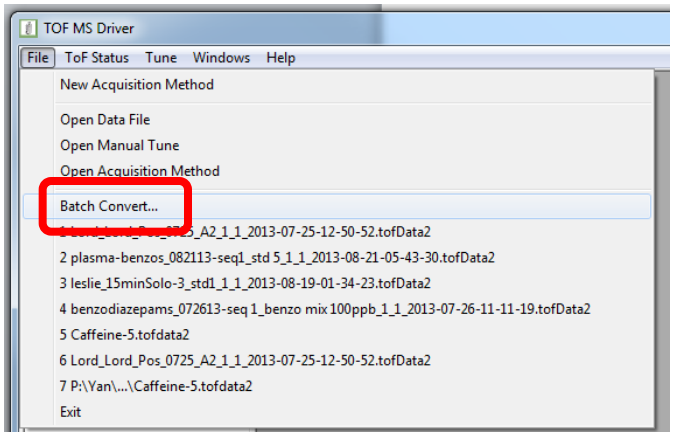


8. Type **5** for **Decimal places** then click **OK**.

Applying Calibration in Post Run Analysis

Improved mass accuracy on data that was acquired using a default calibration (associated with the Tune) can be obtained in post-run analysis by using lockmass. However, known lockmass ions must be present in each spectrum in order to utilize this capability. Multiple files can be processed at once using Batch Convert or files can be re-analyzed separately. This process creates a new .tofline file so, if an original exists and is in the same folder as the .tofdata2 file, it will be overwritten. Be sure the .tofcal2 is also available.

1. In the , select **Batch Convert** from the **File** menu.

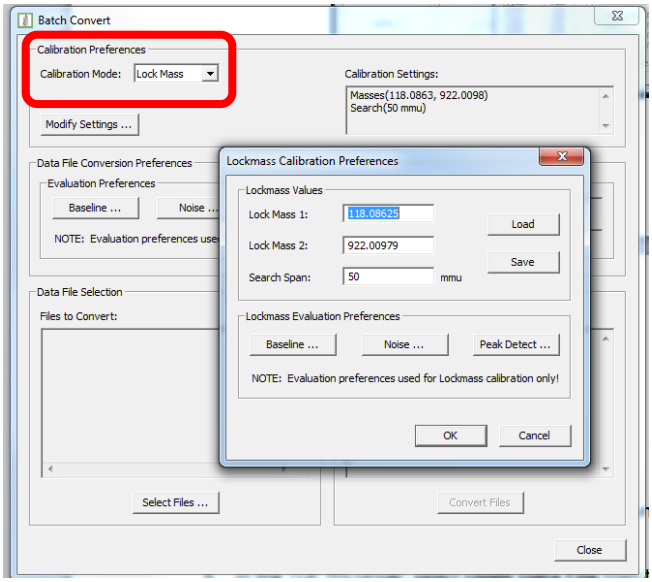


Multiple types of **Calibration Modes** can be applied.

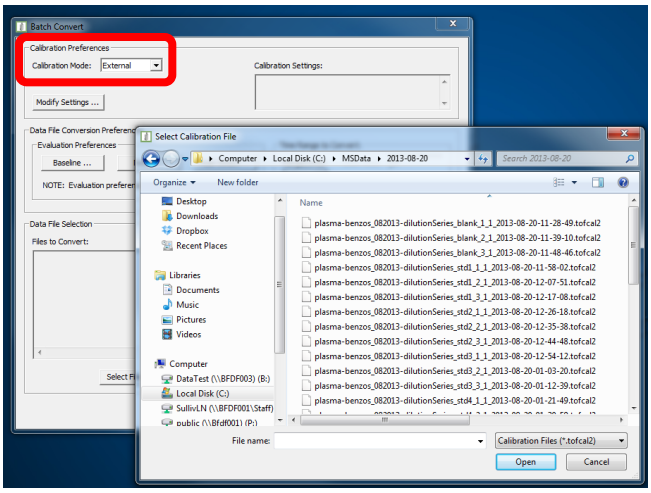
a. **Existing:** Retrieves the Calibration configuration used for the initial acquisition and re-executes calibration using those parameters. The tofcal2 file generated for the run is required. This can be used if adjustments to only the Baseline, Noise or Peak Detect settings are desired while maintaining the existing calibration.

b. **Default:** Utilizes the calibration coefficients found during calibration of the tune parameters.

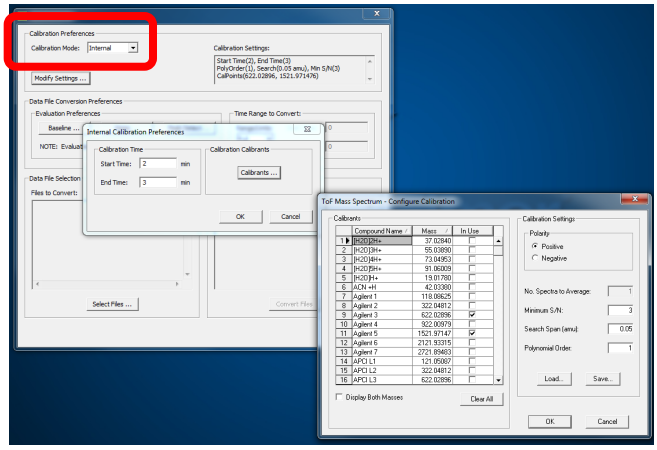
c. **Lockmass:** Utilizes specific peaks to calibrate the remaining data, like internal calibration, but the calibration is applied to the entire run.



d. **External:** Utilizes an independant tofcal2 file for Calibration parameters.

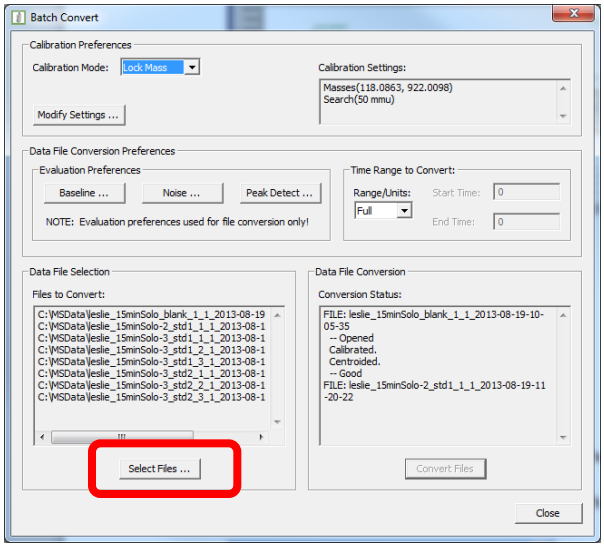


e. **Internal:** Utilizes masses in a specified time period to calculate calibration coefficients.



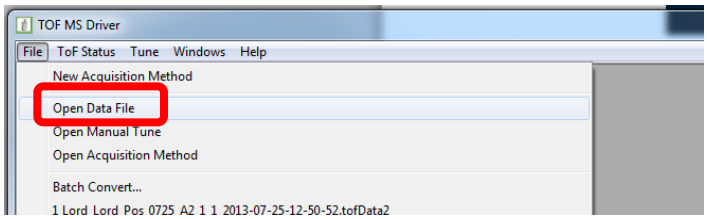
2. Select the desired calibration type and make any necessary updates to parameters. Choose the **Select Files** button and select the tofdata2 files to be processed. Then select **Convert Files**. The right-side display will show progress.

   The new tofline files are written to the same directory as the source tofdata files.

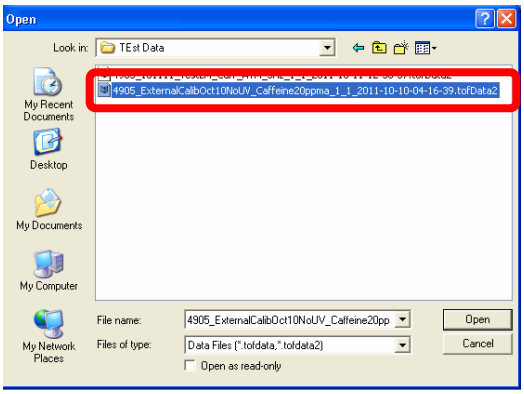


The example shown here illustrates how to use post-run Lockmass on caffeine data within the TOF MS Driver application.

3. In the TOF MS Driver, select **Open Data File** from the **File** menu.



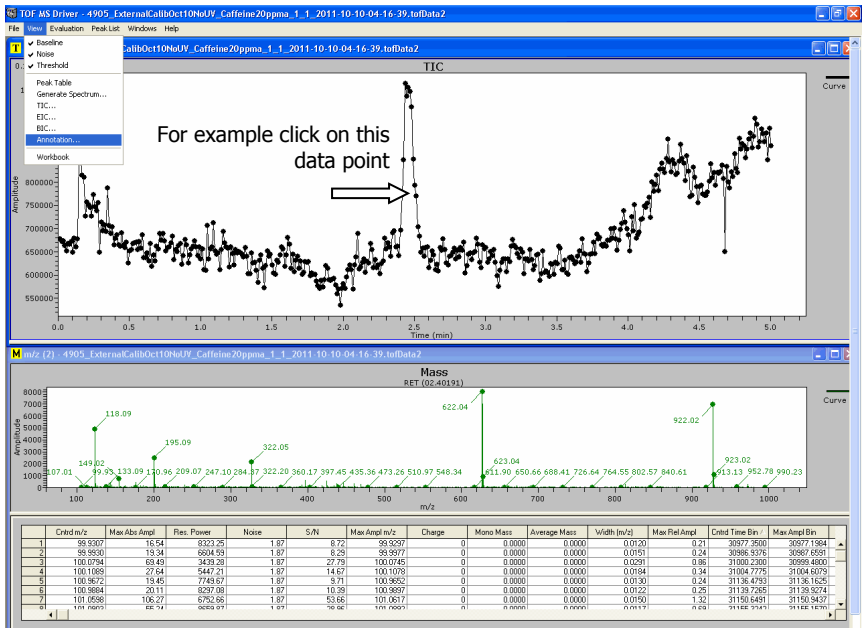
The **Open** dialog displays.



4. Select a data file then click **Open**. In this example we selected:

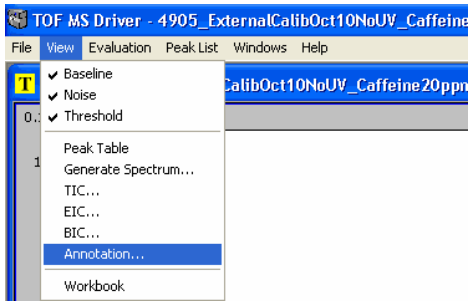
4905_ExternalCalibOct10NoUV_Caffeine20ppma_1_12011-10-10-04-16-39.tofData2

5. Click on a data point in the TIC where you see caffeine (*m/z* 195) and the two lock masses (*m/z* 118 and *m/z* 922) before clicking on the Mass screen.

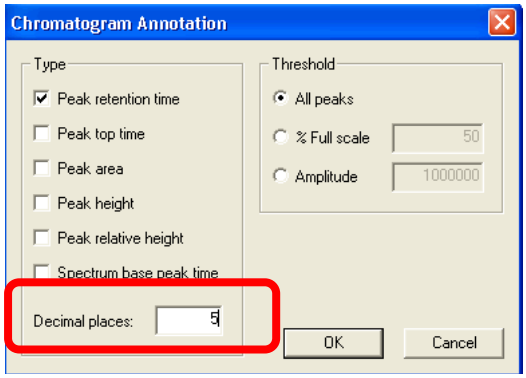


Click on the spectral screen, if the masses display with two decimal points, you should change this to five decimal points.

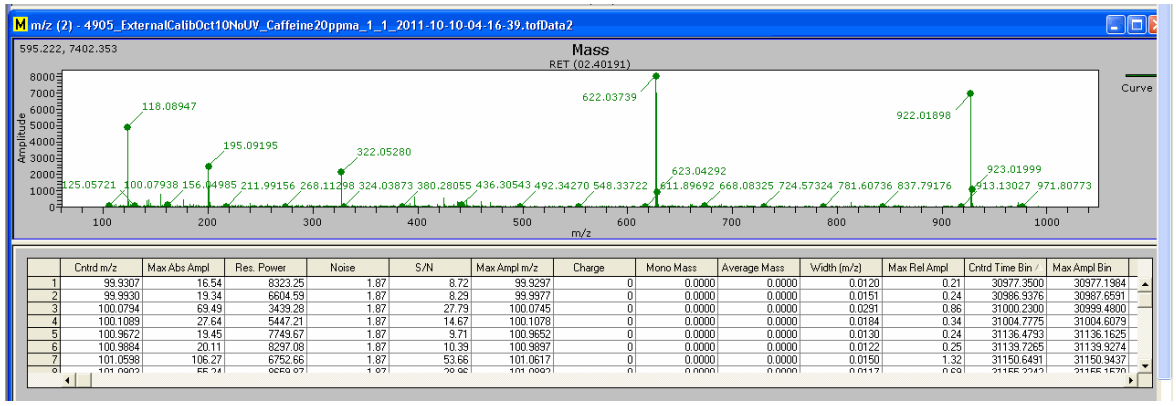
6. Select **Annotation** from the **View** menu.



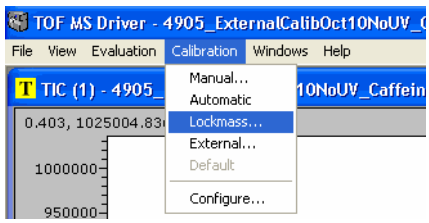
The **Chromatogram Annotation** dialog displays.



7. Type **5** for **Decimal places** then click **OK**.
   Observe that the masses now have five decimal places.



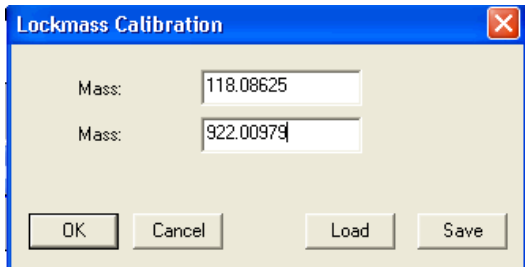
8. Select **Lockmass** from the **Calibration** menu.



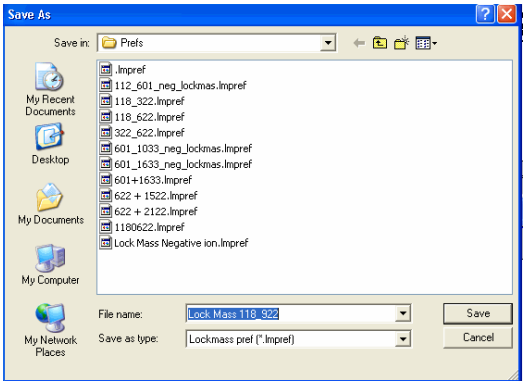
The **Lockmass Calibration** dialog displays.

9. From the lock masses that were infused through the second ESI sprayer, select one mass on each side of the target mass.

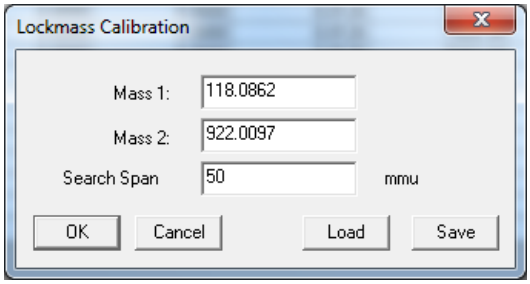
This example shows a target *m/z* 195 (caffeine) that lies between *m/z* 118.08625 and 933.00979 which are selected as the Lockmasses.



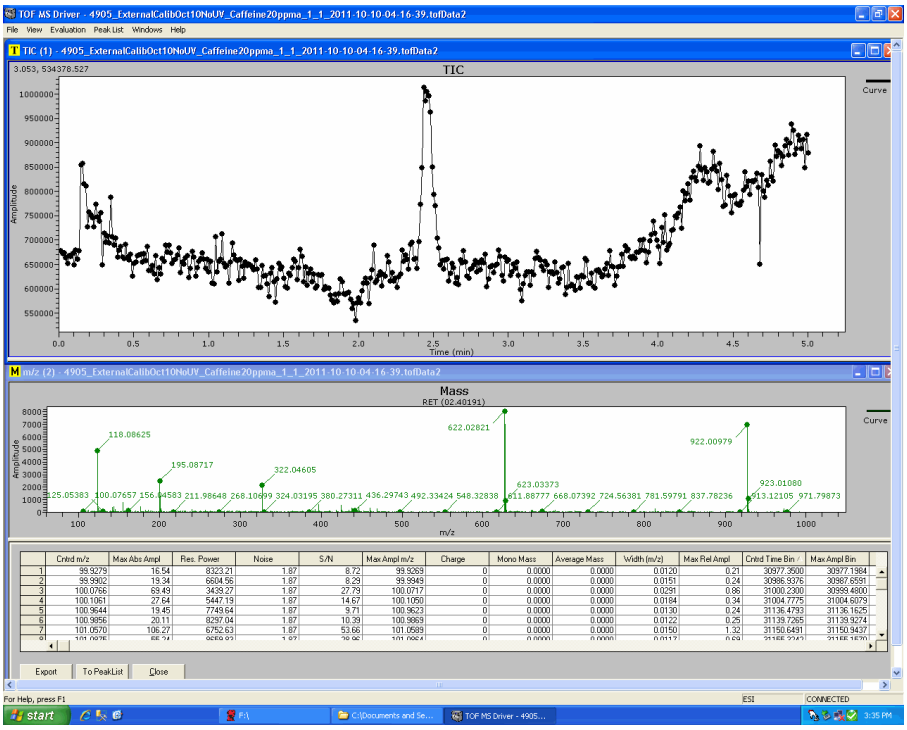
10. To save the displayed masses for future analyses click **Save**.
    The **Save As** dialog displays.



11. Type a **File name**.
    Notice that the file name has the extension **.lmpref** and it is saved in the same directory as your data.



12. Once saved, you can click **OK** to run Lockmass on your data.



As Lockmass runs it creates a file with the extension **.TOFCal2** in the same directory as your data.

After the Lockmass run is complete you can select any data point and observe that the two Lockmasses do not change. The target mass should be accurate to within three decimal places anywhere on the TIC where the target mass resides.

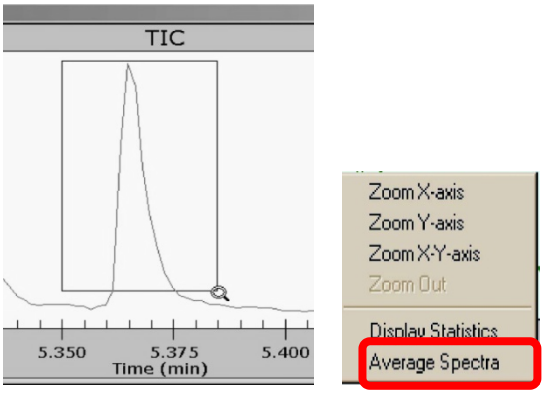
Creating an Average Mass Spectrum in the TOF Driver

Below is an example of how to analyze the TIC peak.

