LIQUID CHROMATOGRAPY/MASS SPECTROMETRY (LCMS)

AXION 2 TOF MS



User's Guide



AxION 2 TOF MS User's Guide

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

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

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

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

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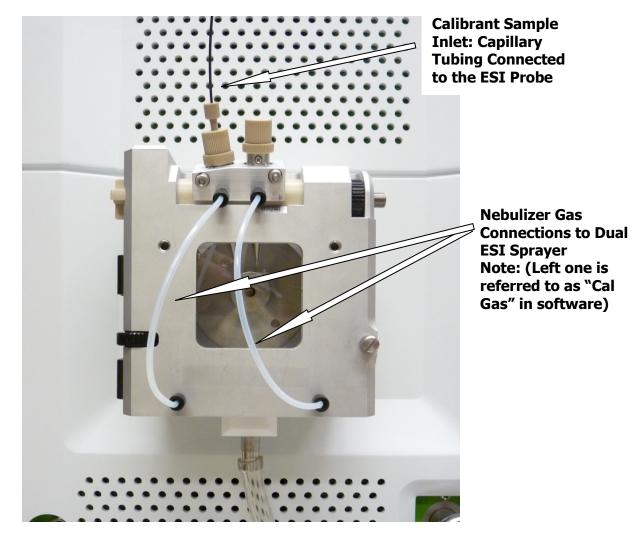
Overview

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

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

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

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



Starting the AxION 2 TOF MS Detector



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

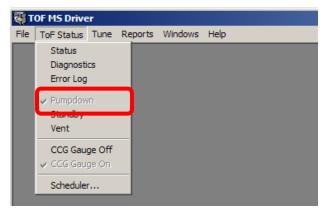
CAUTION! Do not move the instrument with the power on as this may damage the vacuum pumps.

To start the AxION 2 TOF MS system:

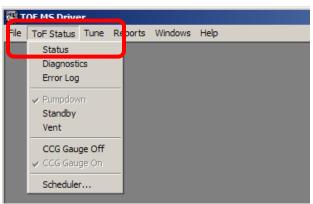
1. Switch the **Power ON/OFF** switch to position **On** (located on the right-side panel). Make sure the power switch on the roughing pump is on so that the Vacuum System will start when **Pumpdown** is selected in the AxION 2 TOF MS driver.

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

- 2. Check that the fans are operating. A cooling air flow should exit at the bottom of the instrument.
- 3. Double-click on the **TOF MS driver** icon on the desktop.
- 4. Select **Pumpdown** from the **TOF Status** menu.



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



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

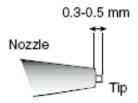
Status (Next update in 6 seconds)	
Source	
Cap Ent: 💻	12 nAmp
Endplate:	191 nAmp
Cylinder	0 nAmp
Drying Gas Temperature: 34	5 °C
APCI Heater Temperature: 01	f
Door: Closed	t l
Instrument Operation	Lamps
Ion Source: Or	n Power: O
ToF Analyzer: Or	n Vacuum: Ο
Vacuum State: Pumpdown	n Ready: O
Pressure	
Foreline: 2.260 mBar	
TOF: 2.90e-007 mBar	Close

When ready, the **Lamps (Power, Vacuum, and Ready)** should all be green. The **Vacuum** lamp changes from flashing to steady light at this stage.

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

Checking the Position of the ESI Probe

- 1. Observe the ESI probe through the inspection window.
- 2. Align it to the center of the Capillary by loosening the lock ring and moving the manifold by turning the position adjustment screw. Tighten the lock ring.
- 3. The marks on the probe assembly can be used for quick positioning. Observe the needle through the inspection window and adjust the tip if necessary.
- 4. Loosen the lock ring.
- 5. Turn the needle assembly adjustment screw until the needle tip protrudes about 0.5 mm from the nozzle.



6. Tighten the lock ring.

Starting Chromera

Configuring Chromera

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

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

Creating a System Database

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

To create a System Database:

 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.

