HUMAN HEALTH

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CHROMERA / FLEXAR SQ 300 MS USER'S GUIDE

Chromera® Chromatography Data System

PerkinElmer®

Chromera and Flexar SQ 300 MS User's Guide

Release History

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Introduction

The **Flexar SQ 300 MS** combined with **Chromera** provides fast and reliable analysis of chemical samples by liquid chromatography mass spectrometry. The system consists of the Flexar SQ 300 MS, LC Pump, and Autosampler; and allows the addition of a column oven and alternate detector, such as a UV/VIS, fluorescence or PDA if desired. These components are connected to a computer running Chromera software that controls procedures and evaluation of results which runs under Windows.

This guide is intended to provide an overview of the workflow to run a SQ 300 MS analysis using Chromera. Before beginning, the SQ 300 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. The screen layout consists of a Navigation pane on the left that contains buttons and tree lists to select various views within the software.

Starting

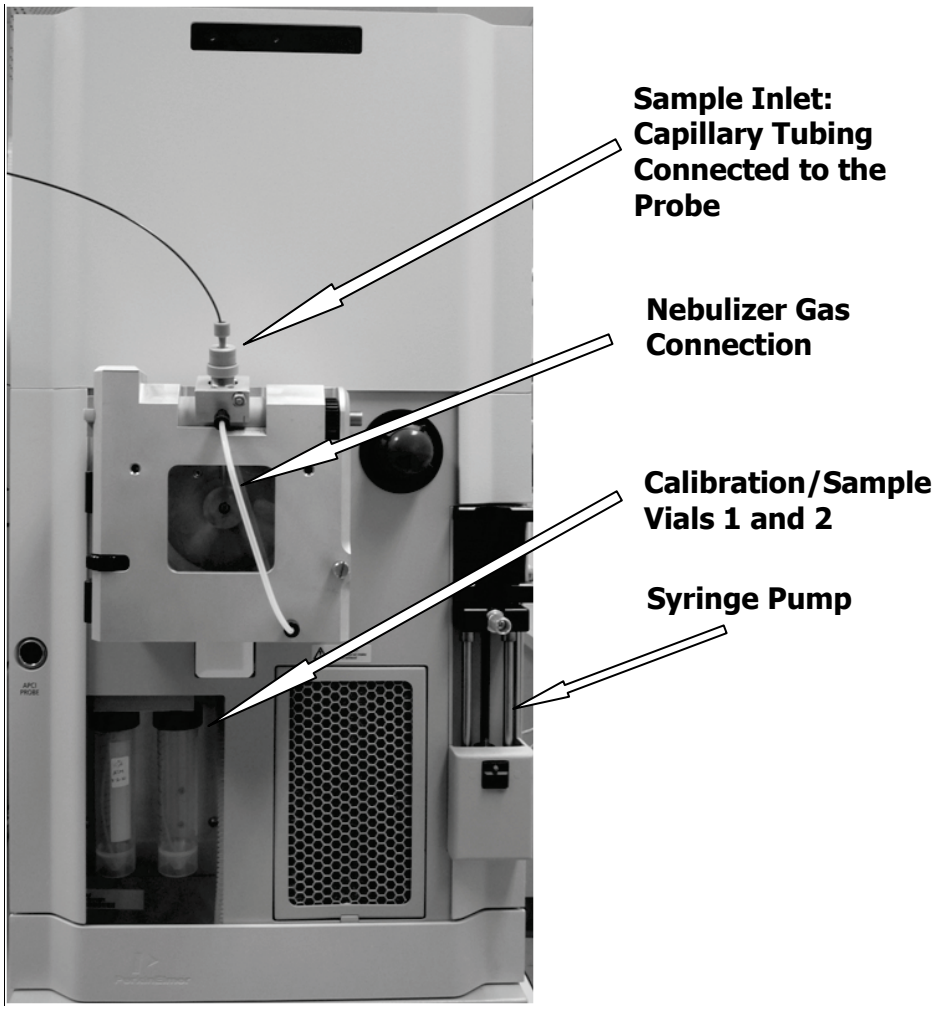
Overview

This document provides basic operating instructions for the Flexar SQ 300 MS instrument including start-up, introduction of samples, data acquisition, and shutdown.

The start-up instructions provided in this chapter assume that Chromera and the SQ 300 MS Driver software, the Flexar SQ 300 MS instrument, and the PC have been correctly installed by a representative of PerkinElmer.

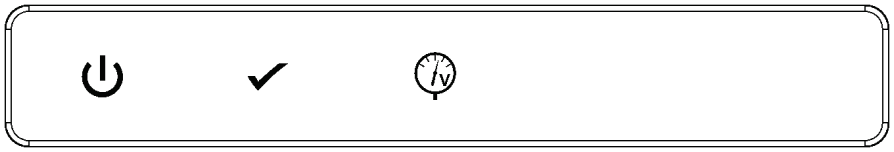
NOTE: *When planning analyses, bear in mind that the instrument needs a minimum of 12 hours from initial installation power-on to establish the required vacuum. However, after venting for routine maintenance, allow 1 hour after pump down and HV activation to allow equilibration of all electronics prior to performing analyses.*

Capillary tubing delivers sample infusion to the probe. Sample can be delivered from an LC system or from the syringe pump.



Flexar SQ 300 MS Status LEDs

The Flexar SQ 300 MS detector status is displayed on the upper front panel through the following indicator lights.



Power On LED	Ready/Error LED	Vacuum LED
Green	Green/Red	Green/Red

LED Functionality

Power On LED	Instrument Status
OFF	OFF
ON (green)	ON

Ready/Error LED	Instrument Status
OFF	OFF
ON (green)	Ready/Running – No Error
ON (red)	Error

Vacuum LED	Instrument Status
OFF	OFF
ON (green)	At vacuum
ON (red)	Not to vacuum

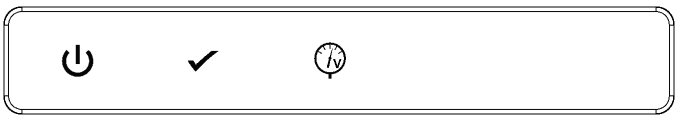
Starting the SQ 300 MS Detector

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 Flexar SQ 300 MS system:

1. Switch the **Power ON/OFF** switch to position **On** (located on the right side panel). The **Power** lamp should light.

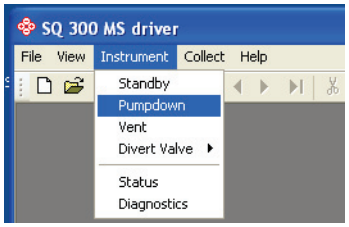


   Make sure the power switch on the roughing pump is on so that the Vacuum System will start when Pumpdown is selected in the SQ 300 MS driver.

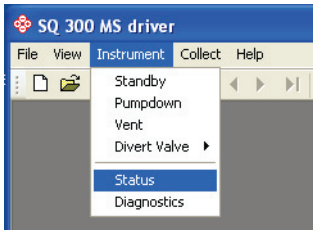
NOTE: *Do not switch the electronics on at this stage.*

2. Switch on power to the PC and login.
3. Check that the fans are operating. A cooling air flow should exit at the bottom of the instrument.
4. Double-click on the **SQ 300 MS driver** icon on the desktop.

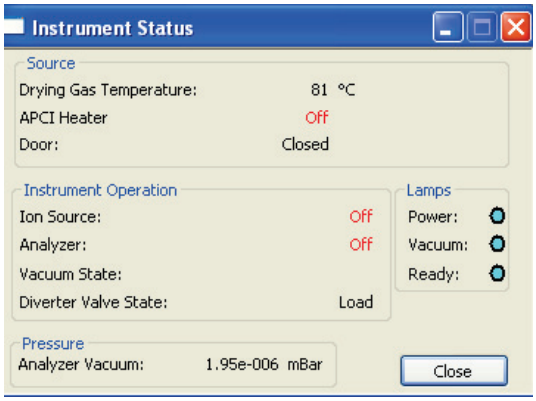
5. Select **Pumpdown** from the **Instrument** menu.



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



7. 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.*

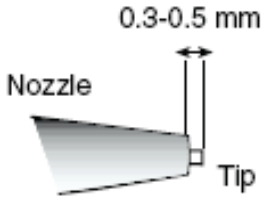
Checking the Position of the ESI Probe

1. Observe the ESI probe through the inspection window and align to the center of the Capillary: loosen the lock ring and move the manifold by turning the position adjustment screw. Tighten the lock ring.

2. 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.

3. Loosen the lock ring.

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



5. 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 you have already configured a system in Chromera Manager you can skip this section.

Creating a System Database

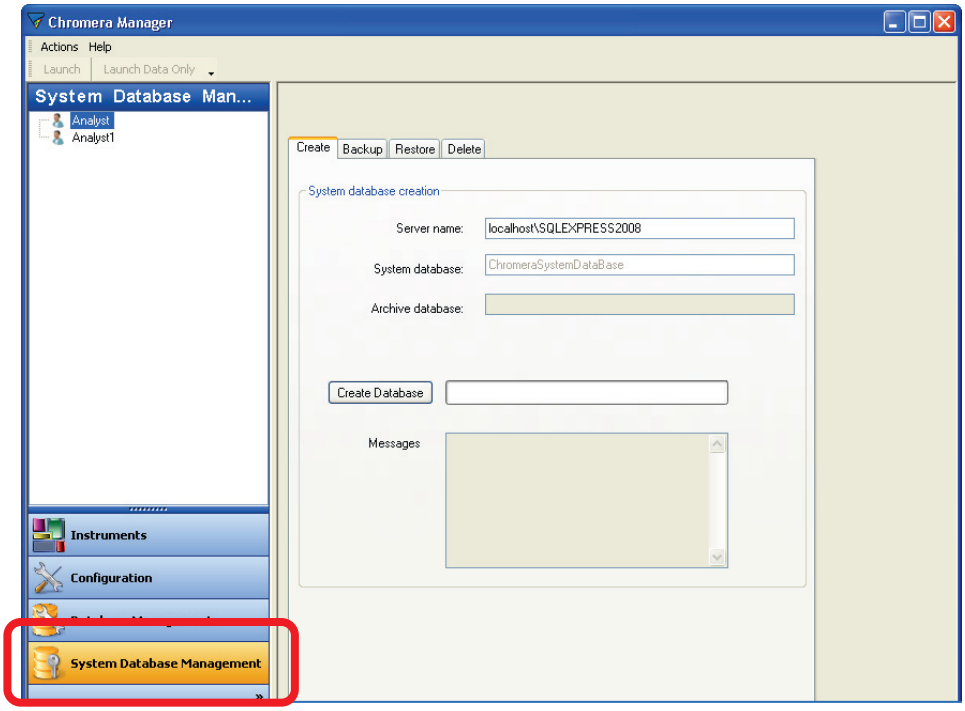
The first time you install Chromera you must create a System Database.

To create a System Database:

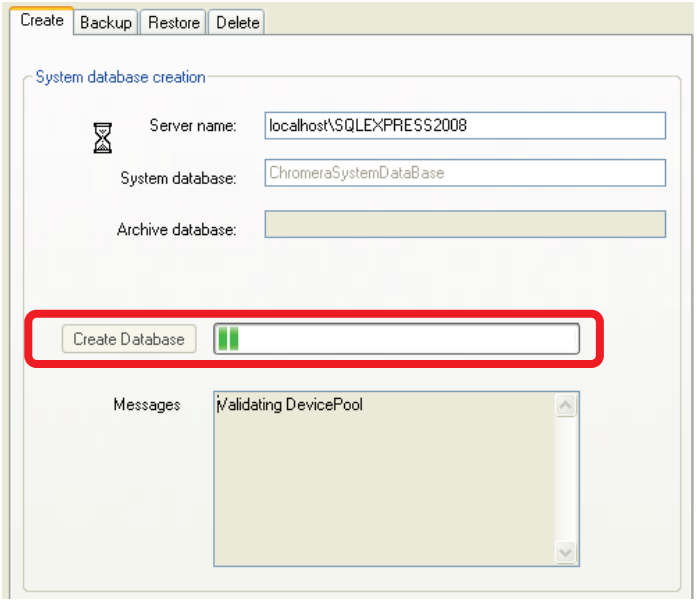
1. Create a **Chromera Manager** shortcut on your desktop.
   Click the Windows **Start** button, then click **All Programs**, locate then right-click on **Chromera Manager,** then select **Send To > Desktop** (create shortcut).

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 SQ 300 MS in a system.

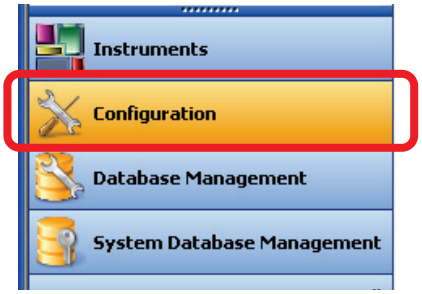
Creating an LCMS Instrument

NOTE: *Prior to creating an Instrument Configuration, make sure all cables are connected between all devices and the Edgeport box (except for the SQ 300 MS Detector and the Flexar PDA, since these devices require Ethernet cable connections).*

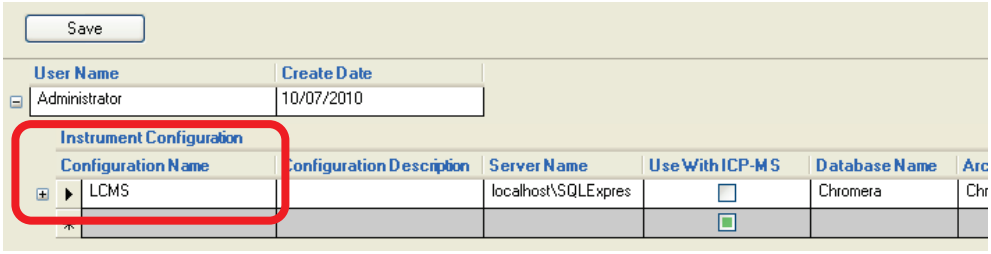
To create an LC instrument:

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

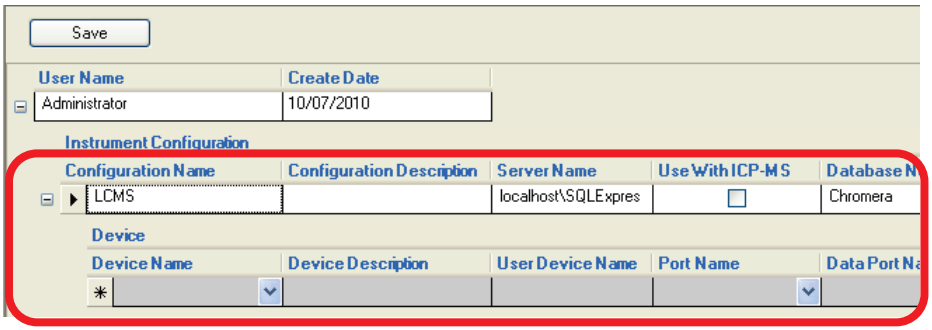
to display the initial **Configuration** screen.



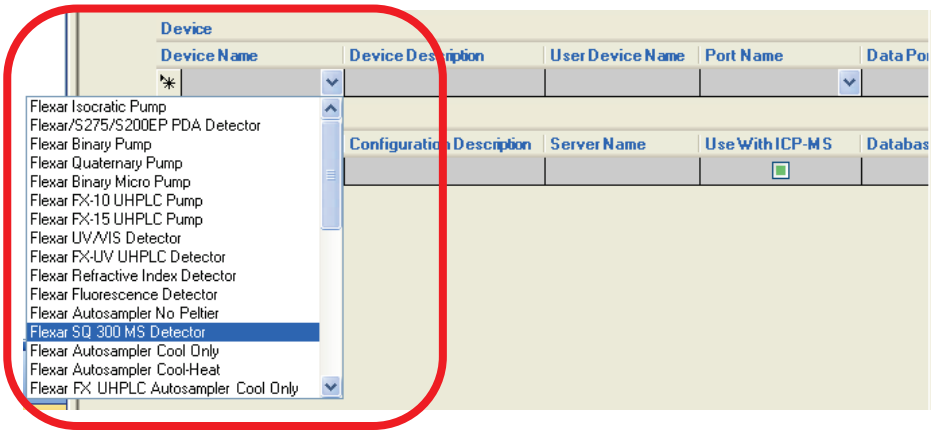
2. Under the **Instrument Configuration** row, click in the box under **Configuration Name** and type an instrument name (this example shows that **LCMS** was typed), then press the **Enter** key.
   A ⊞ next to the row with the name displays.



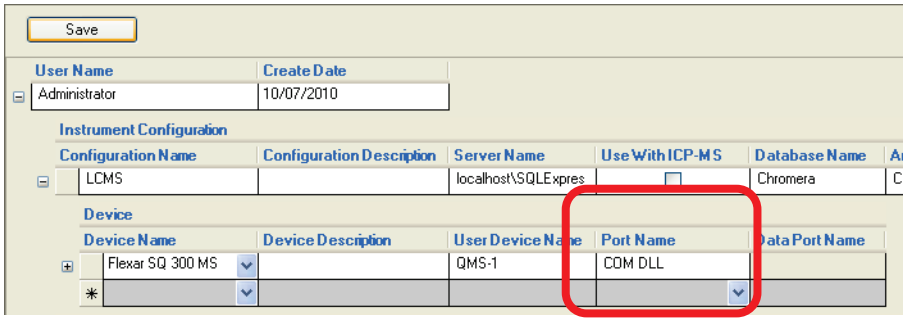
3. Click on the ⊞ and the **Device** row displays.



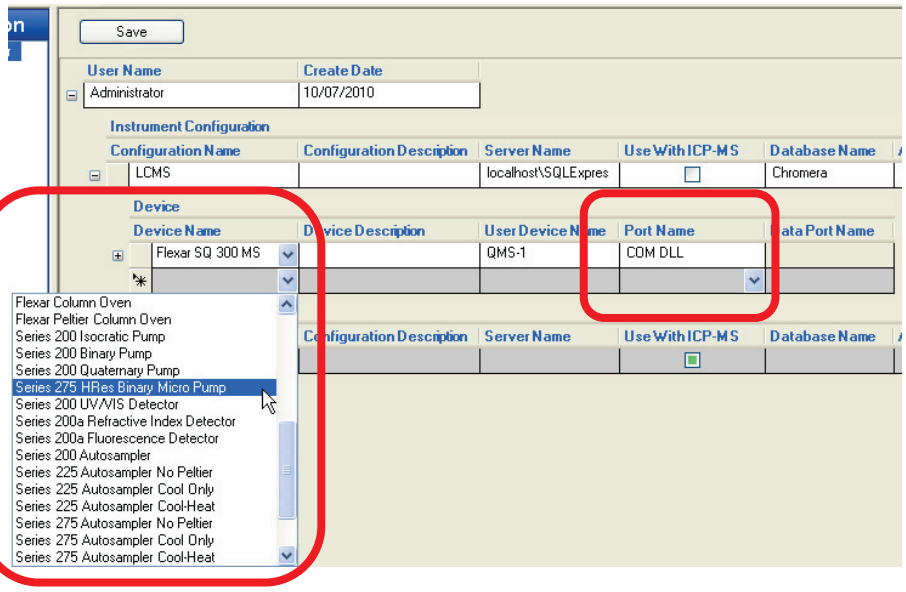
4. Click on the drop-down button in **Device Name** box, and device choices appear. Select the appropriate devices (modules) for the **Instrument** you are creating.
   In this example, select **Flexar SQ 300 MS Detector**.



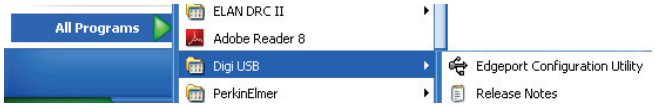
   The **Flexar SQ 300 MS** Detector automatically fills in the **Port Name** field with **COM DLL**.



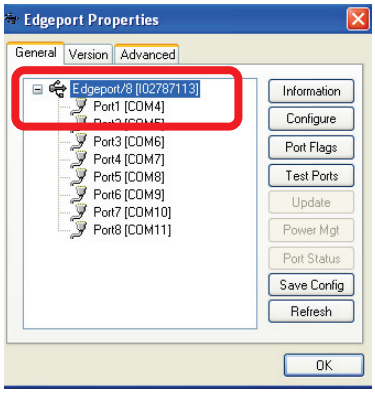
5. Select your LC Pump from the **Device Name** drop-down list.
   In this example, the **Series 275 HRes Binary** pump.



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**.

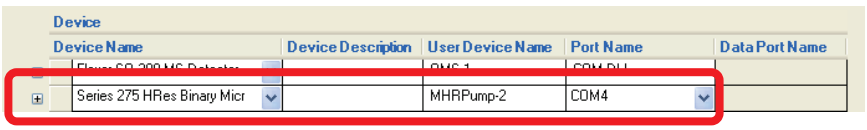


7. The **Edgeport Properties** dialog displays. Click the plus sign  to display a list of the physical Ports (1 – 8) on the Edgeport with the corresponding COM port numbers.



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.

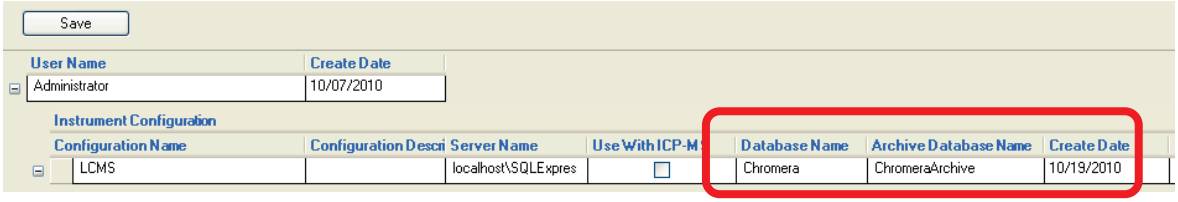
9. Select **COM4** from the **Port Name** drop-down list.
   If your LC Pump is plugged into Port 1 on the Edgeport the corresponding COM port is COM4 as shown in the Edgeport Properties dialog above.



10. Select your LC Autosampler from the **Device Name** drop-down list.
    In this example it is the **Series 225/275 autosampler**.

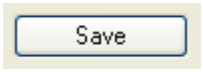
11. Select the **Port Name** for the autosampler.
    If your autosampler is plugged into Port 2 on the Edgeport the corresponding COM port is COM5.

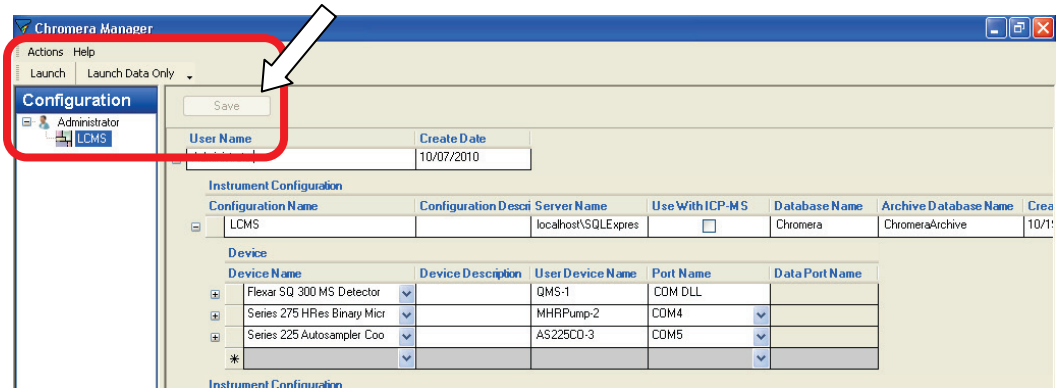
12. Observe the **Database Name** fields. This is where you define the database names.



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.

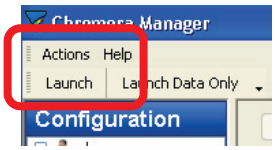
13. When all instrument components have been defined, click the **Save** button located at the top of the screen.



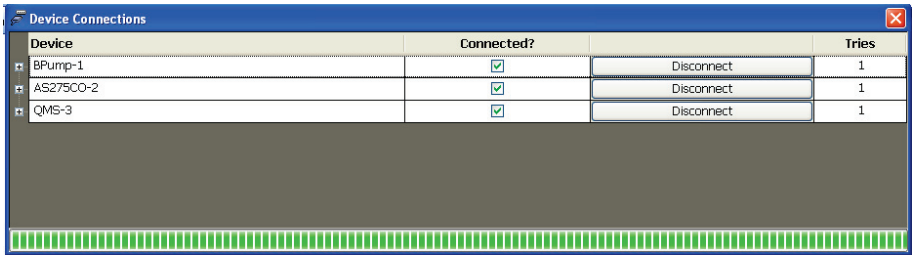


Once you save, the **Configuration Name** (in this example **LCMS**) displays in the **Configuration** pane.

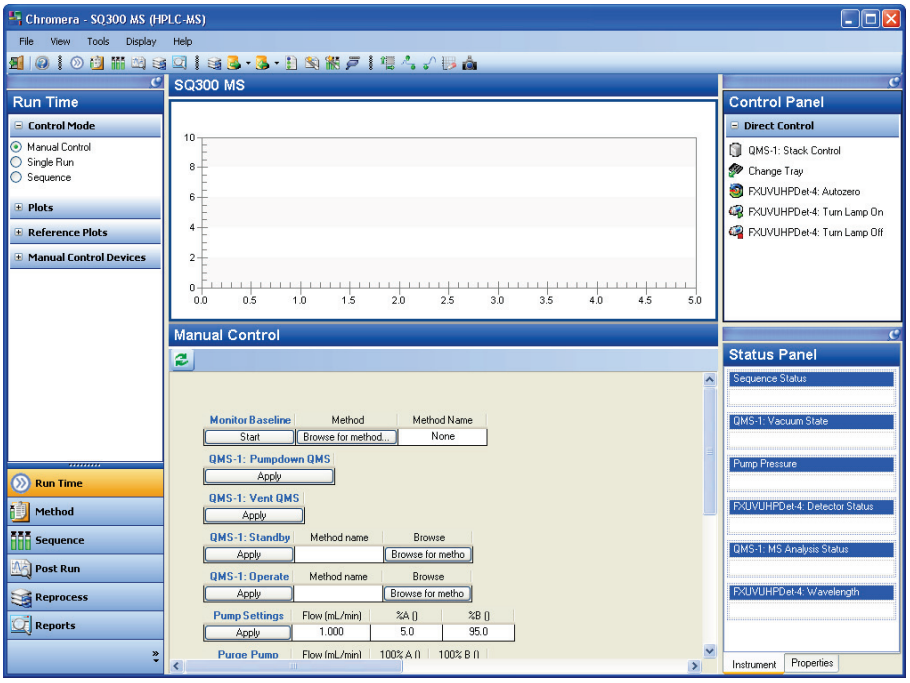
14. Click **Launch** to launch Chromera.



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



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

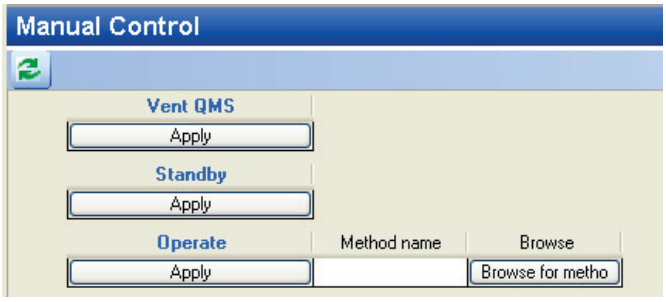


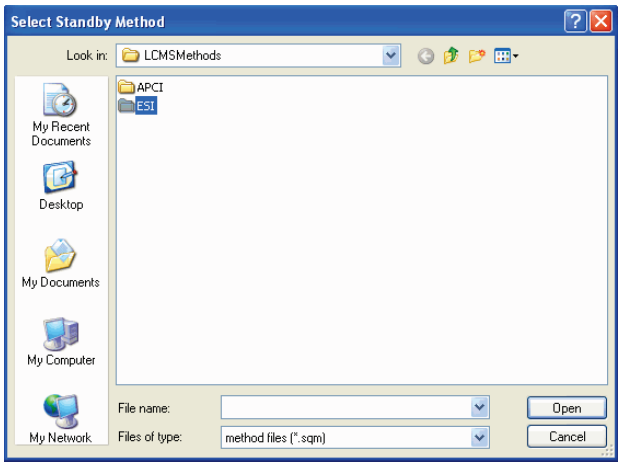
Setting the Operate Method

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.*

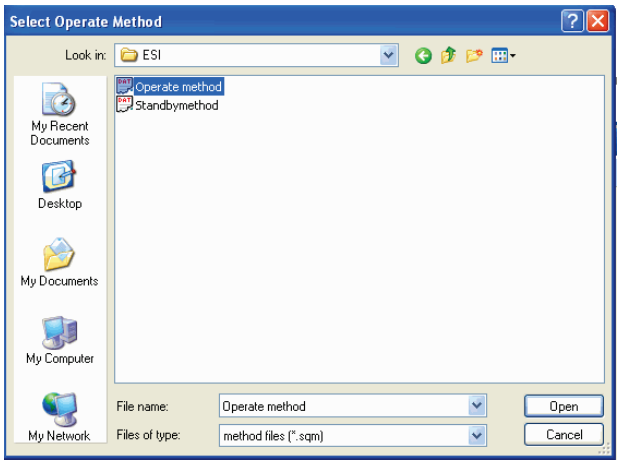
1. In the row below **Operate**, click **Browse for method**.



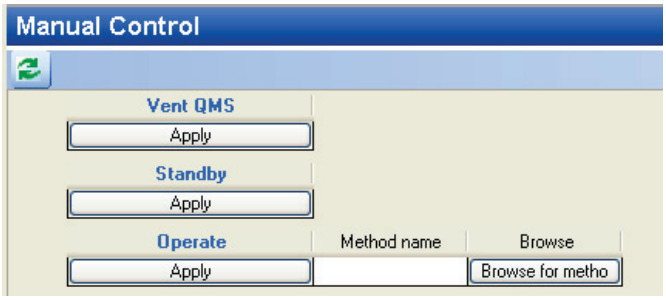


2. Select and open the **ESI** folder.

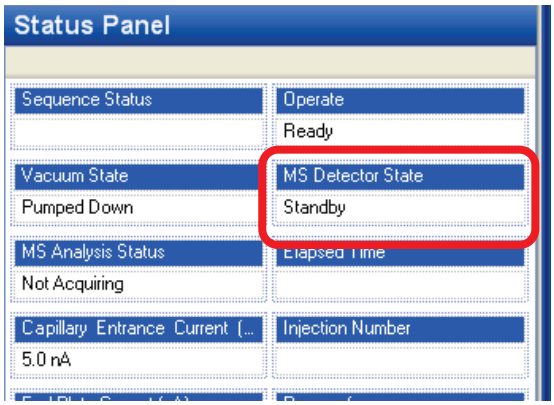
3. Select **Operate method** and click **Open**.



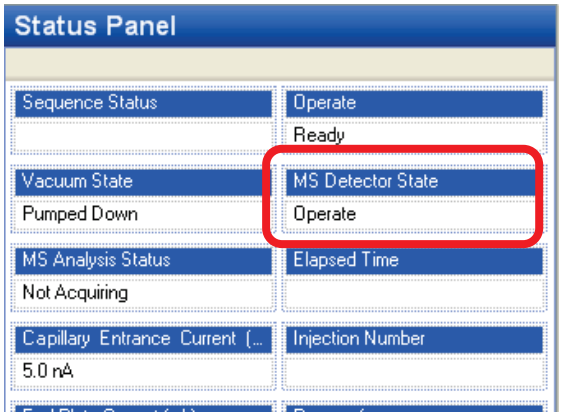
4. Look at the **Manual Control** section of the **Run Time** screen.



5. 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**.



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



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

7. Leave the SQ 300 MS Detector in the **Operate** mode.

Auto Tune on the SQ 300 MS Detector

Tuning the Flexar SQ 300 MS sets ion source and ion lenses parameters to obtain optimal resolution and sensitivity, and involves adjusting the probes. The Flexar SQ 300 MS instrument is tuned at the factory and it will be checked and re-tuned if necessary during installation. Checking the SQ 300's performance by running a Check Tune or Auto Tune is a quick diagnostic procedure if changes in system performance are observed. This Auto Tune procedure simplifies the tuning process.

Upon successful completion, Auto Tune will create calibrated Tunes at 3 different scan speeds, 1k/sec, 5k/sec and 10k/sec. These Tunes will be the foundation upon which you build an MS acquisition method.

Auto Tune is performed through the SQ 300 MS driver.

About the Samples

PKI Tune Mix

Negative Mode Ions: 92, 205, 531 1166, 1466, 2666

Positive Mode Ions: 123, 195, 506, 1022, 1422, 2222, 2522

Calibrants

There are two calibration mixtures that can be employed at the present time. The PKI positive ion tune mix which is good for positive ion tuning and the PKI negative ion tune mix for negative ion tuning.

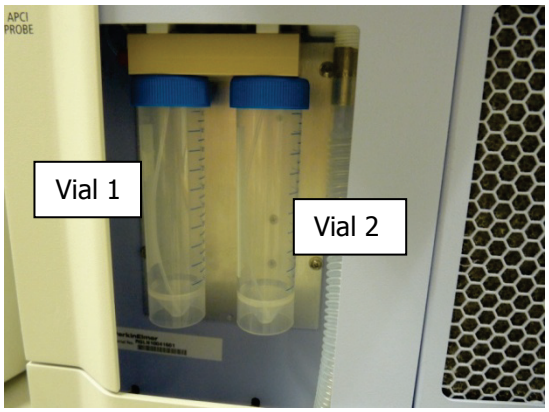
Calibrant Delivery Modes

A calibrant can be introduced into the mass spectrometer by several methods including: using the calibration vials that are located on the front end of the SQ 300 mass spectrometer, using the on board syringe pump (if available) which is located on the front right hand side of the SQ 3000 or by using an external syringe pump.

Preparing the Calibration Vials for Auto Tuning

There are two calibration vials as shown below. Typically use **Vial 1 for Positive Ion Tune mix** and **Vial 2 for a Negative Ion Mix**.

1. To remove calibration **Vial 1**, reach in and unscrew the left side calibration vial as shown below.



Calibration Vials on the SQ 300 MS


Unscrew the calibration vial

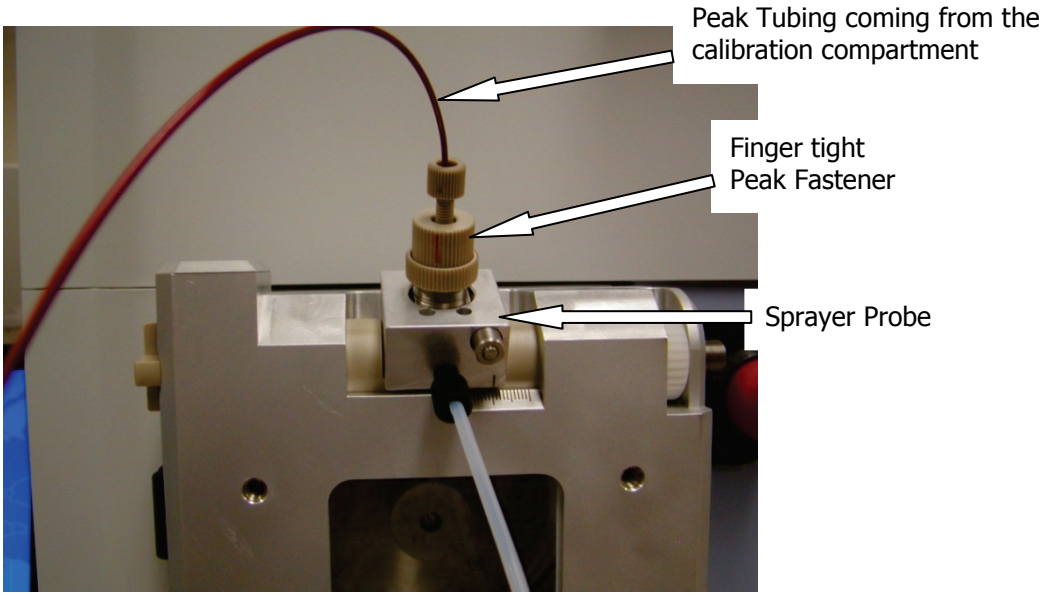
2. Remove the vial and fill the vial with approximately 15 mL of tune mix.


The calibration vial removed


Filling the Calibration vial

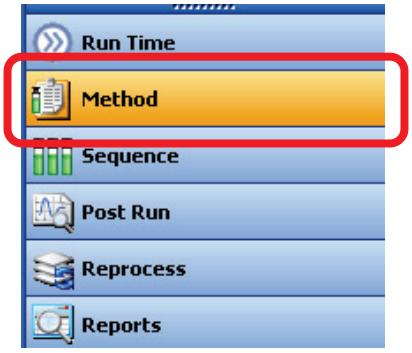
3. Re-insert the calibration vial making sure the small draw tube is inserted into the 50 mL calibration vial. Also make sure that the calibration vial is securely fastened into the blue cap to avoid leaks after pressurization.