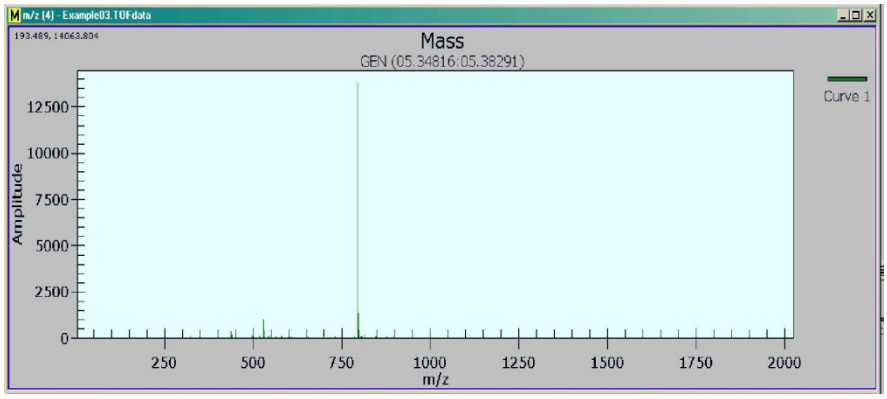
Using the Left Mouse Button Command

1. Left-click and drag a box around the peak of interest.

2. Release the button and select the left mouse button command, **Average Spectra**.



The Mass spectrum will be updated to an average mass spectrum.



Using the Generate Mass Spectrum Dialog

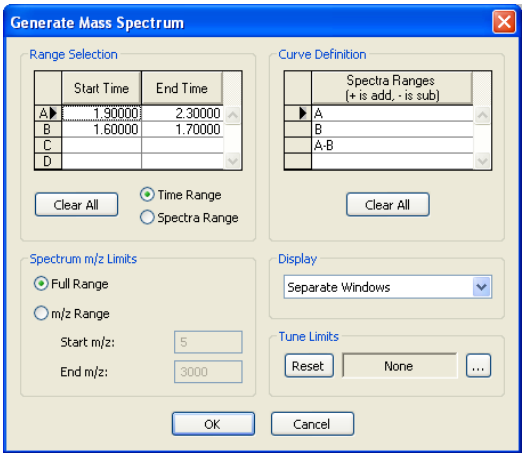
1. To create an average spectrum, activate the TIC spectrum and select **Generate Spectrum** from the **View** menu.

2. In the **Range Selection** section, select whether to use **Time Range** or **Spectra Range** to define the average spectrum.

3. If **Time Range** is selected, enter a **Start Time** and **End Time** in seconds.
   If **Spectra Range** has been selected enter the **first** and **last** spectrum number.

4. Decide how to display the curves in the **Curve Definition** table. Enter the row identification (A,B) to display the curves, simply.

5. Decide how to display the mass spectra in the **Display** section.



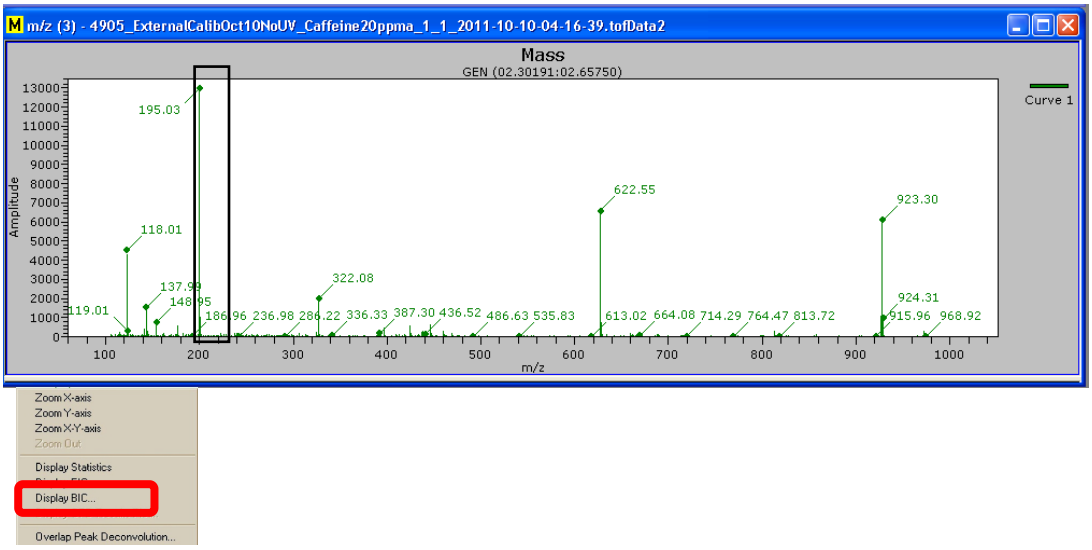
NOTE: *Average spectra are not displayed in the same window as single spectra.*

6. Click **OK**.
   The Mass spectrum will be updated to an average Mass spectrum.

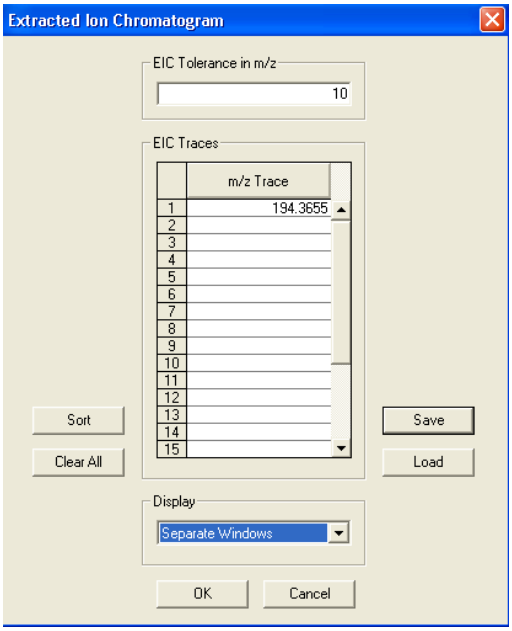
Creating an EIC and BIC from a Mass Spectrum

To create an EIC:

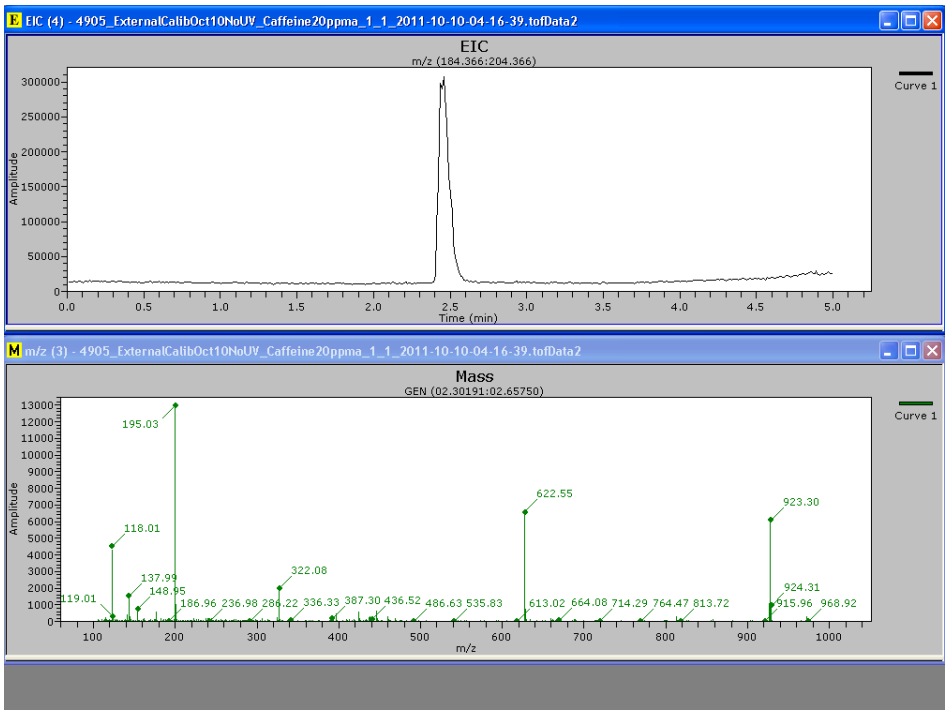
1. Left-click and drag a narrow box around a peak of interest in the Mass spectrum. The width of the box will be the set *m/z* range.



2. Release the mouse button and select **Display EIC** from the menu.
   The **Extracted Ion Chromatogram** (EIC) dialog displays.

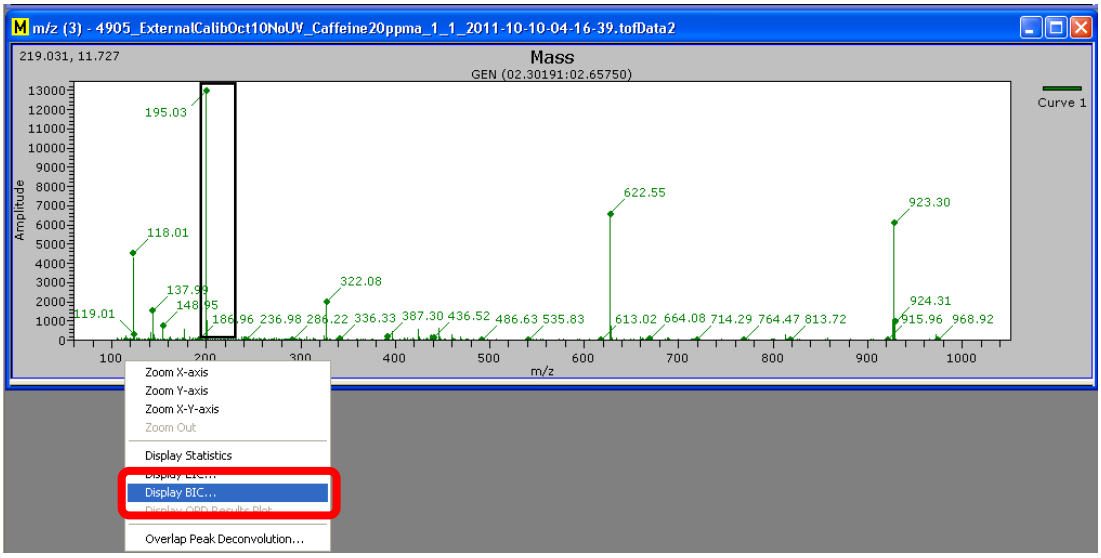


3. Click **OK** and the **EIC** is displayed.
   The EIC displays where in the chromatogram mass peaks occur with *m/z* values within the set *m/z* range.



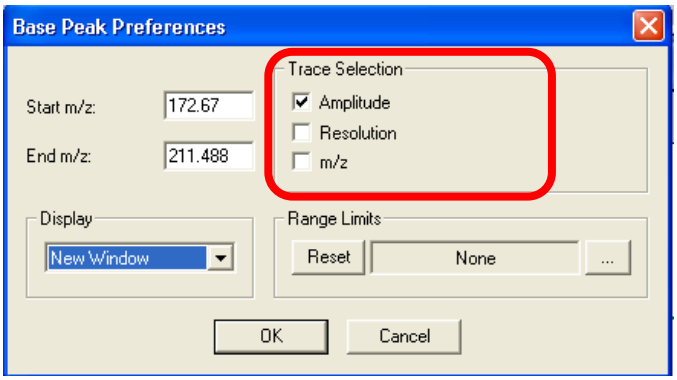
To create a BIC:

1. Left-click and drag a narrow box around a peak of interest in the mass spectrum.
   In this example it is the 195.03 *m/z* peak. The width of the box will be the set *m/z* range.

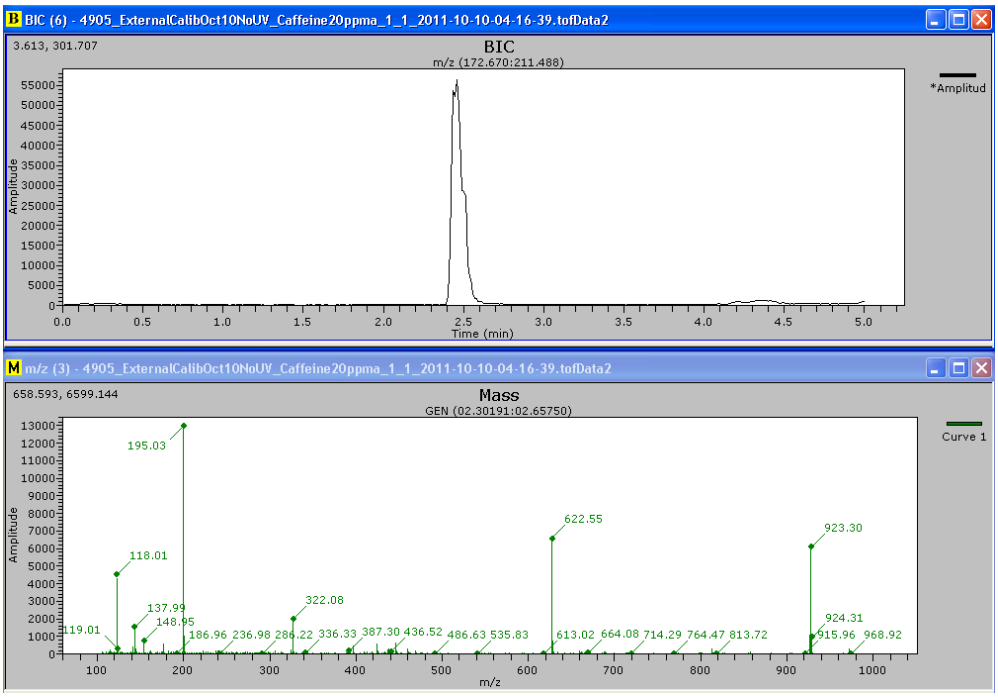


2. Release the mouse button and select **Display BIC** from the menu**.**
   The **Base Peak Preferences** dialog displays.

3. In the **Trace Selection** section only select **Amplitude**.



4. Click **OK** and the BIC is displayed**.**



Processing of Mass Spectra in the TOF Driver Window

NOTE: *In some functions the ability to select "undo" is not available. We recommend creating spectra in a new window before using functions like subtract baseline, subtract threshold, and smoothing. Otherwise, a new spectrum has to be generated in order to revert to the original display.*

Freezing and Thawing Mass Spectra

As you acquire in real time, you can Freeze the Mass spectra window at any point you select and perform functions on that mass without waiting for acquire to complete.

To freeze and thaw Mass Spectra:

1. When a mass spectrum is created using the "hand" the mass spectrum can be frozen by activating the spectrum window and selecting **Freeze** from the **Spectrum View** menu

2. Then, when a new spectrum is created it will be displayed in a new window. The previous spectrum is still available.

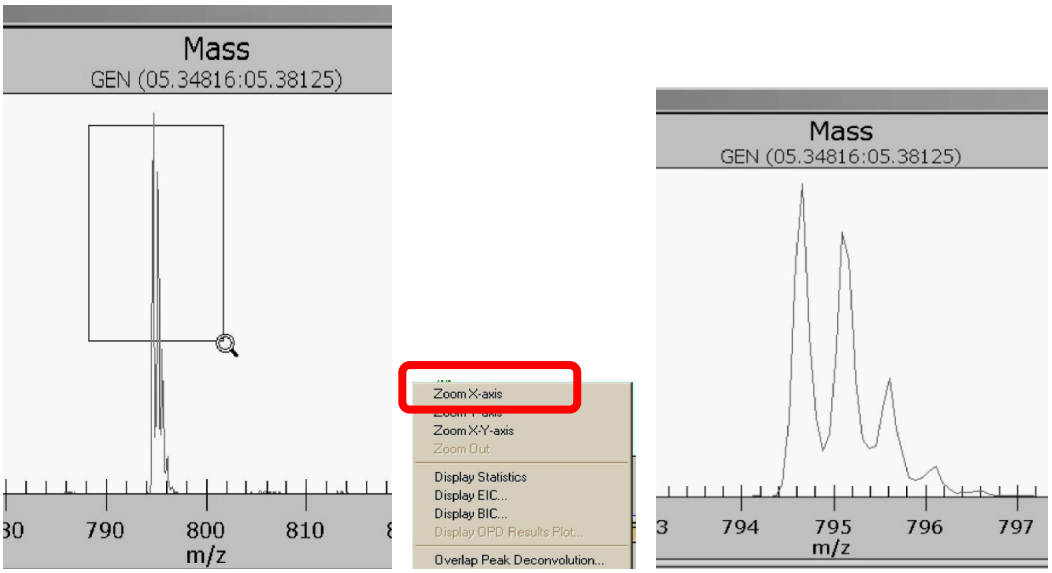
3. To thaw a frozen mass spectrum, activate the spectrum and select **Thaw** from the **View** menu.

Zooming In

To zoom in:

1. Left-click and drag a box around the area of interest.

2. Release the button and select **Zoom X-axis**.



3. Left-click and drag a box somewhere in the spectrum. Release button and select **Zoom Out**.

4. Use the left-mouse button command **Undo Zoom**.

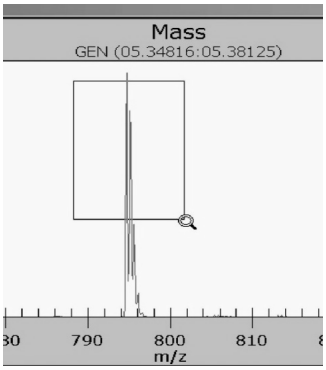
Displaying Statistics

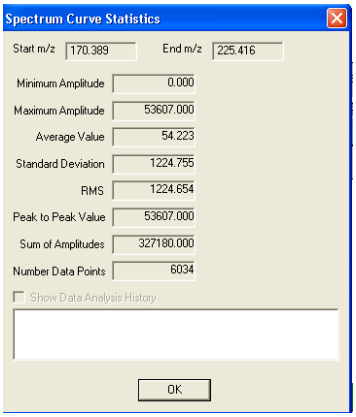
You can display statistics on a spectrum, EIC, TIC, or BIC.

To display statistics:

1. Left-click and drag a box around the area of interest.

2. Release the button and select **Display Statistics**.





Using Right Mouse Click Menus

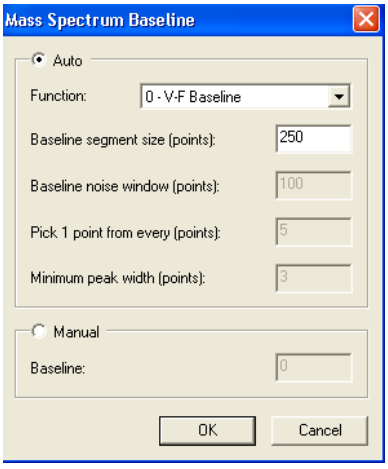
The application obtains a graphical package which includes functions to modify and export graphs. Individual functions can be selected or the **Customization Dialog** can be used.

Baseline Calculations

You can perform baseline calculations on a spectrum, EIC, TIC, or BIC.

To calculate a baseline:

1. When a mass spectrum window is selected, select **Baseline** from the **Evaluation** menu.



2. To calculate an **Auto** baseline with the morphological function, select **Auto** and the **Function** *APB morph.*

3. Enter shortest **Baseline Segment Size**, and **Baseline Noise Window**.

NOTE: *An increased Baseline segment value will flatten the baseline. A decreased value may lead to a baseline which interferes with the peaks.*

The level of the calculated baseline is found in the Peak Information box when Manual peak detection is used.