😽 Chromera Manager		
Actions Help		
Launch Launch Data Only 🖕		
System Database Man	Create Backup Restore Delet	e
	System database creation	
	Server name:	localhost\SQLEXPRESS2008
	System database:	ChromeraSystemD ataB ase
	Archive database:	
Instruments	Create Database	
System Database Management		

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

Create Backup Restore Delet	18
- System database creation	
Server name:	localhost\SQLEXPRESS2008
System database:	ChromeraSystemD ataBase
Archive database:	
Create Database	
Messages Malid	lating DevicePool

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

Creating an LCMS Instrument

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

To create an LC instrument:

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

to



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

		Save						
	Us	er Name	Cre	eate D ate				
E	A	Administrator 10/07/2011						
	ſ	Instrument Configuration			-			
	н	Configuration Name	Со	figuration Description	ServerName	Use With ICP-MS	Database Name	Archive Database Name
	Ð	LC TOF MS			localhost\SQLExpres		Chromera	ChromeraArchive
	-	*	2					

3. Click on the \blacksquare and the **Device** row displays.

Jser Name Administrator	Create Date 10/07/2011				
Instrument Configuration	1	1			
Configuration Name	Configuration Description	Server Name	Use With ICP-MS	Database Name	Archive Database Name
LC TOF MS		localhost\SQLExpres		Chromera	ChromeraArchive
- · ;					
Device					
	Device Description	User Device Name	Port Name	Data Port Name	
Device	Device Description	User Device Name	Port Name	Data Port Name	
Device Device Name	×	User Device Name		Data Port Name	
Device Device Name *	×			D ata Port Name D atabase Name	Archive Database Name

4. Click on the drop-down button in **Device Name** box, and device choices appear. Select the appropriate devices (modules) for the **Instrument** you are creating.

In this example, select **AxION 2** from the drop-down list of devices.

	Instrument Configuration							
	Configuration Name		Confi	guration Description	Server Name	Use With ICP-MS	Database Name	Archive Database Name
	LC TOF MS				localhost\SQLExpres		Chromera	ChromeraArchive
	Device							
	Device Name		Deio	ce Description	User Device Name	Port Name	Data Port Name	
	*	~				×		
NCI 902		^						-
Flexar Binary Pump Flexar Quaternary Pump		-	Con	guration Description	Server Name	Use With ICP-MS	Database Name	Archive Database Name
Flexar Binary Micro Pump								
Flexar FX-10 UHPLC Pump Flexar FX-15 UHPLC Pump								
Flexar UV/VIS Detector								
Flexar FX-UV UHPLC Detector Flexar Refractive Index Detector	,	_						
Flexar Fluorescence Detector	I							
Flexar Autosampler No Peltier								
Flexar SQ 300 MS Detector AxION 2								
Flexar Autosampler Cool Only	2							
Flexar Autosampler Cool-Heat								
Flexar FX UHPLC Autosampler	Cool Only	~						

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

	S	ave					
Us	ser I	Name	Create Date				
🖃 Ad	dmini	istrator	10/07/2011				
	Ins	strument Configuration					
	Co	onfiguration Name	Configuration Description	ServerName	Use With ICP-MS	Database Name	An
		LC TOF MS		localhost\SQLExpres		Chromera	Ch
		Device					
		Device Name	Device Description	User Device Name	Port Name	Data Port Name	
	🕀 🖌 🗛 🖌 🖌		*	TOF-1	COM DLL		
		EL BV 10 LIUD		D/10D	CO144		

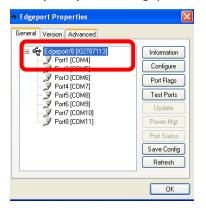
 Select your LC Pump from the Device Name drop-down list. In this example, the Flexar FX-10 UHPLC Pump.