4. Connect the peak tubing that extends from the calibration compartment and use a Peak Finger tight fitting to secure the tubing to the sprayer probe.

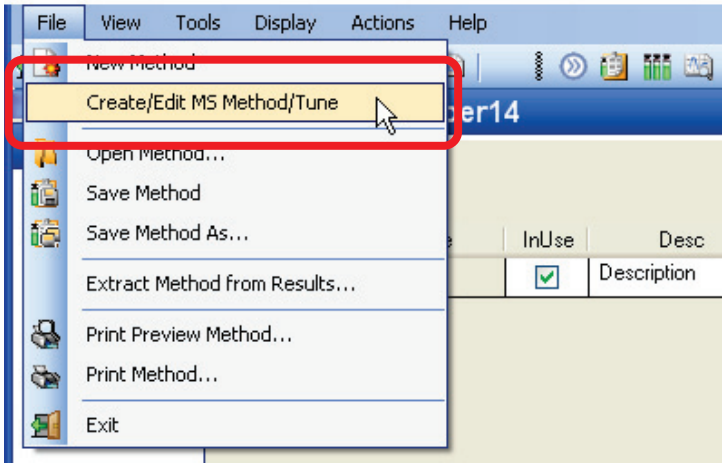


Run an Auto Tune

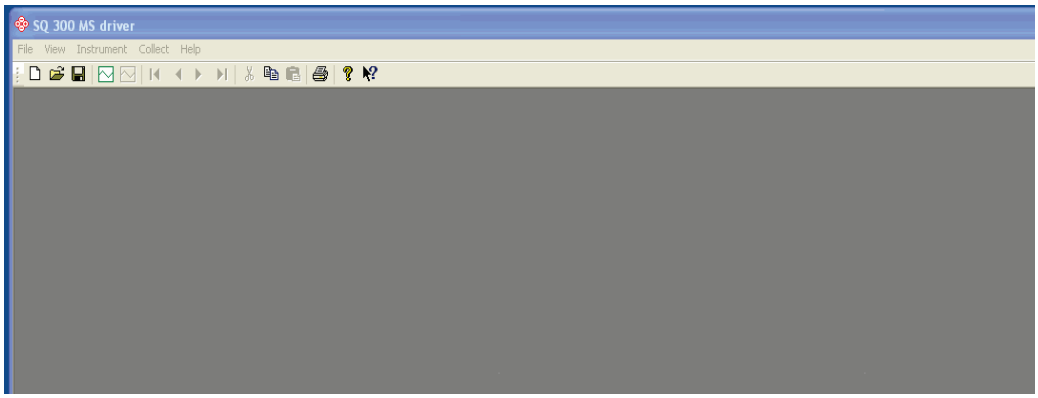
1. Click **Method** to open the **Chromera Method** screen.



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



The **SQ 300 MS driver** screen displays.

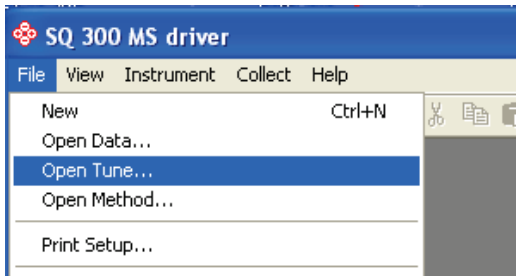


3. If an **Acquisition Method** displays, close the displayed Acquisition Method.

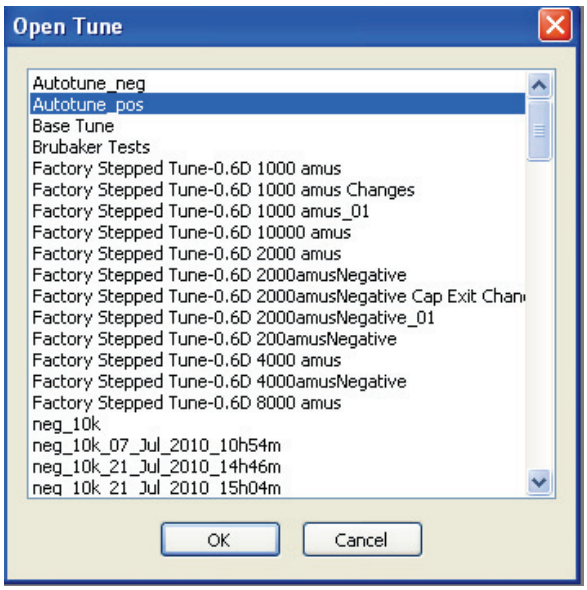
Running Auto Tune from the Calibration Vials

If you are tuning in positive ion mode open the tune Autotune_pos Tune or if you are tuning in the negative ion mode open Autotune_neg Tune.

1. Select **Open Tune** from the **File** menu.

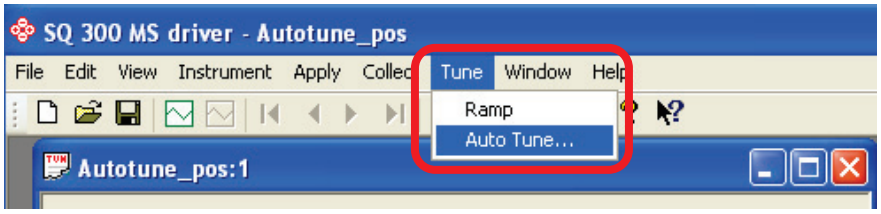


The **Open Tune** dialog displays.

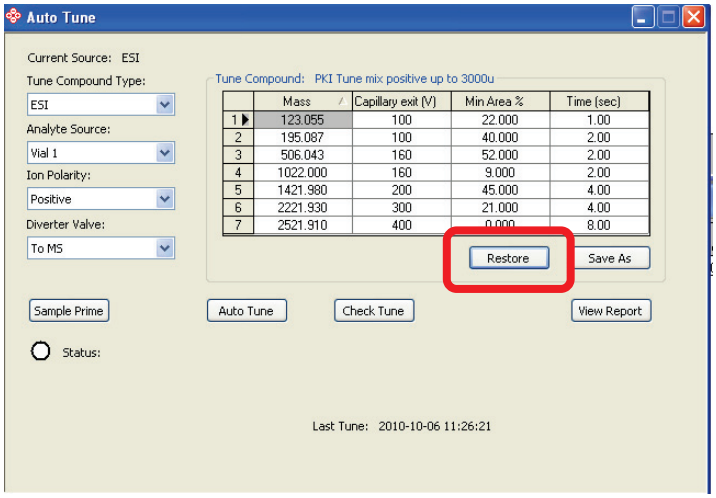


2. Select a tune file and double-click on the tune file to open it.
   The above example shows selecting **Autotune_pos** as the Tune file.

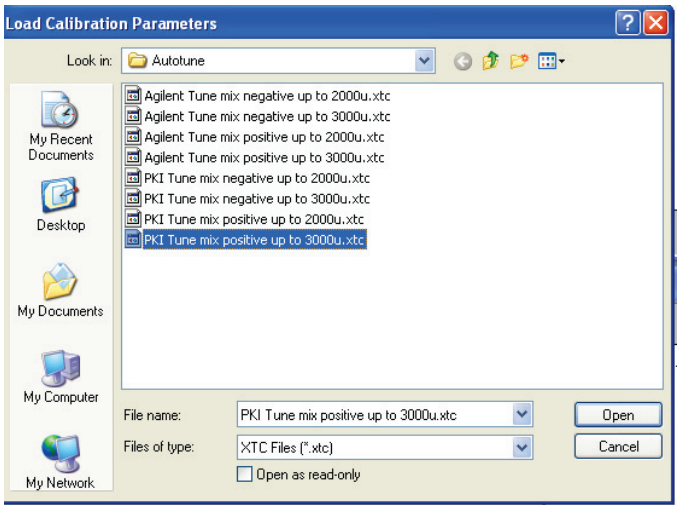
3. Select **Auto Tune** from the **Tune** menu.



The **Auto Tune** dialog displays.



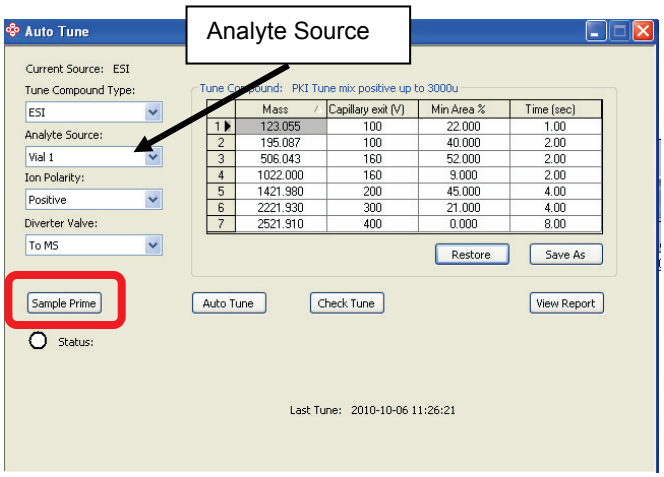
4. In the **Auto Tune** dialog, select a tuning compound reference file by clicking on **Restore.** This displays the **Load Calibration Parameters** screen.



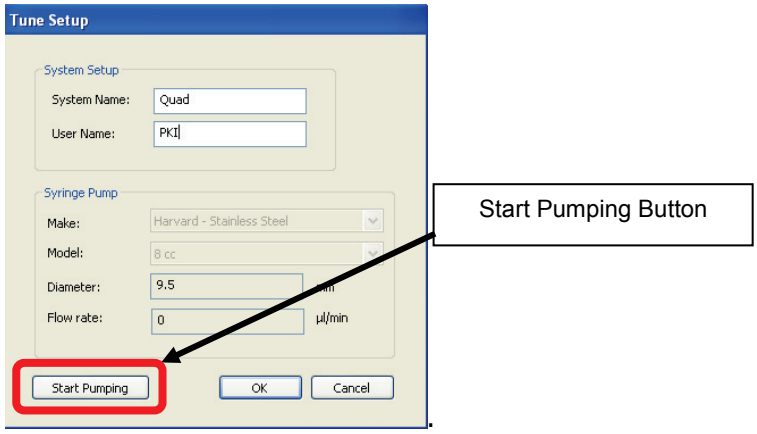
5. For Positive ion, highlight **PKI tune mix positive up to 3000u** and simply double-click on it. If calibration is only required up to 2000u, then the **PKI tune mix positive up to 2000u** file is available to use.

   After running the **PKI tune mix positive up to 3000u** you will run Auto Tune again using the **PKI Tune mix negative up to 3000u**.

6. Select **Vial 1** (with the **Positive Ion Tune Mix**) for the **Analyte Source** from the drop-down menu.



7. Click on the **Sample Prime** button and the **Tune Setup** dialog displays.

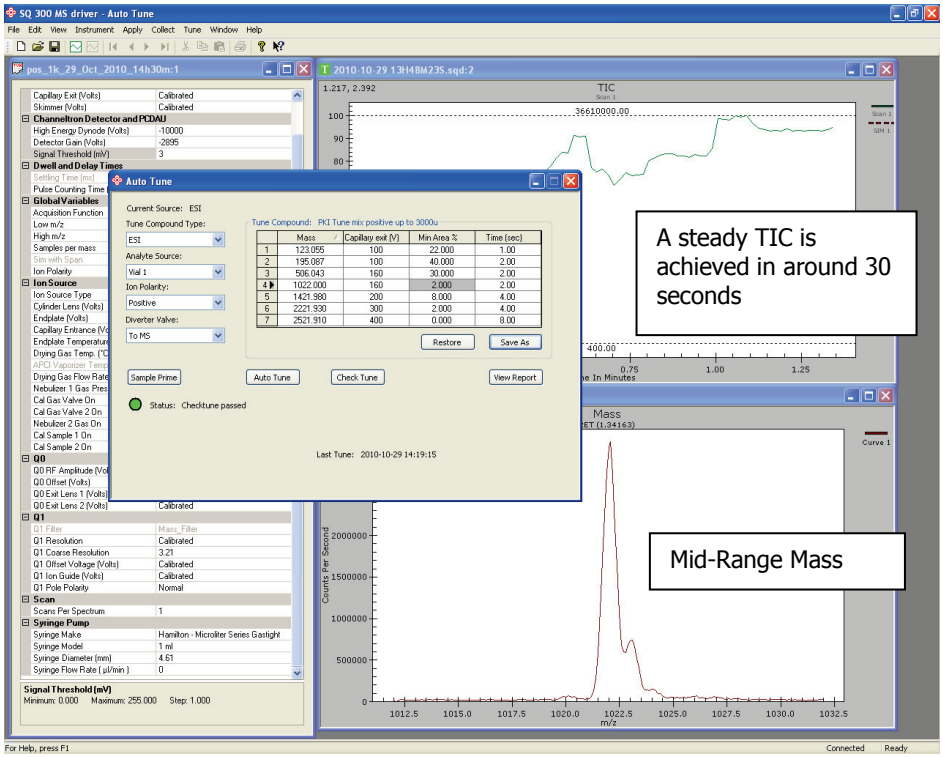


8. Type in the **System Name** and the **User Name**.

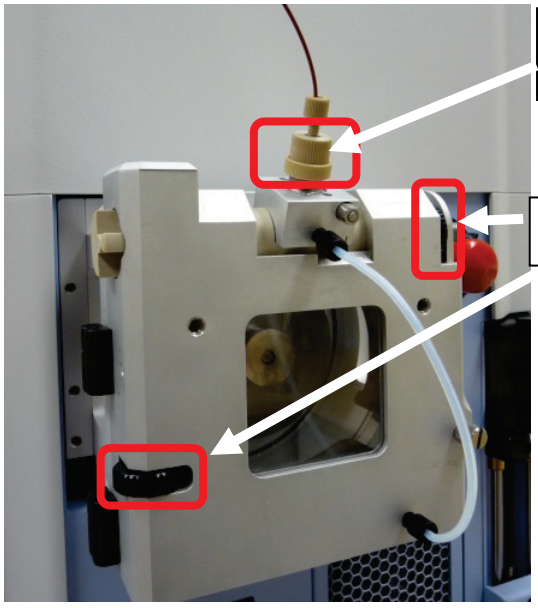
   Note that the **Syringe Pump** selections are grayed out.

9. Click the **Start Pumping** button.

   A mid-range mass is displayed with the corresponding TIC. Keep priming the system until a steady signal intensity is achieved. This generally takes about 30 seconds.

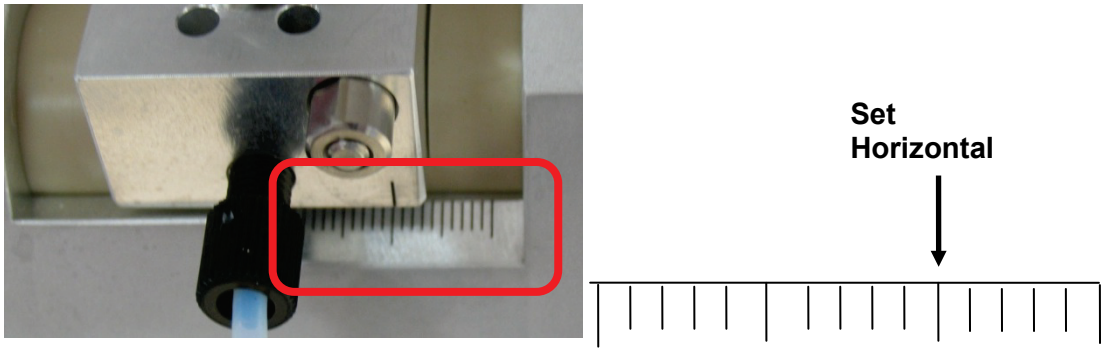


10. While viewing the **Mid-Range Mass**, you can adjust the source position by turning the indicated controls for maximum sensitivity.

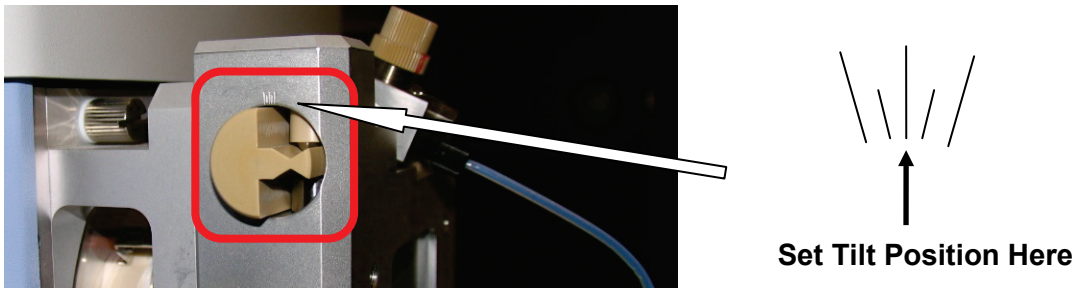


NOTE: After clicking the **Start Pumping** button, make sure you are getting flow through the PEEK tubing. If there is no flow, remove the tubing connection to the probe, cut off about ¼-inch of tubing with a tubing cutter, and reconnect the tubing to the probe. If this fails to work, replace the tubing.

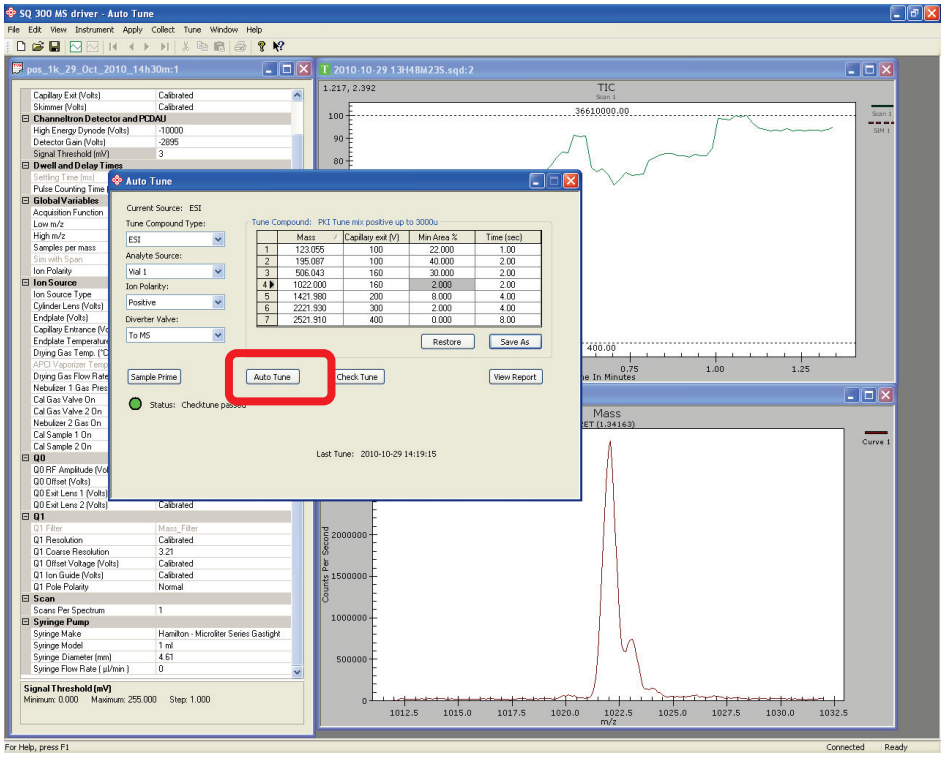
- Set the **source horizontal position** so that the horizontal probe marking is positioned 10 spaces to the right side of the scale as shown below:

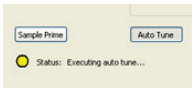


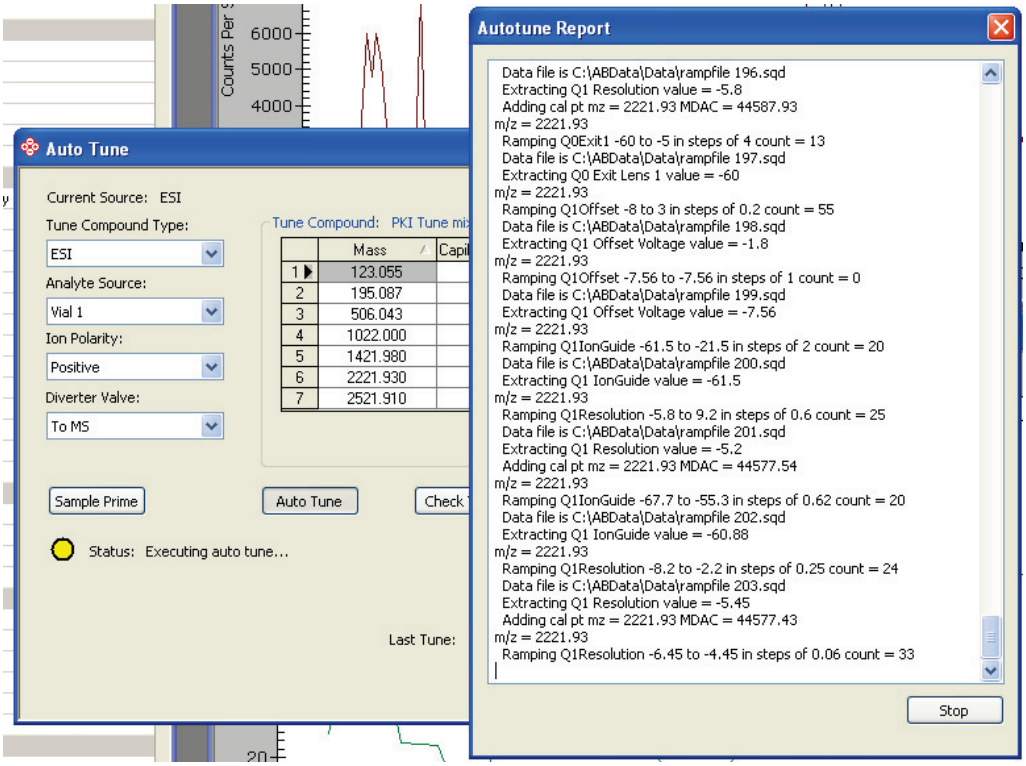
- Set the **Probe Tilt** position as shown below:



11. To start auto tune, click on the **Auto Tune** button. **Each Auto Tune takes approximately 20 min.**
    If the Auto Tune stops abruptly you can re-prime by clicking on the **Sample Prime** button and then click the **Start Pumping** button and wait an additional 30 seconds before running Auto Tune again.



The **Status** light on the Auto tune page will turn **yellow**  and displays **Status: Executing auto tune** and the **Autotune Report** screen displays.



As Auto Tune runs in the positive ion mode, it creates the following three master tunes: **pos_1k, pos_5k,** and **pos_10k** upon completion.

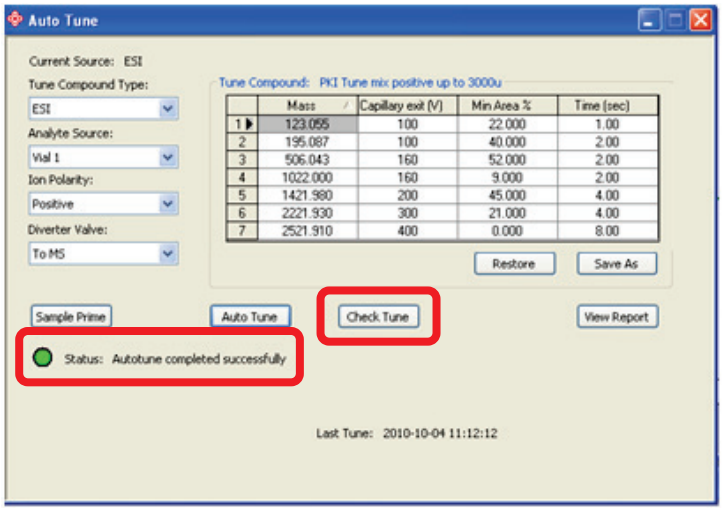
It also creates a copy of each master tune with a date and time stamp appended to the file name. For example:

1. **Pos_1K_04_Oct_2010_16h21m**
2. **Pos_5K_04_Oct_2010_16h21m**
3. **Pos_10K_04_Oct_2010_16h21m**

where:

Pos means: Positive Ion Mode
Neg means: Negative Ion Mode
1K means: a scan rate of 1000 u/sec
5K means: a scan rate of 5000 u/sec
10K means: a scan rate of 10,000 u/sec

The time and date stamp tunes should be used to create any Tunes to be used in a method for data acquisition. The date and time stamp allows the newest Tune to be distinguished from older ones. If Auto Tune runs to completion and passes all criteria, the **Status** light turns **green** and **Status: Auto tune Completed successfully** displays**.**



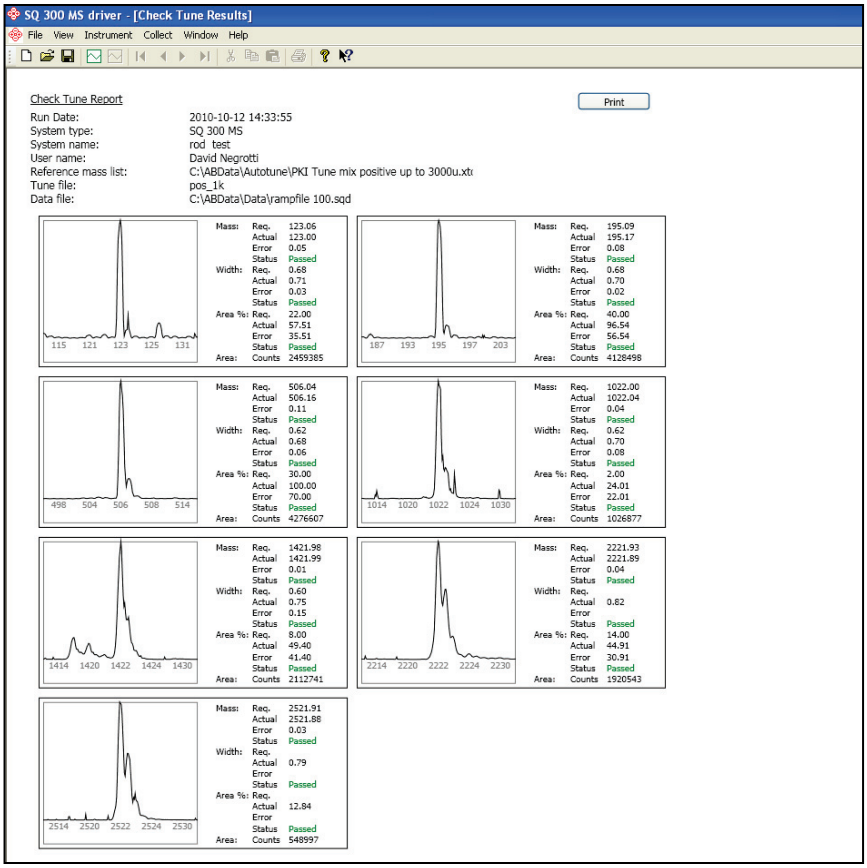
12. After auto tune is complete, click on **Check Tune.** The **Status** light will turn **yellow** and **Status: Executing check tune** displays. If a significant amount of time passes between the completion of Auto Tune and the execution of Check Tune, click the "Sample Prime" button to get the calibration mixture flowing again.

    When check tune is complete a **Check Tune Results** screen displays.

    For each mass there are four evaluation criteria:

    - The **Mass** criterion checks to see if the mass accuracy of a peak is within +/- 0.1 u of the actual mass.
    - The **Width** criterion checks the Full Width at Half Maximum is between 0.5 and 0.7 u.

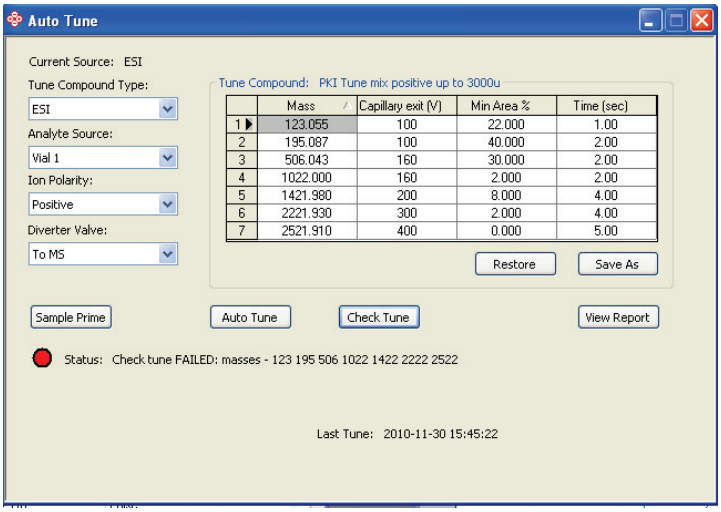
      Note that the Width criteria is expanded up to 0.85 u for high mass ions.
    - The **Area %** criterion checks the relative intensities of each mass in the tune relative the most intense peak in the tuning mix.
    - The report also provides the **Area counts** for each mass after Check Tune is run.



13. The **Check Tune** results above show that the tuning masses all passed the evaluation criteria.

NOTE: ***If check tune fails*** *the* ***Status*** *light will turn* ***red*** *and display* ***Status: Auto tune FAILED: masses*** *– (displays the masses which failed Auto tune) otherwise the status light will be green if all masses passed Auto Tune and Check Tune.*

This example shows the status light is Red and that many tune masses failed the **Check Tune** criteria. Typically, one would just rerun **Check Tune** another time to see if it passes, since running **Check Tune** takes considerably less time than running **Auto Tune.**

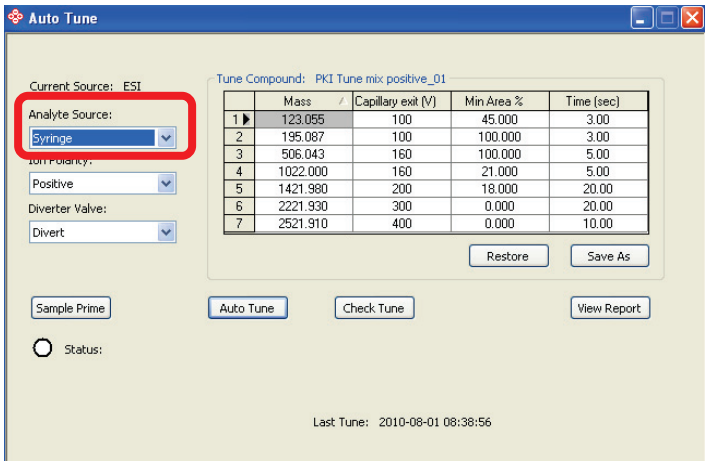


Running Auto Tune Using the Built-in Syringe Pump

Running Auto tune using the built-in syringe pump is very similar to running Auto Tune using the Calibration vials except that the syringe parameters and flow rate must be input as demonstrated in the following screen:

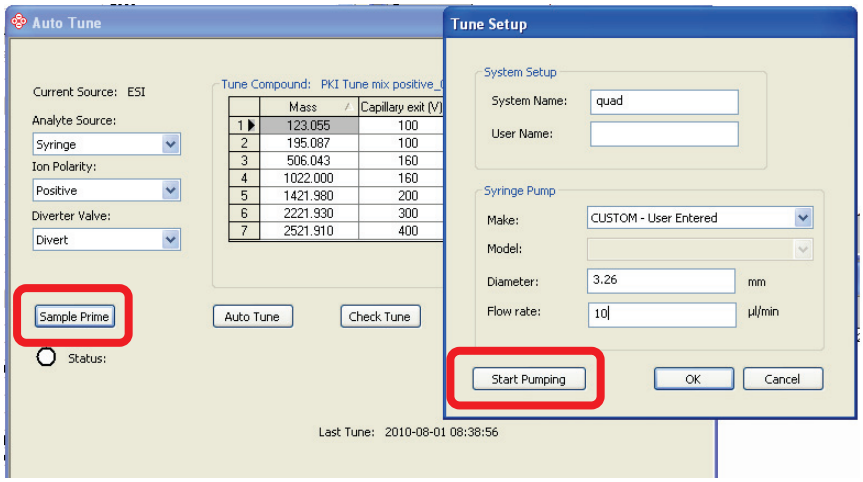
1. Open **Auto Tune** and select **Syringe** from the **Analyte Source** drop-down menu.

   Make sure the syringe is loaded with desired calibrant and that the peek line extends from the syringe tip and connects to the probe sprayer with a peek finger tight connector.



2. Click the **Sample Prime** button.

   The **Tune Setup** dialog displays.



3. Type in the **System Name** and the **User Name**.
4. In the **Syringe Pump** section, select the **Make** of the syringe from the drop-down menu.

   When using a 500 uL Hamilton Syringe select **Custom - user entered** in the drop-down and the set the syringe inner diameter to 3.26 mm and the flow rate to 15 µl/min.

5. Click the **Start Pumping** button and wait until the mid-range mass shown in the scan window has stabilized just as you did before when using the calibration vial.
6. Run **Auto Tune** and **Check Tune** as described earlier in this section.

Initial Process to Configure an Optimal Tune and Method on the SQ 300 MS Detector

Process Overview

Once the SQ 300 is tuned and calibrated, the next step is typically to create a Method specific to the analytes(s) that are to be measured or identified. The following pages describe a detailed process for accomplishing this, specifically so that the operator will understand why the process is conducted and how it will provide the best possible results. Note that the first time this process is followed, it will appear to be rather complex and tedious. However, once it is completed, it can be used for all future analyses with minimal modifications.

The process consists of the following steps:

- Infuse a target analyte into the SQ 300 MS. This will provide a constant signal to the MS, so the analyte can be properly mass labelled and optimized for maximum sensitivity.
- Open a calibrated Tune (of a desired scan speed) and set a few parameters specific to the analyte and analysis that will be run.
- Create a "mini-method" to identify and label ions of interest

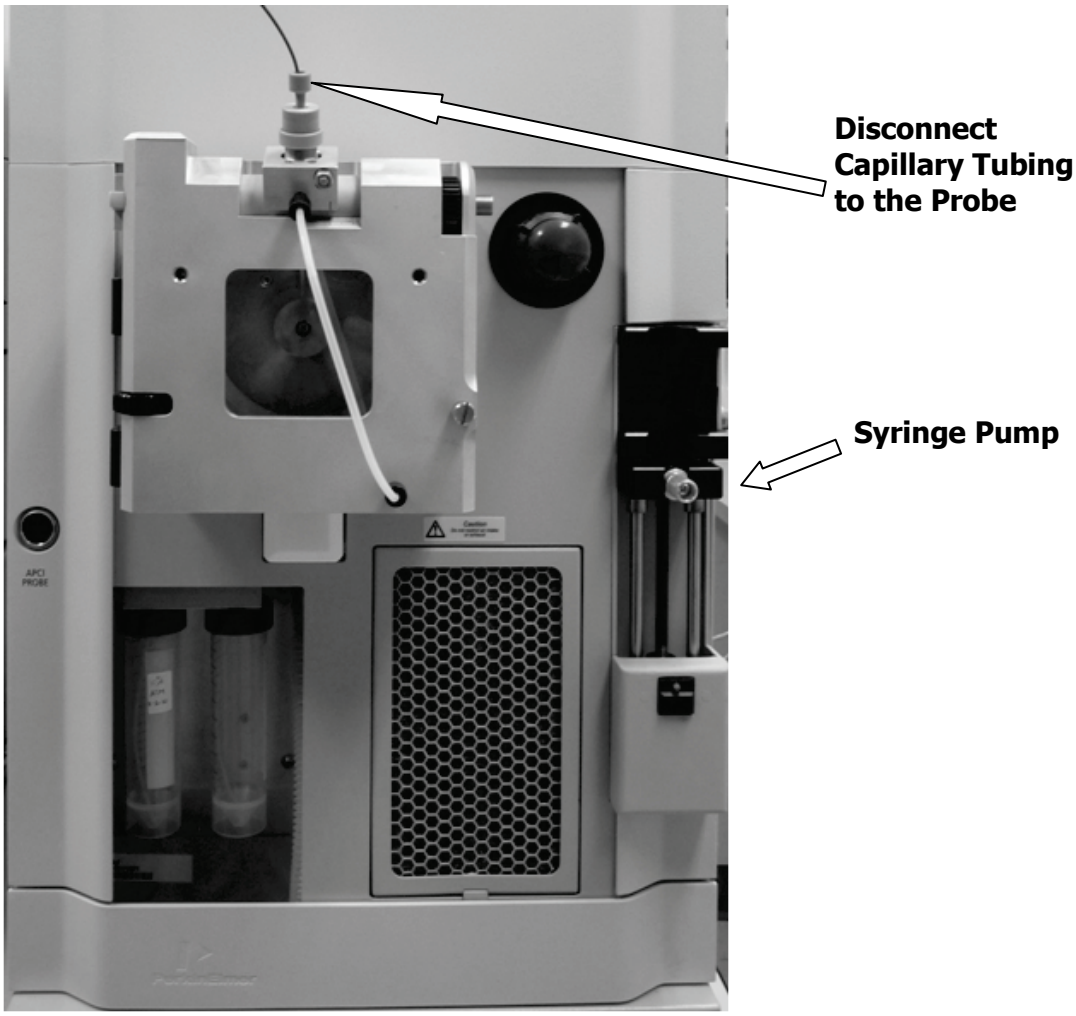
Setting up a Sample Infusion

Infusion is easy to accomplish using the SQ 300 MS Detector's built in syringe pump. Typically, there are two options for accomplishing 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 so as 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 ~100 pg/µl reserpine solution in LCMS grade methanol and water in a 3:1 ratio.