4. Select **Manual** to create a **Manual** baseline by entering a value resulting in a straight line as a baseline.

5. The baseline can be hidden or displayed with the **Baseline** from the **View** menu.

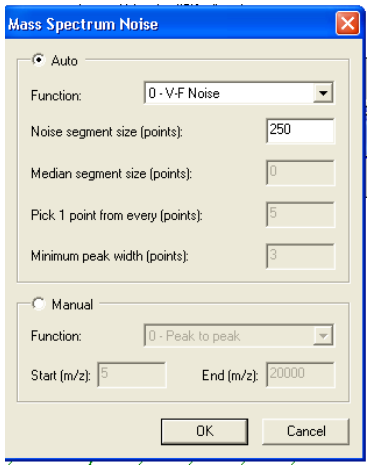
6. The baseline can be subtracted from the chromatogram by selecting **Subtract Baseline** from the **Evaluation** menu.

Setting Spectrum Noise Calculation Preferences

You can set spectrum noise calculation preferences on a spectrum, EIC, TIC, or BIC.

To set mass spectrum noise calculation preferences:

1. When a mass spectrum window is activated, select **Noise** from the **Evaluation** menu.



2. To detect the noise manually, select **Manual** and enter a mass range where there are not any peaks.

3. Select a **Function**: **Peak to peak** or **1-6 Times SD**.

4. In the default version of the signal to noise calculation the following is done:
   - If the noise is **Peak to peak** then the amplitude of the peak top or the centroid is subtracted by the low value of the noise and divided by the peak-to-peak difference.
   - If the noise is **1-6 Times SD** then the amplitude of the peak top or the centroid is subtracted by the mean of the noise and divided by six times the standard deviation of the noise.

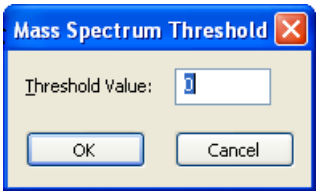
5. Click **OK**.

   The level of the calculated noise is found in the Peak Information box when Manual peak detection is used.

Setting the Mass Spectrum Threshold

To set the mass spectrum threshold:

1. Select a mass spectrum. Select **Threshold** from the **MS Evaluation** menu.



2. Enter a threshold value and decide if the mass spectrum will be subtracted with this value.
   The threshold can be hidden or displayed with the **Threshold** from the **Chromatogram View** menu.

NOTE: *If a new chromatogram has not been created, the "undo" function is not available. To return to the original chromatogram, the data file has to be closed and opened again.*

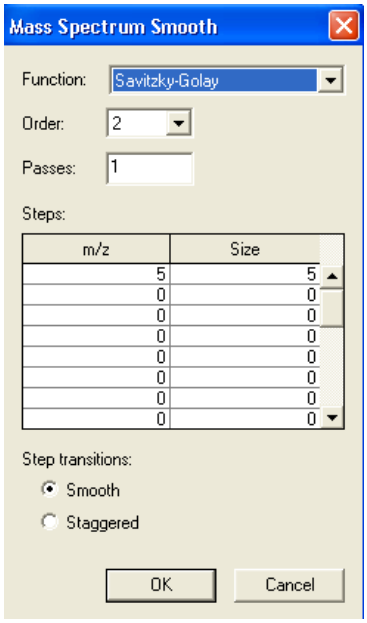
Mass Spectrum Smoothing

You can smooth mass spectrum on an EIC, TIC, or BIC.

To smooth mass spectra:

1. Activate a mass spectrum and select **Smooth** from the **Evaluation** menu.

2. Enter the number of smooths (1-10), window size (0.01-10) and select the function by clicking on the drop-down list.



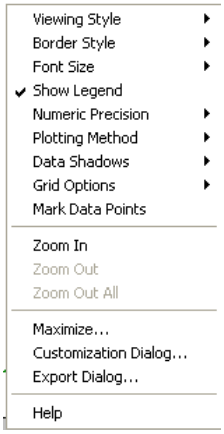
- **Mean:** For each data point in the source spectrum, the processed curve is calculated as the average of the data points within the specified window.
- **Median**: The processed spectrum is calculated as the median of the data points.
- **Savitzky-Golay**
- **Gauissian**

Mass Spectrum Peak Detection

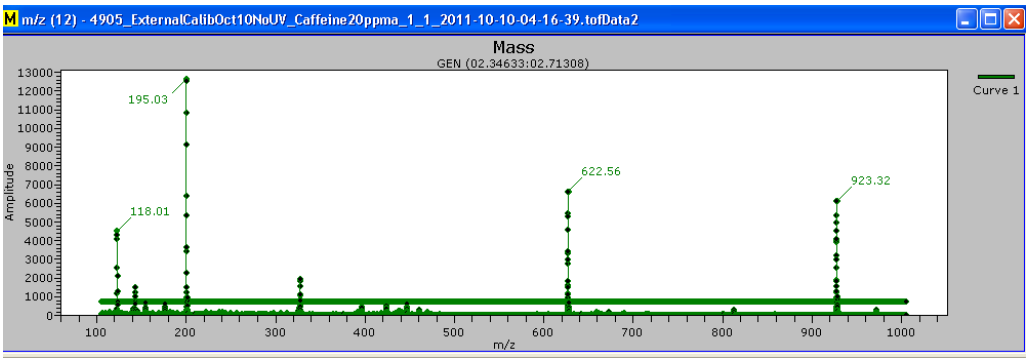
You can set mass spectrum peak detection on a spectrum, EIC, TIC, or BIC.

Manual Peak Detection

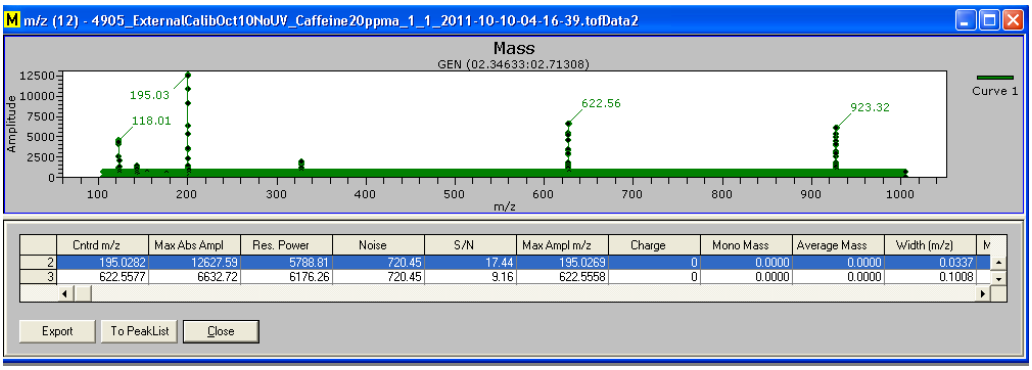
1. To display data points, select the right mouse button command **Mark Data Points**. This makes it easier to see the individual data points in the spectrum.



2. Move the mouse cursor to a data point until the "hand" is displayed.



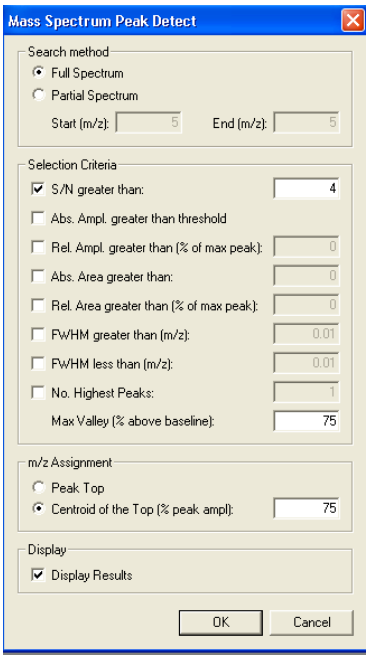
3. Right-click and a **Peak Information** box is displayed in the lower portion of the screen.



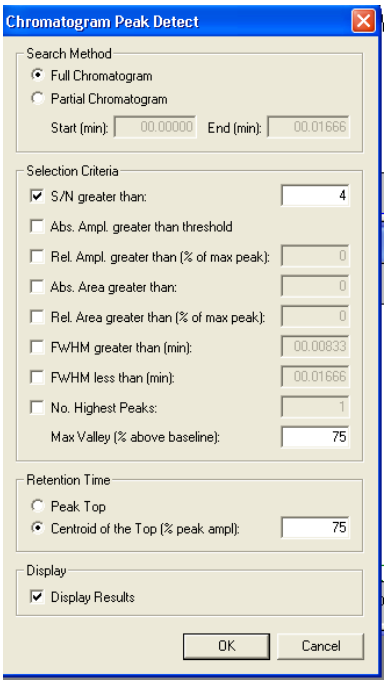
NOTE: *The S/N value is calculated using the centroid amplitude.*

Automatic Peak detection

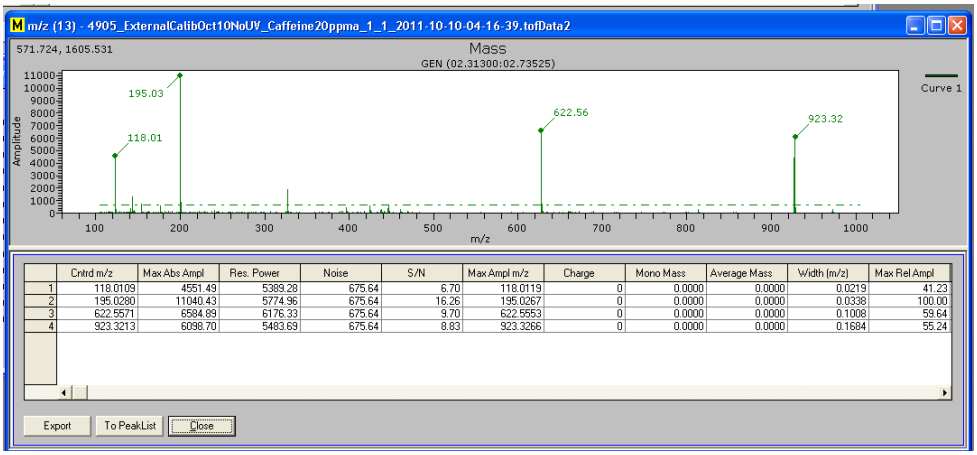
1. Select the mass spectrum.
2. Select **Peak Detect** from the **MS Evaluation** menu.



3. In the **Search Method** section, select **Full Spectrum** or **Partial Spectrum**.
   If partial is selected, enter a **Start** and **End** *m/z* range.
4. In the **Selection Criteria** section, enter a signal to noise limit.
   Peaks with a S/N lower than the entered value will be excluded.
5. In the ***m/z* Assignment** section, select **Peak Top** or **Centroid**.
   When **Peak top** is selected, a spline function will be used to find the top amplitude and its *m/z* value for each mass peak.
   If **Centroid** is selected, a centroid will be calculated using the upper 50% of the peak.
6. To display a peak table, check the box **Display Results**.

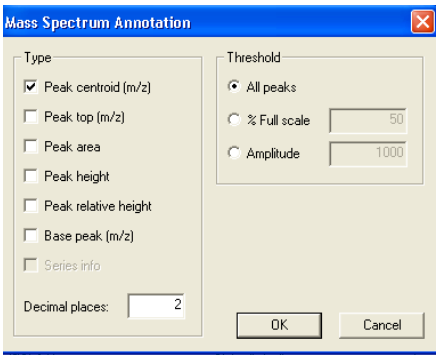


7. Click **OK**.



8. To annotate the *m/z* values in the mass spectrum, select **Annotations** from the **View** menu. Zoom-in for a better display.

   The **Mass Spectrum Annotation** dialog displays.



9. To close the peak table, click the **Close** button below the table.

10. To export the peak table, click the **Export** button below the table. The complete table will be copied to the clipboard.

11. Open Microsoft Excel (or other spreadsheet software) and **Paste** in an empty data sheet.

12. To print the table from Microsoft Excel, select **Print** from the **File** menu.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	
1	Cntrd m/z	Max Ampl m/z	Charge	Mono Mass	Average Mass	Width (m/z)	Res. Power	Noise	S/N	Max Abs Ampl	Max Rel Ampl	Cntrd Time Bin	Max Ampl Bin	Area	Rel Cntrd Ampl	Abs Cntrd Ampl	Peak Start m/z	Peak End m/z	Peak Start Bin	Pea Bin
2	171.6405	171.6425	0	0	0	0.0465	3692.1	0.39	10.11	4	0.43	10294.94	10295	5.13	0.43	3.98	171.5748	171.6763	10293	
3	172.6243	172.6243	0	0	0	0.0452	3823.09	0.39	15.24	6	0.65	10324	10324	7.54	0.65	6	172.5665	172.6921	10322	
4	179.4146	179.4091	0	0	0	0.0631	2843.44	0.59	5.15	3.09	0.33	10522.34	10522.18	5.26	0.33	3.03	179.3682	179.4719	10521	
5	181.3433	181.3436	0	0	0	0.0551	3289.4	0.54	5.54	3	0.32	10577.99	10578	4.54	0.33	3	181.2741	181.4131	10576	
6	188.004	188.0054	0	0	0	0.0482	3902.63	1.35	5.92	8.01	0.87	10767.93	10767.97	10.41	0.87	7.99	187.9358	188.0772	10766	
7	190.1687	190.1702	0	0	0	0.048	3958.34	1.36	5.89	8.01	0.86	10828.94	10828.98	10.35	0.87	7.98	190.0642	190.2421	10826	
8	193.0138	192.9948	0	0	0	0.0567	3402.16	1.48	9.61	19.09	2.06	10908.59	10908.06	37.9	1.54	14.22	192.921	193.1003	10806	
9	194.7189	194.7173	0	0	0	0.0469	4148.07	1.52	4.62	7	0.76	10955.99	10958	8.67	0.76	7	194.6453	194.7893	10954	
10	194.9434	194.939	0	0	0	0.0664	2936.34	1.52	5.33	8.18	0.88	10962.28	10962.15	14.66	0.88	8.08	194.8254	195.0415	10959	
11	204.6533	204.6473	0	0	0	0.0727	2814.42	1.61	8.66	14.15	1.53	11228.49	11228.33	26.49	1.51	13.91	204.5612	204.7458	11226	
12	205.4877	205.4865	0	0	0	0.0502	4089.75	1.67	6.59	11.03	1.19	11251.07	11251.04	14.04	1.2	11.01	205.411	205.559	11249	
13	206.6173	206.6227	0	0	0	0.0744	2775.35	1.67	6.29	10.61	1.15	11281.57	11281.71	20.13	1.14	10.48	206.485	206.7075	11278	
14	220.2375	220.2403	0	0	0	0.0565	3901.1	2.52	10.26	26.04	2.81	11642.89	11642.97	36.99	2.81	25.85	220.0118	220.3182	11637	
15	221.1914	221.1954	0	0	0	0.0629	3514.76	2.52	9.96	25.43	2.75	11667.78	11667.88	39.97	2.73	25.15	221.0849	221.3152	11665	
16	222.1468	222.1514	0	0	0	0.0772	2878.41	2.44	6.76	16.71	1.8	11692.62	11692.76	32.03	1.79	16.47	222.0452	222.276	11690	

Additional Features and Functions

AxION EC ID (Elemental Composition Identification)

This supplemental software package for the AxION 2 TOF helps determine the elemental composition of known ("known unknowns") and unknown ("unknown unknowns") compounds found in a sample analysis. It calculates the elemental composition of the analyte based on the measured exact mass of the observed molecular ion (adduct- typically with a H+ attached) and the relative abundance of the isotope ratios in the molecular ion isotopic distribution. After calculating potential molecular formulas for the analyte, the software links to the PubChem Compound database and lists all the possible compounds (with associated structures) for that composition.

To use this software, you will need an internet connection and the Windows 7 operating system.

New Features

In order to assist users in creation of their own databases, two new tools were added into the software: (1) Formula (Polymer) generator, and (2) Formula Lookup. These two features use historical original approach of atom-combination in generating lists of candidate molecular formulas (contrarily to the default AxION ECID approach of database search). While the Formula Lookup feature is straightforward and simple, the Formula Generator is severely limited by its computational cost, which rapidly makes it too slow when generating molecules with high target mass (above m/z 500), consisting of large number of atom types. Therefore, Formula Generator should not be the "first choice" search in the mass range where PubChem provides many candidates, but it can be used when PubChem truly fails.

Another new feature of the software is the ability for a user to interact with the Neutral Loss screen (previously called CID – collision-induced-dissociation) in order to analyze mass differences between spectral peaks. This document provides examples of use of these new features.

Program start and default DB search

AxION ECID is invoked by highlighting peaks thought by user to belong to a molecular isotope cluster from the M/z peak list of AxION TOF MS Driver software, as shown below.

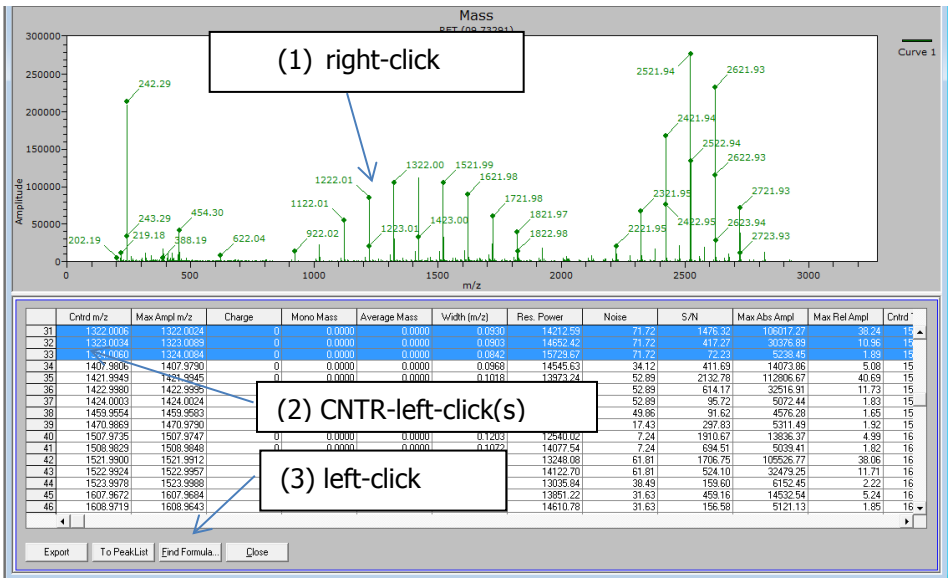


Figure 1. ECID tool is invoked by pressing "Find Formula…" button on the bottom of the peaklist panel of the Mass Spectrum window.

When the tool is started this way, the main window is filled-in with experimental information, and the whole peak-list is loaded into ECID memory in order to allow Neutral Loss Analysis (as illustrated below). It is noteworthy, that ECID uses information provided in peak-list (not the spectrum); therefore the m/z peak-picking preferences will affect the input to the ECID tool.

In this example, experimental target mass is 1322.0006 and PubChem Compound (PC) extract for this mass range is empty, which is listed in the Results panel, if the "Search DB" button is pressed, as shown in Figure 2.