			Ins	trui	ment Configuration	1				
			Cor	nfig	juration Name		Configuration Description	Server Name	Use With ICP-MS	Database Name
				LC	TOF MS			localhost\SQLExpres		Chromera
				De	vice					
				De	viceName		Device Description	User Device Name	Port Name	Data Port Name
			±		AxION 2	~		TOF-1	COM DLL	
			-	木		×			~	
1	NCI 901 NCI 902					~				
	Flexar Binary Pump						Configuration Description	Server Name	Use With ICP-MS	Database Name
	Flexar Quaternary Pump									
	Flexar Binary Micro Pum Flexar FX-10 UHPLC Pu				N					
	Flexar FX-15 UHPLC Pu	mp			k					
	Flexar UV/VIS Detector									
	Flexar FX-UV UHPLC De									
	Flexar Refractive Index [
	Flexar Fluorescence Det									
	Flexar Autosampler No F									
	Flexar SQ 300 MS Detec	ctor								
	AxION 2									
	Flexar Autosampler Cool									
	Flexar Autosampler Cool	rneat				×				

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 1 to display a list of the physical Ports (1 - 8) on the Edgeport with the corresponding COM port numbers.



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- 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.

					C	1			
		er Nai		_	Create Date				
E	Ad	ministra	ator		10/07/2011				
		Instru	ument Configuration						
		Confi	iguration Name		Configuration Description	Server Name	Use With ICP-MS	Database Name	Archive Database Name
		U	C TOF MS			localhost\SQLExpres		Chromera	ChromeraArchive
		D	evice						
		D	evice Name		Device Description	User Device Name	Port Name	Data Port Name	
		±	AxION 2	~		TOF-1	COM DLL]
		•	R			n Hon, o			
		⊕ Þ	Flexar FX UHPLC	Y		FX15ASCH-3	сомз 🗸		
		*		٣			×		

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

11. Select the Port Name for the autosampler.

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

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

_	r Name ninistrator	Create Date 10/07/2011				
	nstrument Configuration					
	Configuration Name	Configuration Description	ServerName	Use With ICP-MS	Database Name	Archive Database Name
	LC TOF MS		localhost\SQLExpres		Chromera	ChromeraArchive
	Device					

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 Save

🗸 Chromera Manager							
Actions Help Launch Launch Data Onl Configure action Administrator LC TDF MS		ave Name		Create Date			
	😑 🛛 Admir	iistrator		10/07/2011			
	In	strument Configuration					
	C	onfiguration Name		Configuration Description	Server Name	Use With ICP-MS	Database Name
		LC TOF MS			localhost\SQLExpres		Chromera
		Device				•	
		Device Name		Device Description	User Device Name	Port Name	Data Port Name
		AxION 2	~		TOF-1	COM DLL	
		Flexar FX-10 UHP	v		FX10Pump-2	СОМ4 🗸	
		Flexar FX UHPLC	~		FX15ASCH-3	сомз 🗸	
		*	v			~	

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

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

/	2	7 Chrom		Manager	
	Ē	Actions	Help		
L	Ī	Launch	Li	unch Data Only	-
		Config	ura	tion	

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

🖉 Device Connections			
Device	Connected?		Tries
🕫 BPump-1		Disconnect	1
# AS275CO-2		Disconnect	1
+ QMS-3		Disconnect	1
	·		

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

Setting the Operate Method

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

- **NOTE:** When you close Chromera you lose the **Operate** method settings. You must reassign it every time you Launch Chromera.
 - 1. In the **Operate** row, click **Browse for method**.

Man	ual Control							
2								
	Monitor Baseline Start	Me Browse fo	thod		iod Name None			
	Pump Setti	ngs	Flow (mL/		%A ()	%B		
	(Apply		0.500		50.0	50).0]
	Purge Pun	ър	Flow (mL/	min)	100% A ()	100% B ()		
	Apply		1.000		~			
	Flush Autosa	mpler	Flush Vol	ume (µL) Numbe	r of Flush Cy	/cles	
	Apply		100	00		1		
	Peltier Tra	ay	Temperat	ure (°C)	Toleran	ce (+/- °C)		
	Apply		20	1	2	2.0		
	Vent TO	F						
	Apply							
	Standby							
	Apply							
ſ	Operate		Method fil	e name	Bro	wse		
	Apply				Browse f	or metho		

The **Select Operate Method** dialog displays.