2. Disconnect the calibration line from the ESI sprayer on the SQ 300 MS and connect a Peek transfer line from the syringe needle to the ESI sprayer.

3. With the syringe prepared for infusion, the next part of the process covered in the next section involves creating a simple software evaluation routine to expedite the data evaluation process.

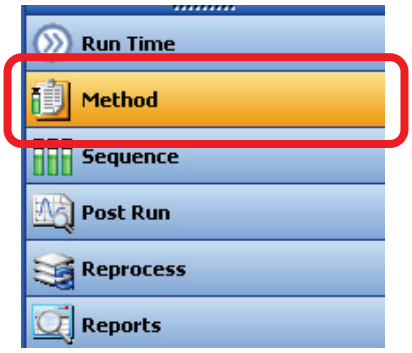


Creating a Peak Detection Mini-Method

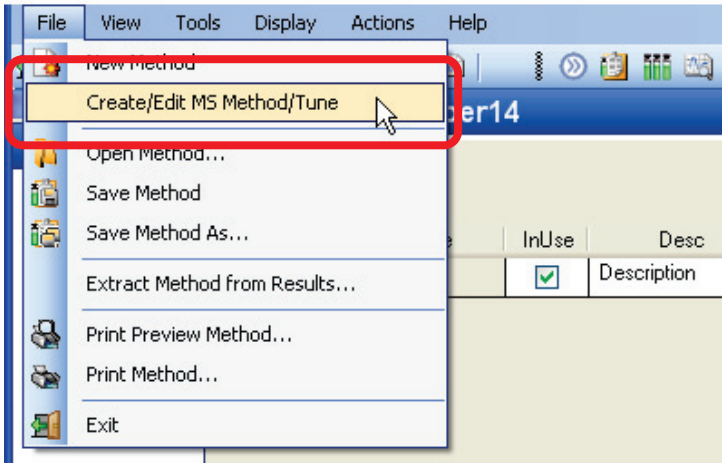
The SQ 300 MS Driver software allows the creation of simple data evaluation routines called mini-methods. Creating this routine will facilitate data evaluation for the process being described here, as well as for many future evaluations.

To create a peak detection mini-method:

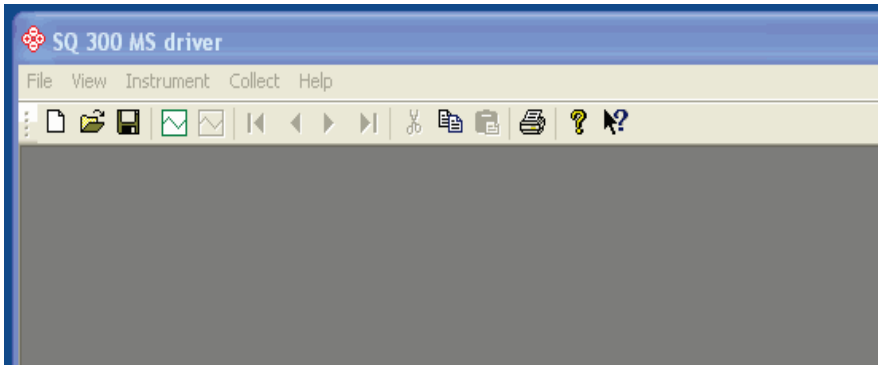
1. Click **Method** to open the **Chromera Method** screen.



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

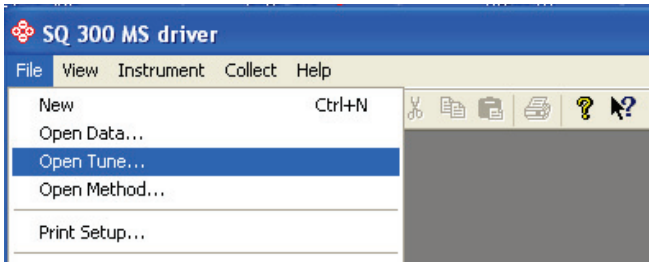


The **SQ 300 MS driver** screen displays.

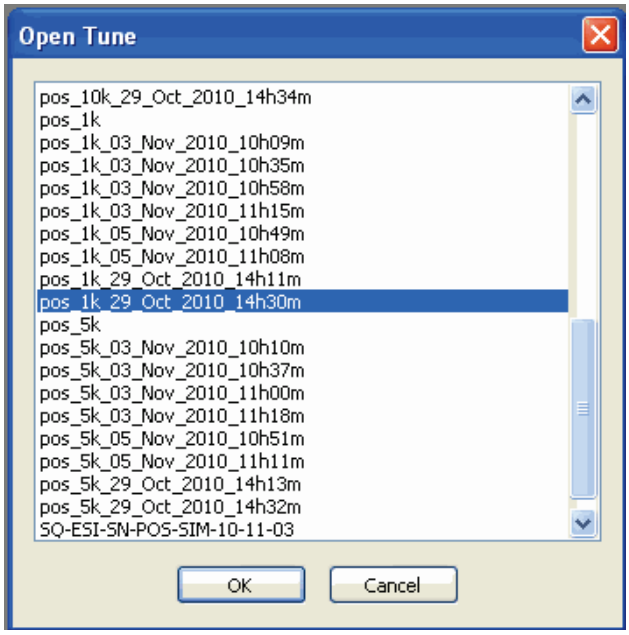


3. If an **Acquisition Method** displays, close the displayed Acquisition Method.

4. Select **Open Tune** from the **File** menu.

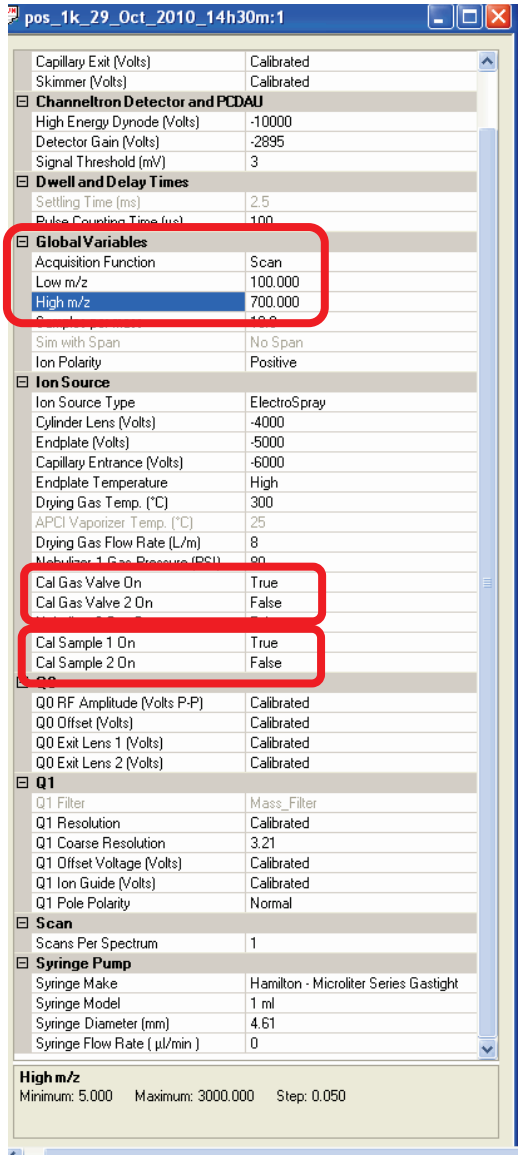


The **Open Tune** dialog displays.



5. Select the most recent **pos_1k tune file** and click **OK** to open it.
   The above example shows selecting **pos_1k_29_OCT_2010_14h30m** as the tune file.

6. A scanning experiment is going to be run, so a few changes need to be made to the calibration Tune created by Auto Tune. Reserpine has a molecular weight of ~608.2, so using electrospray a protonated molecular ion at *m/z* ~609.2, and an isotopic distribution from *m/z* ~609-611 is expected. Set the following parameters:



In **Global Variables**: Set the **Scan** from 100 to 700

- Low *m/z* 100
- High *m/z* 700

Turn off the **Cal flow**:

Set **Cal Gas Valve and Cal Gas Valve 2 On to:**

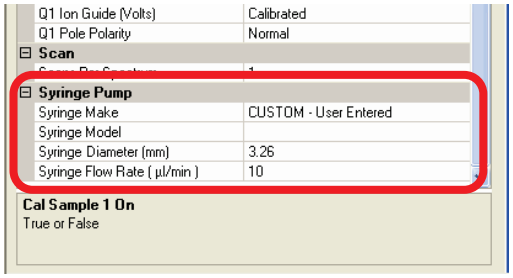
- False (off)

Set **Cal Sample 1 and 2 On to:**

- False (off)

7. Enter the on-board **Syringe Pump** settings for the syringe that will be used.

   In this example, since we are using a Hamilton 500 µl syringe that is not listed in the drop-down list, we select **CUSTOM – User Entered**.



Syringe Make

- CUSTOM – User Entered

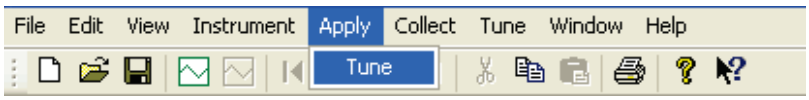
Set Syringe Diameter (mm)

- 3.26

Set Syringe Flow rate (µl/min)

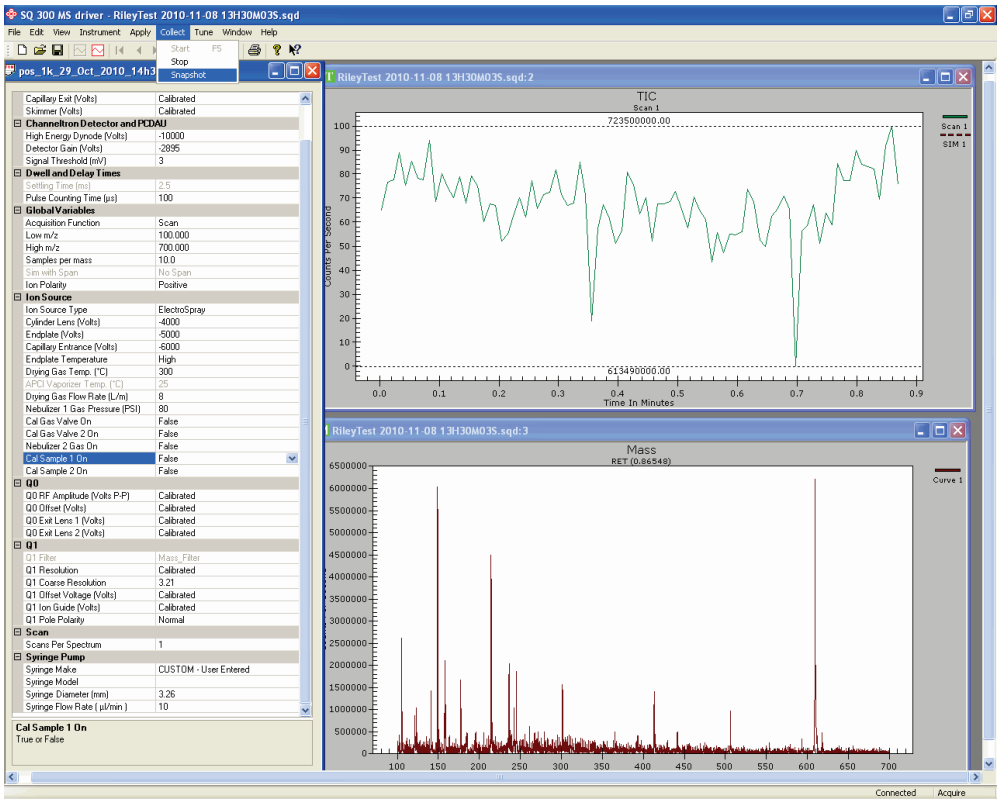
- 15 (Note: Tune window shows 10)

8. To download to the SQ 300 MS all the changes made to the Tune, we must "Apply the Tune" by selecting **Tune** from the **Apply** menu. This will start the syringe flowing at the specified rate.



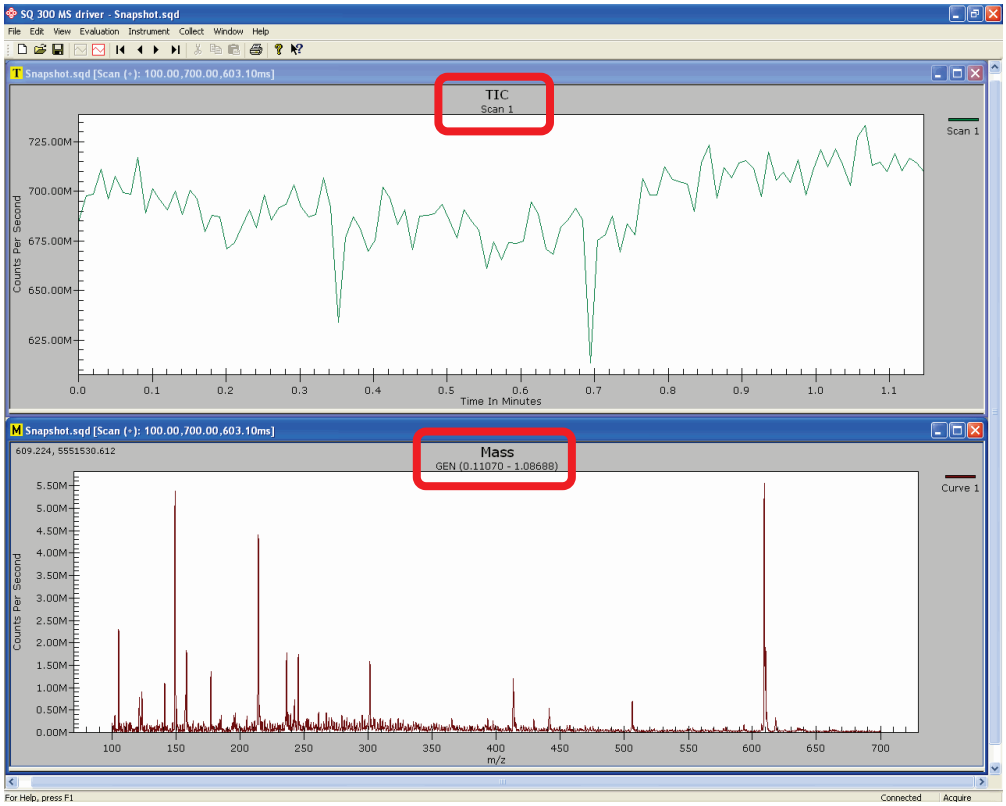
9. To start the data acquisition, click the green run button. 

   Acquire data for 1-2 minutes to allow enough time for the reserpine standard to be pumped all the way to the ESI sprayer, so data can be collected on it.



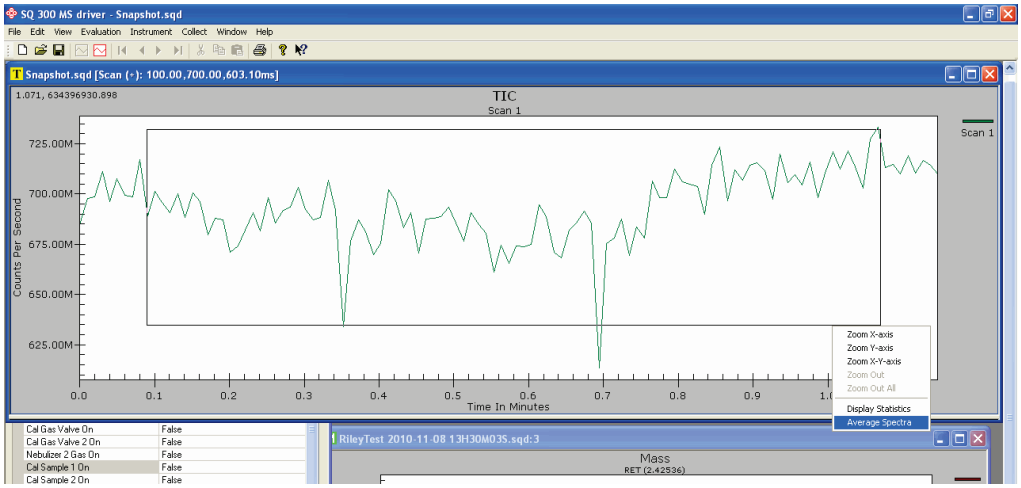
10. Take a "snapshot" of the data by selecting **Snapshot** from the **Collect** menu. A snapshot allows preliminary processing of the data acquired up to the point in time the snapshot was executed.

The **TIC** and **Mass** data evaluation screens appear.



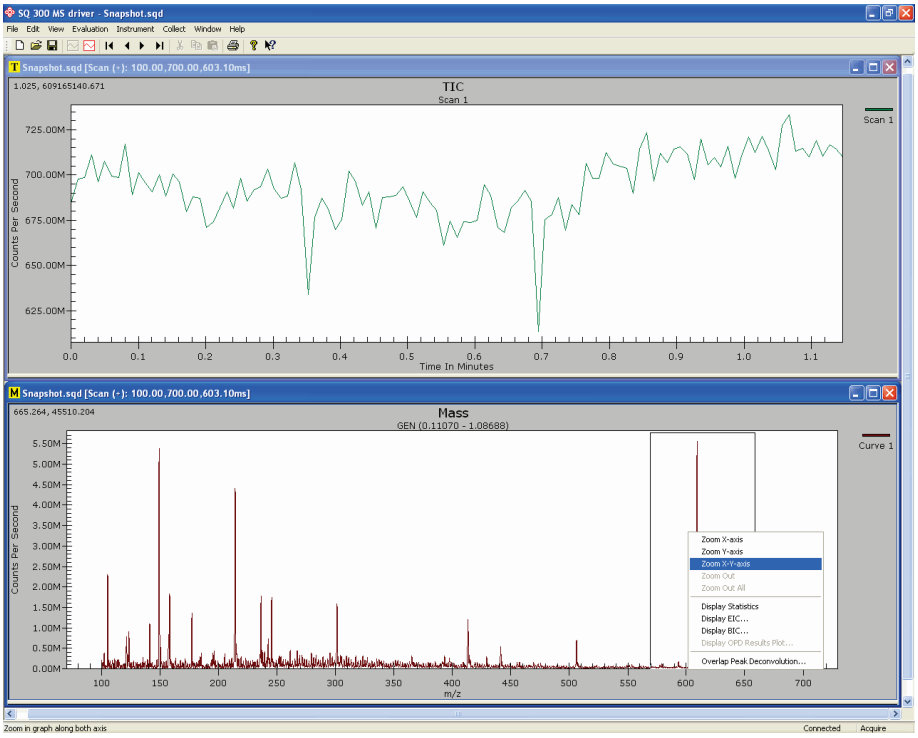
11. Close the **Mass** data evaluation screen.

12. Left-click and drag a box over the **TIC** area as shown below.



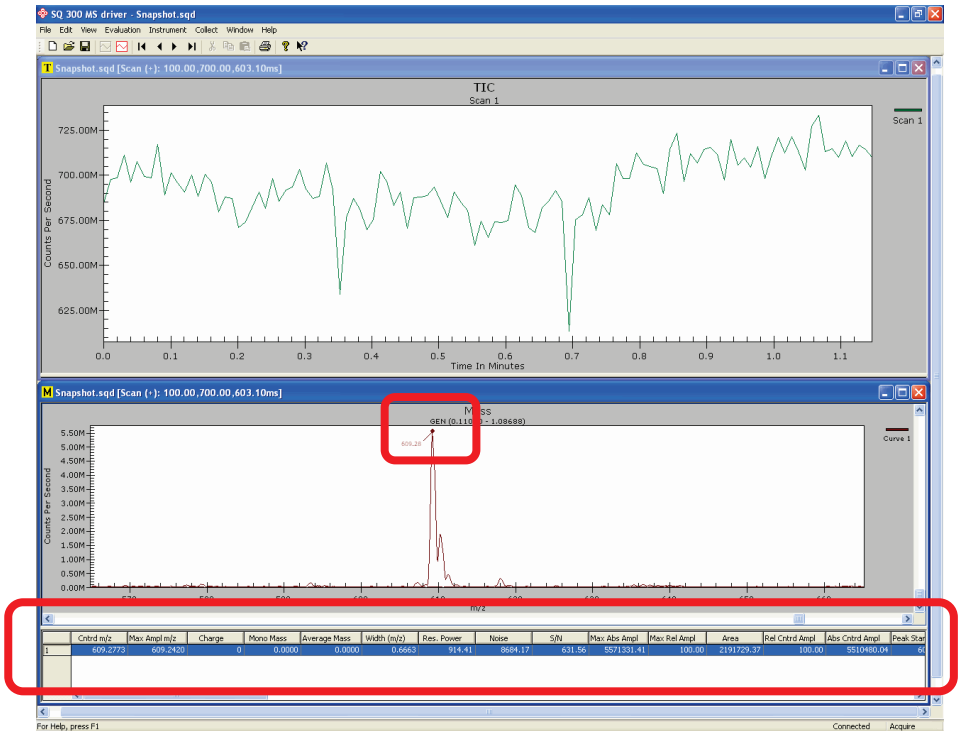
13. When you release the mouse button, select **Average Spectra** from the drop-down list.

The **Mass** data evaluation screen appears.



14. Left mouse click and drag a box around the reserpine protonated molecular ion cluster at *m/z* 609 and select **Zoom X-Y Axis** from the drop-down list.

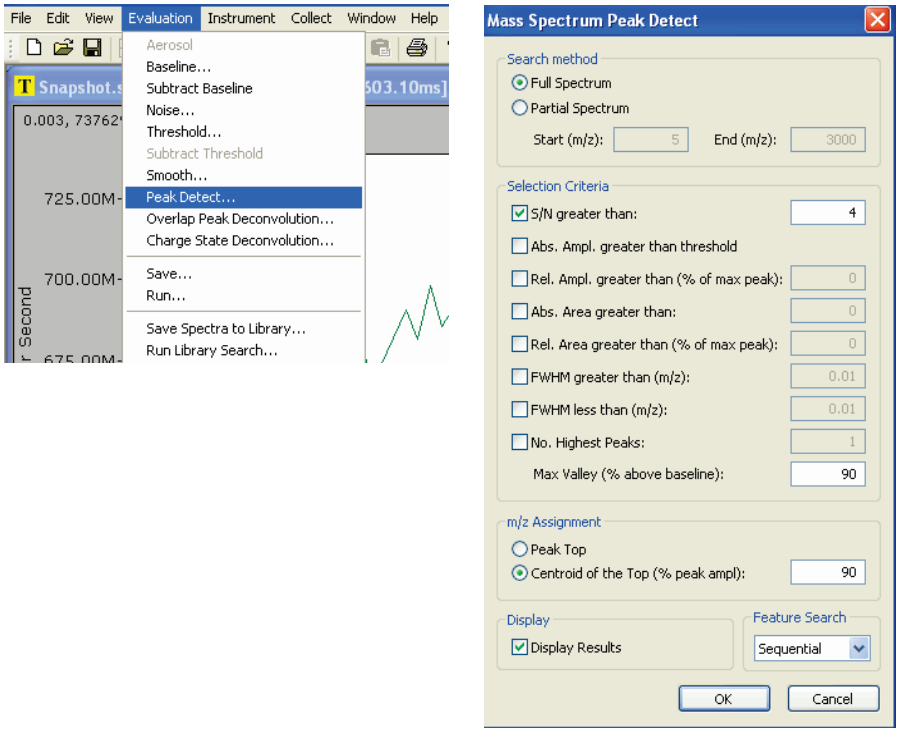
15. Move the cursor to the peak apex (it then turns into a hand), and right-click.



The peak table is displayed on the botton of the **Mass** data evaluation screen.

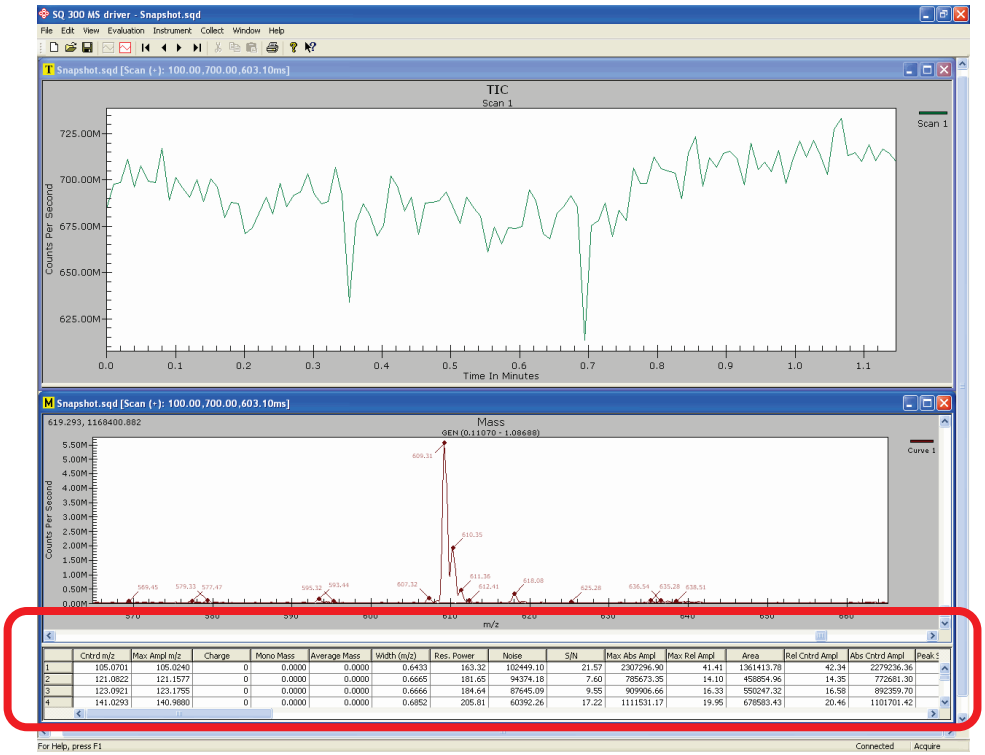
16. A peak detection data evaluation mini-method will now be created to facilitate data evaluation for this and all future analyses. Select **Peak Detect...** from the **Evaluation** menu.

    The **Mass Spectrum Peak Detect** dialog box displays.



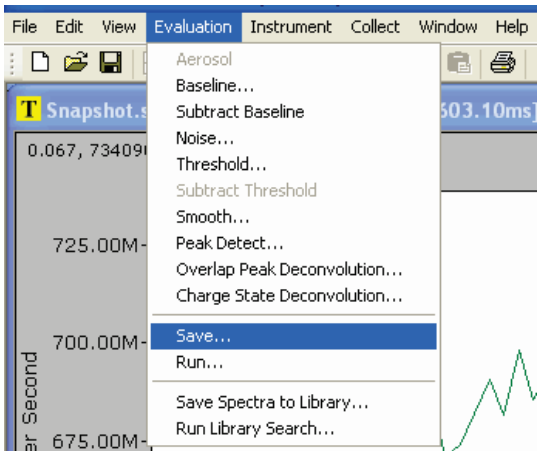
17. Enter the parameters shown in the above screen, then click **OK**.

    A peak table is generated for all identified peaks at the bottom Mass data evaluation screen.



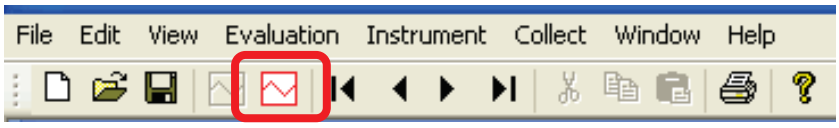
18. Select **Save** from the **Evaluation** menu and the **Save Spectrum Evaluation Method** dialog box displays.

19. Type **Peak Detect** to name the method, then click **OK**.



20. Close the **Snapshot** screens.

21. Stop the acquisition by clicking the **red** button.



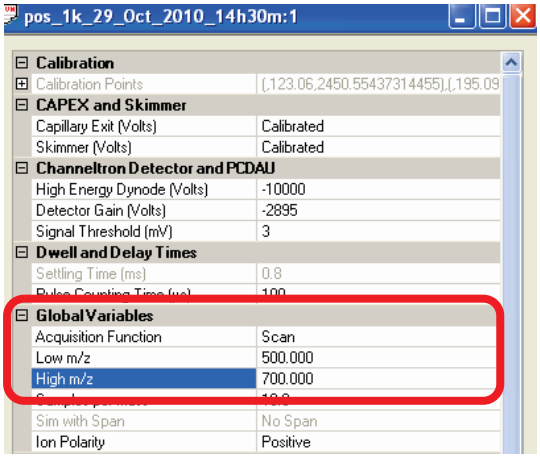
Ramping Parameters- Optimizing the Capillary Exit Voltage

The SQ 300 MS driver SW allows you to ramp certain Tune parameters to determine the best settings for those parameters. However, the Auto Tune 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.

The Capillary Exit voltage operates in two different ways: the first is to set the voltage to a specific value, and the second is to leave it set to "Calibrated". In this case, the software will pick a value based on the values used for the individual calibration ions analyzed during Auto Tune. This means a higher Capillary Exit voltage will be applied to higher molecular weight species.

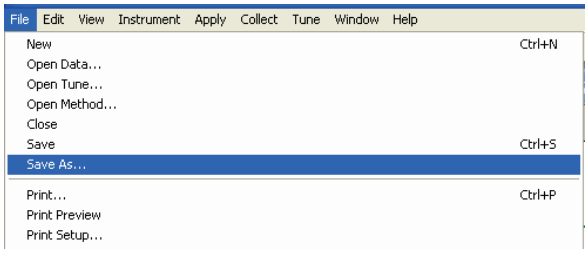
To Ramp the Capillary Exit voltage:

1. In the **Global Variables** section, set to Scan from Low *m/z* **100** to High *m/z* **700**.



Note: the above window shows the Low *m/z* set to 500, not 100.

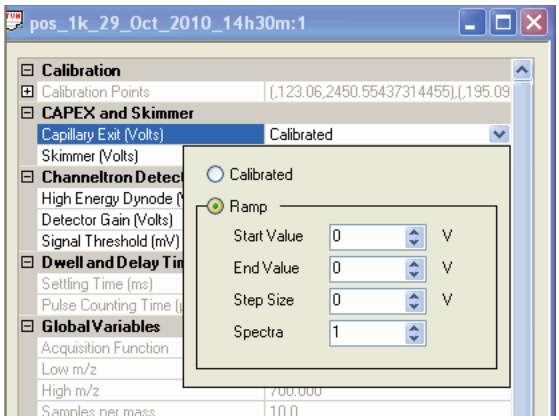
2. Select **Save As ...** from the **File** menu.



3. Save the Tune as **Reserpine Ramp**.

4. Select **Ramp** from the **Tune** menu.

5. Click on the **Capillary Exit (Volts)** and select the **Ramp** function from the drop-down menu.



Set the following Ramp values:

- **Start Value:** 50
- **End Value:** 300
- **Step Size:** 2
- **Spectra:** 3

Note: The settings above are used to show the process in greater detail than is typically required. To expedite this process in the future, the settings should typically be:

- **Step Size:** 10
- **Spectra:** 1

6. Click on **Capillary Exit (Volts)** to apply the values.

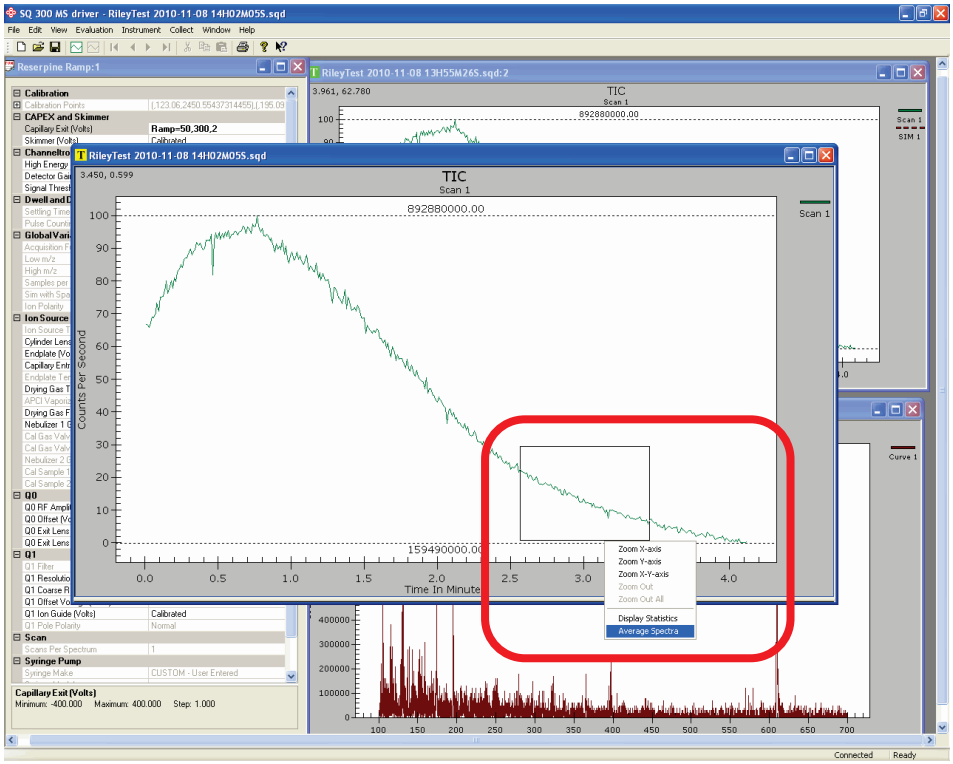
7. Click the green run button  to start acquiring.

   Acquire about 3 to 4 minutes of data.

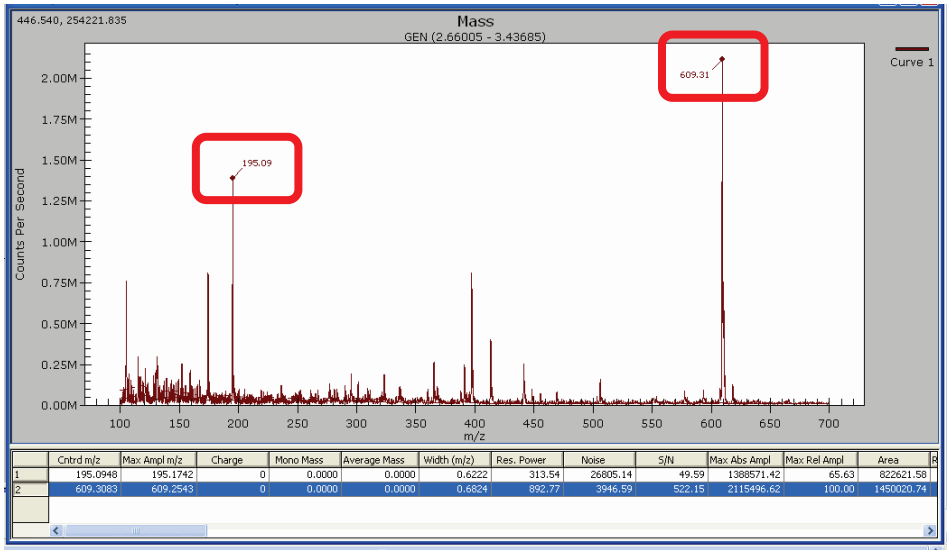
8. Take a snapshot of the data by selecting **Snapshot** from the **Collect** menu.

The **TIC** Snapshot screen displays.

9. Left-click and drag a box over the **TIC** area around 2.5 to 3.0 min. and select **Average Spectra** from the drop-down list.

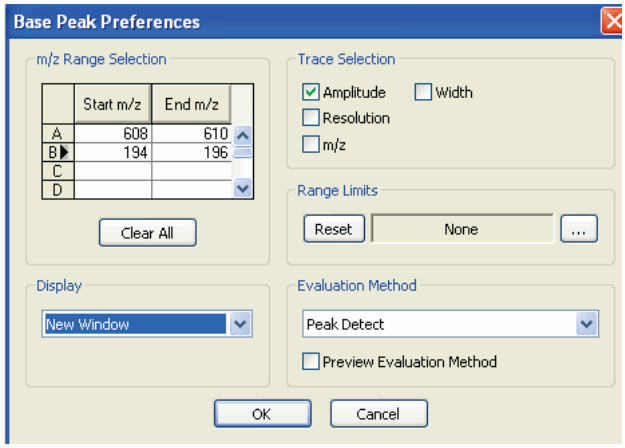


10. Position the cursor at the apex of the 609 peak (the cursor turns to a hand) and right-click.



11. Position the cursor at the apex of the 195 ion (the cursor turns to a hand) and right-click. The 195 ion is a known fragment of reserpine, which will appear with higher capillary exit values.