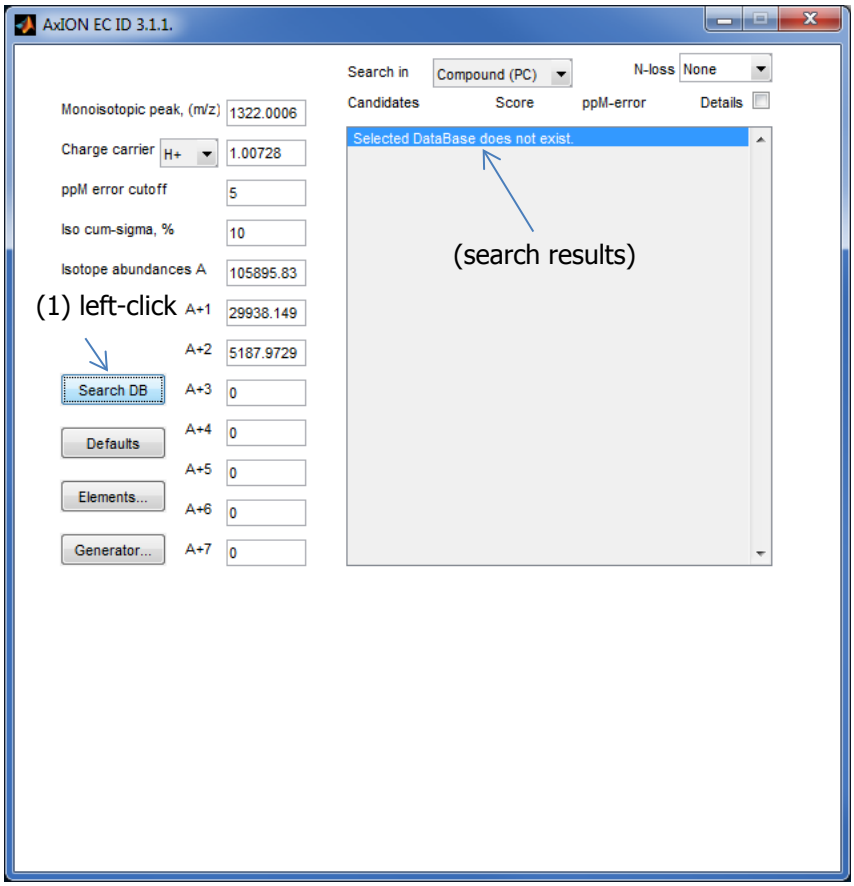


Figure 2. There are no records in Compound Extract DB above m/z of 1000.

Neutral Loss analysis

In the upper-right corner of the ECID window there is pull-down menu control for the Neutral Loss feature ("N-loss"), which refers to tool which calculates mass differences between masses listed in a peak-list, and compares them to a Compound extract neutral molecules, in order to assist user in assignment of relations between mass spectral peaks. Mass difference between peaks can be an indication of their chemical relation, as in the case shown in a spectrum of a polymer, or result from aspects of mechanisms of MS sampling (substitution of a charge agent in electrospray), or ion transfer: in-source collision-induced dissociation, etc.

There are two modes in which neutral losses can be viewed: "P→F" , ("parent to fragments"), stands for listing of mass difference between the target parent ion peak and every other peak in the spectrum, subject to intensity cutoff limits (which are currently not user-selectable, and set for ….). If this option is selected, and a user clicks on a line in the results panel, as shown in Figure 3a, all neutral molecules

listed in Compound (PC) extract whose mass corresponds to experimental mass difference are listed as potential candidates for the neutral loss, as Shown in Figure 4b. Note that the "ppM error cutoff" limit in this case needs to be low, otherwise unknown parent ion "allows" all possible elements to be present in the candidate, making the lists "crowd" the screen. If the parent ion is a found, or generated formula, only elements present in the parent are allowed in the neutral loss. Alternatively, a user can click on the "Elements" button in the left-middle part of the ECID window, to bring-up the element-restriction tool.

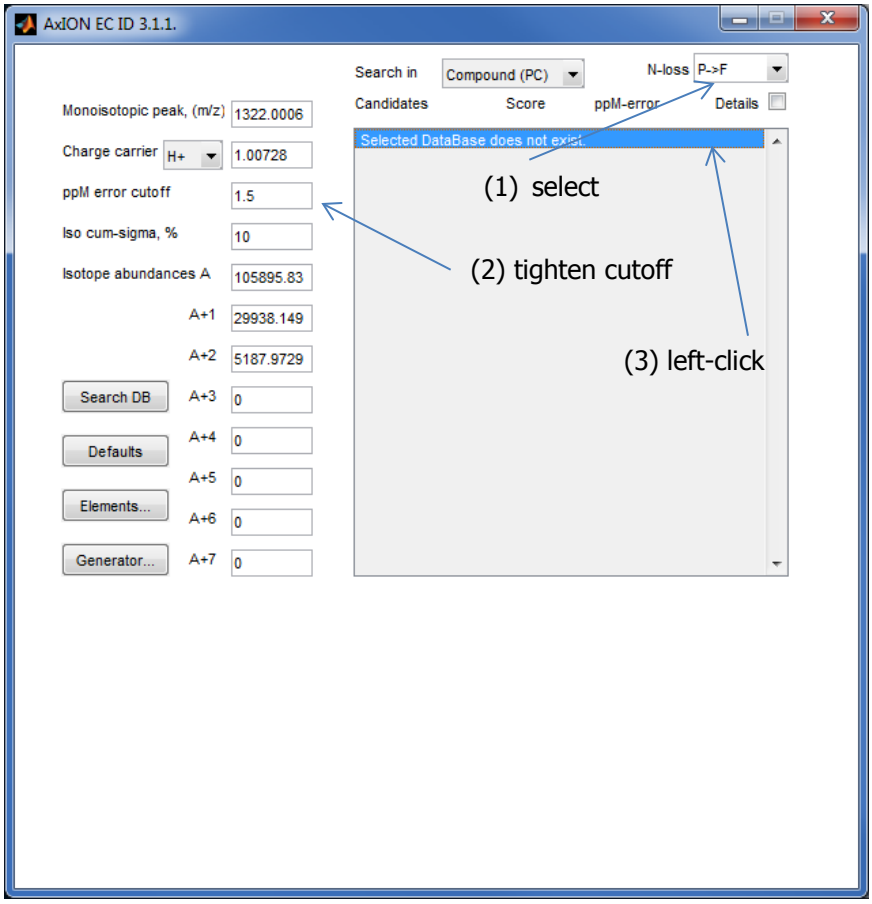


Figure 3a. activation of parent-to-fragments neutral loss utility

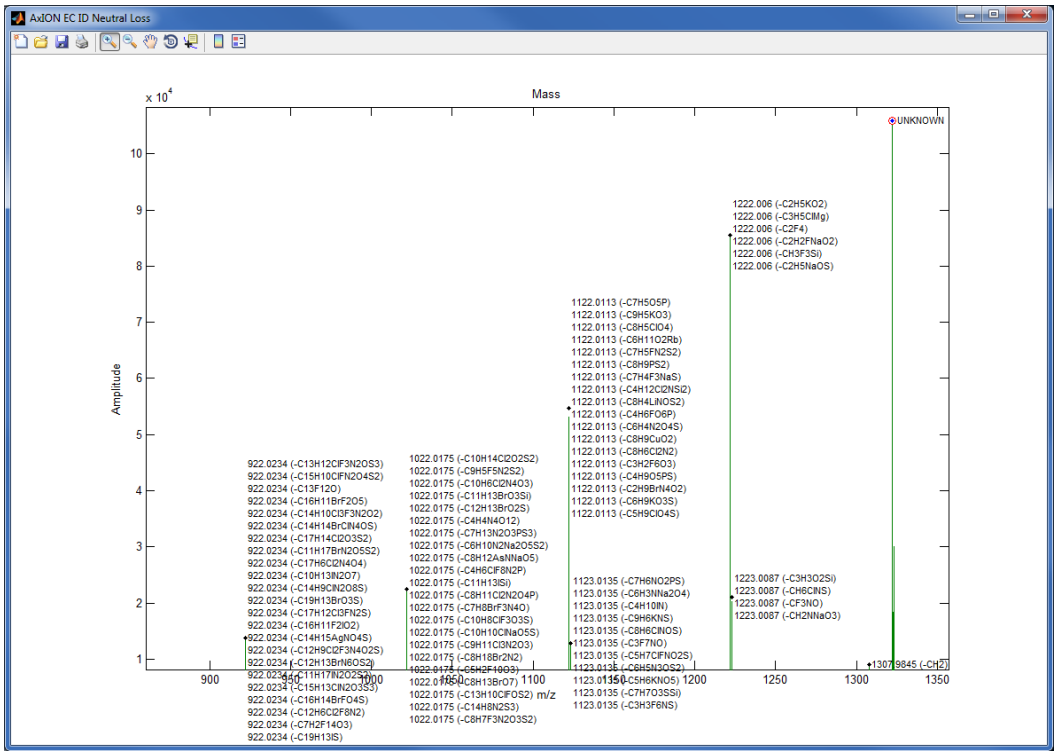


Figure 3b. parent-to-fragments neutral loss display for an unknown parent ion

If the candidate parent ion is unknown, experimental mass listed in ECID "Monoisotopic peak, (m/z)" edit-box is used; if the parent ion is one of the found candidate formulas – its theoretical mass is used. Isotope ratio information is not used.

The zoom  and move  buttons on the top tool menu of the neutral loss window – when selected – allow user not only to change the display of the neutral loss spectrum, but also to limit the neutral loss search only to peaks currently shown in the window. When all the tool buttons are deselected (default state), mouse left-click over the spectrum becomes active for individual mass difference activation. When a user clicks on a pair of peaks, only the neutral loss between the pair of the peaks is listed, as shown in Figure 4.

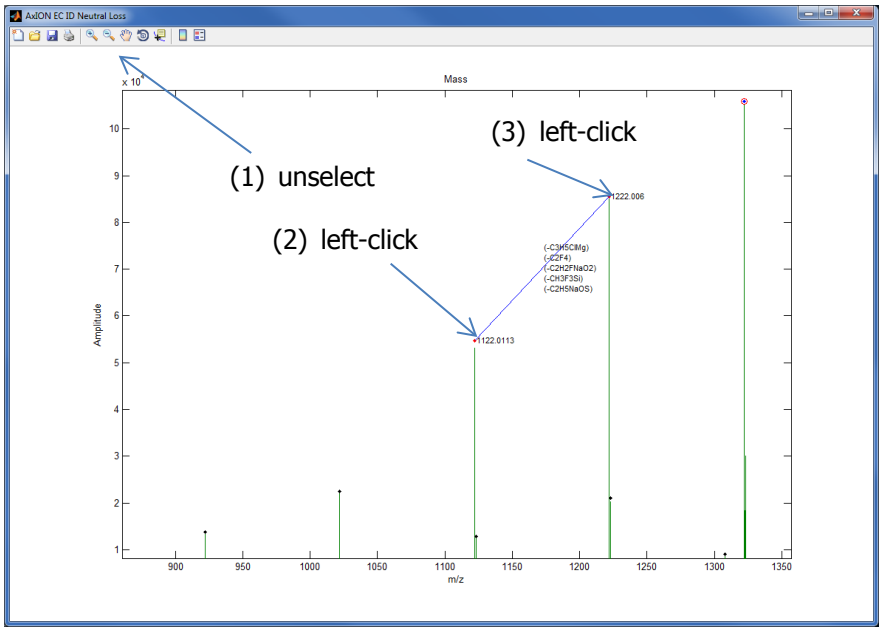


Figure 4. User interactive neutral loss listing.

In the shown example of a target parent ion, the second mode of neutral loss analysis proves useful. Here, "P→F→F" stands for "parent-to-fragment-to-fragment", the representation of sequential neutral losses, which in case of CID would correspond to sequential parent ion decomposition, but in this case reflect the polymer nature of the sample: the "neutral loss" mass is simply the mass of the polymer repeating unit. In other cases it can be an end unit of a branched polymer, etc. Figure 6 shows the result of "P→F→F" listing of neutral losses. Notice that only the top of the spectrum has to be within the zoom, otherwise the sequential loss is calculated between every pair of adjacent peaks, including lower abundance higher isotopes. In this example, both losses list C2F4, which could be our polymer repeating unit.

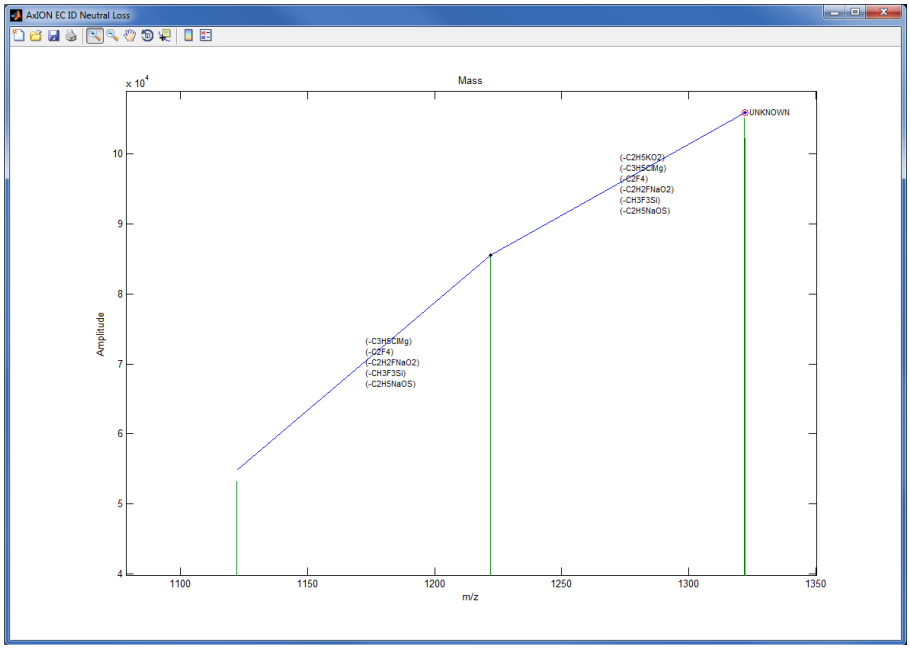


Figure 5. "sequential" neutral loss reveals polymer repeating unit

Polymer and Formula Generator

Having gained some clues into the polymer identity, user can proceed to the atom-combination Formula Generator and Lookup, by pressing "Generator…" button in the left-middle part of the ECID window, which appears on the right of the main window, as shown in Figure 6.

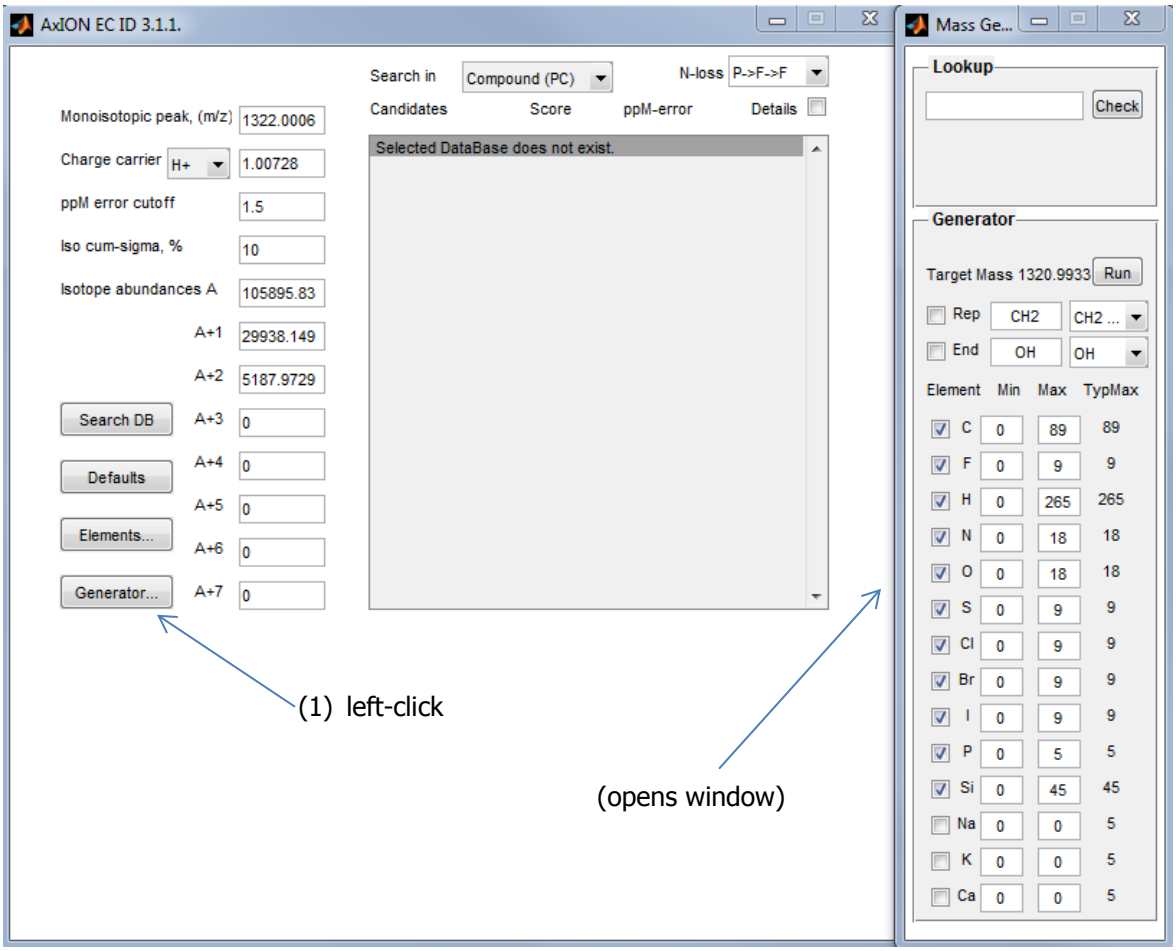


Figure 6. Activation of the Lookup and Generator window

In some cases, user has an idea what the target ion could be. Formula Lookup (top panel) allows user to type in any valid molecular formula and - by clicking "Check" button – report exact theoretical mass and display isotope distribution, as illustrated in the next Section.

Straight-forward formula generation as shown in the default screen shot in Figure 7 is extremely computationally expensive, if all atoms types listed are allowed, and the typical maximum number of each atom type is used. Such search can only be performed in realistic time for target ions below 500 Da. In order to speed it up the concept of a polymer repeating unit is used as a super-atom, which allows to limit the left-over numbers of atom types, as if these atoms were truly on the ends of a polymer. Certainly, this formal approach applies not only for straight polymers; in the context of a formula generation it is simply a way to speed-up combinations search. Example of reasonably short combination generation is shown in Figure 7.