Organize 🔻 New folder				H · 🔳	0
🔆 Favorites	Name	Date modified ~	Туре	Size	
🧮 Desktop	Caffeine Analysis.tofmethod2	9/10/2013 11:08 AM	TOF MS Driver Docu	4 KB	
Downloads	090313-c60.tofmethod2	9/5/2013 5:25 PM	TOF MS Driver Docu	7 KB	
Recent Places	test 144. tofmethod 2	9/4/2013 3:49 PM	TOF MS Driver Docu	6 KB	
📜 Libraries	CalOFF.tofmethod2	8/27/2013 10:31 AM	TOF MS Driver Docu	5 KB	
Documents	EICTest_2timeperiod.tofmethod2	8/27/2013 10:26 AM	TOF MS Driver Docu	12 KB	
J Music	EICTest_4timeperiod.tofmethod2	8/27/2013 9:35 AM	TOF MS Driver Docu	12 KB	
E Pictures	EICTest_3timeperiod.tofmethod2	8/27/2013 9:31 AM	TOF MS Driver Docu	11 KB	
Videos	EICTest_1timeperiod.tofmethod2	8/26/2013 5:37 PM	TOF MS Driver Docu	11 KB	
	15minute_SoloTest.tofmethod2	8/23/2013 2:40 PM	TOF MS Driver Docu	11 KB	
P Computer	082213-benzos_plasma-80.tofmethod2	8/22/2013 7:35 PM	TOF MS Driver Docu	14 KB	
public (\\bfdf001) (Z:)	082213-benzos_plasma-30.tofmethod2	8/22/2013 5:28 PM	TOF MS Driver Docu	14 KB	
	082013-benzos_plasma.tofmethod2	8/22/2013 1:10 PM	TOF MS Driver Docu	11 KB	
👊 Network	DBvsFILETest_081913.tofmethod2	8/19/2013 4:05 PM	TOF MS Driver Docu	5 KB	
ASBFDF002	SWTest_Saving_081913.tofmethod2	8/19/2013 3:51 PM	TOF MS Driver Docu	10 KB	
ASBEDEOOE		8/16/2013 12:23 PM	TOF MS Driver Docu	12 KB	

- Select the method you created in the TOF MS driver, then click **Open**. This example shows **Caffeine Analysis.tofmethod2**
- 3. Look at the Manual Control section of the Run Time screen.

Monitor Baseline	M	1ethod	Meth	od Name	1	
Start	Browse	for method	١	lone		
Vent MS		1				
Apply						
Standby		-				
Apply		1				
Operate		Method fil	e name	Br	owse	
Apply		ADcaffeine	emethod	Browse f	or method	
Pump Setti	ngs	Flow (mL/	min)	%A ()	%B()
Apply		1.000		5.0	95.0	
Purge Pur	np	Flow (mL/	min) 1	00% A ()	100% B ()	
Apply		1.000		2]
Flush Autosa	mpler	Flush Volu	ume (µL)	Numbe	r of Flush Cy	cles
Apply		100	0		1	
Peltier Tra	ay .	Temperatu	ure (°C)	Tolerand	ce (+/- °C)	
Apply		20	INS.	1 .	2.0	

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

Status Panel	
Sequence Status	Operate
Vacuum State Pumped Down	MS Detector State Standby
MS Analysis Status Not Acquiring	Elapsed Time
Capillary Entrance Current (5.0 nA	Injection Number

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

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

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

Status Panel	
Sequence Status	Operate Ready
Vacuum State Pumped Down	MS Detector State
MS Analysis Status Not Acquiring	Elapsed Time
Capillary Entrance Current (50 nA	Injection Number

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

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

Setting the TOF Data Path

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

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



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

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Prefer	ences				
	Save				
Nan	ne				
	IS				
	Device				
	DisplayTitle	Device	DeviceDescription UserDeviceName		
₽	AxION 2			TOF-1	
	Device Preferences	8			
	DisplayTitle		DefaultValue		Units
	MS Acquire Start M	ode	LC-Software St	art 🗸 🗸	
	Location of MS Dat	a Files			Browse

3. Click the **Browse** button.

The Browse For Folder dialog displays.