    When the Ramp completes, the **Base Peak Preferences** dialog displays.



In the **Trace Selection** section, uncheck the following:

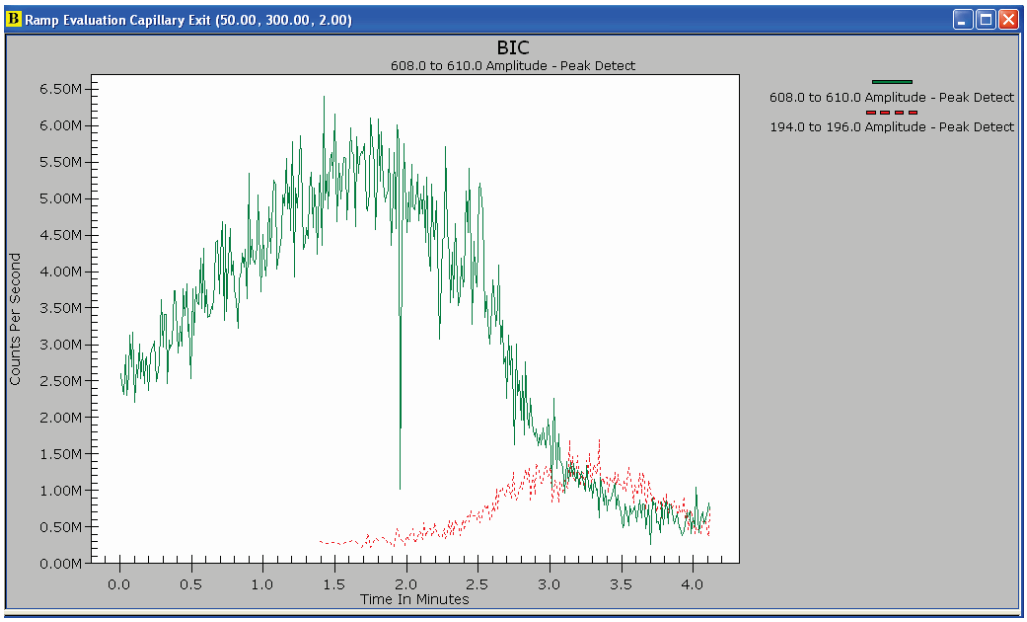
- Resolution
- m/z
- Width

In the **Evaluation Method** drop-down list, select Peak Detect.

12. Set the **Base Peak Preferences** as shown above then click **OK**.

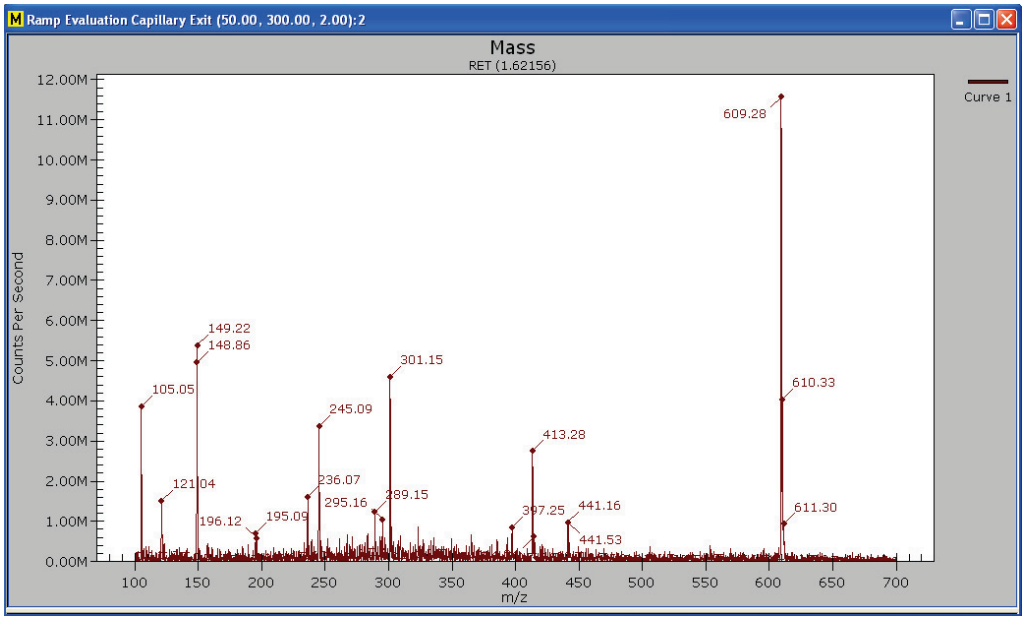
    The two Base Ion Chromatograms (**BIC**) display;

    - the red curve **194.0 to 196.0 Amplitude – Peak Detect** is a reserpine fragmentation ion
    - the green curve **608.0 to 610.0 Amplitude – Reak Detect** is the protonated molecular ion for Reserpine.

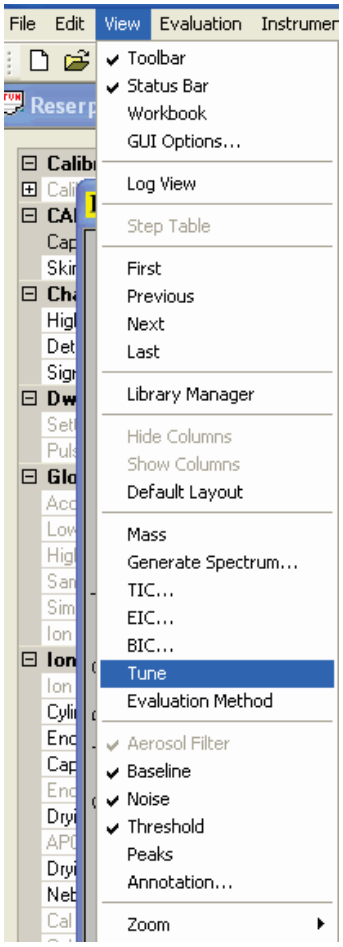


13. On the green curve, move the cursor to the apex of the highest point (it turns to a hand) and left-click.

    The **Mass** data evaluation window displays.

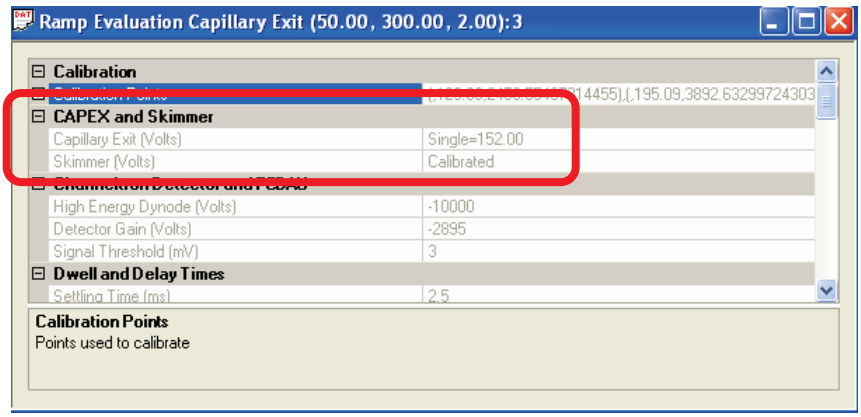


14. Select **Tune** from the **View** menu.



Look at the **Capillary Exit (Volts)** value (the window below shows **Single=152**).

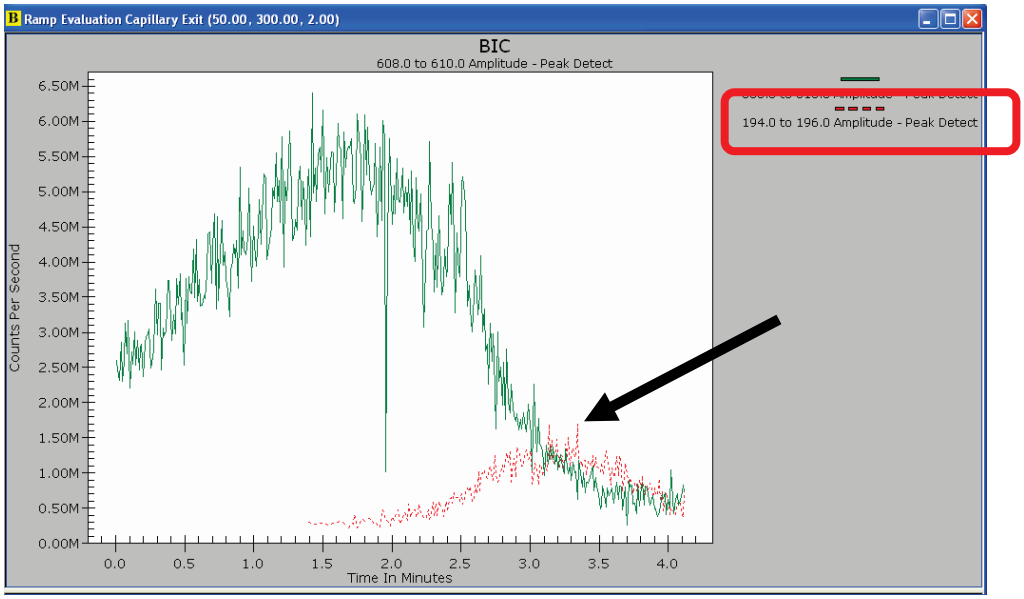
This means that the value of the Capillary Exit voltage at the cursor position, which was the maximum of the curve, was equal to 152 volts. This is the Capillary Exit value that provides the maximum intensity for the reserpine protonated molecular ion.



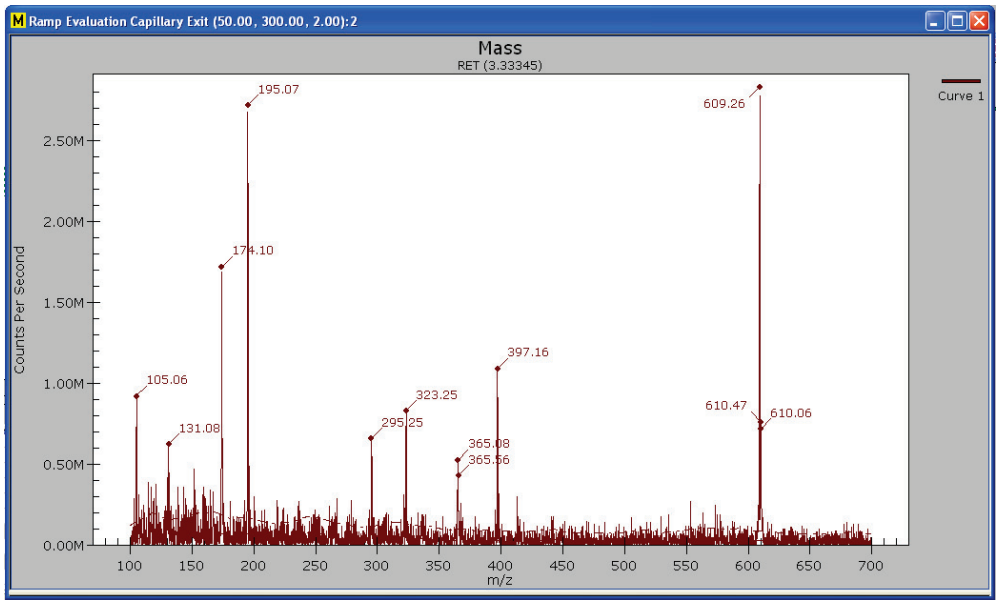
With a typical "bell shaped curve" as is demonstrated here, any value near the maximum will be perfectly acceptable. So in this example, any value in between 150 to 160 volts will provide the maximum signal intensity for reserpine.

15. Close all **Snapshot** screens except the BIC and main screen.

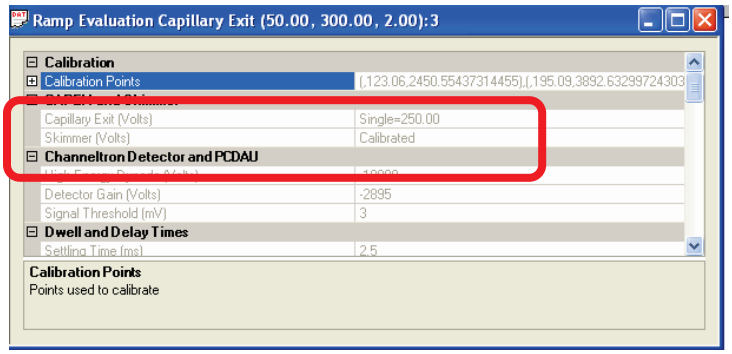
16. Now we wish to examine our reserpine fragment ion a bit. In the BIC screen, click on the red line (**194 to 196 Amplitude – Peak Detect**).
    This makes the red line a solid line.



17. Left-click on the highest point of the red curve for the fragment ion.
    The **Mass** window displays.

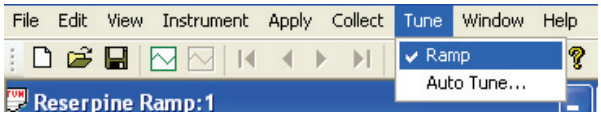


18. Select **Tune** from the **View** menu.
    Note that the **Capillary Exit** value is **Single=250**.



This indicates that to produce the maximum intensity *m/z* 195 fragment ion, a **Capillary Exit** voltage of ~250 volts should be used.

19. Select **Ramp** from the **Tune** menu to deselect it.



20. Stop the analysis, close all **Snapshot screens**, and close the **SQ 300 MS driver**.

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!)

Creating Methods and Sequences

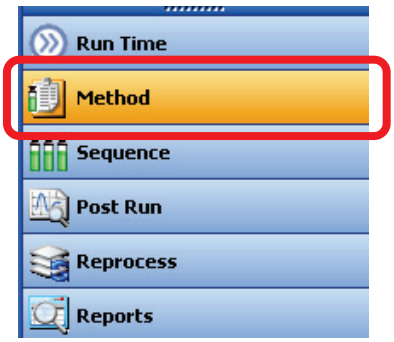
Creating an MS Method

The foundation for an optimal MS Method is starting with a mass calibrated Tune (with a date/time stamp). These are created every time Auto Tune is completed and under normal operating conditions, they can remain stable and usable for months. However, if there is any doubt regarding whether the MS is still properly calibrated, the easiest thing to do is to run a Check Tune to verify that the MS is operating to specification. If Check Tune indicates that retuning is required, simply run Auto Tune to recalibrate the system.

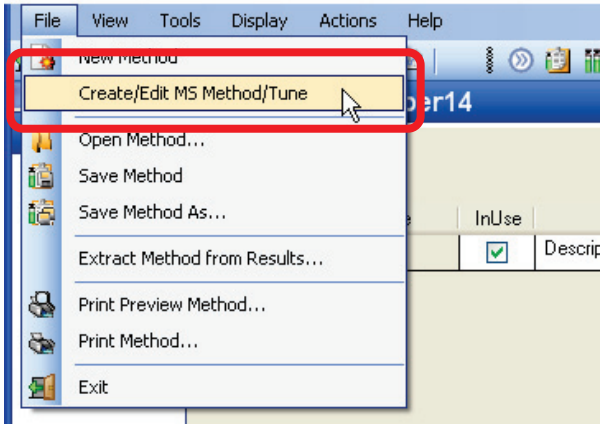
The following example shows how to create an MS method that will acquire both a Scan and a SIM for a given period of time, alternating between the two acquisition modes. For SIM acquisitions, the correct mass calibrated Tune to optimize for the acquisition will always be the **1k Tune** (with date/time stamp). For Scan acquisitions on real world samples, selection of the correct mass calibrated Tune depends on the scan speed required to get enough sample points to properly profile the chromatographic peak. Typically, at least 10-15 data points (i.e., in this case, Scans) are required to properly profile a peak, and the mass range required for the scan is another factor requiring consideration. The user should select from the **1k**, **5k** and **10k** Tunes (with date/time stamps) that will best meet the scanning requirements.

To create an MS method:

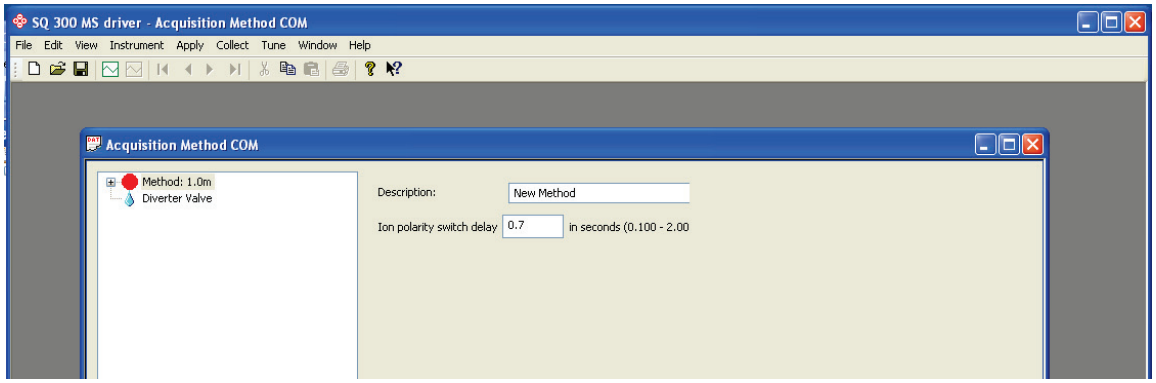
1. Click **Method** to open the **Chromera Method** screen.



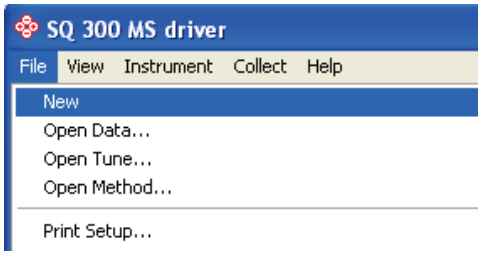
2. Select **Create/Edit MS Method/Tune** from the **File** menu.



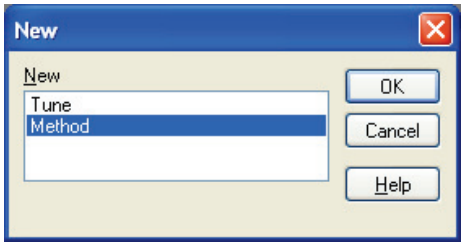
The **SQ 300 MS driver** screen displays.



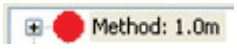
3. Select **New** from the **File** menu.



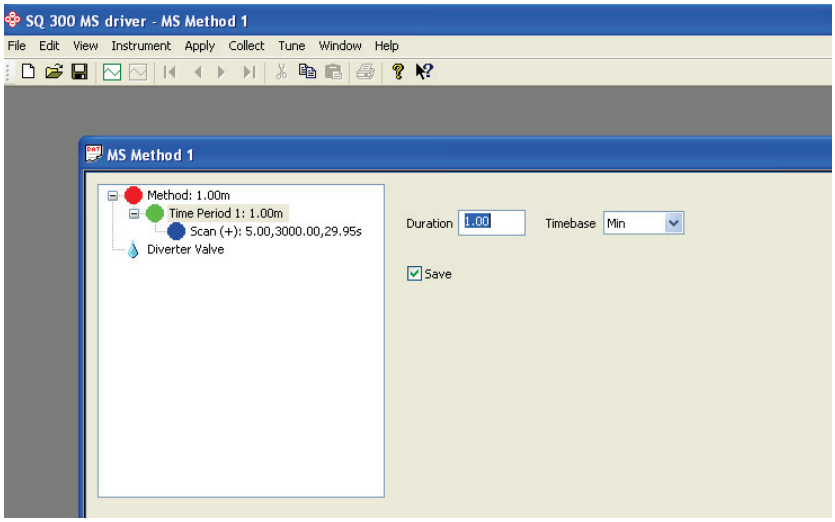
The following dialog displays.



4. Select **Method** then click **OK.**
   The following method screen displays.

5. Click the plus sign 

to expand the method row and display **Periods**.

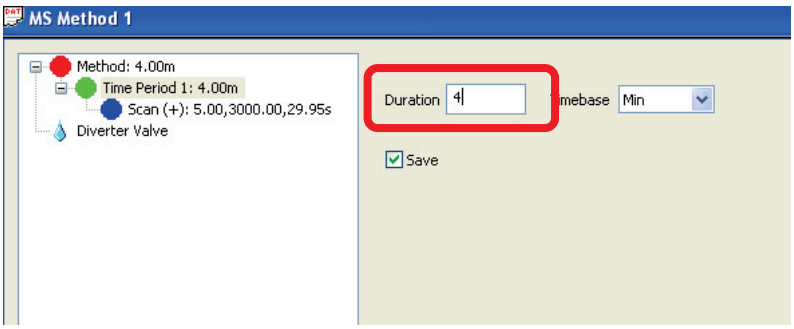


6. Give the method a name by selecting **Save a**s from the **File** menu.
   In this example, the method name is **MS Method 1**.

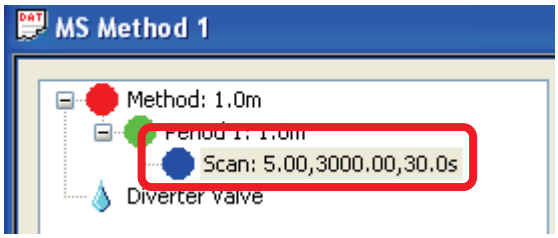
7. Click on the green **Time Period** dot and set the run **Duration** to equal that in the Chromera HPLC method.

NOTE: *At this point in time, it is up to the user to insure that the MS **Time Period** equals the the run **Duration** specified in Chromera. Otherwise, the timing issues may arise during the running of a sequence.*

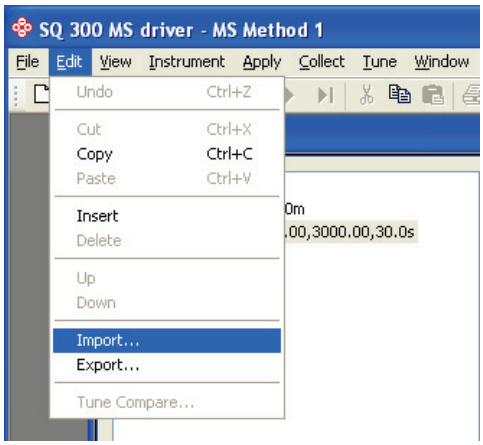
Since you have not yet created a Chromera HPLC method, set the run duration to 4 minutes then set your HPLC method to a 4 minute run duration time.



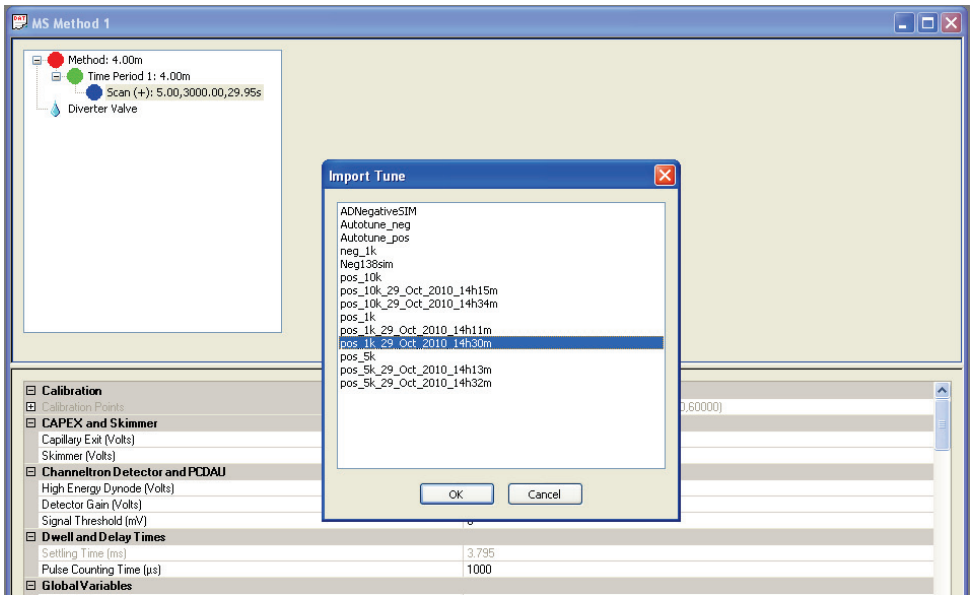
8. Click on **Scan** (the blue dot).



9. Select **Import** from the **Edit** menu.

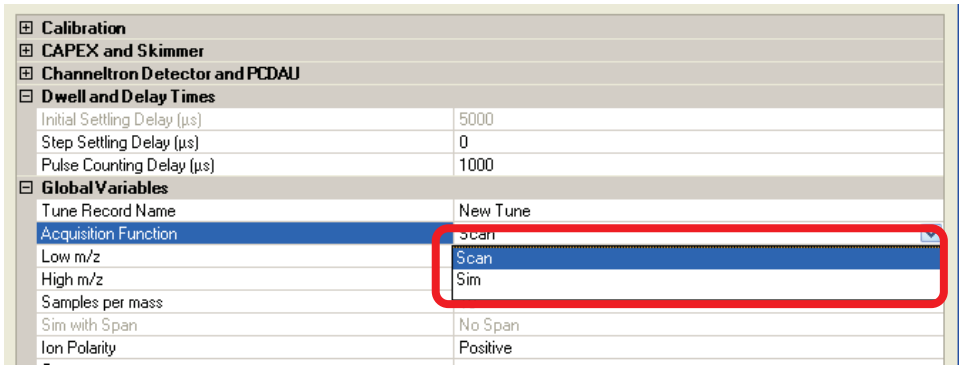


The **Import Tune** dialog displays.



10. Select the most recent **pos_1k** tune file (with a date/time stamp), then click **OK**.
    This example shows **pos_1k 29 Oct 2010** is selected as the most recent pos_1k tune file.

11. In the **Global Variables** section, click on **Acquisition Function,** select **SIM** from the drop-down list, and then press **Enter**. This will convert the **Tune** from a scanning acquisition to a SIM acquisition.



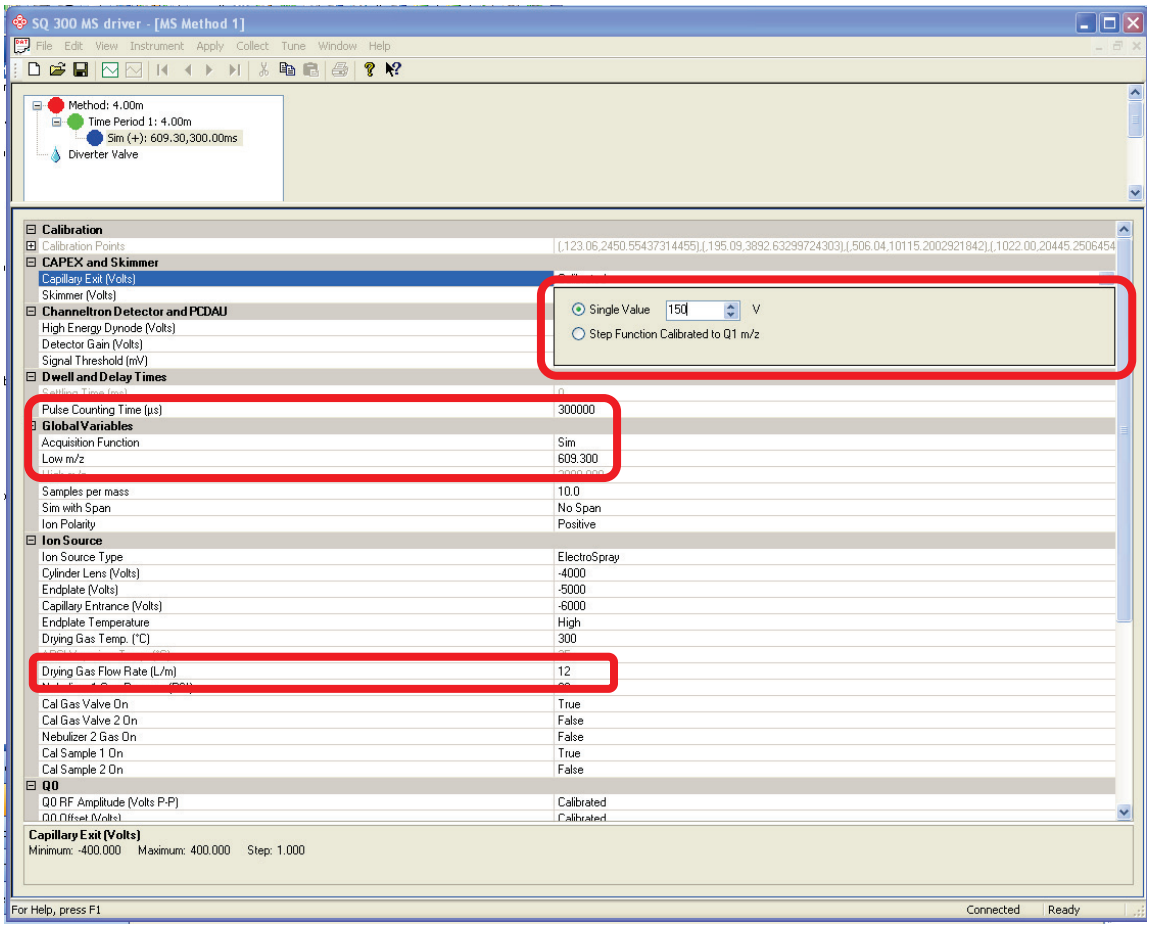
12. To set the specific SIM mass, select **Low m/z,** type your value, and press **Enter**. This example uses *m/z* **609.3**.

13. Set the **Pulse Counting Time (µs)** to **300000**.
    This is a 300 msec dwell time, which will give excellent signal to noise. It is possible to acquire data with much shorter dwell times to achieve higher sampling rates, such as those required for UHPLC separations or if the acquisition requires monitoring many SIM ions "simultaneously" (current limit is 38).

14. Set the **Drying Gas Flow Rate (L/min)** to **12**.
    The optimal drying gas flow is dependent on a number of factors, such as the LC flow rate, composition of the mobile phase and the temperature setting of the **Drying Gas**.

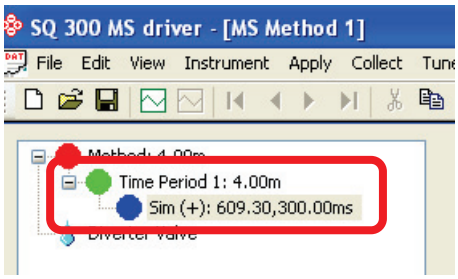
15. Click on **Capillary Exit (Volts)** and from the drop-down list and select **Single Valve**. Enter a value of **150**.

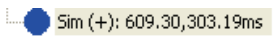
NOTE: *This is a pre-determined value known to be optimal for the compound used in this example. The alternative selection for* ***Capillary Exit*** *(Step Function…) uses a calculated value for the mass selected that is derived from the optimal values determined for the masses in the calibration mix during the Auto Tune procedure.*



16. Left-click on **Capillary Exit (Volts)** again to set this value.

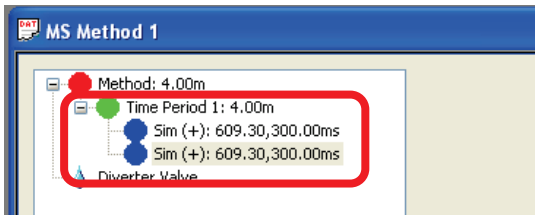
17. Select **Save** from the **File** menu to save the method.
    Notice that in **Time Period 1**, the **Sim** (+) displays the set values.



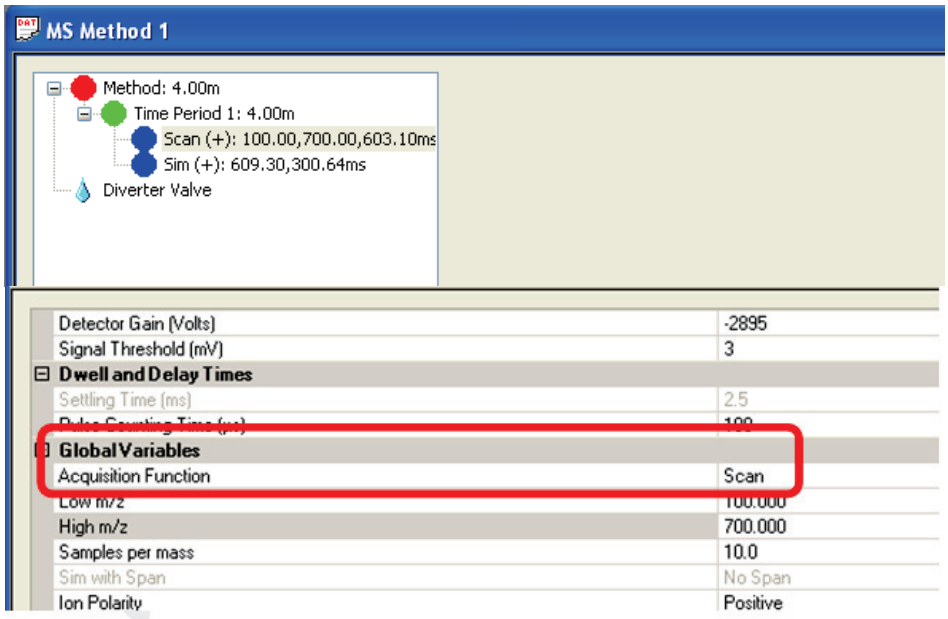
18. Click on the blue dot next to **Sim** ()

19. Select **Copy** from the **Edit** menu, click on the **Time Period** (green dot), then select **Paste** from the **Edit** menu.
    This makes both blue dots **Sim (+)**.

NOTE: *This is a quick way to build a Method requiring multiple acquisition experiments. Simply copy previously defined Tunes as this minimizes the number of changes required for each Tune in the Method.*



20. Click on the top **Sim** blue dot and change it to **Scan** in **Acquisition Function** of the **Global Variables** section.

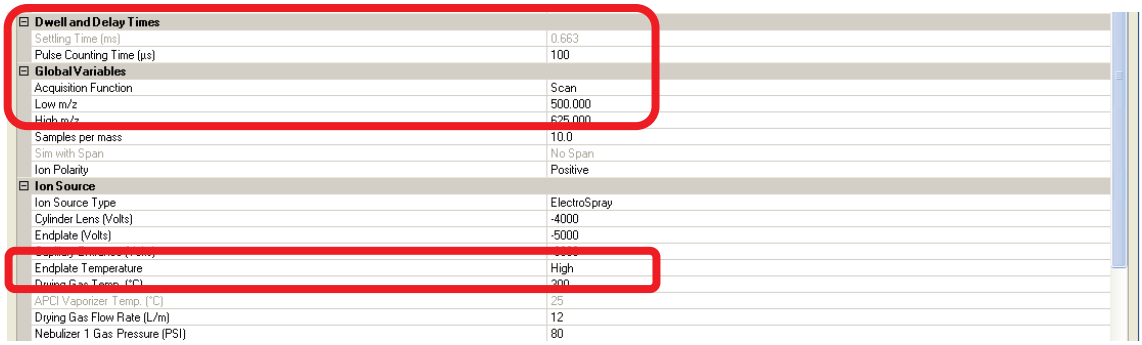


21. Set the **Low *m/z* to 500** and the **High *m/z* to 625.**

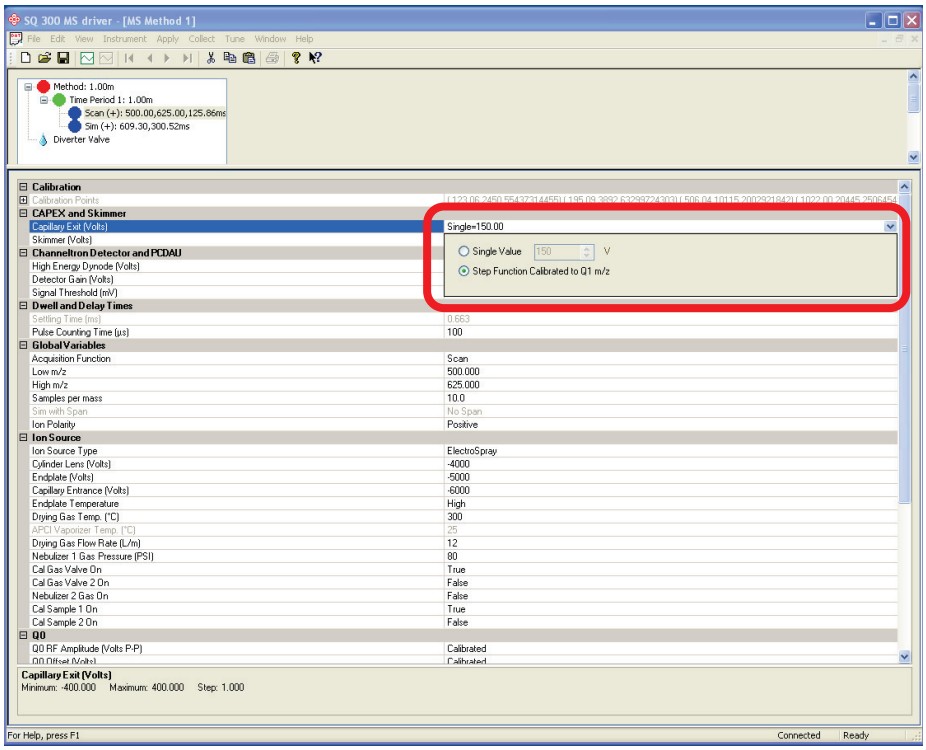
22. Set the **Pulse Counting Time (µs)** to **100.**

NOTE: *The pulse counting (dwell) time entered here applies to <u>each</u> data point within the scan. In this example, the scan range is m/z 500-625, and there are 10 samples per mass, resulting in 1251 total data points acquired per scan, <u>each</u> of which will be measured for 100 µs.*

23. Set the **Drying Gas Flow Rate (L/min)** to **12**.



24. Click on **Capillary Exit (Volts)** and from the drop-down list select **Step Function Calibrated to Q1 m/z**.



25. Left-click on **Capillary Exit** again to set this value.

26. Select **Save** from the **File** menu.

    This example MS acquisition Method, comprised of two Tunes (1 SIM and 1 Scan) that will cycle continuously until the end of Period 1, is now defined and saved.