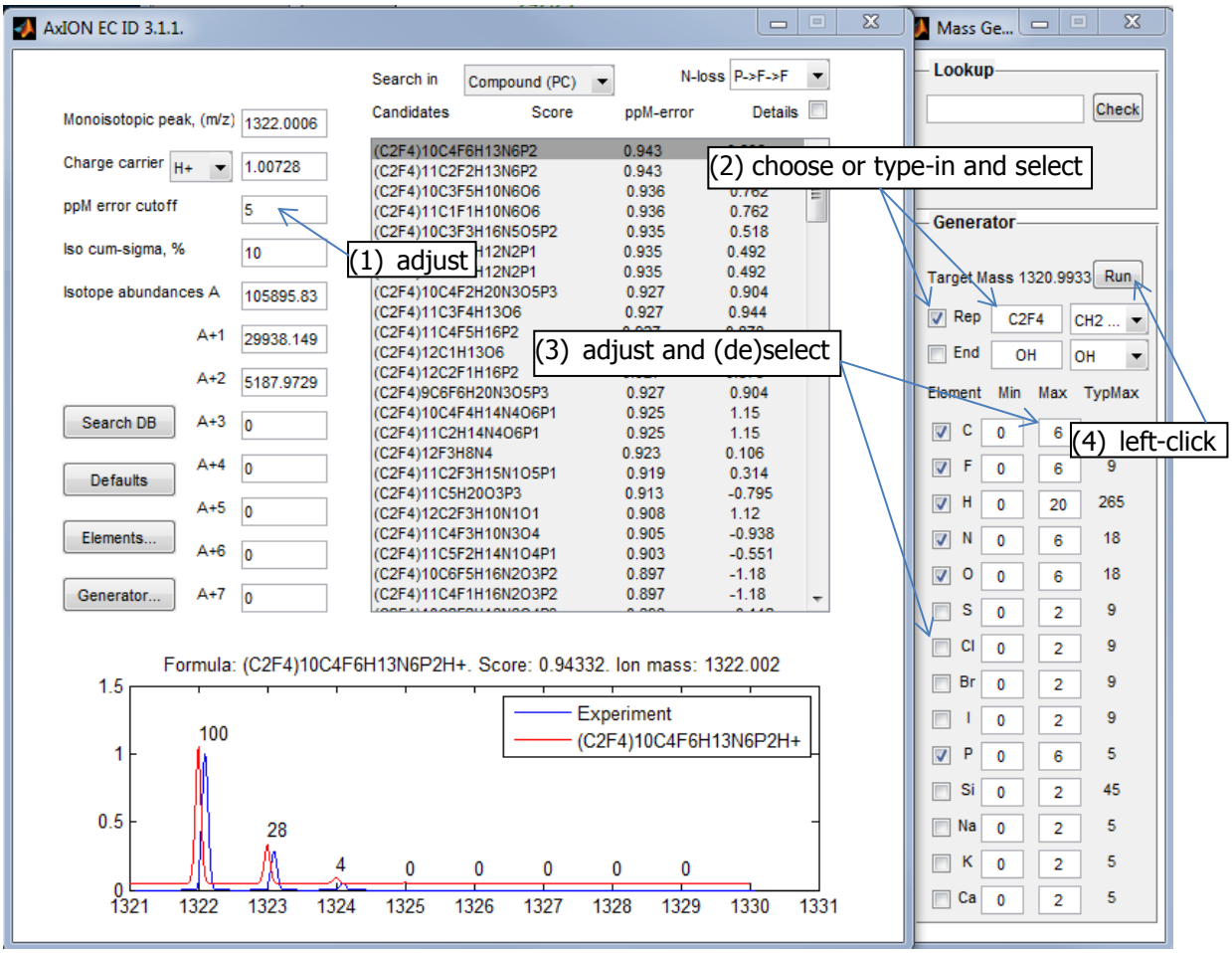


Figure 7. Polymer-like formula generation.

Careful examination of candidate results reveals redundant formulas, if too many atoms are allowed on the end units; these redundant formulas differ by number of repeating units, while the atoms of the repeating units are still listed in the formula, but assumed to be in the part of the molecule other than the repeating chain. From the stoichiometry stand point these formulas are identical. In order to get rid of them, and to significantly speed-up the generation – lower the "Max" number of atoms of appropriate types.

Atom types allowed in Polymer and Formula generator usually are a small subset of "Elements…" allowed ion the main ECID periodic table tool. As a stronger restriction, Generator only uses its own allowed atoms. Explicit "end" unit choice is reserved for future use; in the current software release it is ignored: end units are calculated by direct formula generator.

Formula Lookup

As mentioned above, the upper panel of the Generator window allows to type-in any molecular formula and check its lowest-mass isotope mass and isotope distribution. But it also can be used to estimate what is on the ends of a polymer. In the shown case of –C2F4- chains, user can look up an accurate mass of just the repeating chain as shown in Figure 8.

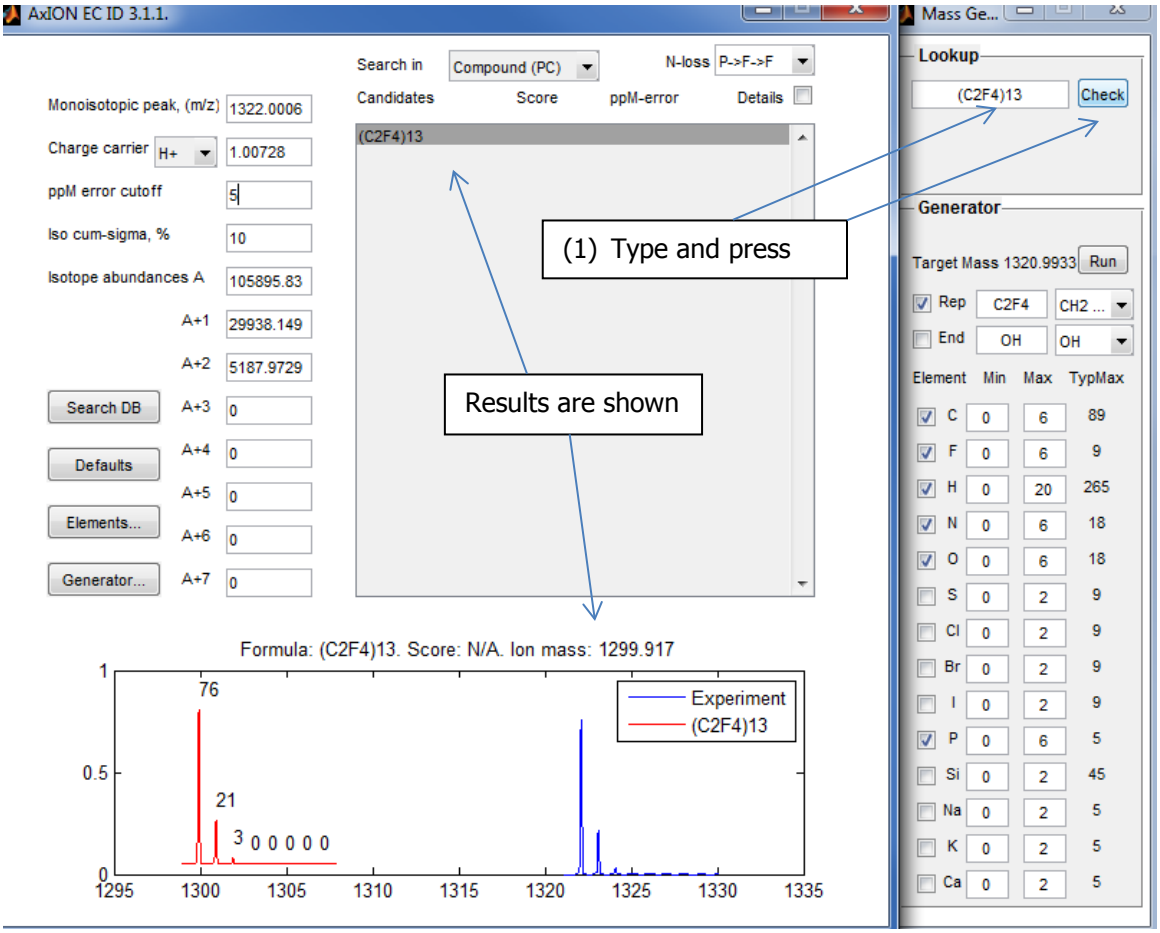


Figure 9. Using Formula Lookup feature

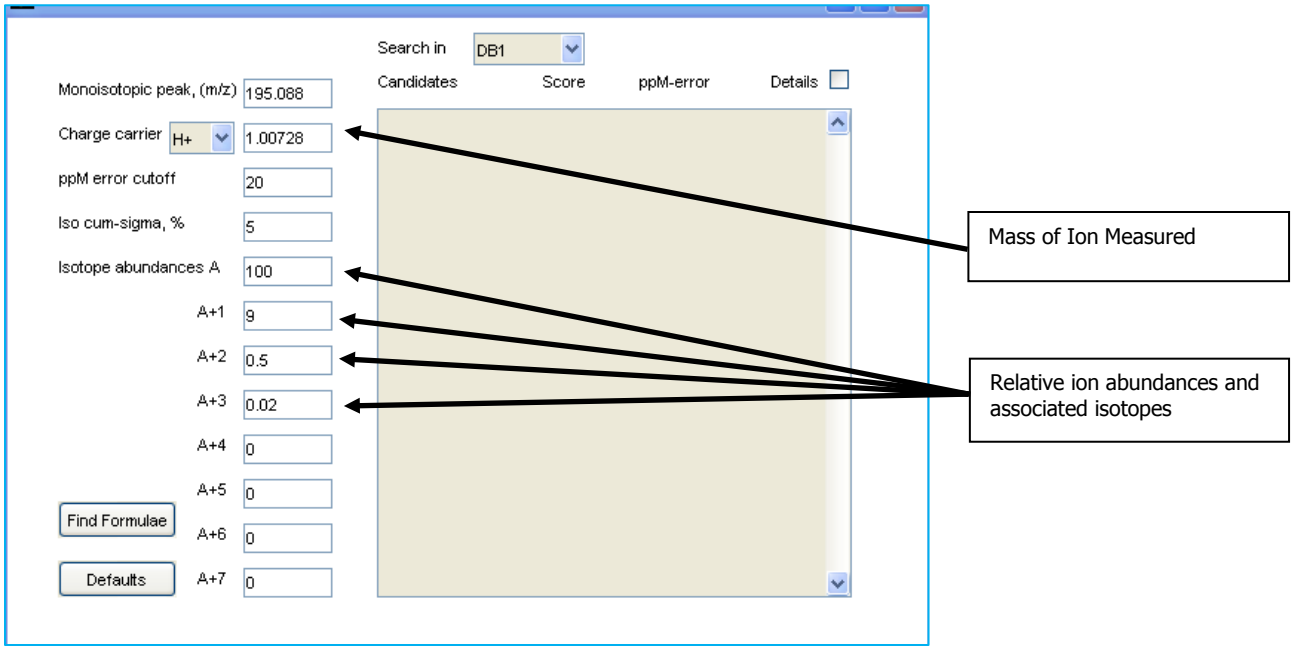
As shown in the spectral lower part of results panel, formula typed in the Lookup box is calculated exactly, with no account for the "Charge carrier" listed in the upper-left part of the main ECID window. However, "+" and "-" will be recognized when typed directly at the end of a formula in the Lookup panel, resulting in respective subtraction or addition of an electron, respectively. In the given example, user sees that the difference between the maximum number of repeating units (13) and the experimental target mass of 1322.0006 equals 1322.0006-1299.917=22.0836, hardly indicating anything straight-forward. The next lowest number of repeating units is 12, suggesting the combined mass of 1199.923, and the total end unit mass of 122.0776. This mass can be manually typed into the "Monoisotopic mass" of the main ECID window for direct interrogation by default database search (isotope ratios will have to be adjusted, of their acceptance limits – expanded).

Running AxION EC ID

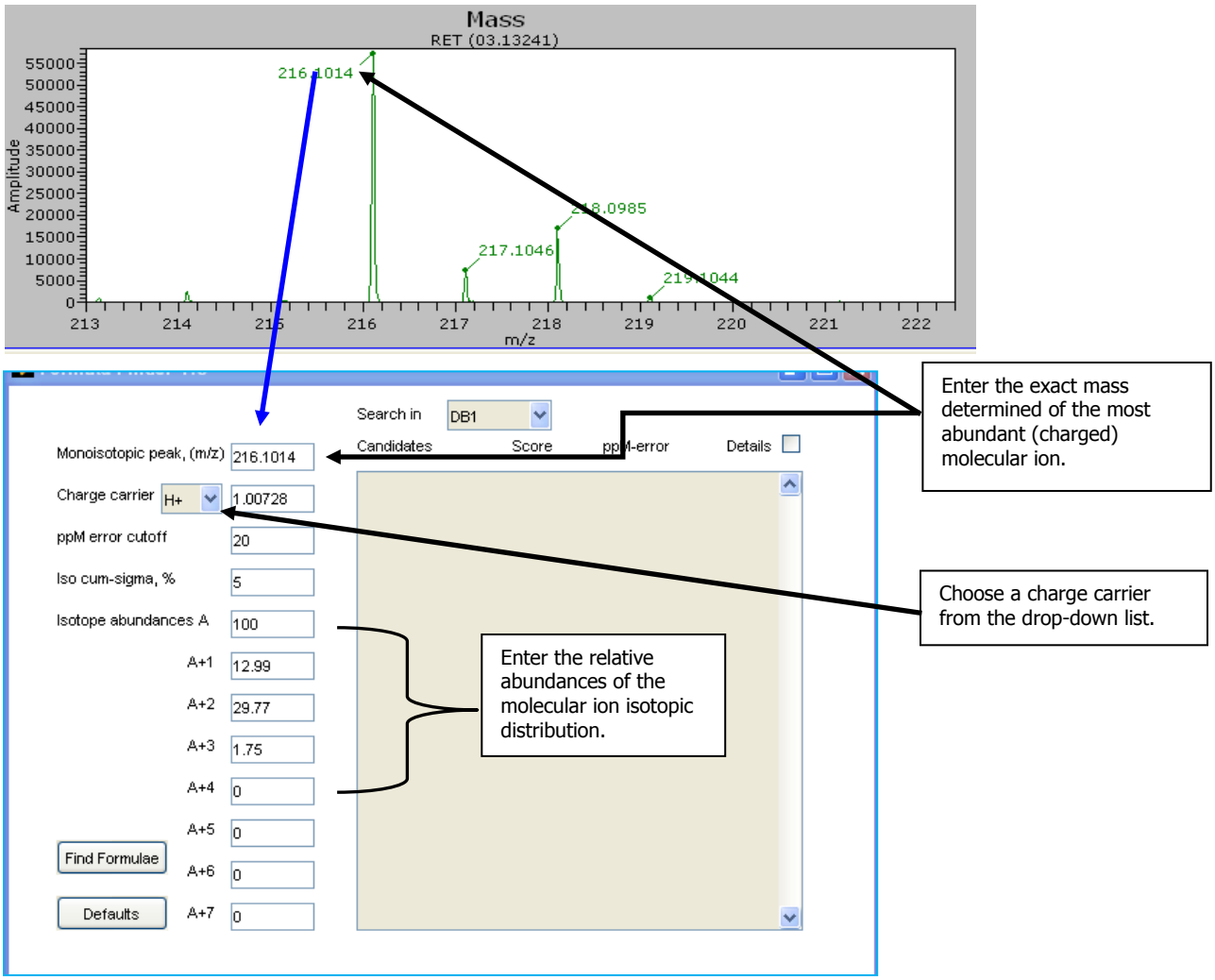
The following example takes you through a simple analysis to show how AxION EC ID can be used to find possible compounds and their structures.

1. Open AxION EC ID by clicking on this icon on your desktop.

The following default window appears:



2. Input the exact mass of the most abundant molecular ion and the observed % ratios of the molecular ion isotopic cluster as measured by the AxION 2 TOF.



In the example above, the exact mass of the ion measured is *m/z* **216.1014** and relative % ion abundance of ions A, A+1, A+2, A+3 are **100, 12.99, 29.77 and 1.75** respectively. The relative ion intensities can be obtained from the peak table associated with the spectrum observed.

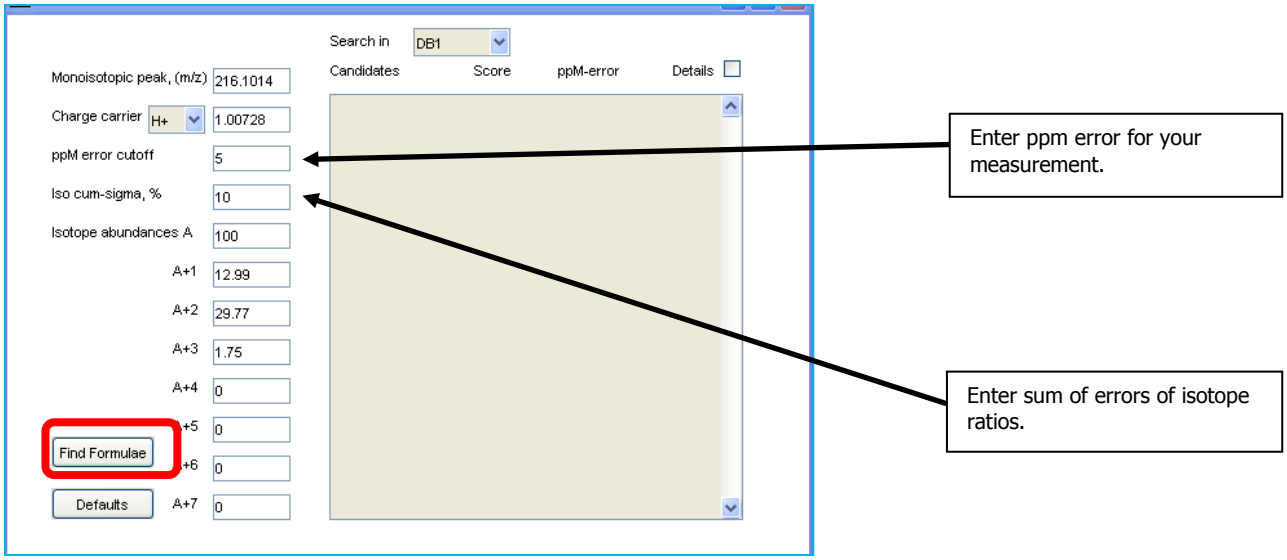
3. Choose a **Charge carrier** from the drop-down list.

   For electrospray or APCI analyses, the charge carrier species typically would be $+(H^+)$ for positive ion analysis and $-(H^+)$. for negative ion analysis.

   Additional **Charge carriers** options will be discussed in a later part of the manual.

4. Enter the expected mass error of your measurement in the **ppM error cut-off** field and **Iso cum-sigma, %** (expected sum of errors of all isotope ratios from measurement).