Browse For Folder	<u>? × </u>
Location of MS Data Files	
 Image: Second se	
- Mobata	
Make New Folder OK Can	

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

- 5. Click the Make New Folder button. The New Folder displays.
- 6. Right-click on New Folder and rename it MSData.

7. Click **OK.**

The location of MS data files are now set to C:\MSData.

DisplayTitle	DefaultValue	Units
MS Acquire Start Mode	LC-Software Start	-
Export Chromatograms	No	-
Automatically Centroid	No	-
Location of MS Data Files	C:\MSData	Browse

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



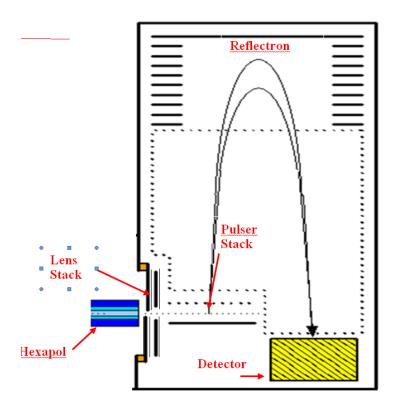
The TOF data will now be written to C:\MSData as defined in the preferences.

AutoTune the AxION 2 TOF MS Detector

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

Overview of the Tuning Procedure

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



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

About the AutoTune Algorithm

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

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

How the AutoTune Algorithm Runs

Look in:	Autotune	- 🗧 📩 🖬
<u>Ca</u>	Name 🔺	- Date modified - Type
1	AutoRefine	8/23/2013 1:43 PM TOFSCR 2 File
Recent Places	DAUnormalize	8/23/2013 1:43 PM TOFSCR2 File
	DetectorNormalize	8/23/2013 1:43 PM TOFSCR2 File
	LensTune	8/23/2013 1:43 PM TOFSCR2 File
Desktop	TrapPulse	8/23/2013 1:43 PM TOFSCR2 File
	TuneMethod	8/23/2013 1:43 PM TOFSCR2 File
Libraries Computer		
Network		▼ Open

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

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

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

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

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

About the Samples

Tune Mix (PerkinElmer Part Number ZG2421A)

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

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

Calibrants

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

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

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

Calibrant Delivery by Syringe Pump

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

_ 🗆 🗵

Data Acquisition

Saved Count:

Optics / Flight Tube

Trap Enhancement

µl/min ▼

Units:

Pump is OFF

mm

Spectra Acquired

Spectra Saving is OFF

Acquisition is OFF

DAU

Comments

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

👔 Manual Tune - Typical Tune Pos 8kV

Pulse

100

3002

Positive

8 Apply

Ontics Syringe Pump

Manufacturer: Hamilton - Microliter Series Gastight 💌 500 µl ▼ Diameter: 3.26

•

-

Primary Variable

Spectra Per Sec.: 1

Acq. Function:

Low m/z:

High m/z:

Ion Polarity

🖄 Calibrate

Ion Source

Ramping

Syringe

Mode

Liquid 10,00

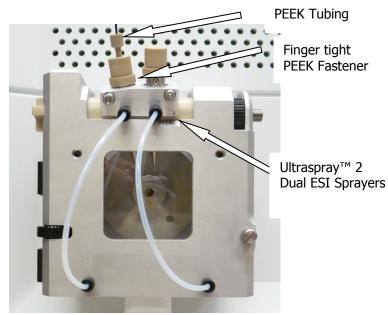
÷

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

Preparing for AutoTune

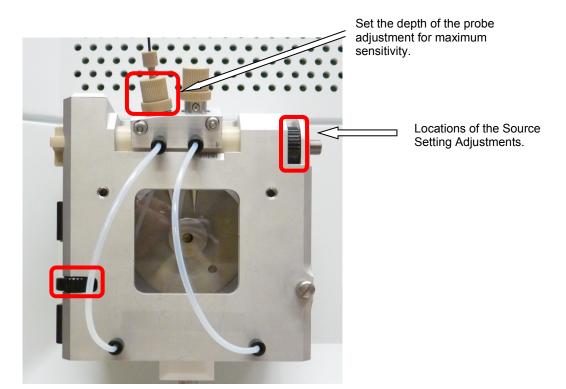
Typically use the Positive Ion Tune mix.