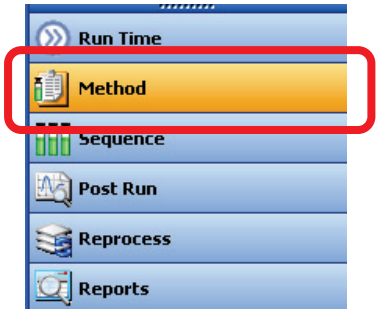
27. **Close** the **SQ 300 MS driver**.

Creating a Chromera Method

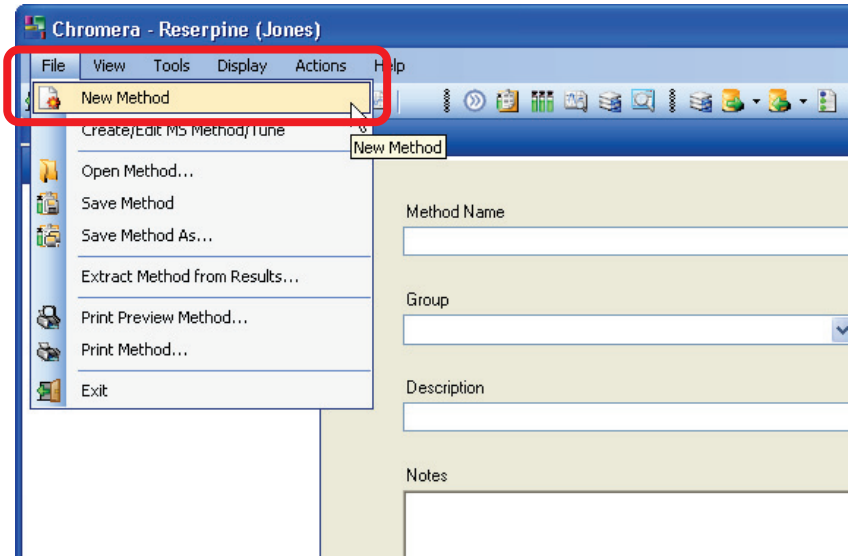
After creating the MS method, open Chromera to create a Chromera method. The Chromera method will define all the operating requirements for all the other components in the Chromera configuration.

To create a Chromera method:

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

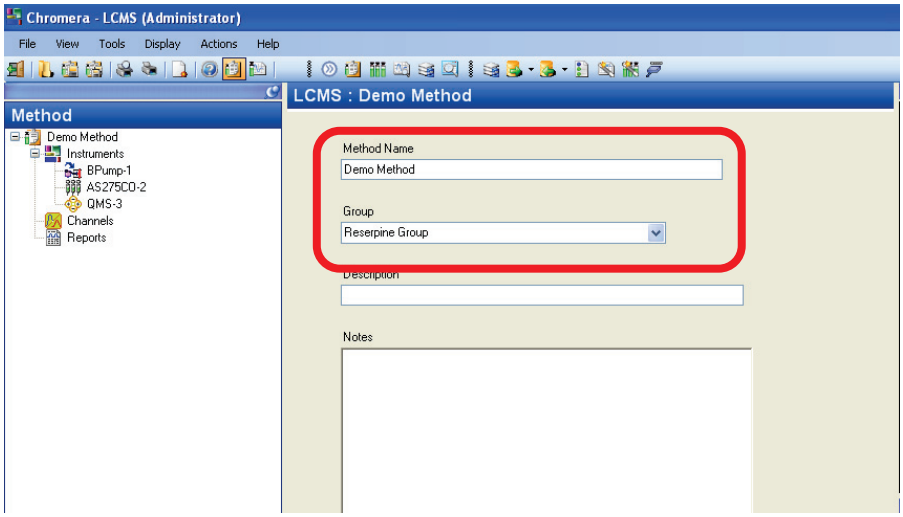


2. Select **New Method** from the **File** menu.



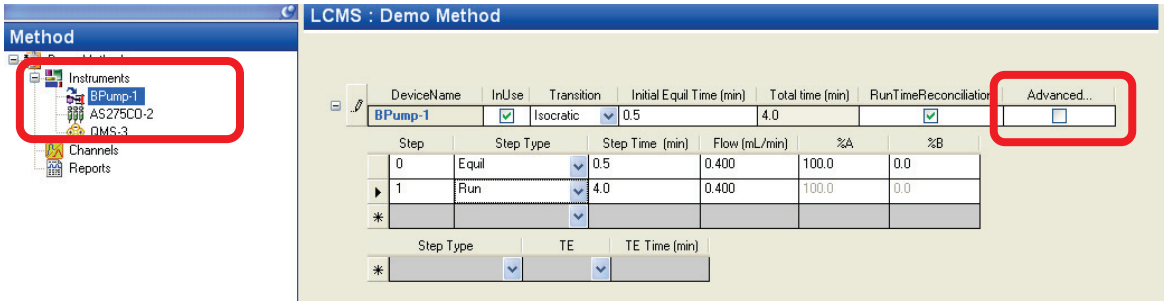
3. Type a **Method Name** and a **Group**.
   Optionally, you can also enter a **Description**.

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

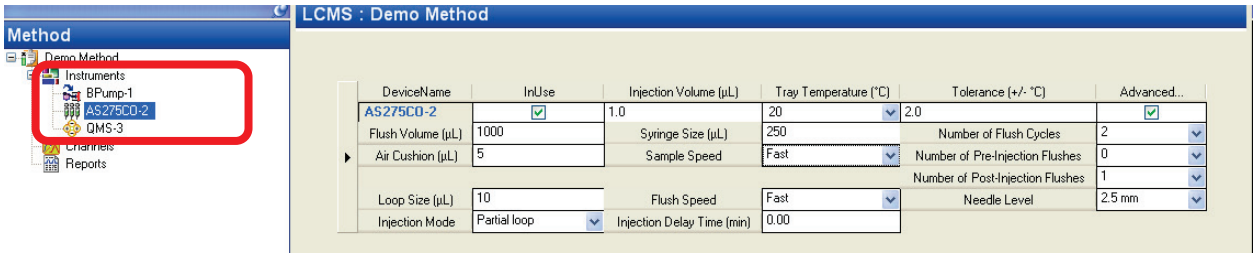


4. Select **Save Method** from the **File** menu.

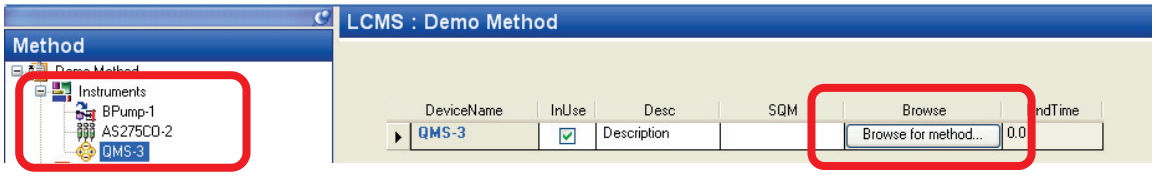
5. Enter your instrument parameters by clicking on each instrument.
   Click on **BPump-1** and enter the pump parameters. Click **Advanced** to show additional parameters.



6. Click on **AS275CO-2** and enter the autosampler parameters. Click **Advanced** to show additional parameters.

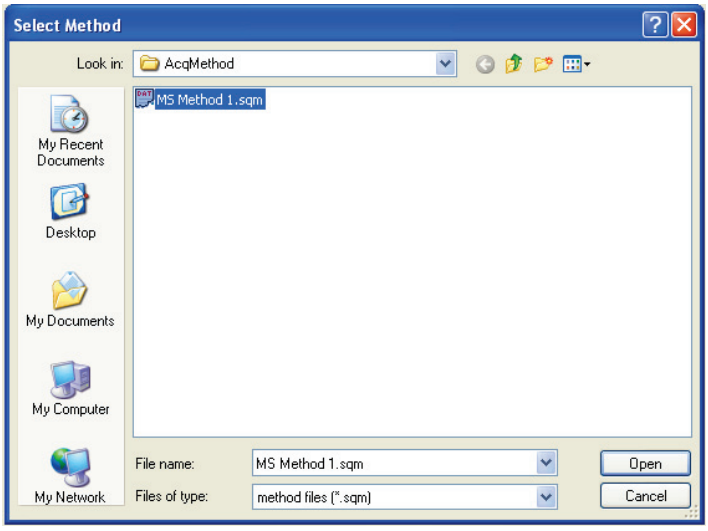


7. Click on **QMS-3** in order to link the MS Method previously defined in the SQ MS driver software to the Chromera method being defined.

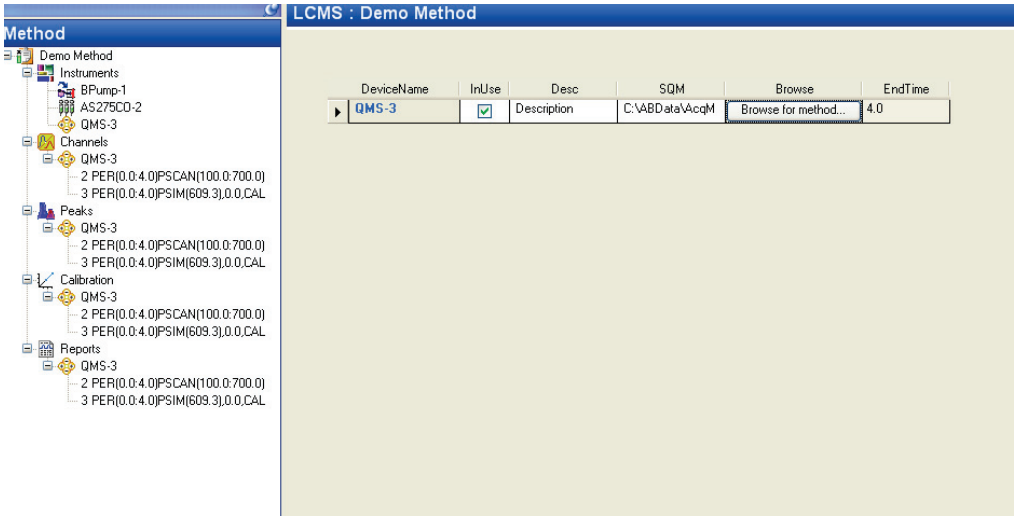


8. Click **Browse for Method**.

The **Select Method** dialog displays.



9. Select the method you created in the SQ 300 MS driver, then click **Open**.
   This example shows **MS Method 1.sqm**



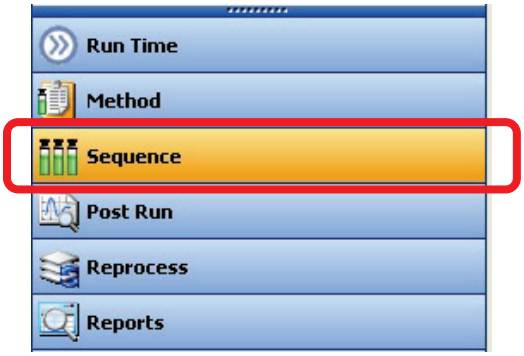
10. Select **Save Method** from the **File** menu.

Creating a Chromera Sequence

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

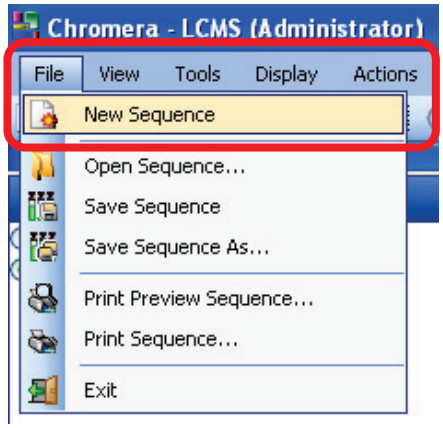
To create a Chromera sequence:

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



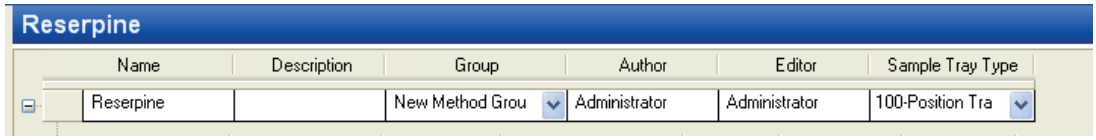
The Sequence screen opens with the last run sequence displayed.

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

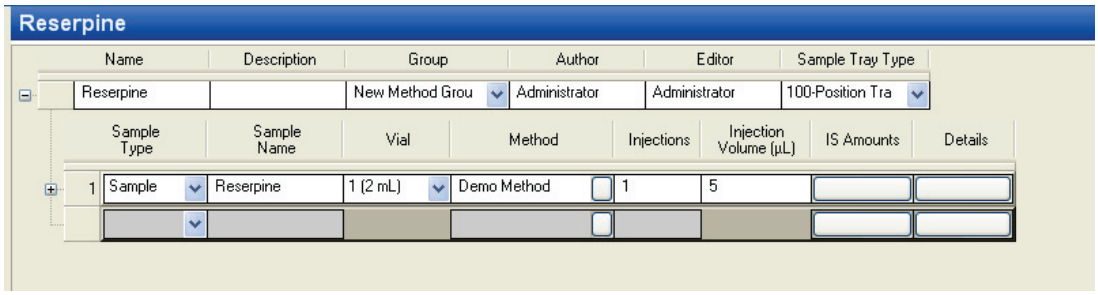


A blank sequence screen displays.

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



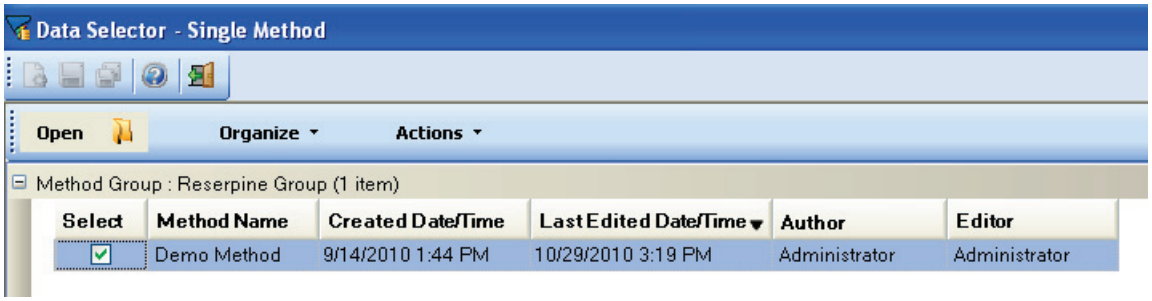
4. Click the plus sign to display the sequence parameters.



5. Enter the Sequence Parameters.
   - Select the **Sample Type** from the drop-down list. This example shows **Sample**.
   - Type a **Sample Name**. This example shows **Reserpine**.
   - Type the number of **Injections**. This example shows **1** injection.
   - Type the **Injection Volume (µL)**. This example shows **5** µL.

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

in the **Method** field.
The **Data Selector – Single Method** dialog displays.

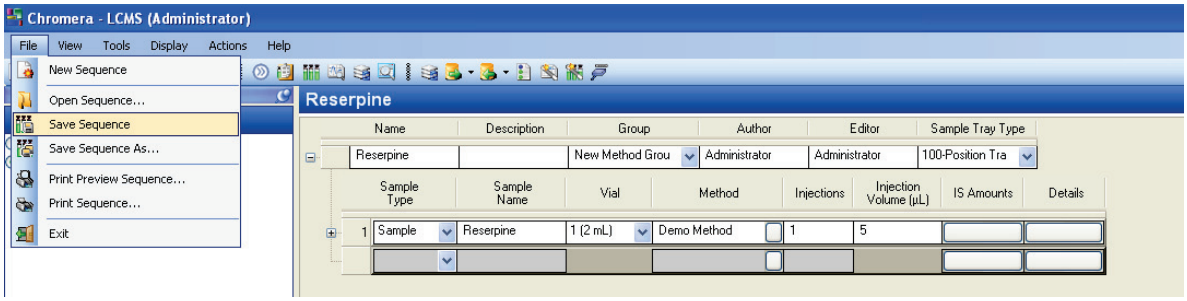


7. Since you saved the method in the **Reserpine Group**, click the plus sign to expand the **Method Group: Reserpine Group**.
   This displays all methods saved in the **Reserpine Group.**

8. Click in the **Select** box to select the method. This example shows **Demo Method** is selected.

9. Click **Open** to enter this method in the sequence.

10. Select **Save Sequence** from the **File** menu.



Starting Data Acquisition

Preparing for an Analysis

Prepare the system with the mobile phase, column, and sample listed below for the analysis. The analysis conducted for the example shown on the following pages utilizes an isocratic HPLC method, which is delivered from a single mobile phase reservoir ("A" in the example).

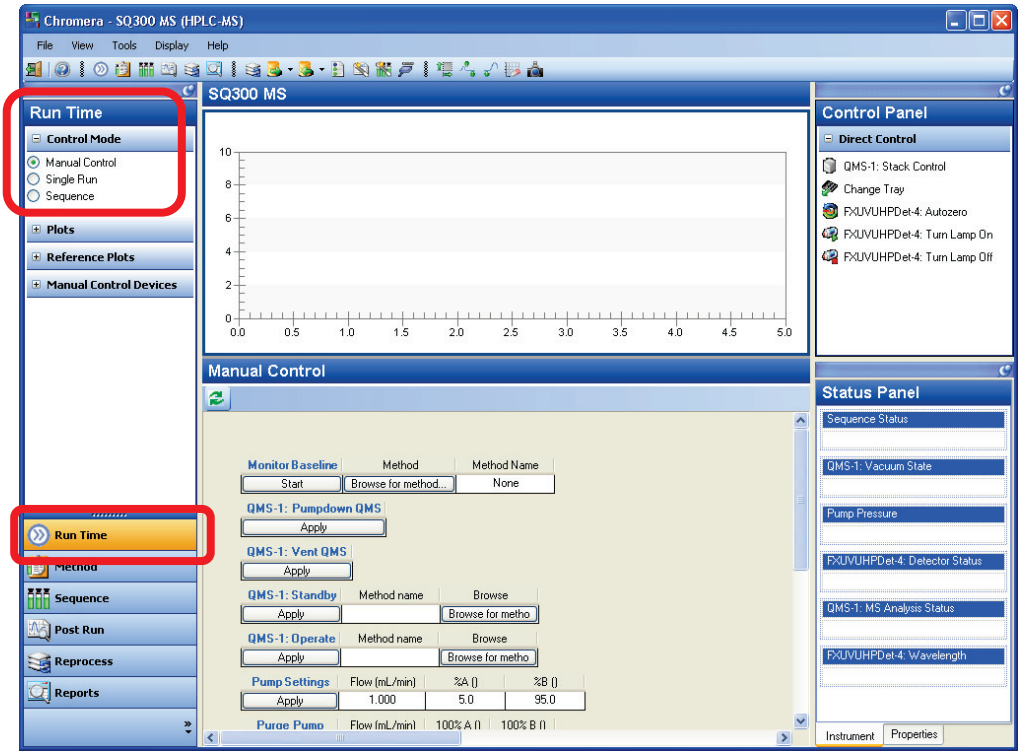
- Mobile Phase: 5 mM ammonium formate in 75/25 Methanol/Water
- Sample: 100pg/µL reserpine
- Column: 3x3 CR C18 column and column holder

Equilibrate the System

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

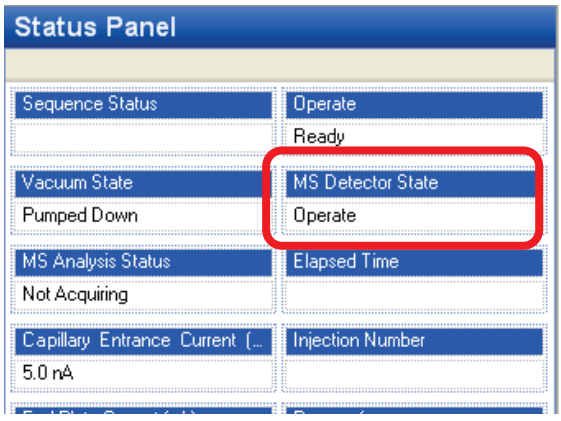
To equilibrate the system:

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



2. Make sure the SQ 300 MS in the **Operate** mode**.**

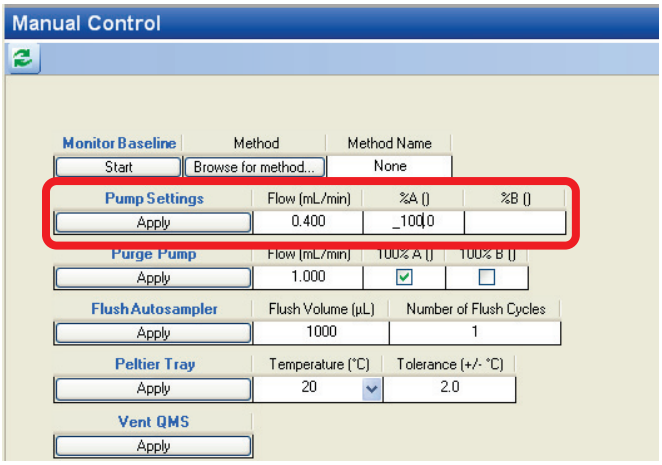
   Observe that in the **Status Panel**, the **MS Detector State** displays **Operate**. If not, Look at the **Manual Control** section of the **Run Time** screen.



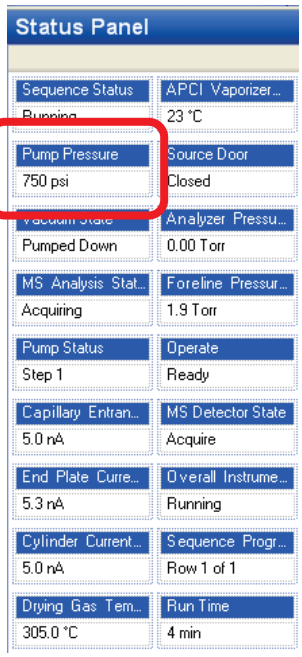
3. In the **Operate** row click **Apply.**

4. Make sure the chromatographic tubing is connected between the LC system and the SQ 300 MS detector.

5. Enter your **Pump Settings (0.4 mL/min and 100% A)**.



6. Click the **Apply** button to start the pump.
   Monitor the pump pressure in the **Status Panel**.

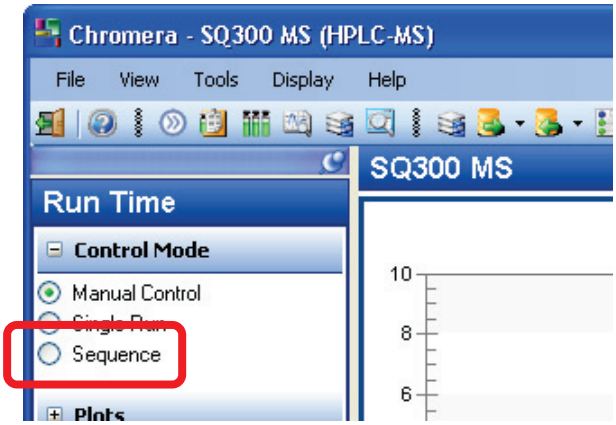


Running a Sequence

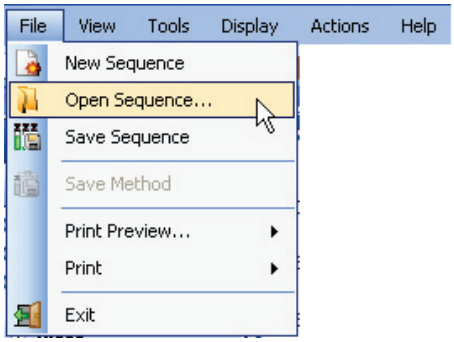
Once the system has reached equilibration, you can load and run the sequence.

To run a sequence:

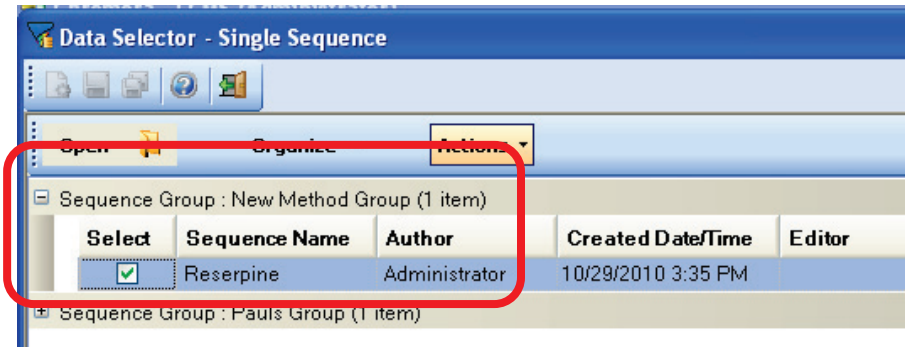
1. Click **Sequence** for the **Control Mode**.



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

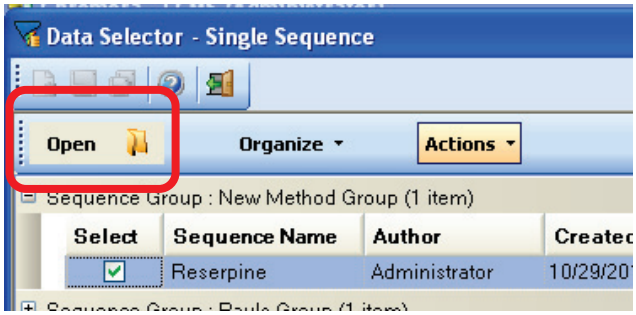


The **Data Selector – Single Sequence** screen displays.

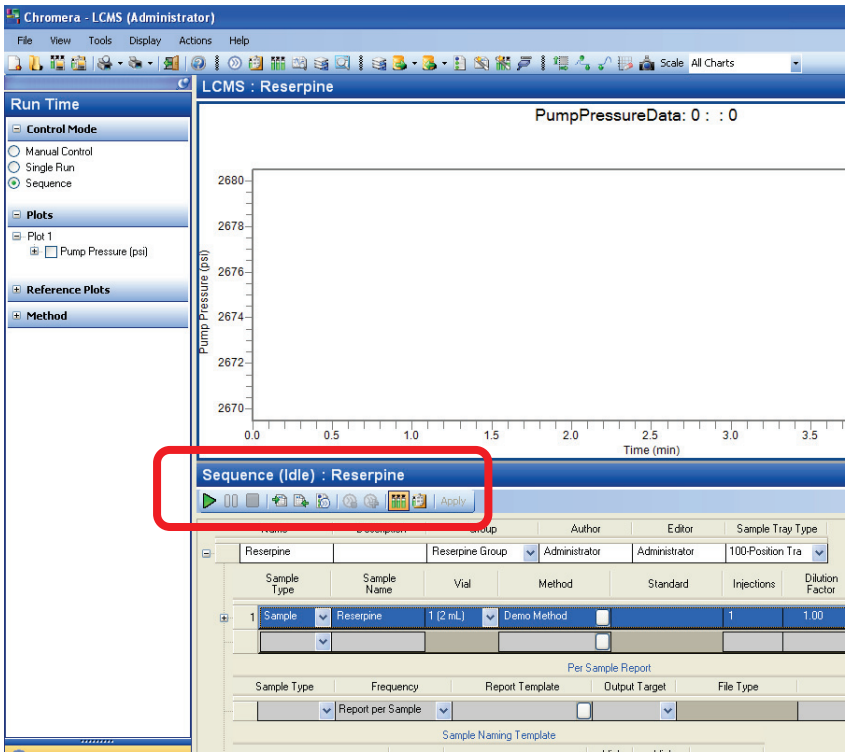


3. Since you saved the sequence in **New Method Group**, click the plus sign  to expand the **Sequence Group: New Method Group**. This displays all sequences saved in this group.
4. Click in the **Select** box to select the sequence. This example shows the Sequence named **Reserpine** is selected.

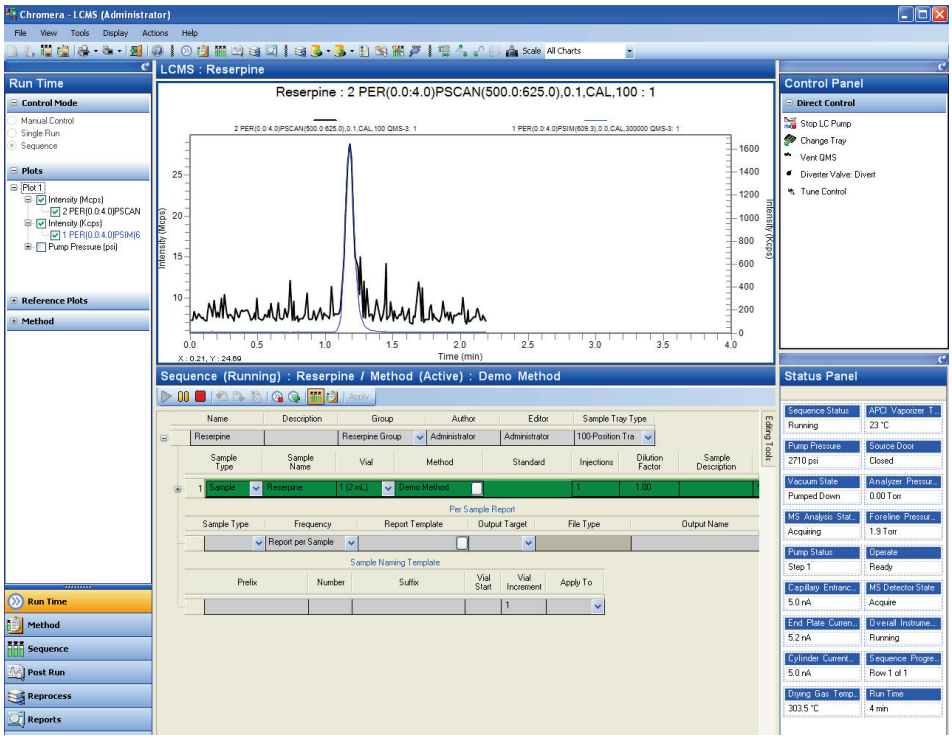
5. Click **Open** to open this sequence.



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

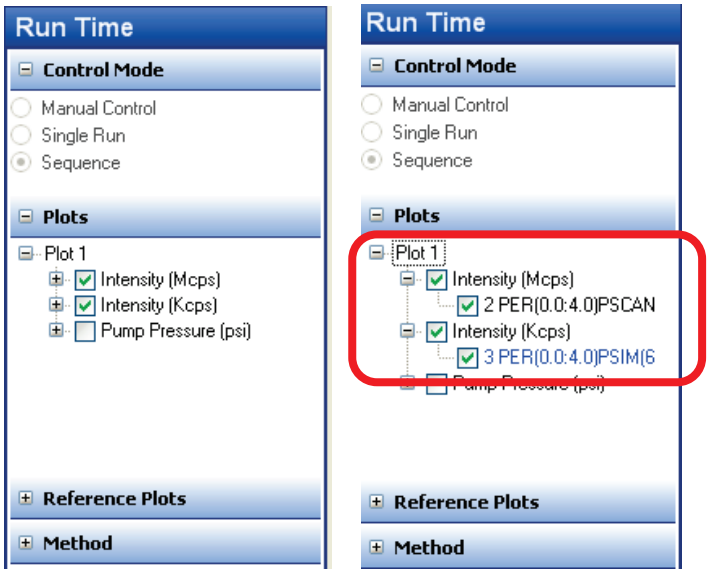


6. Click on the green **Start** ▶ button. The sequence starts to run.

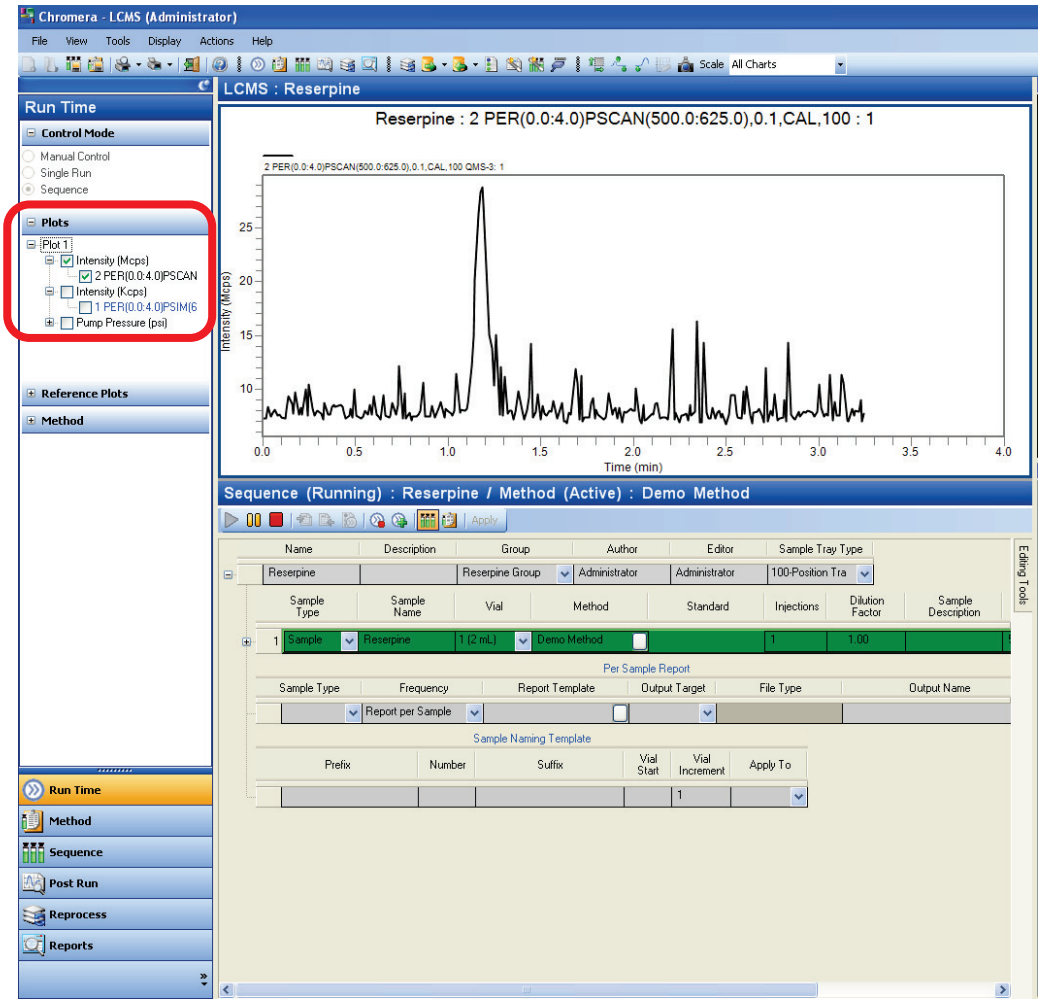


The running sequence is displayed as a green line. Observe the two chromatographic traces. The Total Ion Chromatogram or TIC, which is the sum of intensities for all ions observed in each scan is is displayed as a black line, and the SIM of *m/z* 609.3 is displayed as a blue line.

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



8. During an analysis, you can view the SIM or Scan individually by unchecking the **PER (0.0:4.0)PSIM(6...** box. This example shows only the Scan.