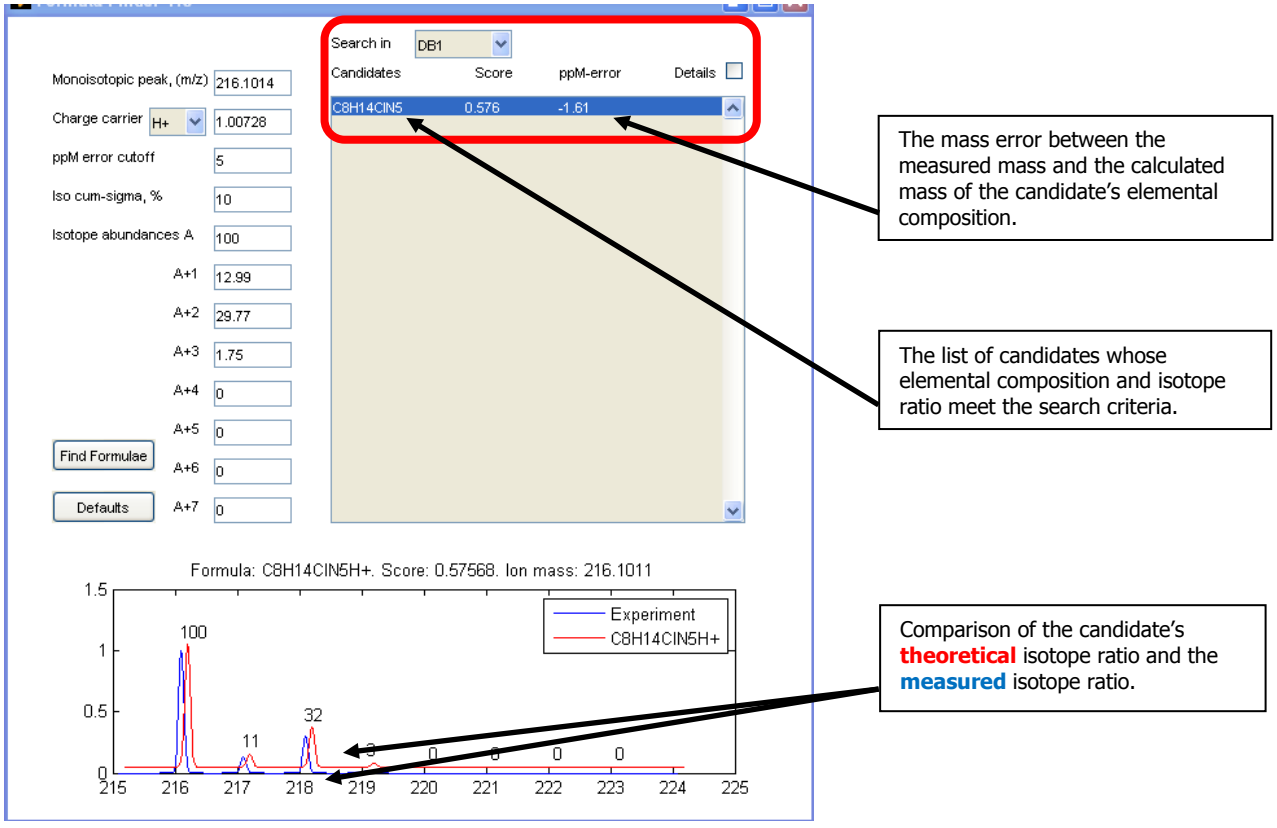
   Typical values for the AxION 2 TOF are about **5 ppM mass error** and 10% **Iso cum-sigma, %** using a default (external) calibration. This type of mass accuracy can be obtained on a temperature equilibrated system that had been pulsing over the acquisition mass range for at least 1-2 hours. However, there are other factors that also affect observed mass accuracy, so please consult the AxION 2 User Manual for additional information. Enter these values as shown below:



5. Click the **Find Formulae** button.

   A window appears with a list of **Candidates** that meet the search criteria for mass accuracy and isotopic abundances.

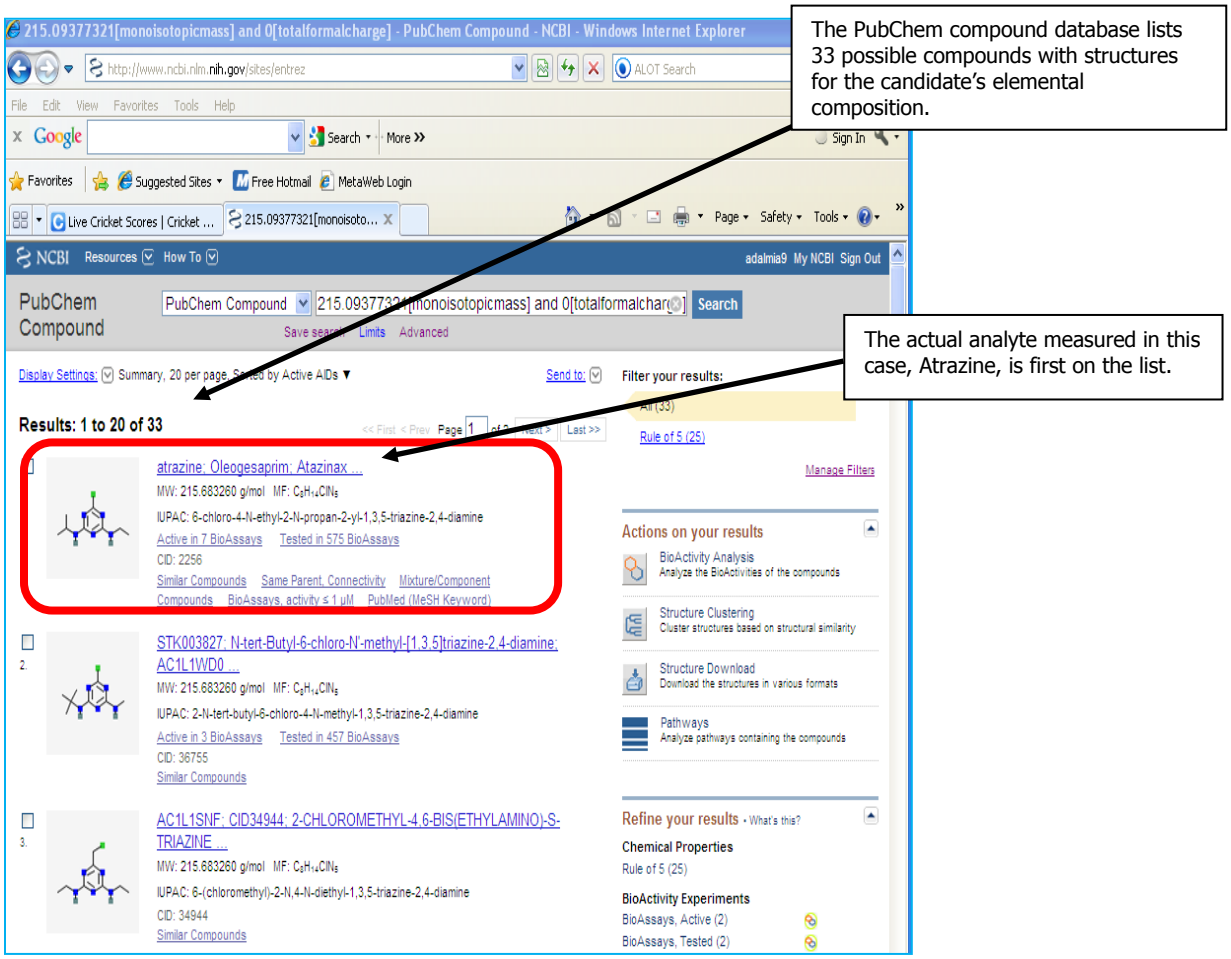
   The window shows for each candidate the **ppM error** (between the measured mass and the expected mass) and the **Score** (from 0 to 1.000) for the given elemental composition. The higher the score, the better the match of the candidate to the unknown.



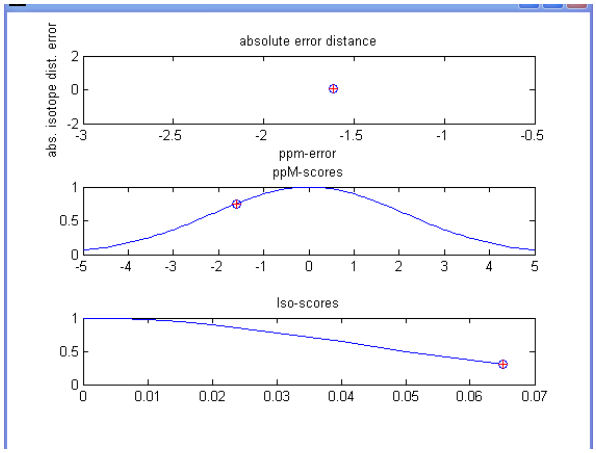
6. To get structures for a given elemental composition, click in the **Details** checkbox, highlight the elemental composition, and click again on it. This opens a new window from the internet browser

containing the **PubChem Compound** database. This window lists the names of all possible compounds and structures with that elemental composition in the database.

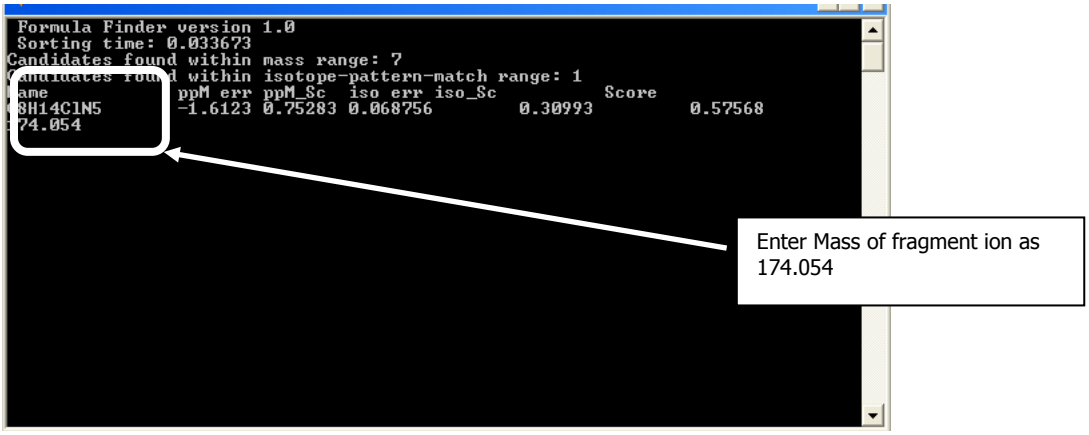
The following example shows a list of 33 compounds having the elemental composition **C8H14ClN5**. The compound analyzed in this case, atrazine, appears first on the list.



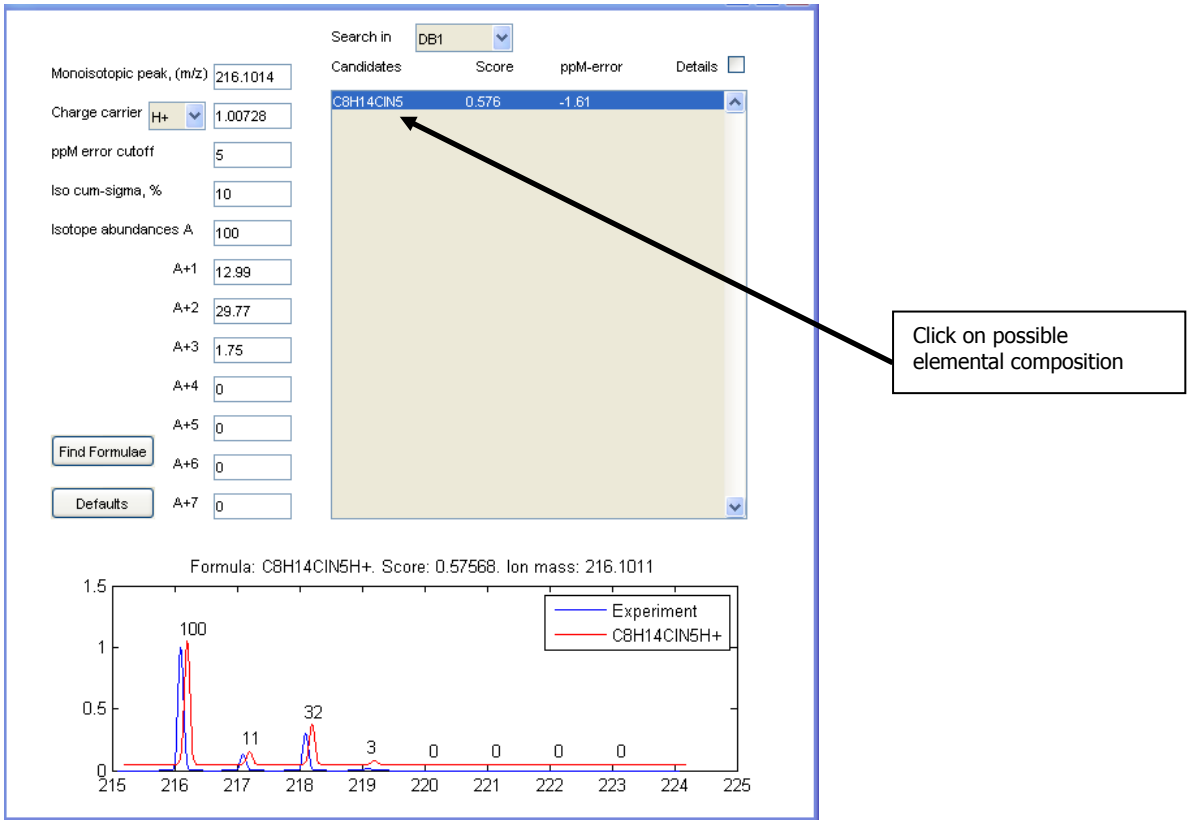
Another window displays with **Formula stat Details** for ppm mass error and absolute isotope distribution error, ppM scores and isoscores.



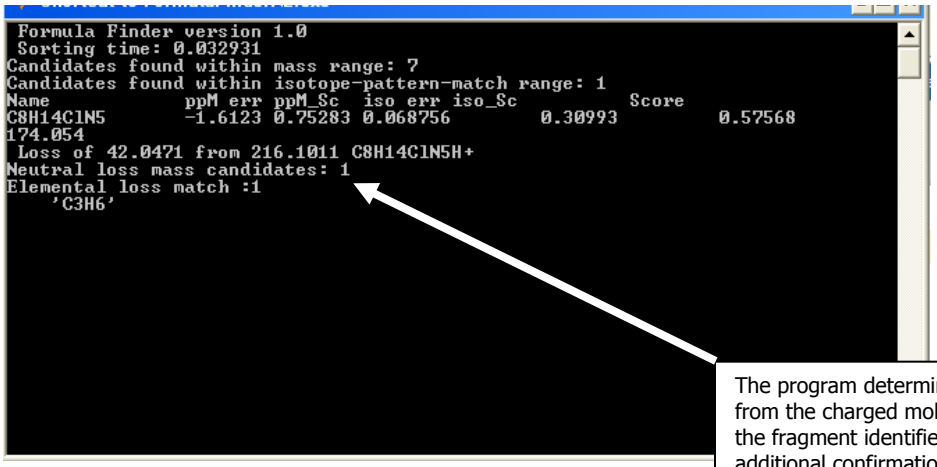
7. If the exact mass of a fragment ion is known, then you can enter it into following window. After typing it, press the **Enter** key to provide further confirmation of the given elemental composition.



8. Click on a given elemental composition in the **AxION EC ID** window to see if this composition can have this fragment as shown below-:



The following window will appear.



The program determines that a neutral loss from the charged molecular ion could yield the fragment identified. This provides additional confirmation of the given elemental composition.

Charge Carrier Options

The software provides you with several **Charge carrier** options. These are available to address the different potential adducts that can be formed during, and ionization processes that can take place in, LCMS analyses. The use of H^+ as a charge carrier has already been demonstrated in the previous example.

For positive ion mode adducts (in addition to H^+), the available charge carriers options are NH_4^+, Na^+, K^+. The charge carrier for a radical cation is listed as "+", which represents the addition of an electron. For negative ion mode analyses, the loss of H^+ was previously mentioned, and the software also provides an option for the charge carrier Cl^-. For other potential charge carriers not listed, such as such as Li^+ in positive mode or formate (or the addition of an electron) in negative mode, the user can simply input the specific mass of the charged species in "Blank" option for charge carrier window. The list of all charge carrier options and their masses are shown below:

Charge Carrier	Mass/Dalton
H^+	1.00728
Na^+	22.9892
K^+	38.9632
+	0.0005
NH_4^+	18.0338
$-H^+$	-1.00728
Cl^-	34.9694
Blank	Input mass for adduct such as formate, lithium or mass of an electron for radical anion

The example below shows the use of a formate ion as a charge carrier during the analysis of vomitoxin by LCMS.

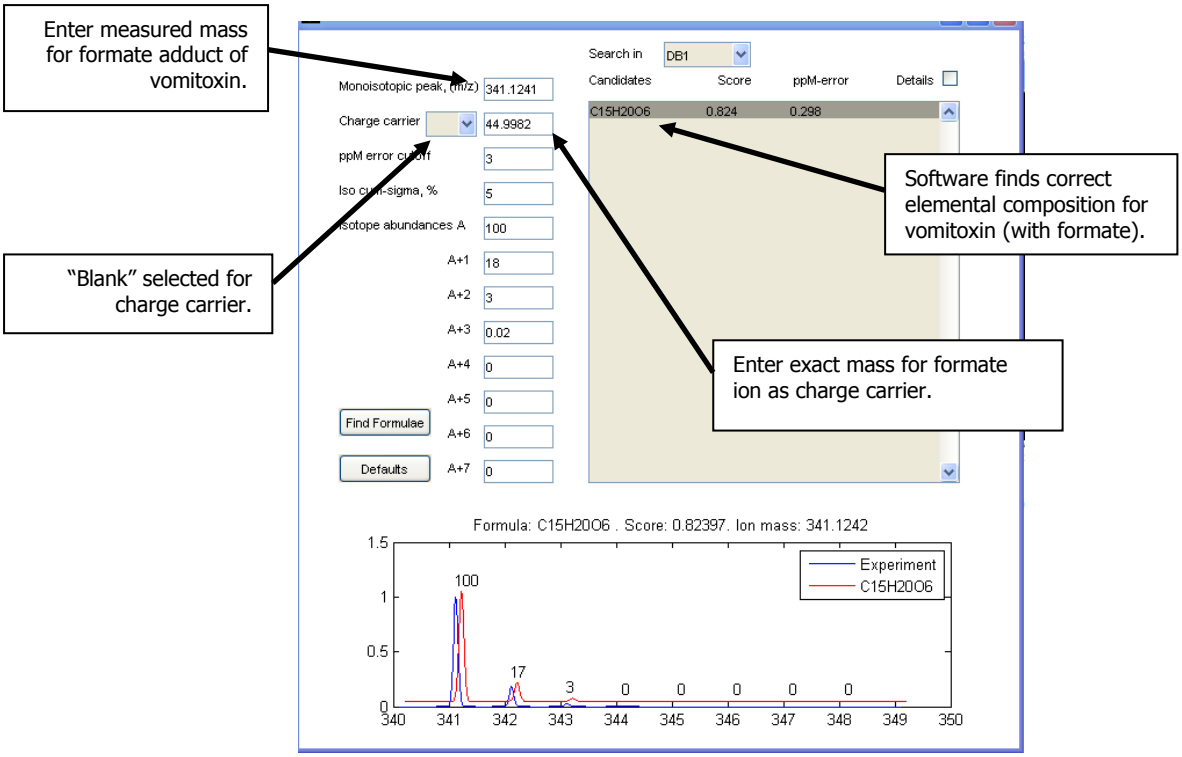
1. Select the "blank" option from the charge carrier drop down menu.

2. Enter **44.99820** u for the mass of a formate ion in the charge carrier input cell.

Since the expectation is that formate is the charge carrier, the normal isotopic profile of vomitoxin would have the addition of formate to each isotope. Consequently, the most abundant, monoisotopic mass (with formate addition) needs to be entered, as well as the observed isotopic ratio. The tolerances for mass accuracy (ppM) and allowed error in measured relative isotopic ratios are also required.