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

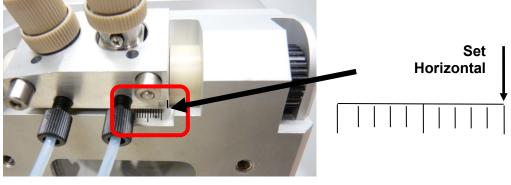


Running AutoTune

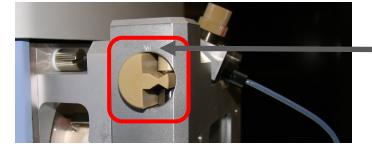
Set you source positions before you start the AutoTune procedure.



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



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



- \\|//

Set Tilt Position Here

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



TOF MS Driver

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

1 T	OF MS Drive	er		
File	ToF Status	Tune	Windows	Help
N	ew Acquisitior	n Metho	d	
0	pen Data File			
0	pen Manual T	une		
0	pen Acquisitio	on Meth	od	

The **Open Tune** dialog displays.

	Probe: 3 - DUAL	_ESI Polarity:	_
			, _
	-		
	User Name 🛛 🛆	Tune Name	Modified
	AUTOTUNE	Typical Tune Neg 8kV	9/1/2013 10:00:00 pm
18	AUTOTUNE	Typical Tune Pos 8kV	9/1/2013 10:00:00 pm
	AUTOTUNE	Typical Tune Pos 11kV	9/1/2013 10:00:00 pm
20	AUTOTUNE	Typical Tune Pos 5kV	9/1/2013 10:00:00 pm
21	AUTOTUNE	Typical Tune Neg 5kV	9/1/2013 10:00:00 pm
22	AUTOTUNE	Typical Tune Neg 11kV	9/1/2013 10:00:00 pm
20	PACIONI	vent	3/1/2013 10:00:00 pm
24	guest	SRPos10KV-07252013	8/12/2013 4:41:47 pm
25	guest	TW Lens tune Pos 8kV	11/27/2012 10:35:47 am
26	guest	ADneg8kvESI	8/30/2012 12:58:46 pm
27	guest	Start lens tune Tune Neg 8kV	11/28/2012 12:01:08 pm
28	guest	SR_ESI positive 25 jun 2013-8kv	7/25/2013 5:07:33 pm
29	guest	Vent DSA	8/1/2013 9:00:56 am
30	guest	AD5KVdemo	8/10/2012 10:39:05 am
31	guest	TW ESI 8 KV Positive11-15-2012	11/16/2012 11:11:49 am
32	guest	DSA calibrated Pos 10kV	11/27/2012 9:51:09 am
- 33	guest	TW Poslon Pulse ESI -5kv 2012-08-17	8/17/2012 11:31:05 am
	-		

There are six **AUTOTUNE** files displayed.

6. Select Typical Tune Pos 8kV.

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

7. Click Open.

The Manual Tune - Typical Tune Pos 8kV dialog opens.