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

Analyze Results in Post Run

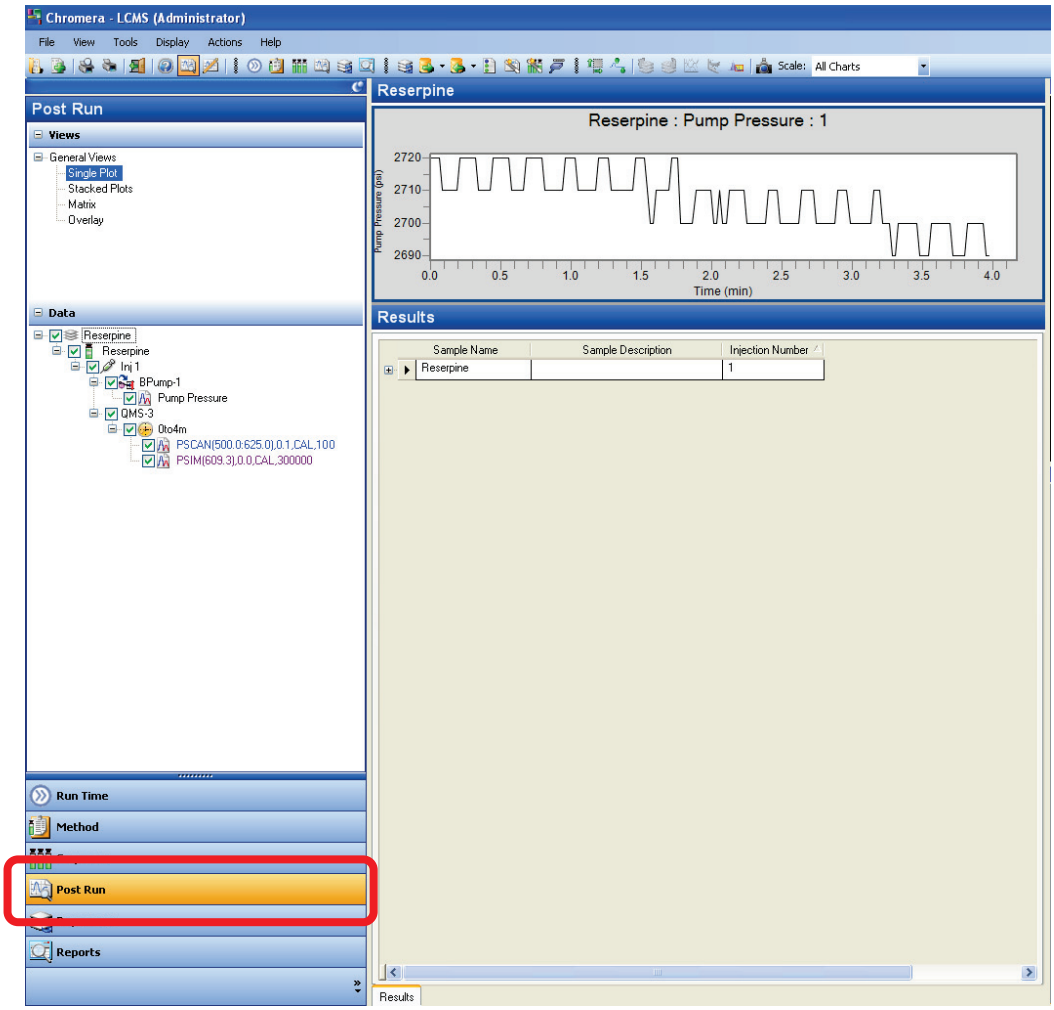
Viewing the Results in Post Run

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).

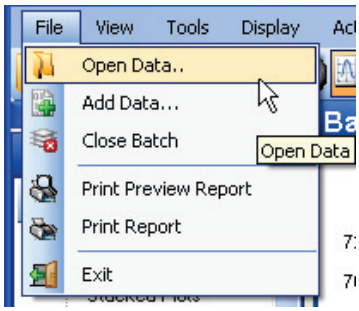
To view results in Post Run:

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



The data trees for Reserpine are displayed in the **Data** pane.

You could also search for previously stored data by selecting **Open Data** from the **File** menu.

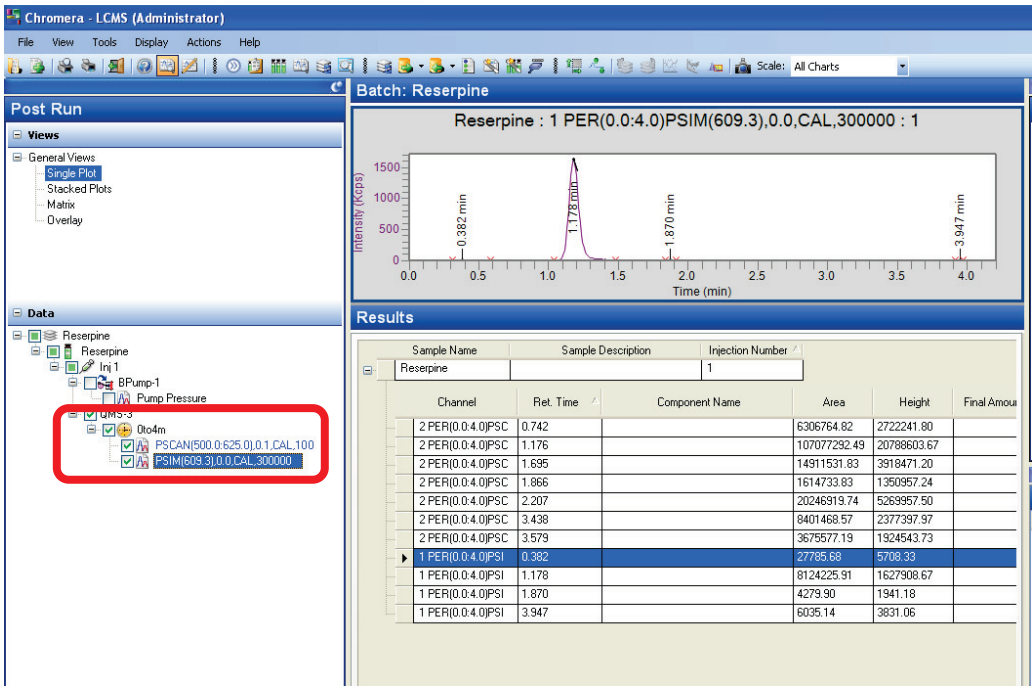


This displays the **Data Selector**. Search and select the data you want to analyze then click **Open**.

2. Uncheck the pump pressure **(BPump-1)**.

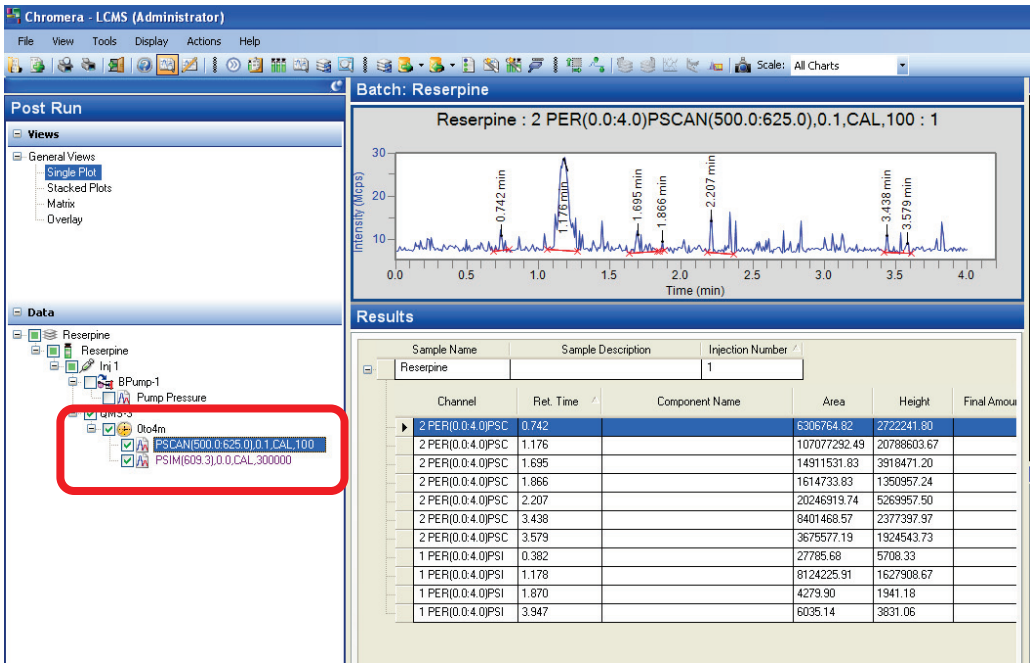
3. Click on **SIM**.

   A SIM chromatogram is displayed in the top plot window and the SIM data are displayed in the **Results** pane.

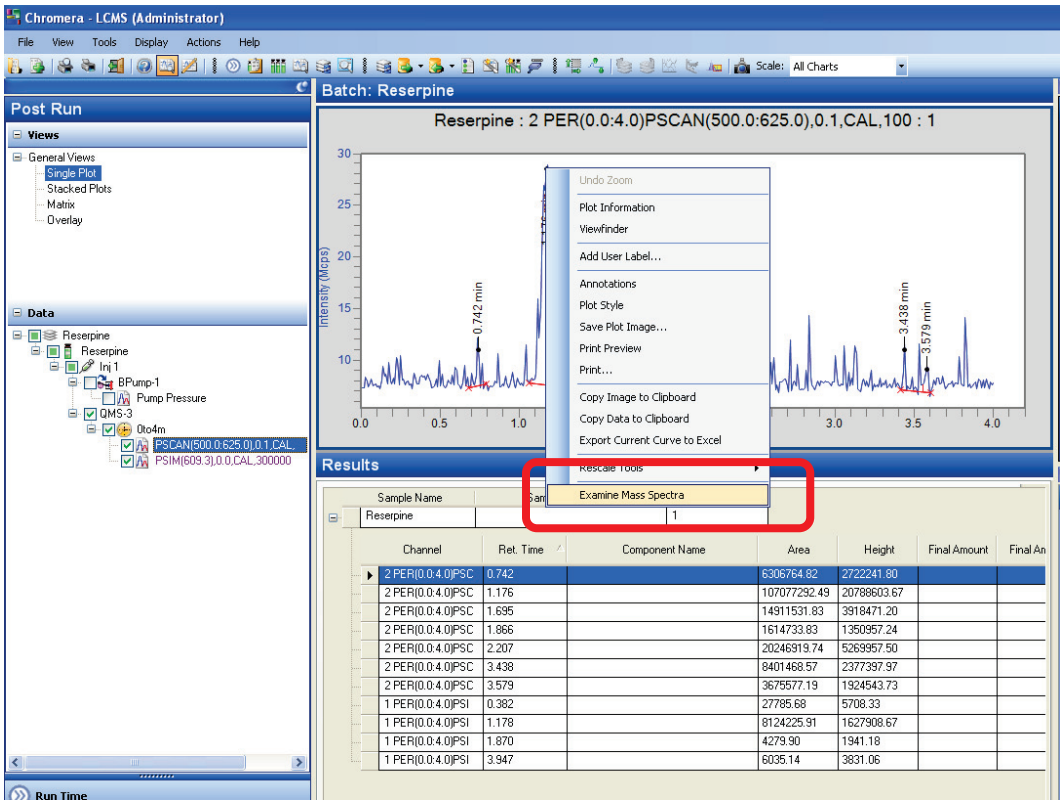


4. Click on **Scan.**

   The TIC (total ion chromatogram) is displayed in the top plot window and the TIC chromatographic data are displayed in the **Results** pane.



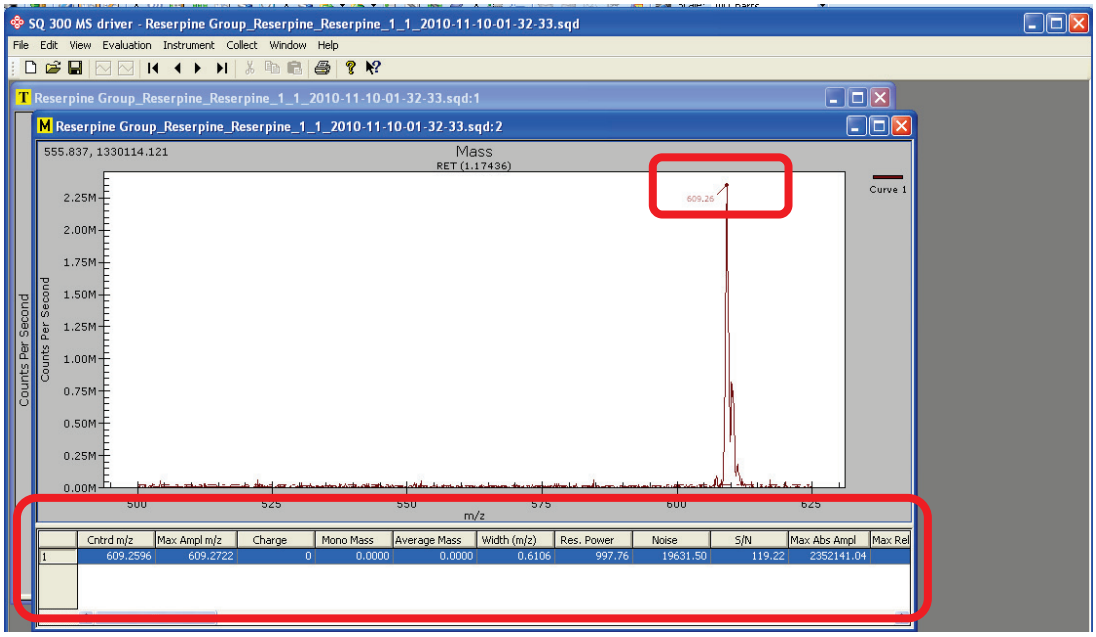
5. Move the mouse pointer to the apex of the peak (it turns to a hand) at retention time ~1.2 min. and then right-click.



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

The spectrum from the selected retention time opens in the lower portion of the SQ 300 MS driver window, and a copy of the TIC is displayed in the top portion of the window.

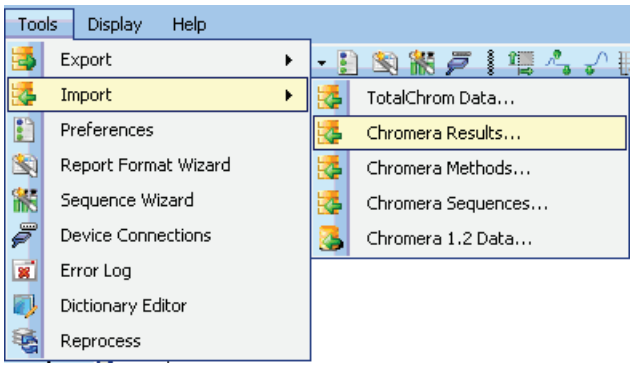
7. Move the mouse pointer to the apex of the *m/z* **609.3** peak (it turns to a hand) then right-click to display the peak table on the bottom of the window. The peak table provides some statistical data on the identified peak including absolute intensity, the peak width, etc.



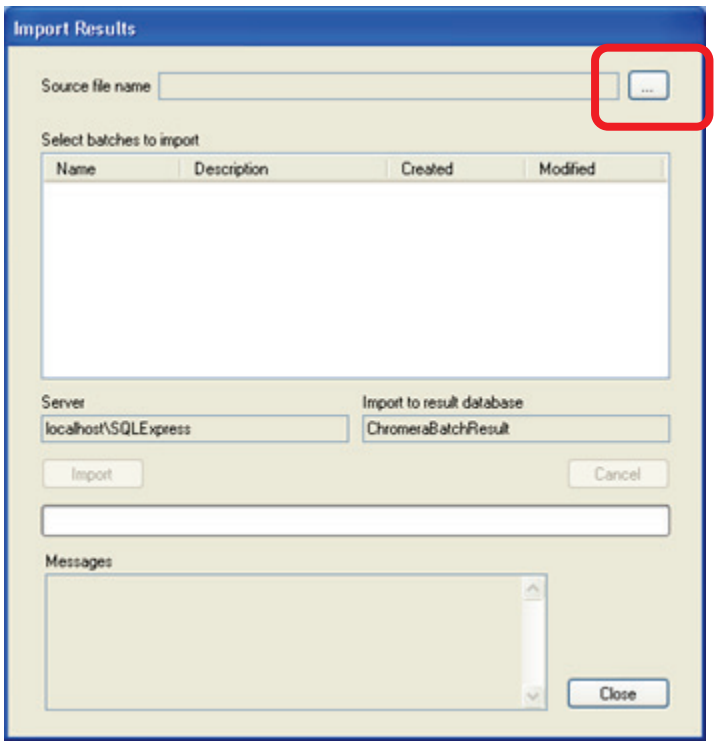
Importing Chromera Data and Methods

To import the data into Chromera:

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

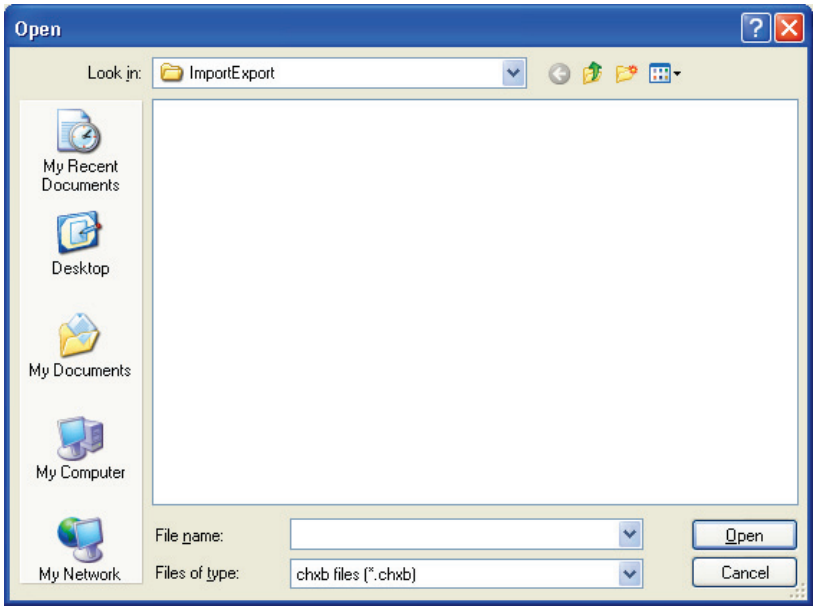


The **Import Results** dialog appears:



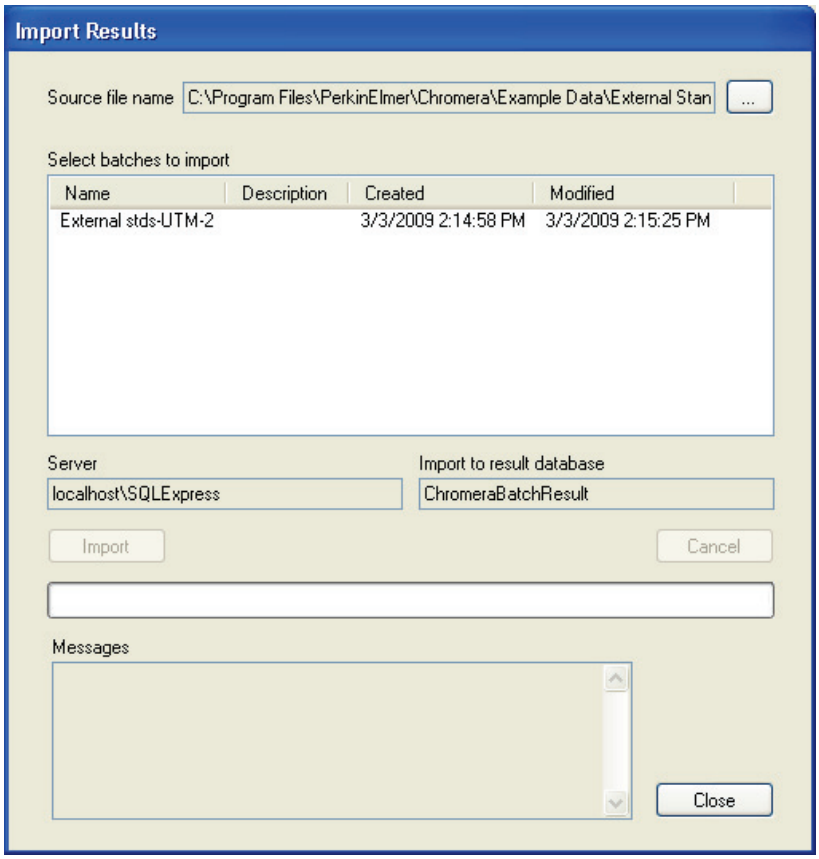
2. Click the browse button  to the right of **Source file name**.

The **Open** dialog appears:

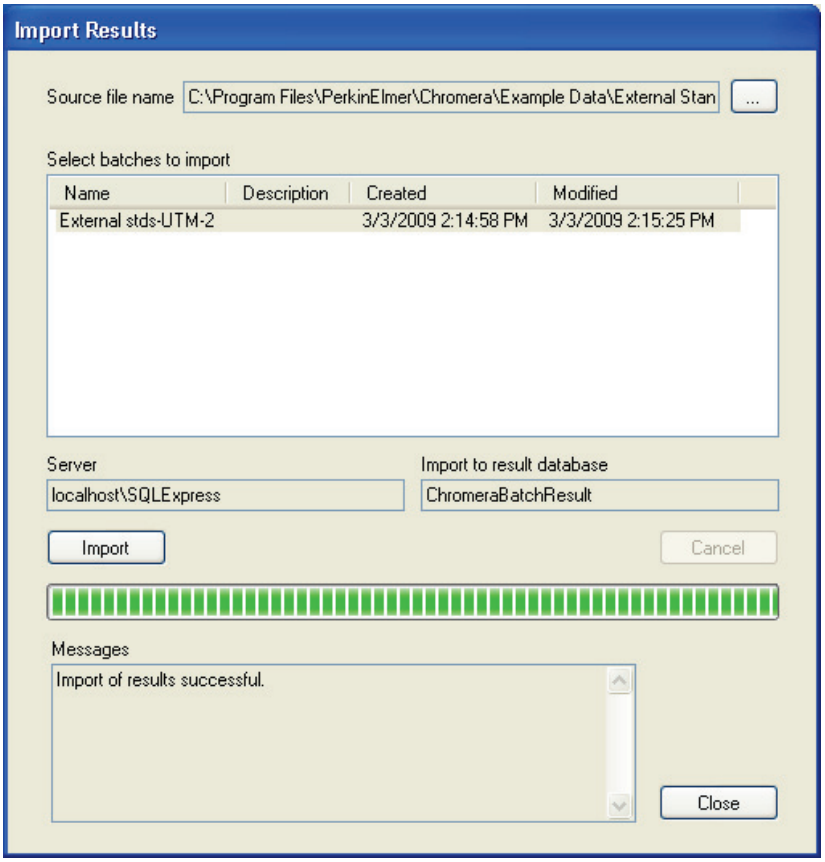


3. Navigate to the directory containing your data.

4. 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.



5. 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.



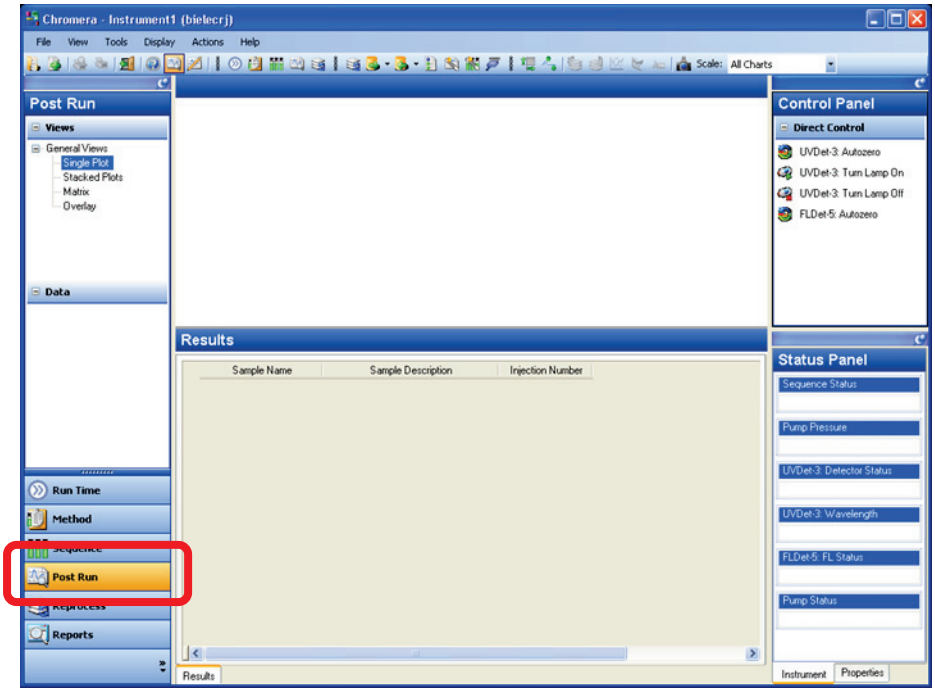
6. Click the **Close** button.

Using the Data Selector

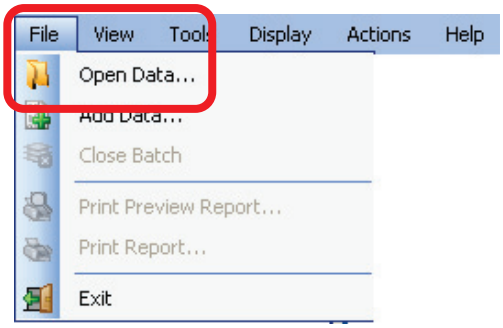
Now that data has been imported into Chromera you can open it using the **Data Selector**. To open a data file using the Data Selector follow this procedure:

1. Click on the **Post Run** button 

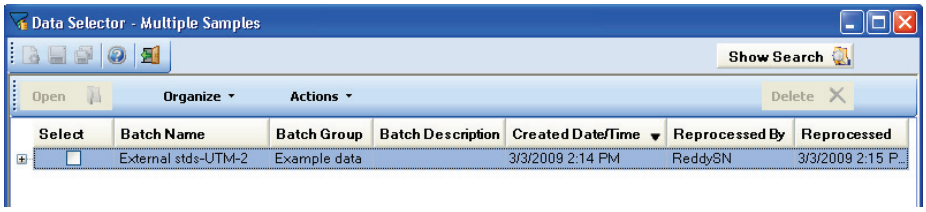
   The **Post Run** environment opens:



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

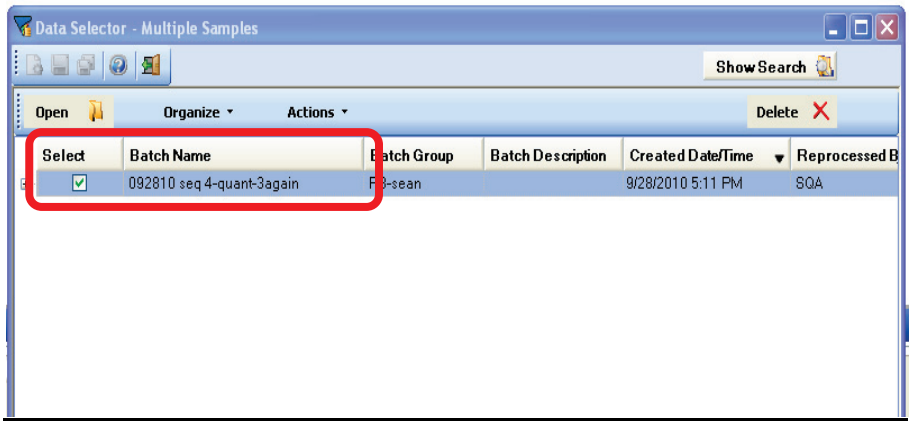


   The **Data Selector** opens.



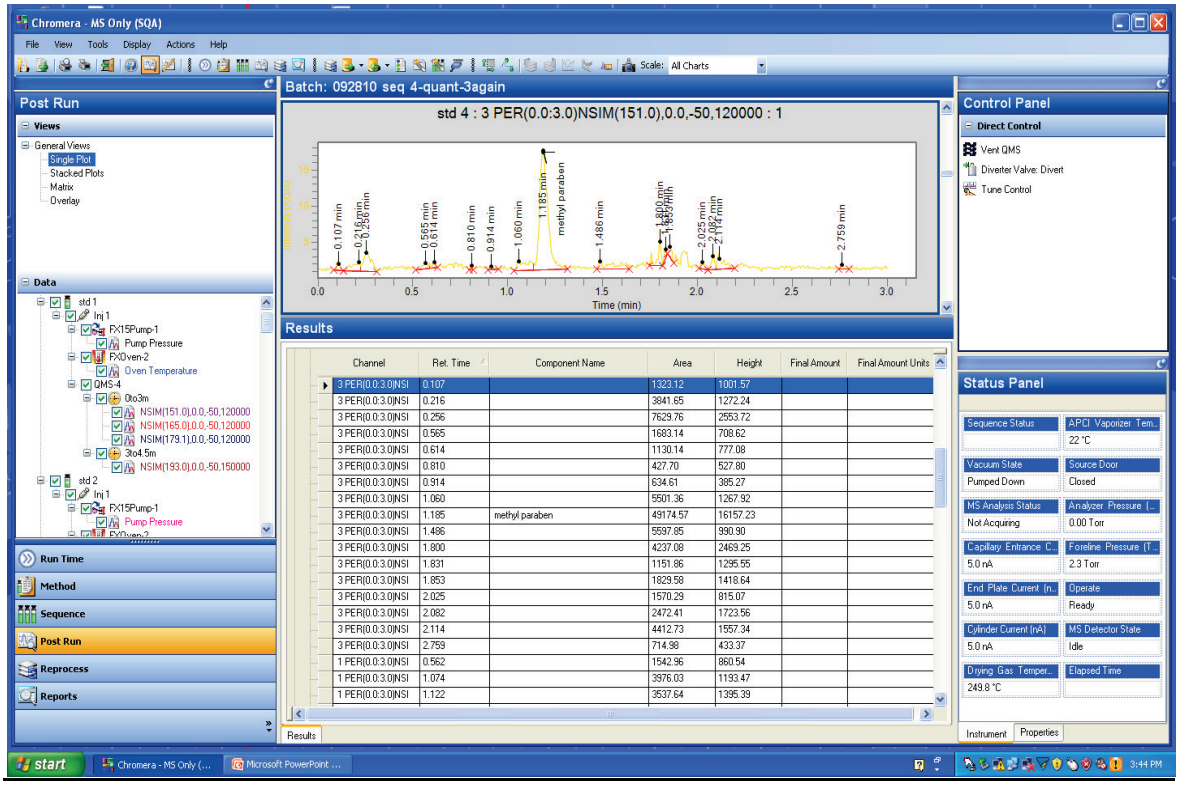
3. Click on the plus sign to expand the batch and choose one or more samples from a batch.

   The data are stored in Batches, which are, in turn, stored in groups. Use the **Data Selector** to choose data for guiding the method editing. The operation of the **Data Selector** is the same throughout the software.



4. Click in the check box to **Select** the **Batch** of data (this example shows **092810 seq 4-quant-3again**), then click the **Open** button.

   This selects all of the samples under the batch. The **Post Run** screen displays the **Example Data**.



Searching a Library to Identify Unknowns

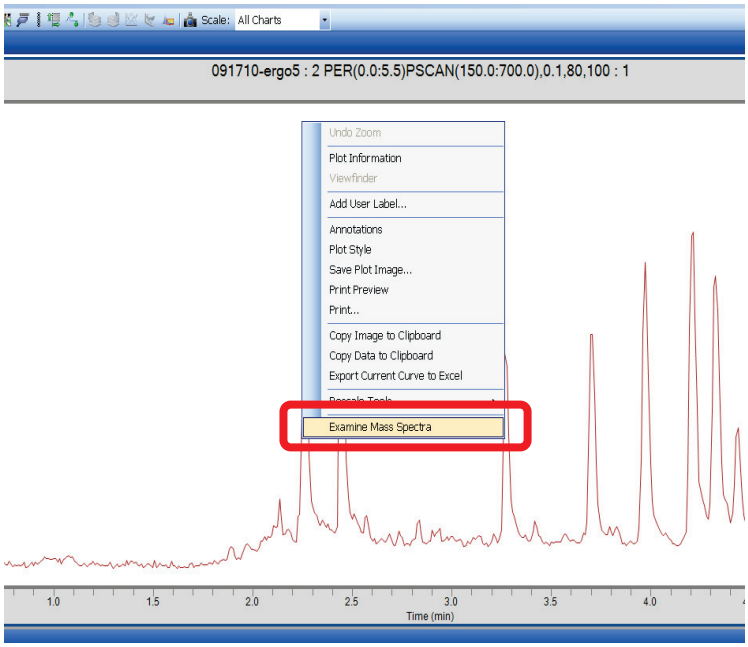
It is important to note that both electrospray (ESI) and atmospheric pressure chemical ionization (APCI) are very soft ionization techniques, especially when compared to more traditional techniques, like electron impact used for GC/MS analysis. Consequently, API mass spectra typically have very little information beyond the protonated molecular ion isotopic cluster, especially after proper background subtraction is performed to remove chemical noise. However, additional "characteristic" fragmentation information can be obtained by increasing the capillary exit voltage, as demonstrated in the ***Ramping Parameters- Optimizing the Capillary Voltage*** section of this guide. Providing additional fragmentation ions will increase the probability of success when trying to compare unknown spectra to previously acquired library spectra.

This example assumes there are previously acquired and stored spectra in the library which can be searched for comparison to the unknown spectra. The following steps summarize the procedure for searching a library to identify unknowns.

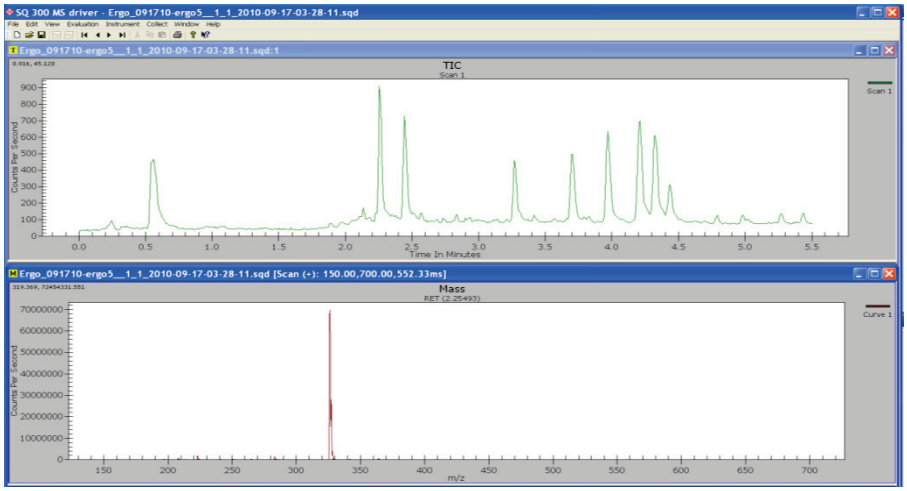
- Display the mass chromatogram of the sample run.
- Select the peak of interest representing the unknown.
- Display the mass spectrum in the SQ Driver.
- Perform a library search.

To search a library to identify unknowns:

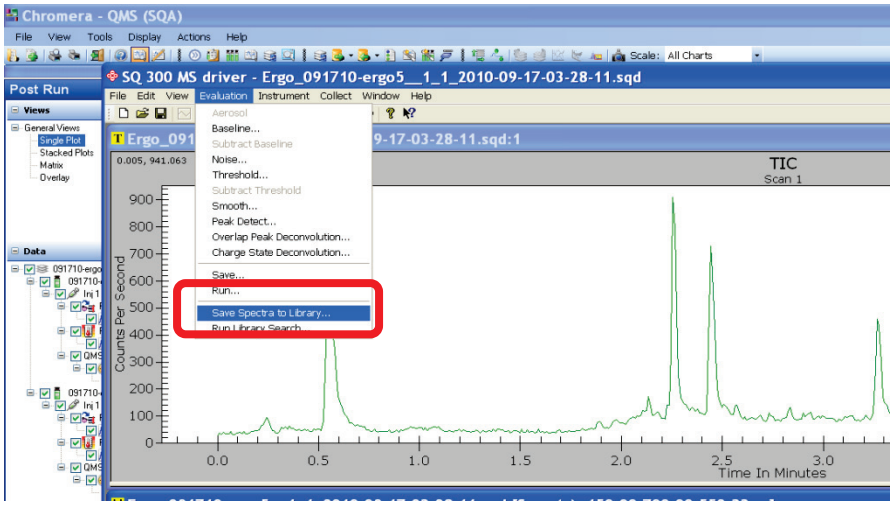
1. In Chromera, open the chromatogram of interest in **Post Run**.
2. Move the cursor to the apex of the peak of interest.
3. Right-click on the apex of the unknown peak of interest and select **Examine Mass Spectra** from the pop-up menu.