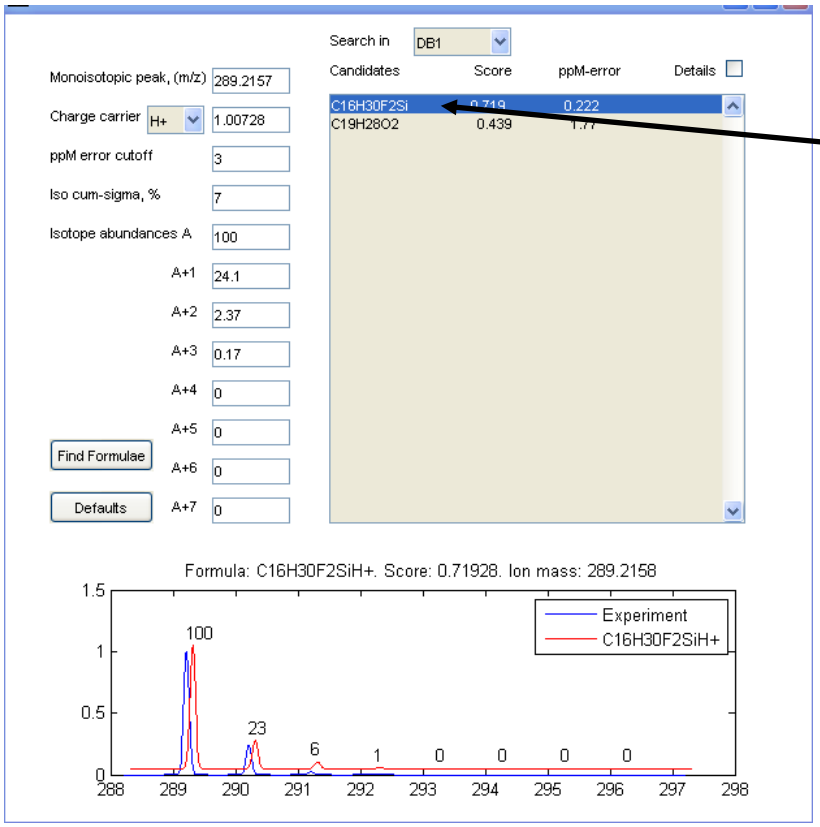
3. Click the **Find Formulae** button.

After entering all information, the window will look like as given below and should provide the right elemental composition for vomitoxin with formate ion addition.



Depending on the measured mass, the ppM error and the sum of errors of relative isotope ratios, the software might identify more than one possible elemental composition. To reduce the number of potential choices, you can use the exact mass of a fragment ion, as demonstrated in the previous example. If this is not possible, you can also eliminate some formulae options by evaluating certain candidates that have elements with somewhat "unique" isotopic distributions.

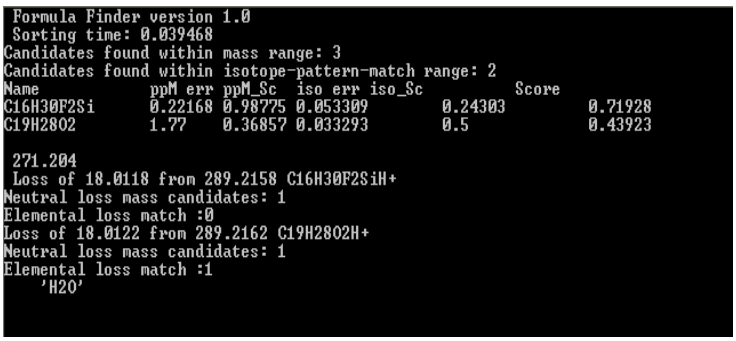
The following example uses data from a testosterone analysis and demonstrates how a specific element with its isotopic distribution can be used to eliminate a potential elemental composition.



The formula with highest score, as a formula candidate, can be eliminated if you know that compound does not contain Si.

Also, if you know the mass of fragment ion, and by following directions described earlier, certain elemental compositions can be eliminated by checking if it could be fragment ion of given elemental composition. You would need to enter the mass of frament ion in the window below and press enter.

Fragment ion eliminated the First formula which was wrong

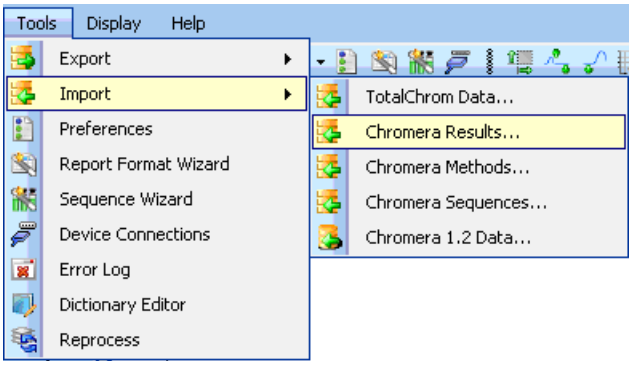


Now if you go back to the previous window, highlight and click the compound, then highlight and click the compound in the secong line. Observe the results displayed in the black AxION EC ID window. This window displays Elemental loss mass candidate number to help you determine which composition to eliminate.

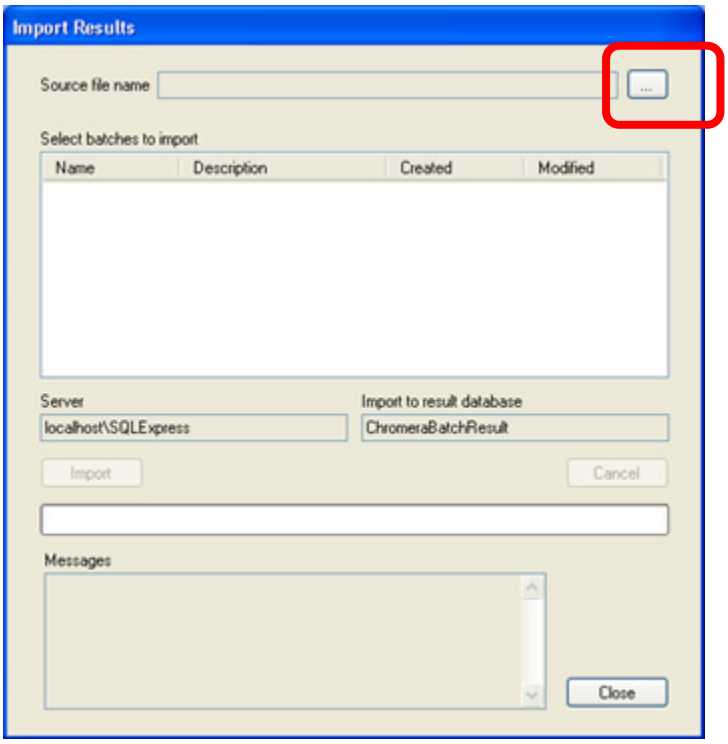
Importing Chromera Data and Methods

To import the data into Chromera:

1. Select **Import** from the **Tools** menu then select **Chromera Results...**

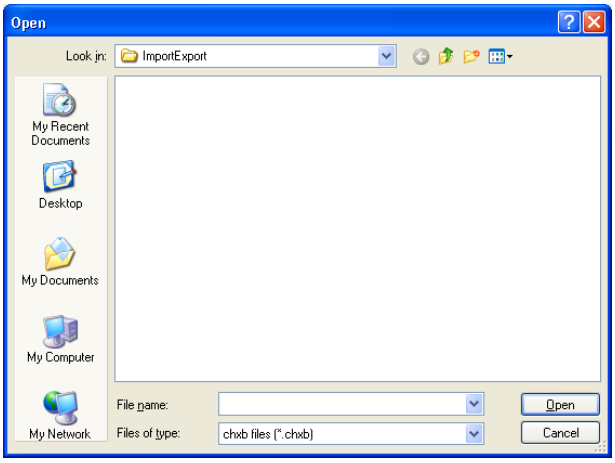


The **Import Results** dialog appears:



4. Click the browse button ... to the right of **Source file name**.

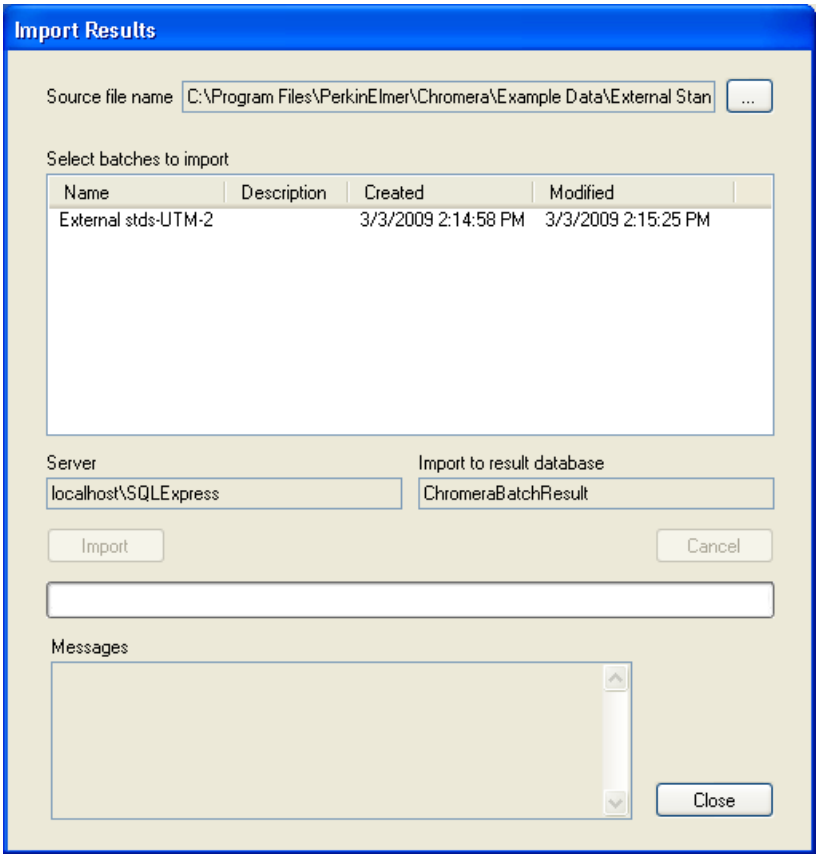
The **Open** dialog appears:



5. Navigate to the directory containing your data.

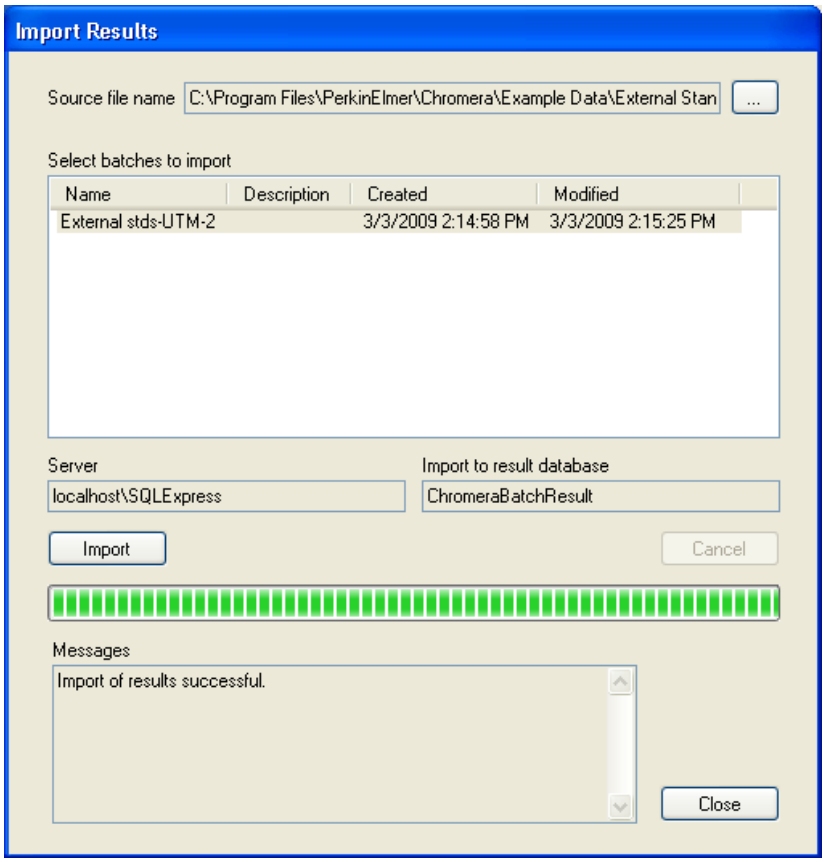
6. Select the **data file** then click **Open**.

   This example shows data file name (**External std-results.chxb)**, that appears in the **Select batches to import** list.



7. Select **External stds-UTM-2** then click the **Import** button.

The progress bar shows the import progress. Upon completion, the message **Import of results successful** appears in the **Messages** box.

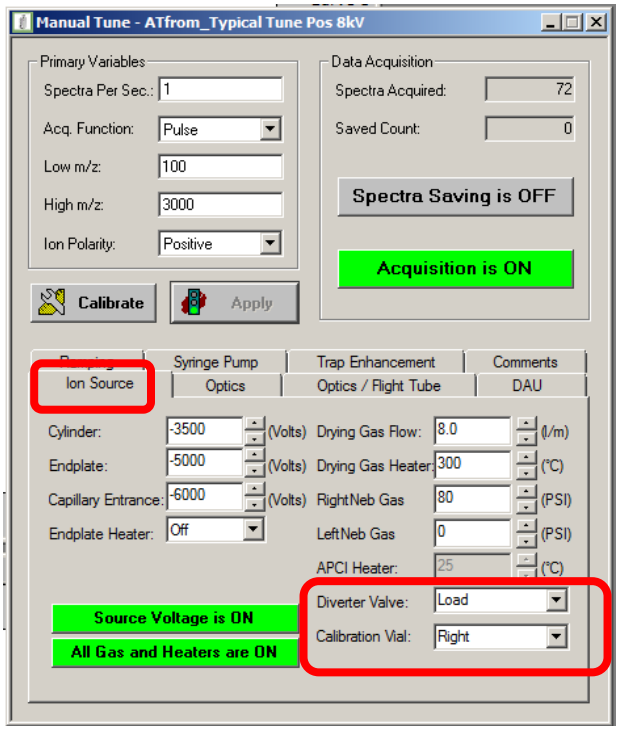


8. Click the **Close** button.

Setting the Calibration Vial and the Diverter Valve

The **Calibration Vial** and **Diverter Valve** are controlled from the **Ion Source** tab in the Tune dialog. To specify settings for these peripherals in the Method, utilize the peripheral settings in the Method Editor.

- Select the function from the **Diverter Valve** or **Calibration Vial** drop-down list you want to perform.



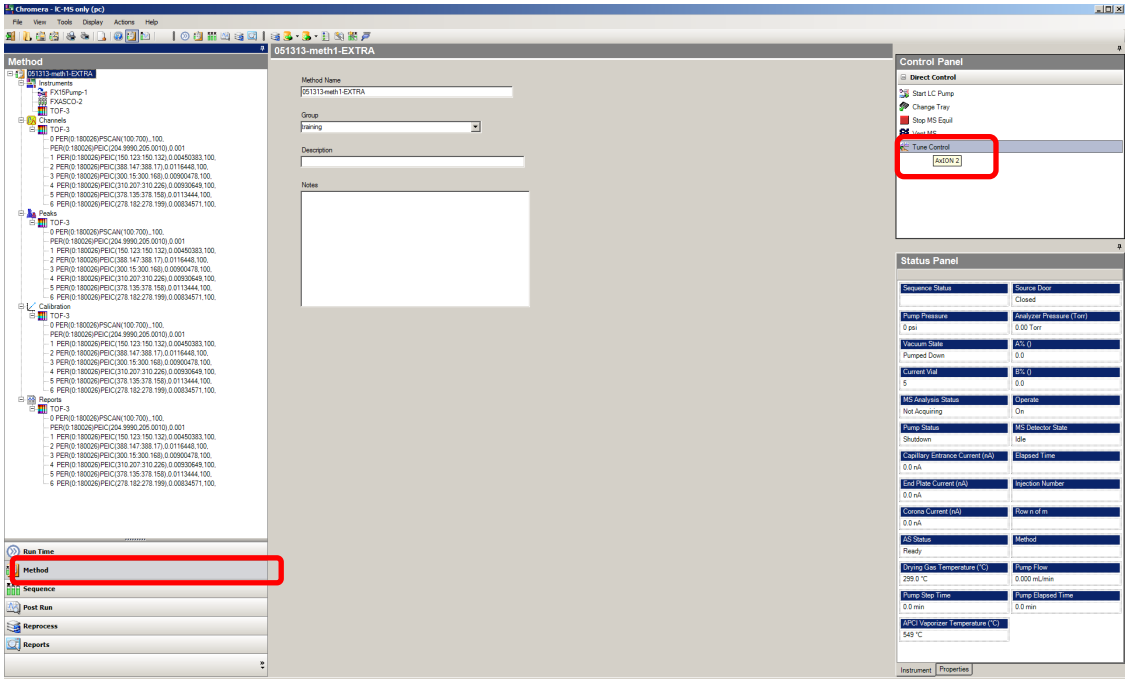
QuickStart Guide for LC-AxION 2 TOF MS Analysis Using "on the fly" Lockmass

1. Prepare Calibration Mix Reagent with Caffeine as described on page 24.

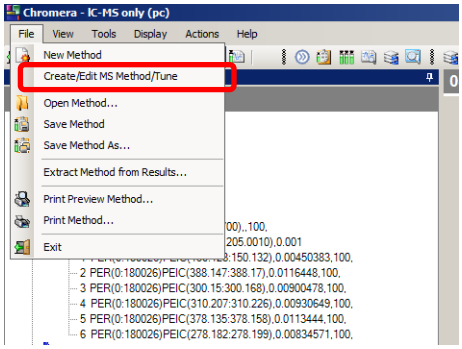
2. Launch the appropriate instrument configuration, then the application, in Chromera Manager.

3. Select the **Method** section. Then Select **Tune Control** in the right-side Control Panel. This allows TOF MS Driver software to take control of AxION 2 TOF.

   Chromera will not control the TOF instrument until the TOF MS Driver application is closed and, in Chromera, **Tune Control** changes to **Stack Control** in the meantime.

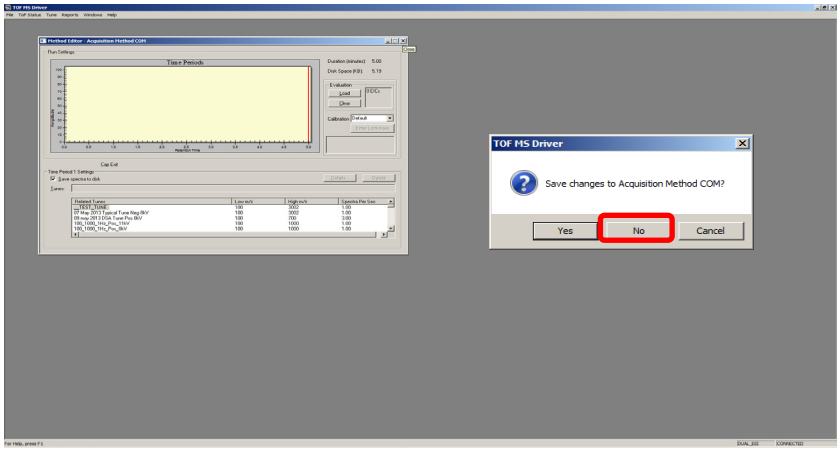


4. In Chromera select **File**, then **Create/Edit MS method/Tune**. This opens the TOF MS Driver application.



5. Minimize Chromera or select the icon in the Windows Taskbar to maximize the TOF MS Driver application.

The Application automatically displays the Method Editor with Acquisition Method COM loaded. Close this method without saving.



6. Select **File** then **Open Manual Tune**. Select then **Apply** the appropriate Tune for the work being done. Check the Mass Range and ensure the Acquisition Rate (labeled **Spectra/Sec**) is between 3-5. A 3 GHz acquisition rate is more than sufficient as the UHPLC peaks are wide enough (3-4 secs) that sufficient data points are collected over the peak.