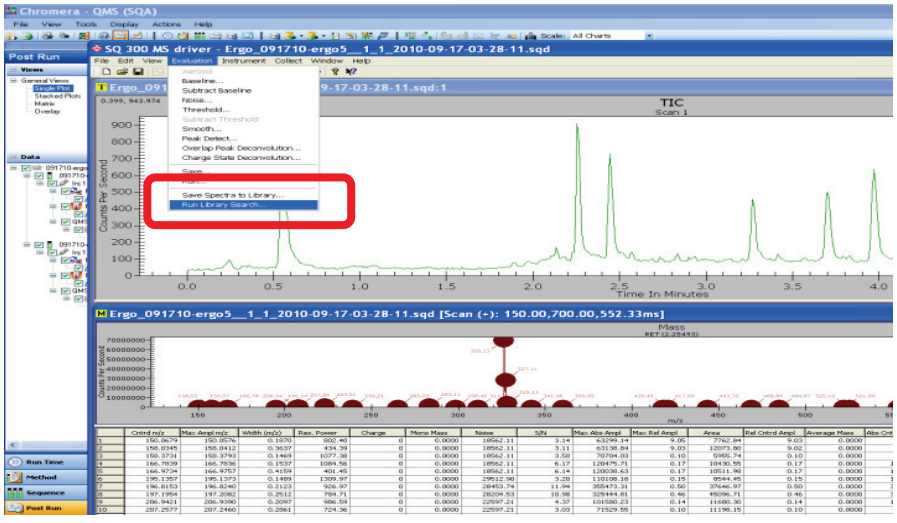
The SQ 300 MS driver window is displayed with the **Mass** spectrum window selected.



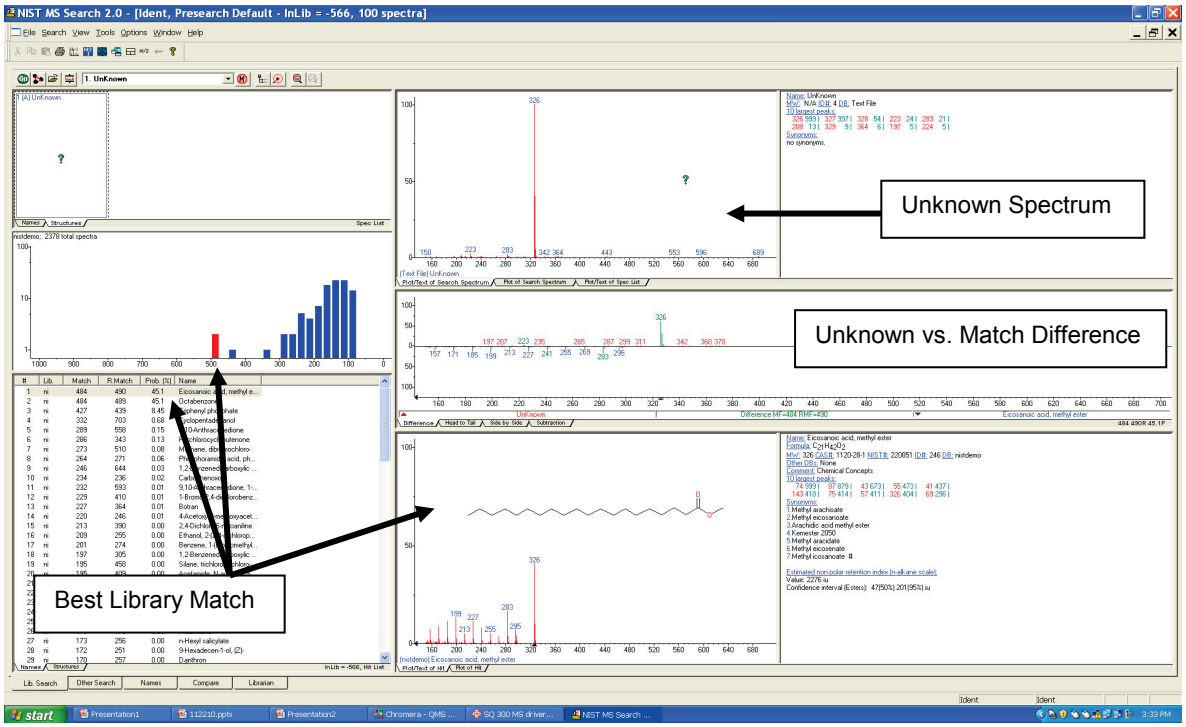
4. Select **Save Spectrum to Library** from the **Evaluation** menu.



5. Run a library search by selecting **Run Library Search** from the **Evaluation** menu.



6. The search results are then displayed.

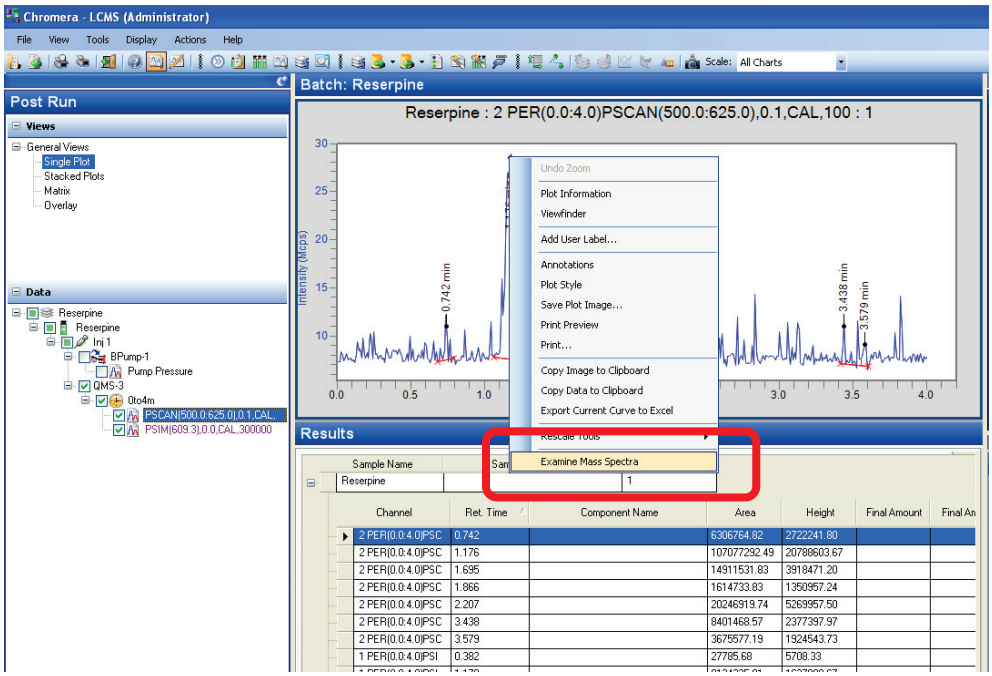


For additional information on library searching, refer to the NIST manual which should be installed on the hard drive and is also available on line.

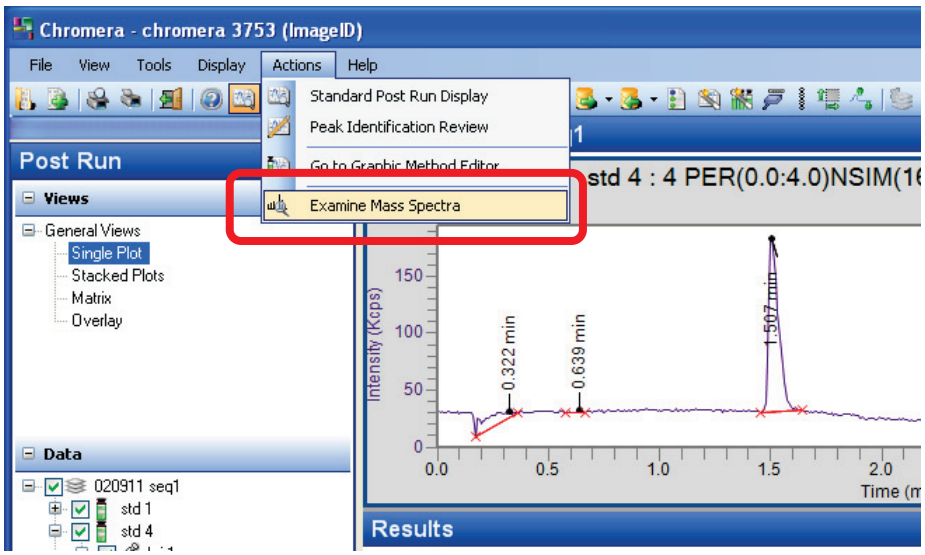
Viewing Spectra Results in the SQ 300 MS Driver Window

NOTE: *Some of the following examples show data from a time-of-flight (TOF) mass spectrometer. However, the commands demonstrated may be applied to the quadrupole data generated by the SQ 300 MS detector.*

1. As demonstrated earlier, moving the mouse pointer to a point on the chromatogram in Chromera and then right-clicking and selecting **Examine Mass Spectra** from the drop-down list will open the SQ 300 MS driver processing window.

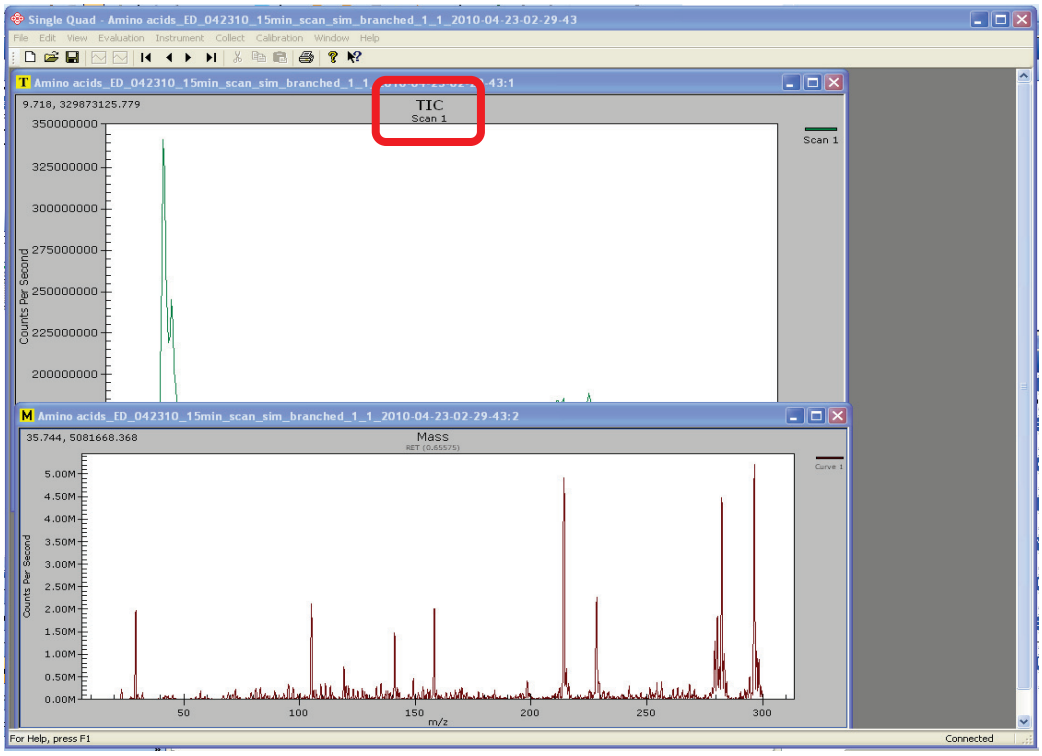


2. Another way to enter the mass spectral processing domain (demonstrated on a different data file) is to select **Examine Spectra** from the Chromera **Actions** menu.



The spectra open in the **SQ 300 MS driver** window.

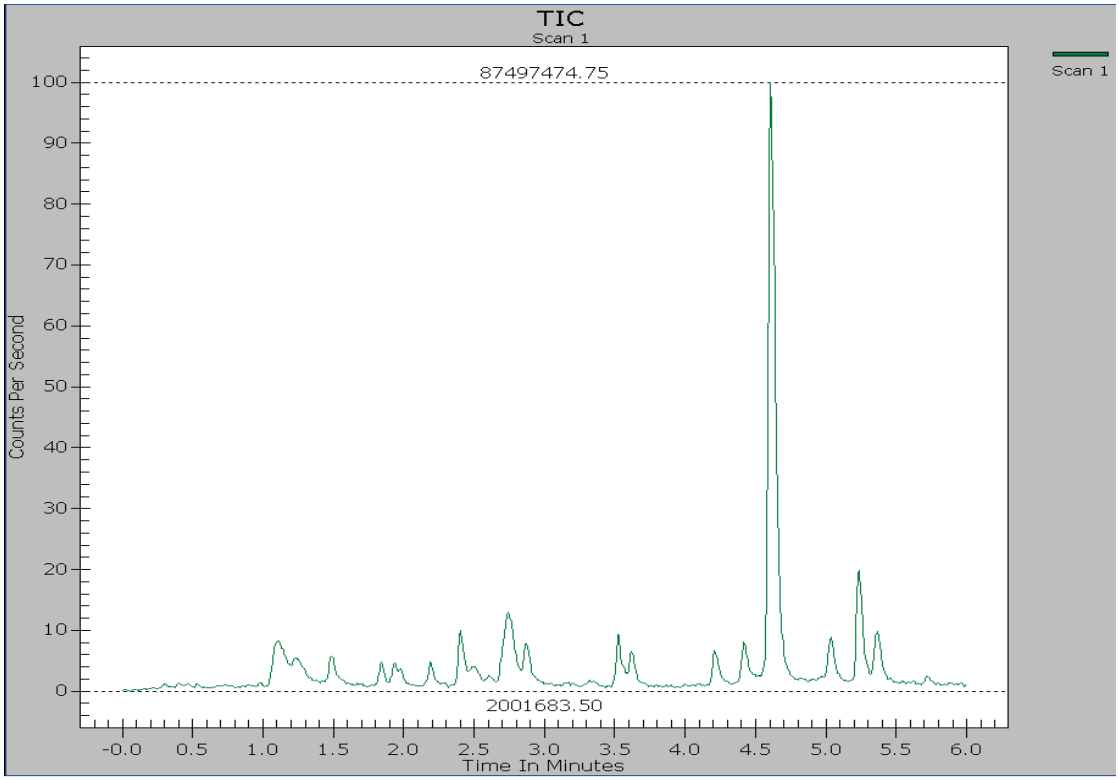
3. The **SQ 300 MS driver** window displays a Total Ion Chromatogram **(TIC)** in the upper half of the window. If the mouse was right-clicked in the Chromera chromatogram (as in the first example above), the spectrum from that retention time will be displayed. If no point in the Chromera chromatogram is selected, then the first spectrum from the acquisition will be displayed.



About the Total Ion Chromatogram (TIC)

The TIC is a chromatogram where each data point represents the sum of intensities of all ions detected for each 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 where the amplitude is UV absorbance.

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.



Creating an Extracted Ion Chromatogram (EIC)

The EIC is a form of a limited mass range TIC. An EIC is extracted from scan data, and typically used where specific ion(s) of interest, or small mass ranges are being searched for. It is possible to display several mass ranges in the same EIC chromatogram.

NOTE: *If specific ions of interest are being monitored for quantitative purposes, the best sensitivity (i.e., lowest detection limits) will be obtained by monitoring the ions in SIM acquisition mode, rather than extracting them from scan data acquired over a large mass range.*

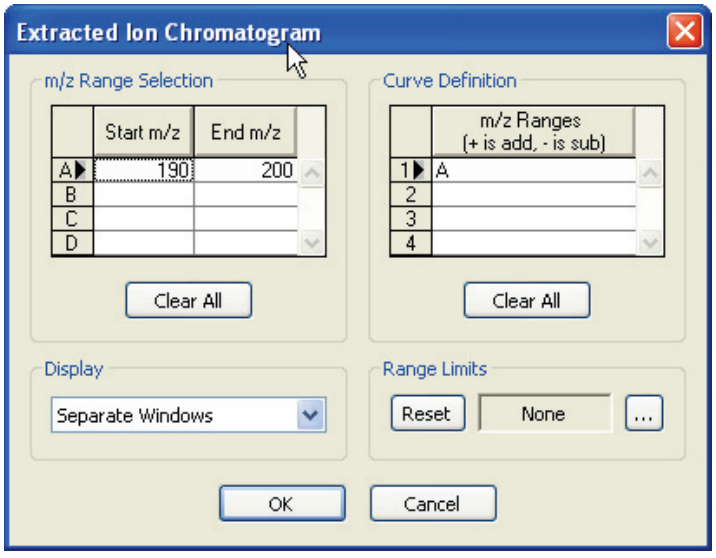
An EIC can be generated in two ways:

- Using the EIC dialog.
- Using a "box" procedure in a generated mass spectrum.

Using the EIC Dialog

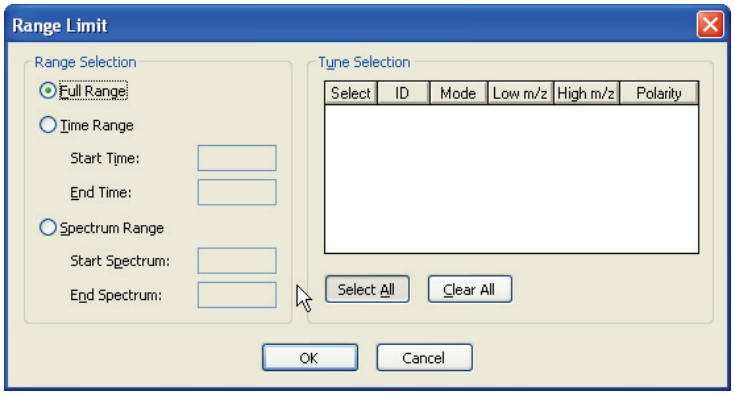
To create an Extracted Ion Chromatogram (EIC):

1. Select the TIC in the SQ 300 MS driver window
2. Select **EIC** from the **View** menu.
3. Enter the first mass range in the first row of the ***m/z* Range Selection** table by clicking in the cell and entering a value.



4. Enter the second mass range in the second row of the ***m/z* Range Selection** table.
5. Decide how to display the curves in the **Curve Definition** table.
   This allows you to perform simple algebraic operations (addition or subtraction) on the mass ranges selected. Enter the row identification (A,B) to display the curves in a simple way.
6. Select the **Range Limit** dialog.

7. For the **Range Selection**, select **Full Range**, **Time Range** or **Spectrum Range**.

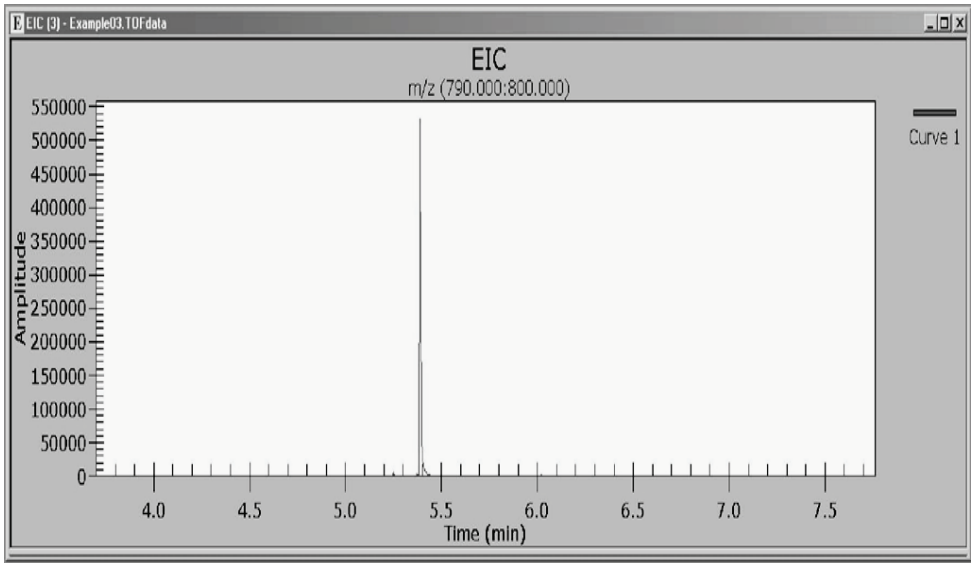


8. Select how to display the created EIC.

NOTE: *An EIC cannot be displayed in the same window as a TIC.*

9. Click **OK** to close the **Range Limit** dialog and click **OK** to close the **Extracted Ion Chromatogram** dialog**.**

   The **EIC** displays.



Creating a Base Ion Chromatogram (BIC)

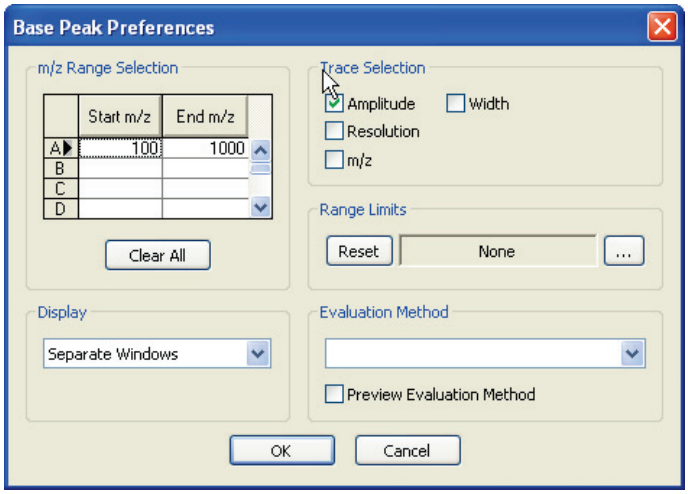
The BIC is a form of a limited mass and time range ion chromatogram where mass, peak intensity/area and resolution are displayed as a function of time or spectrum number.

A BIC can be generated in two ways:

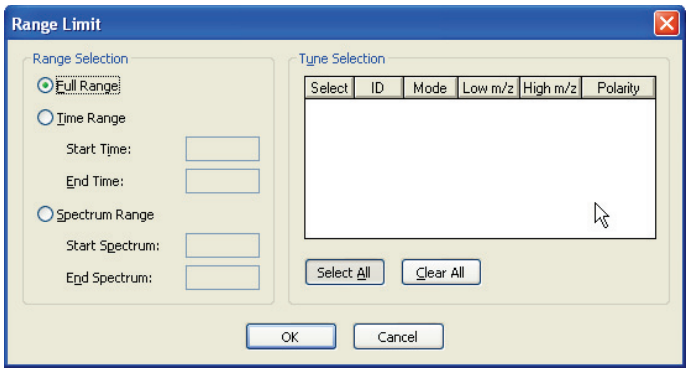
- Using the BIC dialog.
- Using a "box" procedure in a generated mass spectrum.

To create a Base Ion Chromatogram (BIC):

1. Select a TIC in the SQ Driver window
2. Select **BIC** from the **View** menu.
3. In the ***m/z* Range Selection** section enter the *m/z* range to cover the mass peak to be studied.



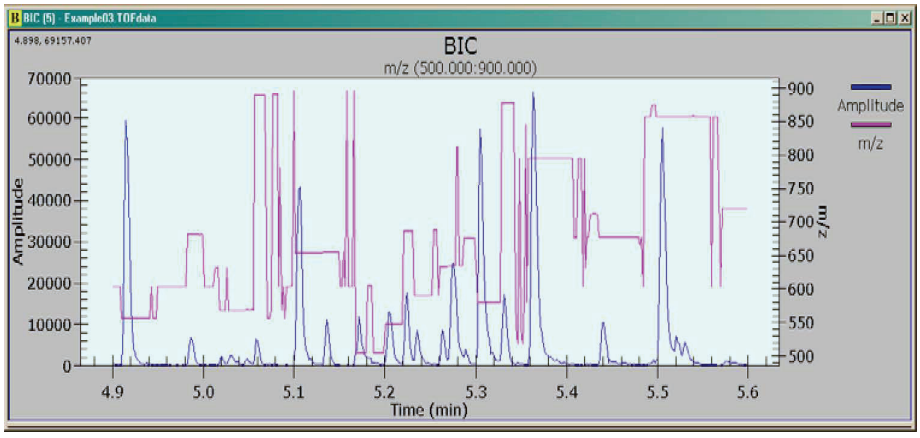
4. In the **Display** section, select how to display the chromatogram.
5. In the **Trace Selection** section, select the **Traces** to be displayed.
6. Select the **Evaluation Method** from the drop-down list.
7. Click the **Range Limits** search button  . The **Range Limit** dialog displays.



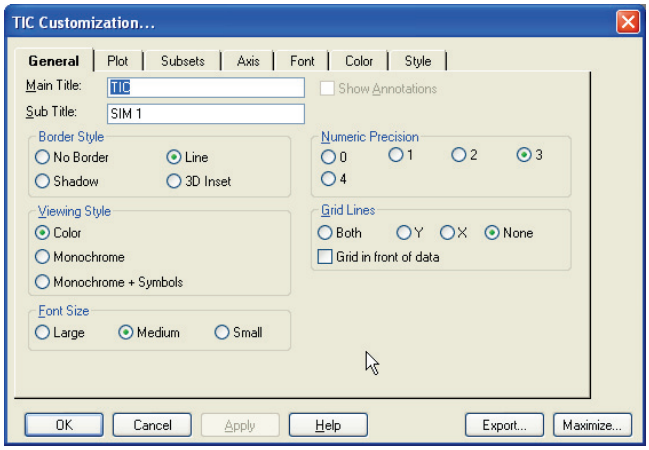
8. In the **Range Selection** section, select to display **Full Range**, **Time Range**, or **Spectrum Range**.
   - If you select **Time Range**, enter the number of the first and last spectrum.
   - If you select **Spectrum Range**, enter the number of the first and last spectrum.

9. Click **OK** to close the **Range Limit** dialog, then click **OK** to close the **Base Peak Preference** dialog.

   The **BIC** will display mass, intensity and resolution for the selected base peak as a function of retention time.



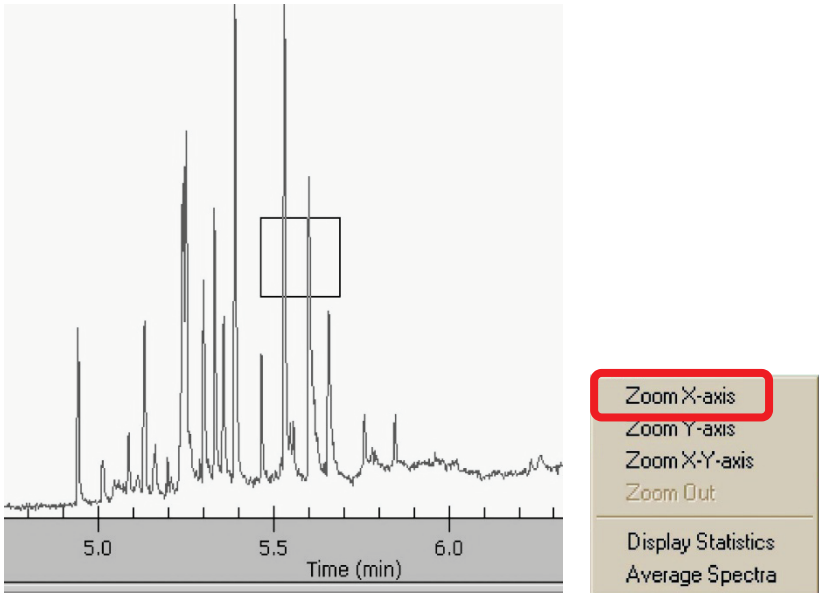
10. To display intensity only, right click and select **Customization Dialog** from the menu to display the **Customization** dialog. In the **Subsets** tab, select Intensity and click **OK**.



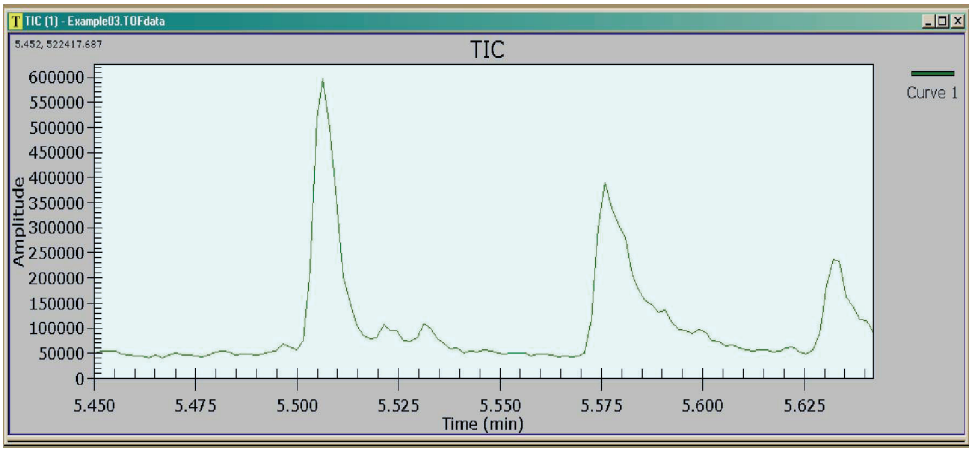
Processing of Ion Chromatograms

To zoom in:

1. Left-click and drag a box around the area of interest, release button and select **Zoom X-axis**.



The zoomed area appears.

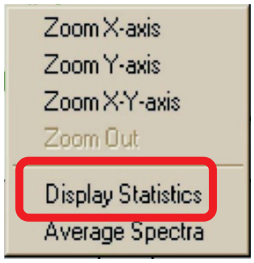


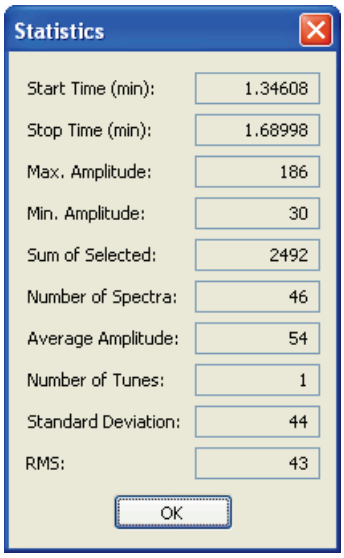
2. Left-click and drag a box somewhere in the chromatogram, release the button and select **Zoom Out**.

3. Use the right mouse button command, **Undo Zoom**.

Displaying Statistics

- Left-click and drag a box around a peak of interest, release the button and select **Display Statistics**.



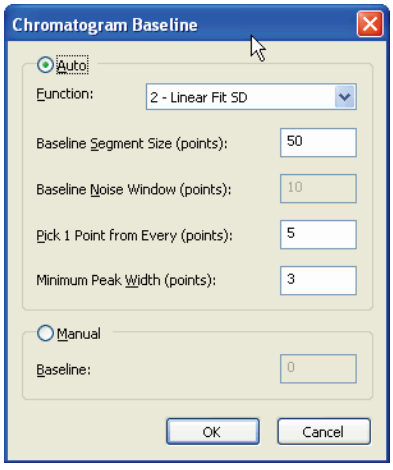


About Right Mouse Click Menus

Each graph view contains a graphical package which includes functions to modify and export graphs. Individual functions can be selected or the **Customization Dialog** can be used.

Baseline Calculation

1. When a TIC, EIC, or BIC 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.*

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

5. A **Manual** baseline can be created by entering a value resulting in a straight line as a baseline.

6. The baseline can be hidden or displayed with the **Baseline** from the **View** menu.
   The baseline can be subtracted from the chromatogram with the menu command **Subtract Baseline** from the **Evaluation** menu.

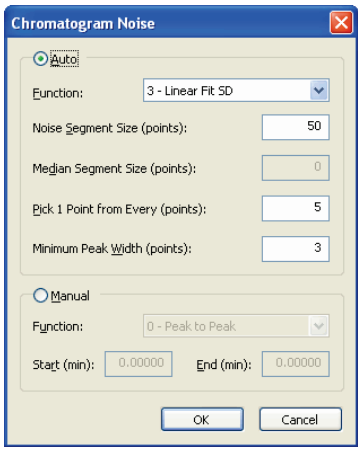
Setting the Chromatogram Noise Calculation Preferences

1. When a TIC, EIC or BIC window is selected, 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.

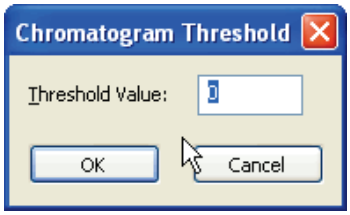
3. Select Peak to peak or Root mean square.
   In the default version of the signal to noise calculation the following is done:
   - If the noise is Peak to peak, the amplitude of the peak top or the centroid is subtracted by the low value of the noise and divided by the peak-to-peak difference. Click **OK**.

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



Chromatogram Threshold

1. Activate a TIC, EIC or BIC and select **Threshold** from the **Evaluation** menu.



2. Enter a **Threshold value** and decide if the ion chromatogram will be subtracted with this value.

3. 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.*

Chromatogram Smoothing

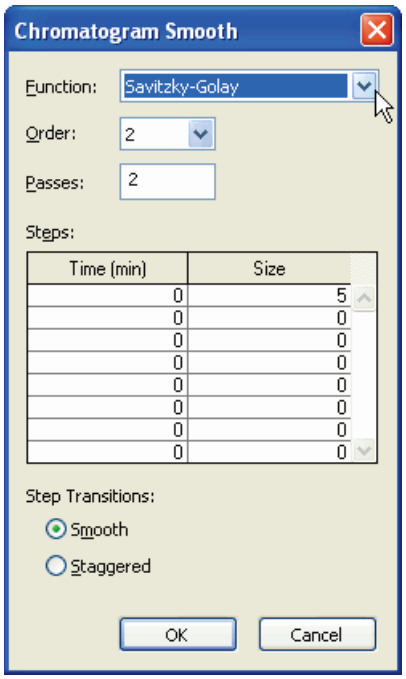
1. Select a TIC, EIC, or BIC.

2. Select **Smooth** from the **Evaluation** menu.

3. Enter number of smooths (1-10), window size (0.01-100) and select a **Function** from the drop-down list.

   0-Mean: For each data point in the source curve the processed curve is calculated as the average of the data points within the specified window.

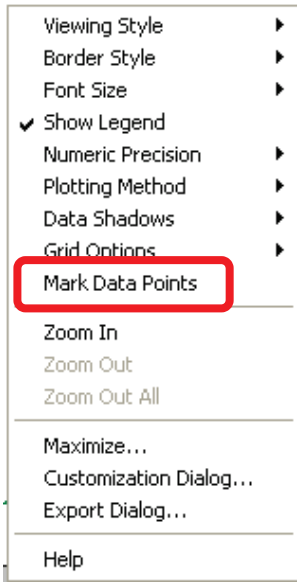
   1-Median: The processed curve is calculated as the median of the data points.



Chromatogram Peak Detection

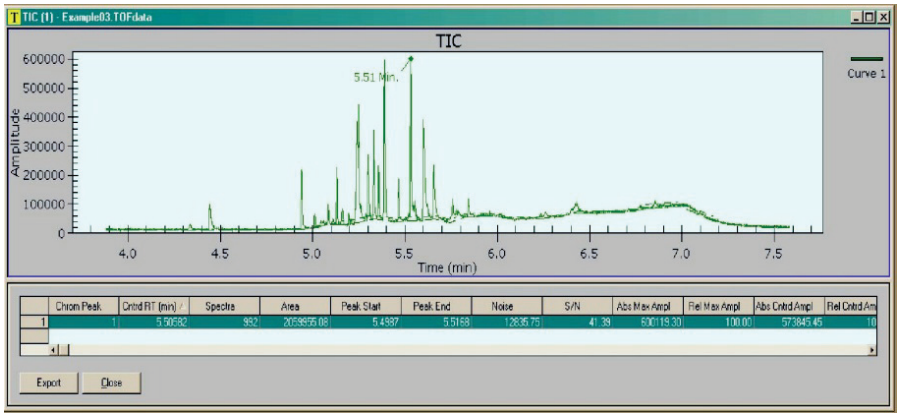
Manual Peak Detection

1. Using the right-mouse button select **Mark Data Points**.



Data points appear on the peaks.

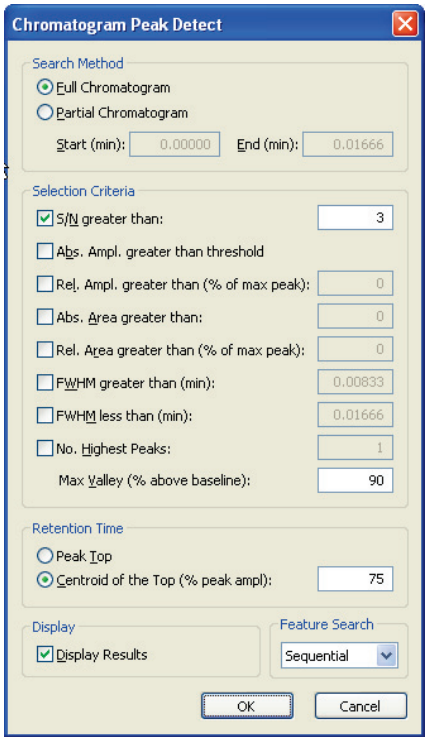
2. Move the cursor to a data point until the "hand" displays.
3. Click the right-mouse button and display peak detect results in the **Peak Table.**



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

Automatic Peak Detection

1. Select a TIC or an EIC.
2. Select **Peak Detect** from the **Chromatogram Evaluation** menu.
   Peaks with a signal-to-noise ratio lower than this limit will be excluded from the peak table.



3. Under **Search Method**, select **Full Chromatogram** or **Partial Chromatogram**. If partial, enter a range in seconds.

4. Under **Selection Criteria**, enter a signal-to-noise limit.

5. Under **Retention Time**, select **Peak Top** or **Centroid.**

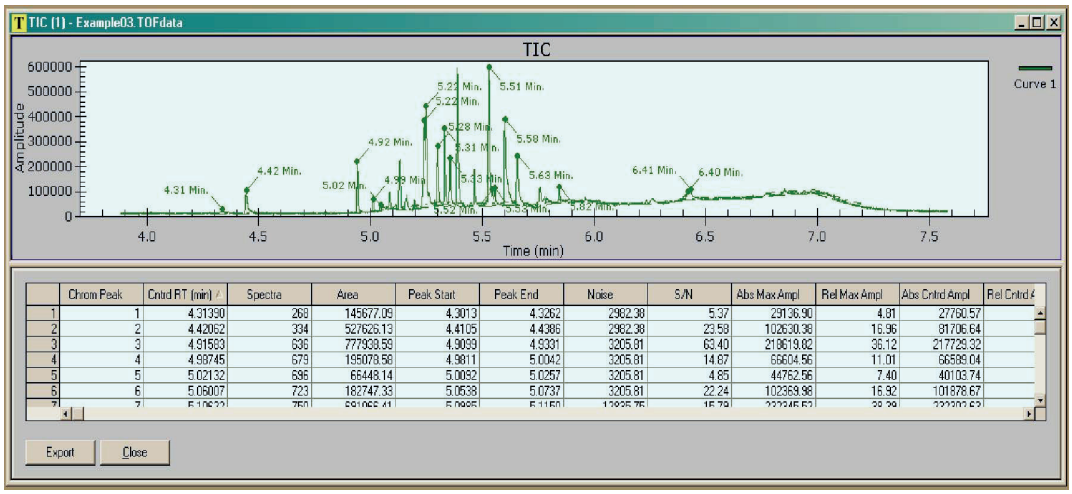
   When **Peak top** is selected the spline function will be used to find the top amplitude and retention time for each chromatogram peak.

   If **Centroid** is selected you must enter a value in percent of the peak amplitude from where the centroid is calculated.

6. Click **OK**.

7. Click **Close** below the table to close the peak table. To export the peak table, click the **Export** button below the table. The complete table will be copied to the clipboard

8. Open Microsoft Excel or another data handling software and paste in an empty data sheet.



	Chrom Peak	Cntrd RT (min)	Spectra	Area	Peak Start	Peak End	Noise	S/N	Abs Max Ampl	Rel Max Ampl	Abs Cntrd Ampl	Rel Cntrd A
1	1	4.31390	268	145677.09	4.3013	4.3262	2982.38	5.37	29136.90	4.81	27760.57	
2	2	4.42062	334	527626.13	4.4105	4.4386	2982.38	23.58	102630.38	16.96	81706.64	
3	3	4.91583	636	777938.56	4.9099	4.9331	3205.81	63.40	219619.02	36.12	217729.32	
4	4	4.98745	679	195078.58	4.9811	5.0042	3205.81	14.87	66604.56	11.01	66589.04	
5	5	5.02132	696	66448.14	5.0092	5.0257	3205.81	4.85	44762.56	7.40	40103.74	
6	6	5.06007	723	182747.33	5.0638	5.0737	3205.81	22.24	102369.98	16.92	101878.67	
7	7	5.10622	750	691066.44	5.0985	5.115	12835.75	15.79	232345.53	38.39	232302.66	

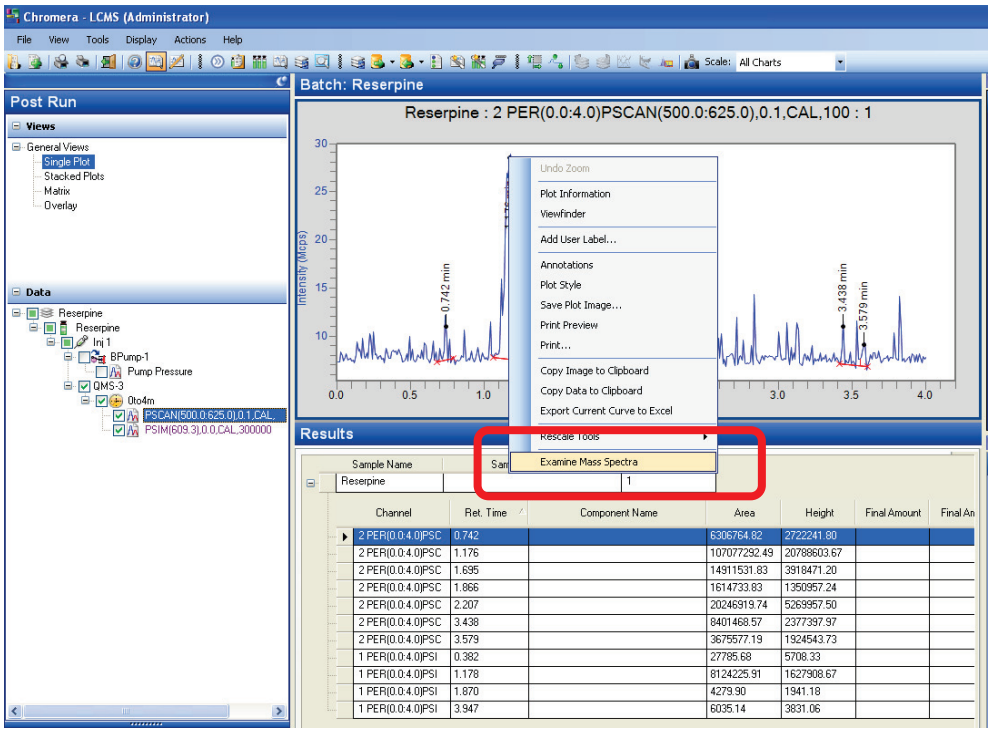
9. Select **Print** from the **File** menu in Microsoft Excel to print the table.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Chrom Peak	Cntrd RT (min)	Spectra	Area	Peak Start	Peak End	Noise	S/N	Abs Max Ampl	Rel Max Ampl	Abs Cntrd Ampl	Rel Cntrd Ampl	Width	Chrom No.	Baseline	
2	1	4.3139	268	145677.1	4.3013	4.3262	2982.38	5.37	29136.9	4.81	27760.57	4.84	0.0137	3	11752.33	
3	2	4.42062	334	527626.1	4.4105	4.4386	2982.38	23.58	102630.4	16.96	81706.64	14.24	0.0086	3	11374.67	
4	3	4.91583	636	777938.6	4.9099	4.9331	3205.81	63.4	219619.8	36.12	217729.3	37.94	0.0065	3	14614.7	
5	4	4.98745	679	195078.6	4.9811	5.0042	3205.81	14.87	66604.56	11.01	66589.04	11.6	0.0069	3	19147.2	
6	5	5.02132	696	66448.14	5.0092	5.0257	3205.81	4.85	44762.56	7.4	40103.74	6.99	0.0056	3	24650.69	
7	6	5.06007	723	182747.3	5.0538	5.0737	3205.81	22.24	102370	16.92	101878.7	17.75	0.0042	3	30558.77	
8	7	5.10622	750	691066.4	5.0985	5.115	12835.75	15.79	232345.5	38.39	232302.6	40.48	0.0059	3	29543.4	
9	8	5.13522	768	268524.8	5.1249	5.1514	12835.75	4.52	58032.03	14.55	86899.53	15.14	0.009	3	29904.64	
10	9	5.21562	812	1693419	5.201	5.2208	12835.75	26.89	384728.1	63.57	378609.6	65.98	0.0095	3	36168.42	
11	10	5.22472	824	1464628	5.2208	5.2324	12835.75	31.05	443981.2	73.36	435630.1	75.91	0.0066	3	37141.08	
12	11	5.2758	853	923213.7	5.2688	5.2853	12835.75	16.67	262738.2	46.72	268839.4	46.11	0.0069	3	44928.93	
13	12	5.30608	872	986041.7	5.3003	5.3233	12835.75	22.71	355790.1	58.79	340898.1	59.41	0.0054	3	49429.53	
14	13	5.33183	886	682070.9	5.3233	5.3433	12835.75	14.18	231387.1	38.23	229008.6	39.91	0.006	3	48945.89	
15	14	5.36447	905	2033844	5.3548	5.3878	12835.75	40.97	605188.3	100	569758.3	99.29	0.0058	3	43676.12	
16	15	5.44082	953	503641.8	5.4342	5.4491	12835.75	11.17	200021.7	33.05	184061	32.08	0.0057	3	40728.16	
17	16	5.50582	992	2059955	5.4987	5.5168	12835.75	41.39	600119.3	99.16	573845.5	100	0.0064	3	42546.57	
18	17	5.52165	1003	268468.4	5.5168	5.5267	10453.15	5.07	108530.4	17.93	96390.01	16.8	0.0082	3	43518.12	
19	18	5.5322	1009	239130.8	5.5267	5.54	10453.15	4.66	111775.3	18.47	92895.51	16.19	0.0054	3	44167.7	
20	19	5.5792	1034	2029260	5.5682	5.5963	10453.15	23.64	389953.3	64.44	293482.3	51.14	0.0087	3	46360.21	
21	20	5.6334	1068	888621.3	5.6243	5.6608	10453.15	14.27	243966.2	40.31	197367.5	34.39	0.007	3	48248.09	
22	21	5.73607	1121	442385	5.712	5.7451	10453.15	5.5	116524.8	19.58	97953.44	17.07	0.0079	3	40662.43	
23	22	5.82057	1183	224372.3	5.8145	5.8261	10453.15	5.18	119120.6	19.68	110552.7	19.27	0.0063	3	55480.63	
24	23	6.39515	1530	210007.6	6.3884	6.4016	5985.43	4.53	100253.5	16.57	94847.8	16.53	0.013	3	67761.95	
25	24	6.40945	1538	270988.3	6.4016	6.4182	5985.43	4.41	106713.7	17.96	94313.85	16.44	0.0115	3	67943.2	
26																
27																
28																

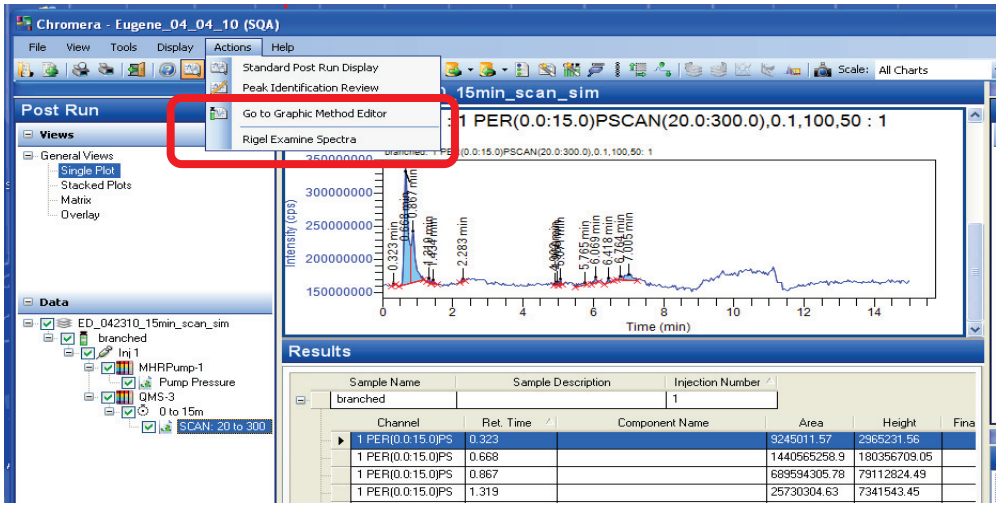
Evaluating Mass Spectra

Evaluating Acquired Mass Spectra

1. As demonstrated earlier, moving the mouse pointer to a point on the chromatogram in Chromera and then right-clicking and selecting **Examine Mass Spectra** from the drop-down list will open the SQ 300 processing window.



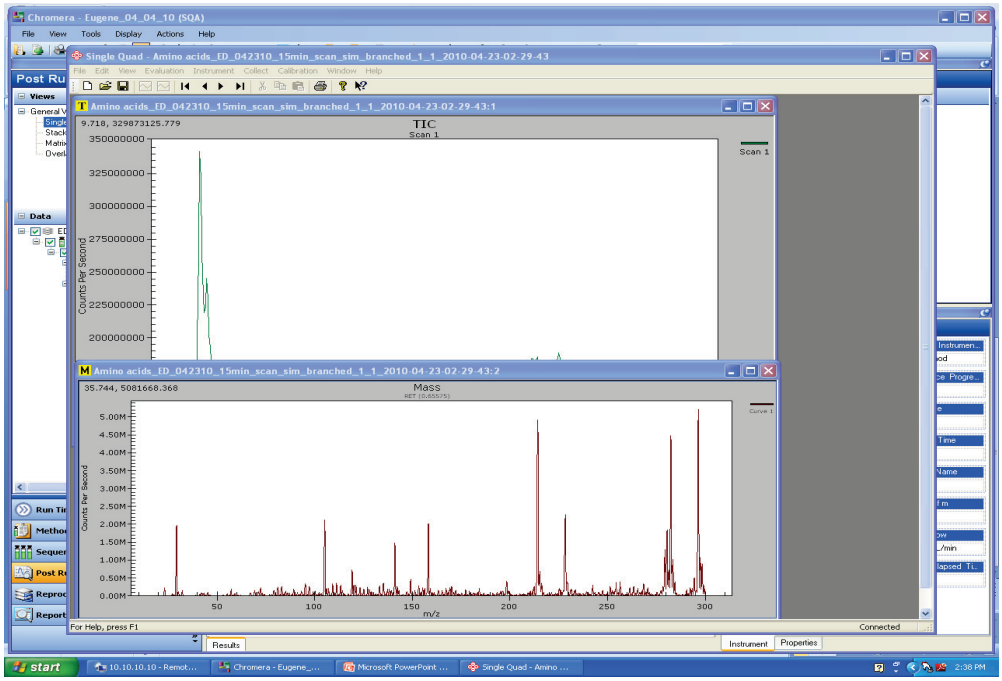
Another way to enter the mass spectral processing domain (demonstrated on a different data file) is to select **Examine Spectra** from the Chromera **Actions** menu.



The spectra open in the SQ 300 MS driver window.

The SQ Driver will display 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 chromatogram is selected, then the first spectrum from the acquisition is displayed.



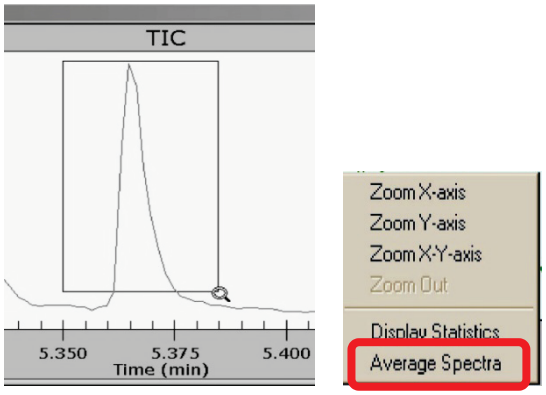
When a data file is opened a TIC window and a Mass spectrum window will be displayed.

Creating an Average Mass Spectrum

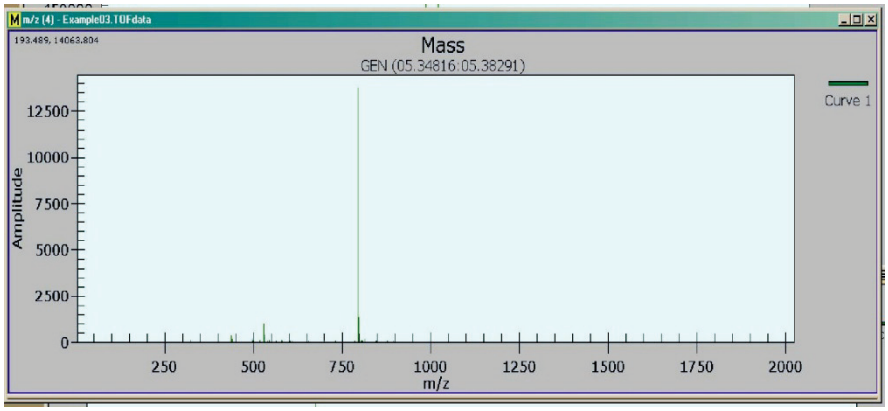
Below is an example of how to analyze the TIC peak.

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**.



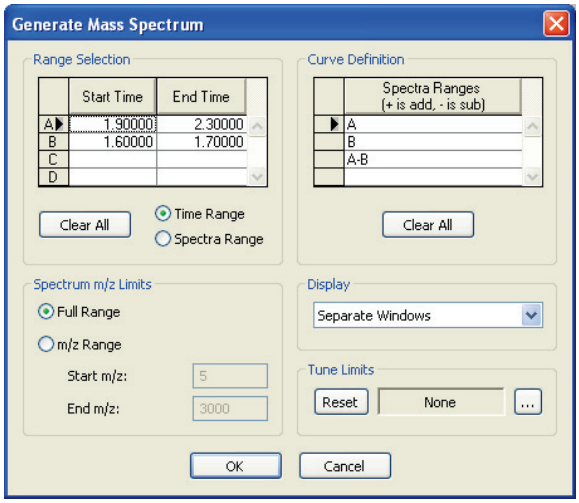
The Mass spectrum will be updated to an average mass spectrum.



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.*

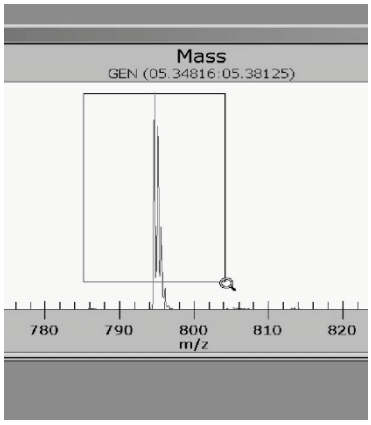
6. Click **OK**.

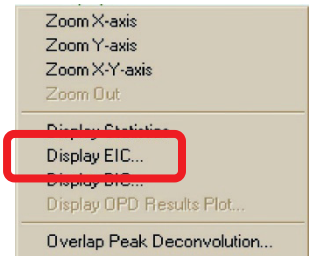
7. The Mass spectrum will be updated to an average Mass spectrum.

Creating an EIC and BIC from a Mass Spectrum

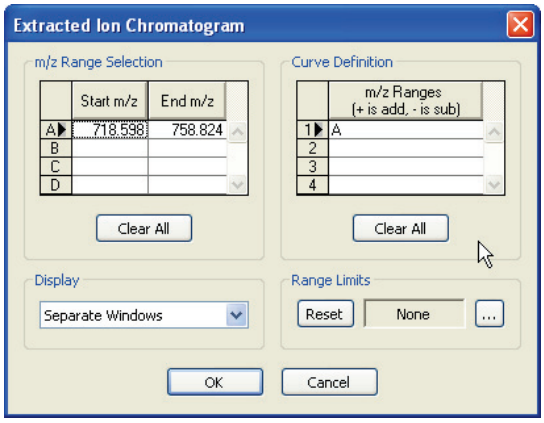
To create an EIC:

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

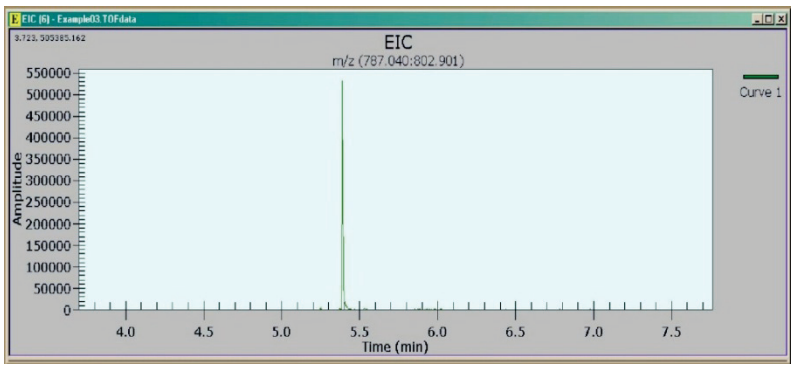




2. Release the button and select **Display EIC** from the menu.
   The **Extracted Ion Chromatogram** dialog displays.

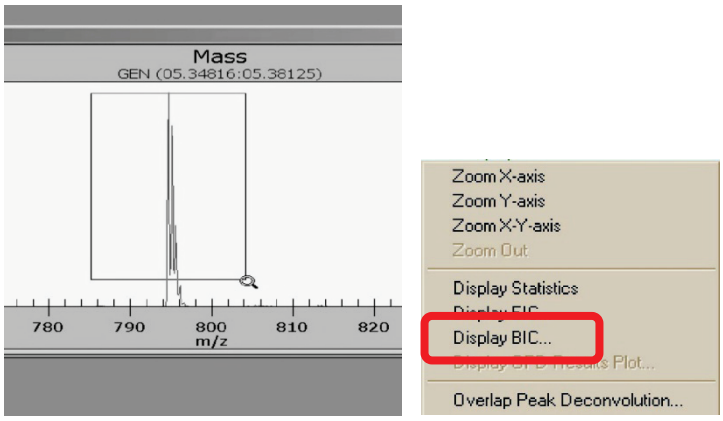


3. Click **OK** and the **EIC** is displayed.
   The EIC displays where in the chromatogram mass peaks occur with *m/z* values within the set *m/z* range.

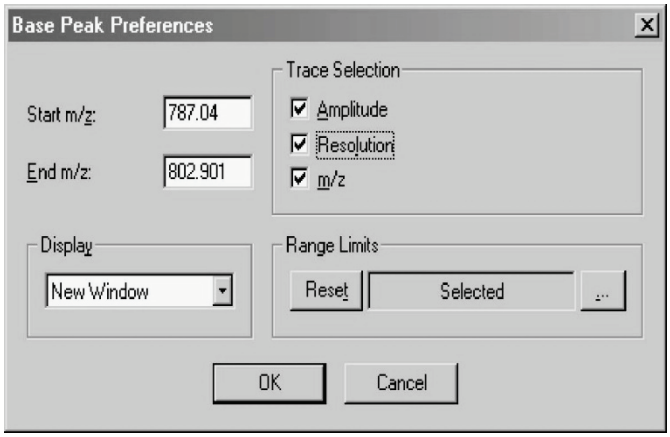


To create a BIC:

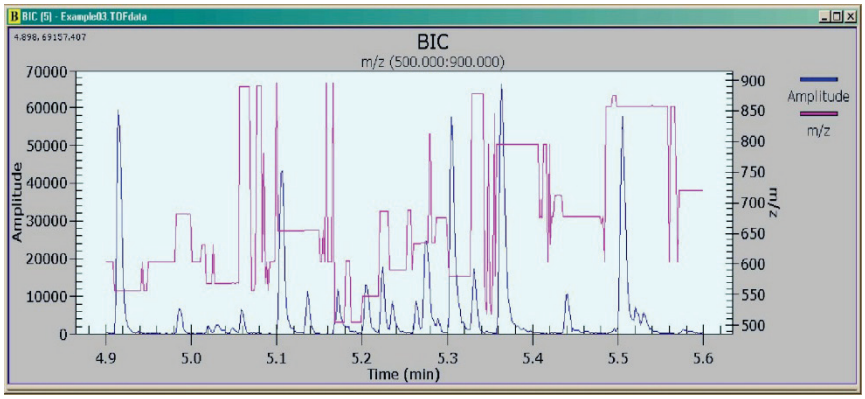
1. Left-click and drag a box around a peak of interest in the mass spectrum. The width of the box will be the set *m/z* range.



2. Release the button and select **Display BIC** from the menu**.**



3. Press **OK** and the BIC is displayed**.**



Processing of Mass Spectra

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

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.

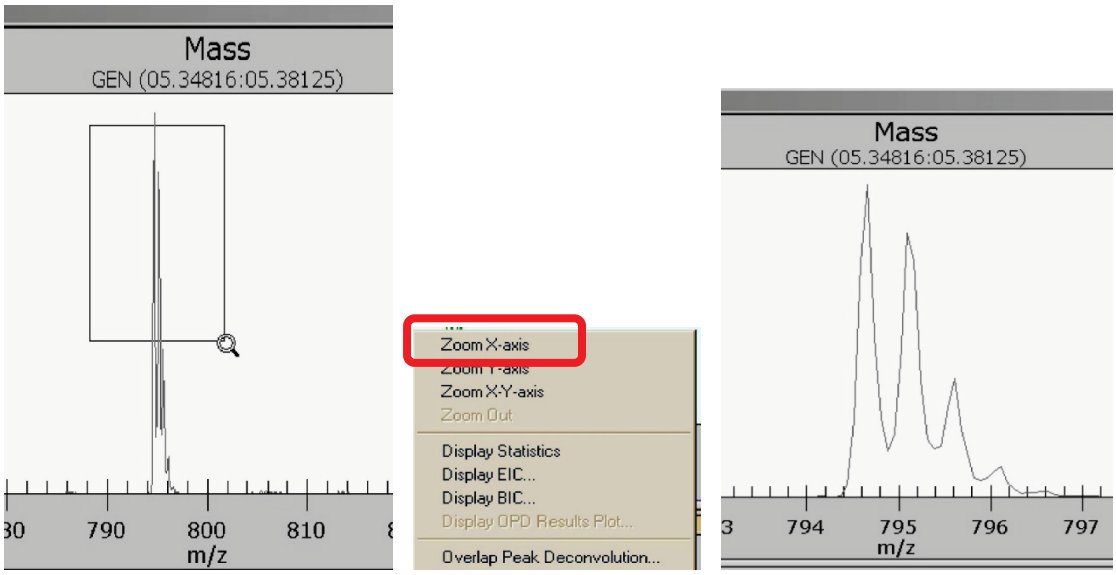
3. To thaw a frozen mass spectrum activate the spectrum and select **Thaw** from the **View** menu.

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**.



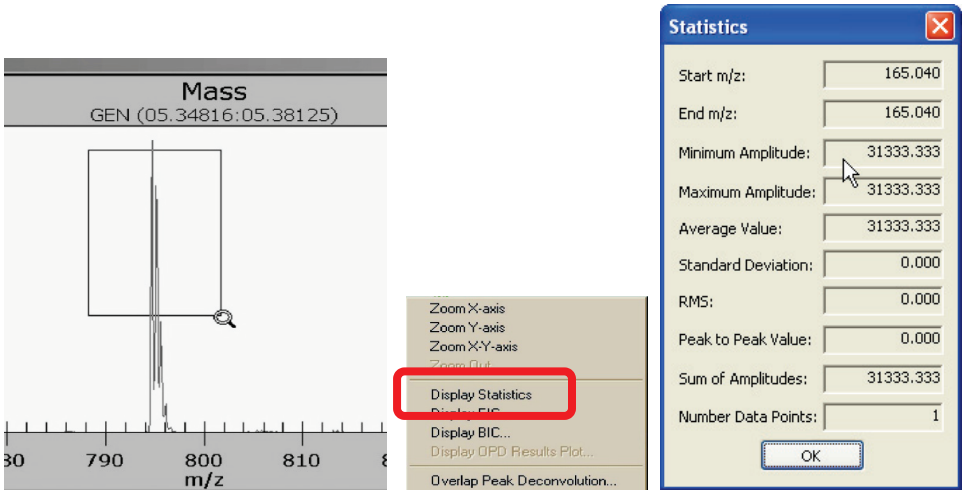
3. Left-click and drag a box somewhere in the spectrum. Release button and select **Zoom Out**.

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

Displaying Statistics

To display statistics:

1. Left-click and drag a box around the area of interest.
2. Release the button and select **Display Statistics**.



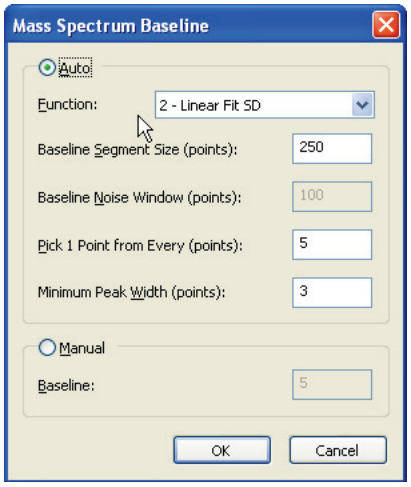
Using Right Mouse Click Menus

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

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.

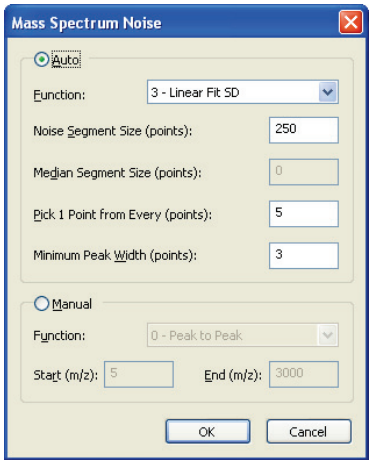
5. The baseline can be hidden or displayed with the **Baseline** from the **View** menu.

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

Setting Spectrum Noise Calculation Preferences

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 **Root mean square**.

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 **Root mean square** 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.

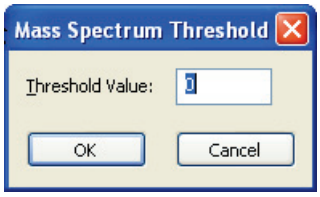
5. Click **OK**.

6. 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
2. Select **Threshold** from the **MS Evaluation** menu.



3. Enter a threshold value and decide if the mass spectrum will be subtracted with this value.

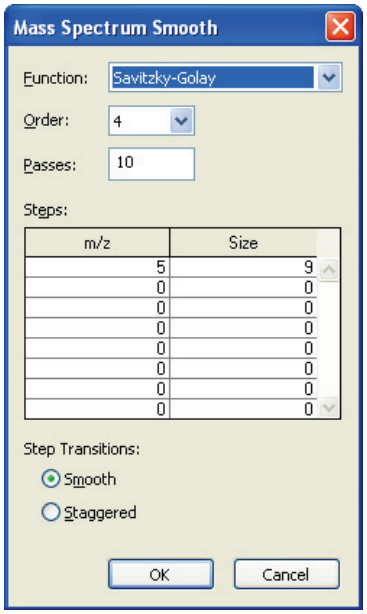
Mass Spectrum Smoothing

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.

   0-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.

   1-Median: The processed spectrum is calculated as the median of the data points.



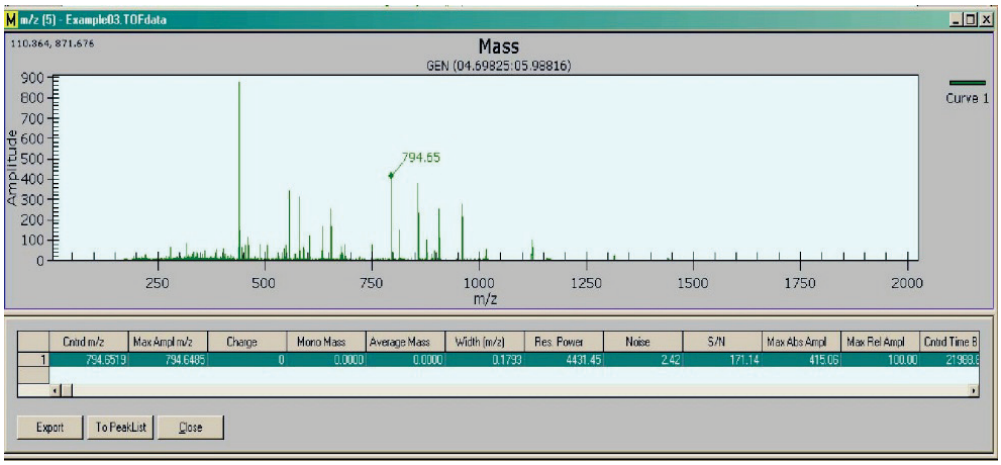
Mass Spectrum Peak Detection

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-mouse click and a **Peak Information** box is displayed.

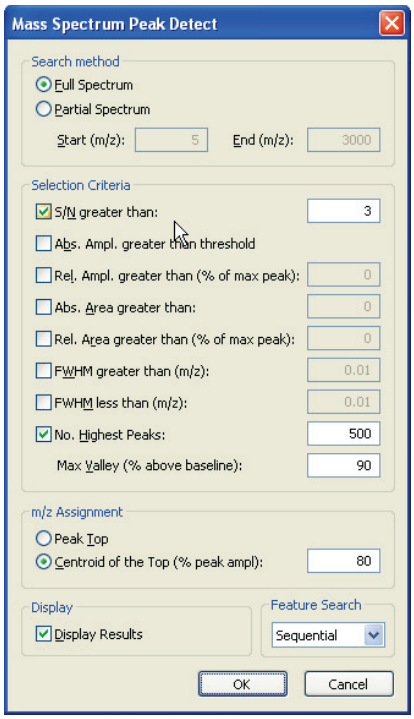


NOTE: *The S/N value is calculated using the centroid amplitude.*

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**.

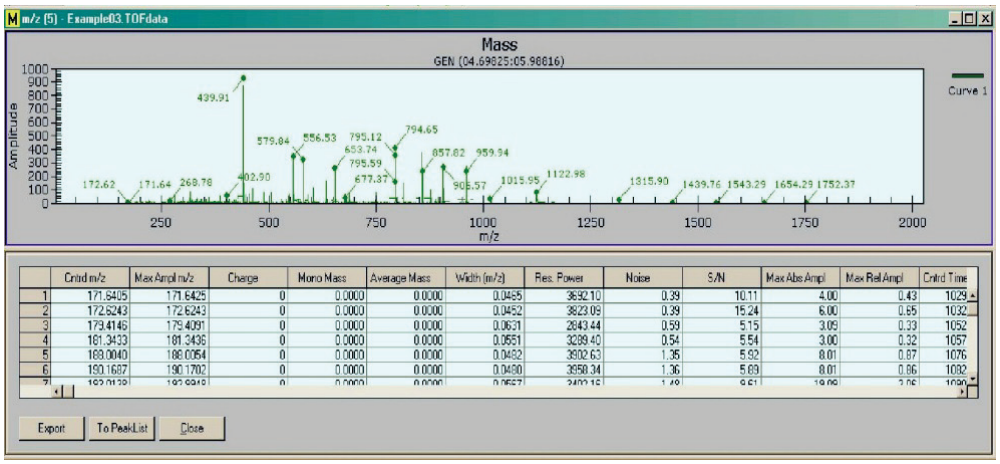
7. Click **OK**.

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

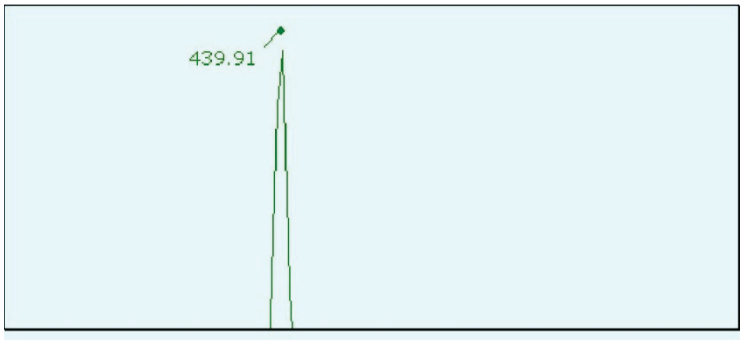
9. To close the peak table, click the **Close** button below the table.

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

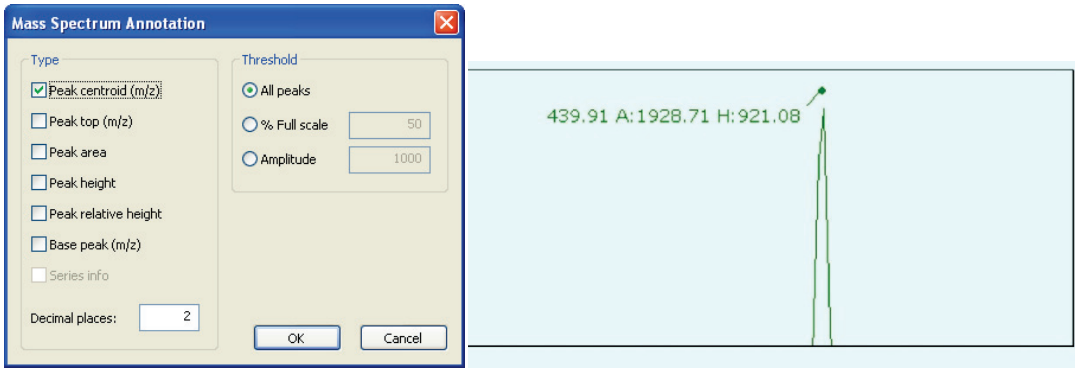
11. Open Microsoft Excel (or other spreadsheet software) and **Paste** in an empty data sheet.



The following example shows a default peak annotation.



The following example shows an enhanced peak annotation.



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	172.5685	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	187.9356	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	10906	
9	194.7189	194.7173	0	0	0	0.0489	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	204.6633	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	204.7468	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	222.1458	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	

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