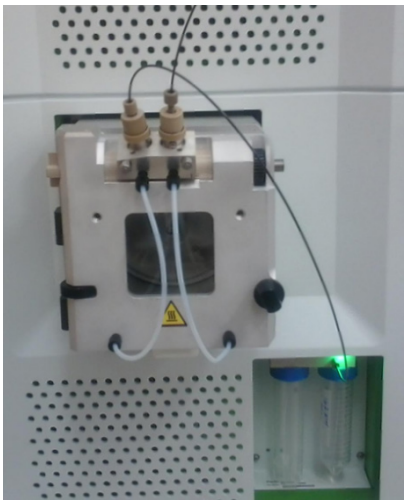
7. In the **Manual Tune** window, turn on the **Calibrant Vial** or **Syringe** and also turn on **Acquisition**.

8. Once the signal appears steady, select **Calibration** then **Configure** from the main menu. Select the desired calibration points (at least 4) and calibration settings. Execute Calibration and save it if acceptable. See section **Default Calibration** on page 36 for detailed instructions.

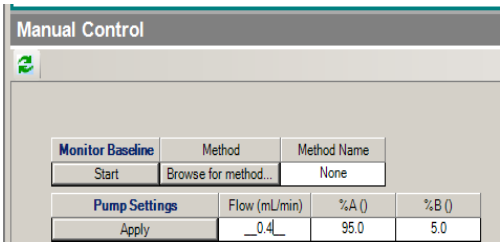
9. In the manual tune make the following updates for lockmass and save the tune. These changes adjust for HPLC flow of 0.4 mL/min to aid in de-clustering and desolvating the molecules:
    - Increase the **Drying Gas Heater** to 350-400 °C
    - Change the **Drying Gas Flow** to between 12-14 L/min

NOTE: *It is not necessary to set these values back to previous to recalibrate. This is a one-time adjustment to the tune.*

10. Move the calibrant inlet to the left probe of the Dual ESI ion source and connect the LC effluent to the right probe of the source.



11. Without closing the TOF MS Driver, navigate back to Chromera and start the LC pump. Flow rate should be set to between 0.4 and 0.5 mL/min.



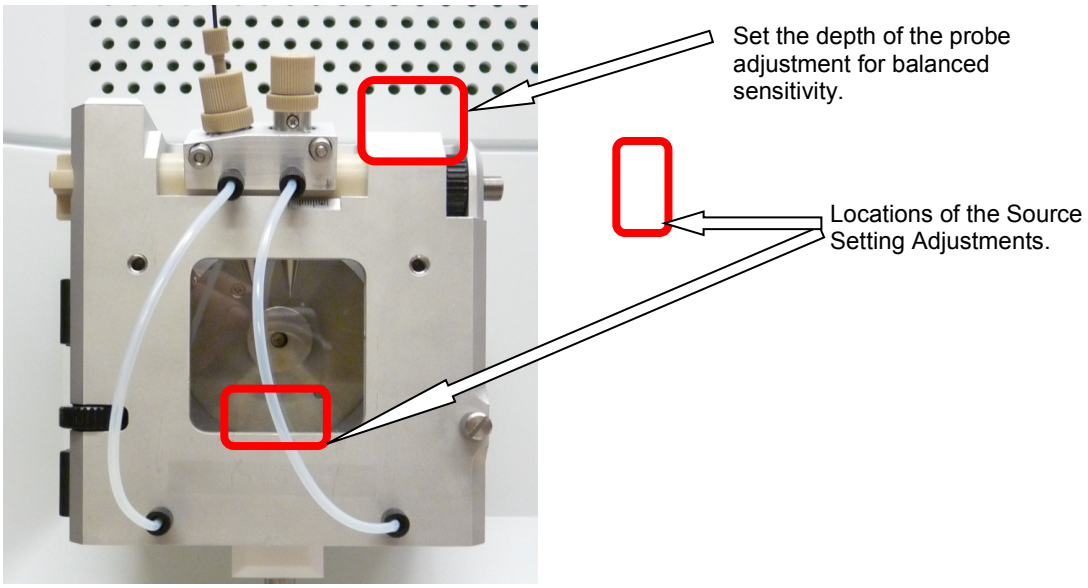
IMPORTANT: *Please do not exceed 0.4-0.5 mL/min especially when using a highly aqueous solvent. Greater than recommended flow will result in condensation on end plate. Even using the highest setting on drying gas and source temp the condensation can be quite high. Higher flow rates also diluted the calibrant solution and may make it impossible to get sufficient counts in each spectrum for successful lockmass.*

12. Without closing Chromera, navigate back to TOF MS Driver and confirm the lockmass calibrant ions are of sufficient signal counts with the HPLC flow also active.

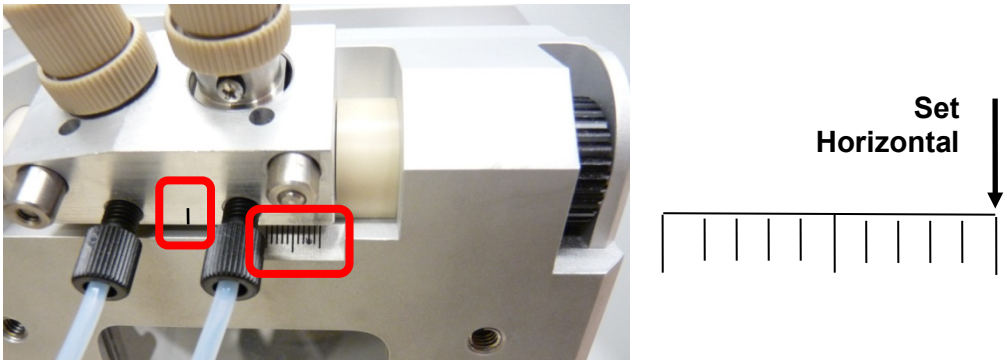
13. Adjust the positions of the dual sprayers to get the best signal of the analyte and the lockmass calibrants.

    a. The calibrant ions 195.0876 (from caffeine) and 622.0290 are used as lockmass ions for this particular example. 118.08625 and 922.00979 can also be used as lock mass calibrants instead, however.

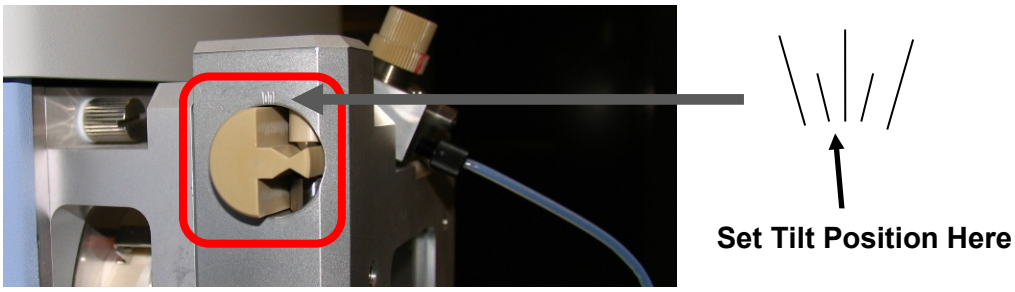
    b. The goal of these adjustments is to ensure the counts on the lock mass calibrants are at least 3000 or greater (as high as 10-20,000 is acceptable). The peak shape is also important i.e. no peak splitting should be seen on top of peak so that centroid of the peak is correctly assigned. Peak splitting usually occurs if the counts are too low.



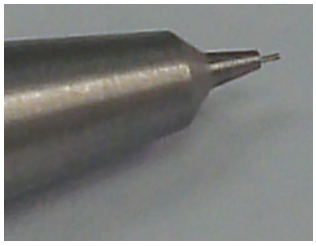
c. The "x" position refers to the horizontal movement of the probes. The position of the probes relative to each other are fixed hence adjusting one probe adjusts the other probe automatically in the "x" direction. The mark on the moving block (on the right side of the right probe) should be roughly between 10 and 11 of the "x" scale.

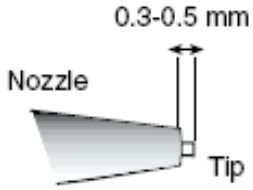


d. The "y" direction refers to the tilt of the probes. With the HPLC flow of 0.4 L/min, the tilt is usually kept in the middle of the scale. The "y" position along with the drying gas heater temperature and drying gas flow prevent condensation of the HPLC solvent on the end plate. The "y" position can be tilted closer towards the entrance (instead of leaving at the middle position) to get better signal as long as there is no condensation of liquid on the end plate.



e. The "z" position refers to the capillary inside of the probes. Each probe can be moved in and out. The "z" positions of both probes need to be adjusted so that the signal of the lockmass ions is the highest for the established x and y positions. The plumes generated from both probes interact and adjusting the z position will affect (suppress or occasionally enhance) the signal of the lockmass ions. Adjust the right probe first then the left probe while watching the screen for best signal of lockmass ions.





NOTE: *The process of adjusting the "z" position may have to repeat this a couple times to establish the best position. Once this is done you do not have adjust again unless the signal for the lockmass ions is poor even after x and y positions have been adjusted.*

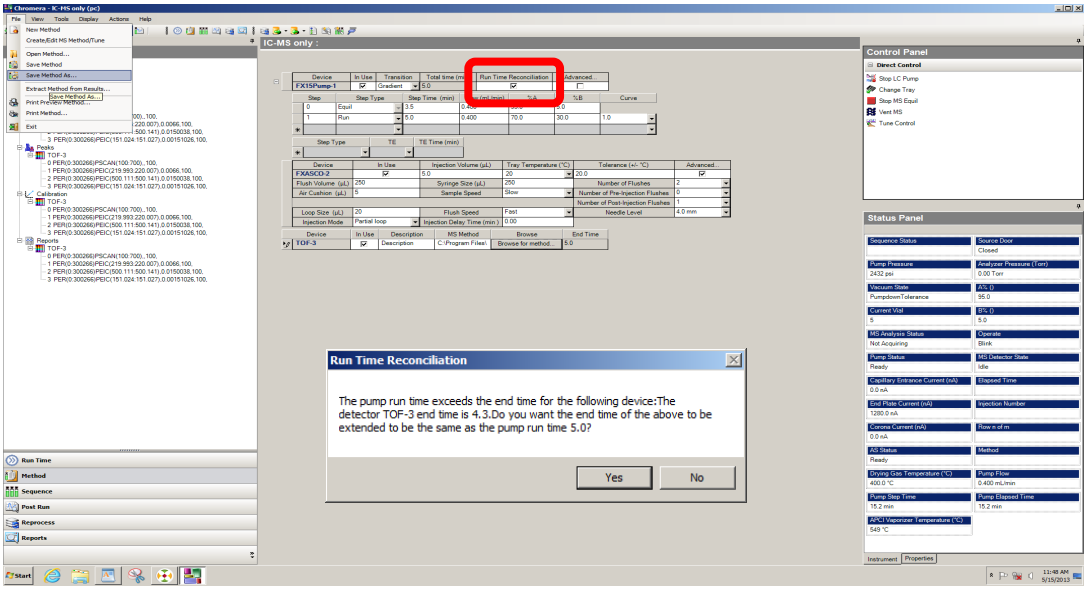
14. Perform a final check of the Lockmass ions for sufficient counts and acceptable peak shape. Ensure the tune file is saved and then close it.

15. From the main TOF MS Driver menu select **New Acquisition Method**.

16. Configure and save the method, associating the tune that was just used in the previous steps. Refer to the section titled **Creating an MS Method**, starting on page 60. Be sure to include the lockmass parameters in the method. The lockmass ions entered are to the accurate 4th decimal place with a search span of 50 mmu.

17. Close TOF MS Driver and navigate back to Chromera.

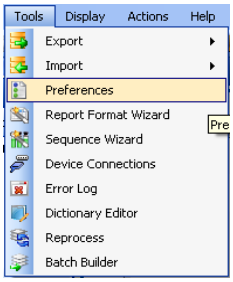
18. Create a new Chromera Method. Refer to section **Creating a Chromera Method**, starting on page 68. Ensure the LC run time configured matches the *desired* total time in the TOF acquisition method and that **Run Time Reconciliation** is checked.



19. Save the Chromera method with a unique name and associate it to a group. Reconcile the TOF method time when prompted.

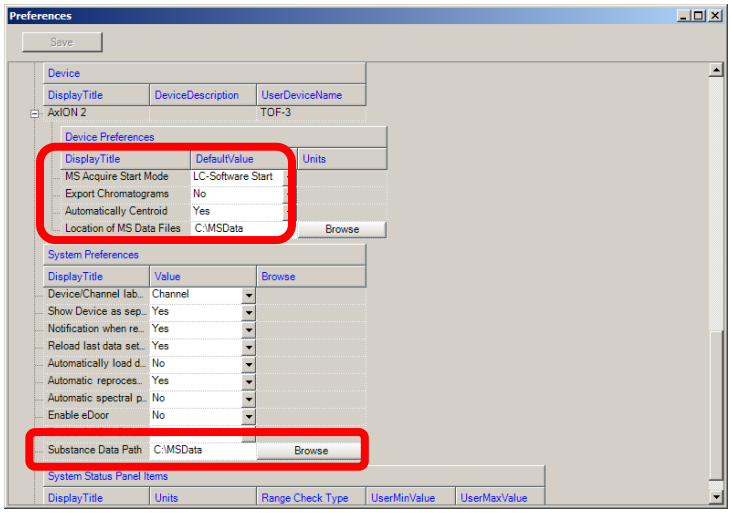
20. Update the settings in Chromera so centroiding is automatically performed on each data file after it is collected.

    a. Under **Tools** select **Preferences**.

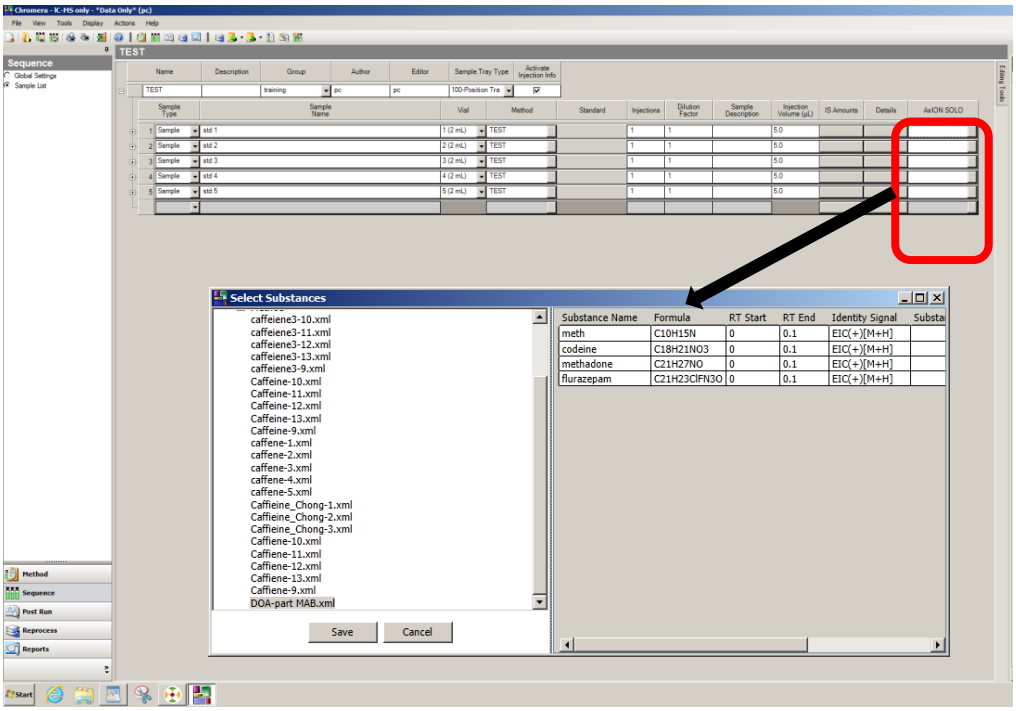


    b. Enable the centroiding function in the AxION2 subsection by selecting **Yes** for **Automatically Centroid**.

    c. Optionally, the incorporation of a substance list in the data files (tofline) for use in AxION SOLO can be enabled. Select **Yes** for **Enable AxION Solo/XPO**. The path for locating the substance list also needs to be specified in **Substance Data Path**. The actual substance list applied is defined per run.

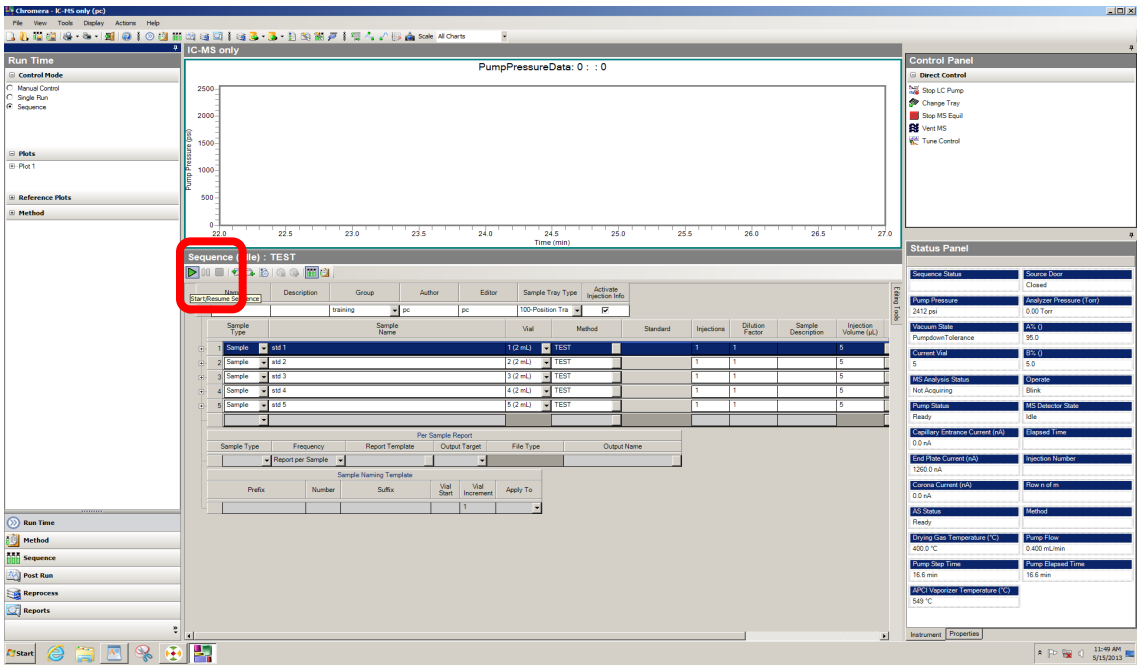


21. Create and save a Sequence using the Chromera method that was just created. Refer to the section titled **Creating a Chromera Sequence**, starting on page 72.



- **Optional:** Embed a target list in data files for analysis by AxION SOLO. For each run definition, select the AxION SOLO button and navigate to the directory where the substance list xml file is saved. Open the substance list and save. Fill down the column for rest of the samples. This sequence can be saved and then analyzed. The centroided data files embedded with the xml target list when opened in solo will have the target list associated with them.

22. Open the sequence to run. Refer to the section titled , starting on page 79. Start the run by selecting the green "play" button.



PerkinElmer
710 Bridgeport Avenue
Shelton, CT 06484-4794, U.S.A.

Internet: http://www.perkinelmer.com
email: info@perkinelmer.com