Primary Variables—		Data Acquisition		
Spectra Per Sec.:	1	Spectra Acquire	ed: [C
Acq. Function:	Pulse 💌	Saved Count:	Γ	C
Low m/z:	100			
High m/z:	3002	Spectra	Saving	is OFF
Ion Polarity:	Positive 💌			
. 549		Acquis	ition is	OFF
Calibrate	🎒 Apply			
Ramping Ion Source	Syringe Pump Optics	Trap Enhancemen Optics / Flight Tub		Comments DAU
	Opucs	Optics / Hight Tub	e	DAU
	The second secon			
Cylinder:	3500 - (Volts) Drying Gas Flow:	5.0	
Cylinder: Endplate:	-5000 (Volts		1.1.1.1	
Endplate:	-5000 · (Volts) Drying Gas Heater	1.1.1.1	
and the second second	-5000 · (Volts) Drying Gas Heater	300	(°C) (PSI)
Endplate: Capillary Entrance	-5000 (Volts -6000 (Volts) Drying Gas Heater) Nebulizer Gas	300 80	(°C) (PSI)
Endplate: Capillary Entrance Endplate Heater:	-5000 × (Volts -5000 × (Volts -5000 × (Volts Off ×) Drying Gas Heater) Nebulizer Gas Auxiliary Gas	300 80 0.0	(°C)
Endplate: Capillary Entrance Endplate Heater:	-5000 (Volts -6000 (Volts) Drying Gas Heater) Nebulizer Gas Auxiliary Gas APCI Heater:	300 80 0.0 25	(PSI)

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

Applying Tune	
Progress	
00:30 Remaining	

- 9. Click the **Ion Source** tab and set the **RightNeb Gas** or **LeftNeb Gas** to 80psi, depending on which probe is used for calibrant delivery.
- **NOTE:** The gas must be on when running dual sprayers.
 - 10. Select the **Calibration Vial** containing the Tune Mix and click the **Apply** button to activate the vial *or* turn on the syringe pump by selecting the **Pump is OFF** button in the **Syringe Pump** tab.

🚺 Manual Tune - Typical Tune Pos 8kV	_ _ _ ×
Primary Variables	Data Acquisition
Spectra Per Sec.: 1	Spectra Acquired: 0
Acq. Function: Pulse	Saved Count: 0
Low m/z: 100	
High m/z: 3002	Spectra Saving is OFF
Ion Polarity: Positive 💌	
🔊 Calibrate 🚺 Apply	Acquisition is OFF
Ramping Syringe Pump	Trap Enhancement Comments
Ion Source Optics	Optics / Flight Tube DAU
Cylinder: -3500 (Volts) Drying Gas Flow: 8.0
) Drying Gas Heater: 300 (°C)
Capillary Entrance: -6000) RightNeb Gas 80 (PSI)
Endplate Heater: Off	LeftNeb Gas 0 (PSI)
	APCI Heater: 25 (°C)
Source Voltage is ON	
All Gas and Heaters are ON	Calibration Vial:

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

🧃 Manual Tune - T	ypical Tune Pos 8kV		
Primary Variables -		Data Acquisition	
Spectra Per Sec.:	1	Spectra Acquired:	6
Acq. Function:	Pulse 💌	Saved Count:	0
Low m/z:	100		
High m/z:	3002	Spectra Sav	ving is OFF
Ion Polarity:	Positive 💌		
N		Acquisitio	n is OFF
Calibrate	Apply Apply		
Ramping	Syringe Pump	Trap Enhancement	Comments
Ion Source	Optics	Optics / Flight Tube	DAU
- Ion Guide Inter	face Optics (Volts)	Flight Tube (Volts)	
Lens 1 Trap:	20.0 🚔	Pulse Lens Volts:	540.0 🌻
Lens 1 Eject:	0.0 🚔	Pulse Lens 1 Bias:	-0.200 🚔
Lens 2:	-5 🖤	Pulse Lens 3 Bias:	28.0 🚔
Lens 3:	0	Flight Tube:	-8000.0 🚔
Lens 4:	-80 🚔	Reflectron:	1700.0 🌻
		Detector:	2750 🚔

- **NOTE:** If the instrument had been in positive ion mode for at least two hours, let the instrument pulse for one hour to equilibrate the electronics and hardware. If the polarity was just changed, open the Tune and allow the instrument to pulse for at least 2 hours before running AutoTune in the current polarity.
 - 12. While the instrument is pulsing, infuse the tune mix at 10μl/min into the mass spectrometer and make sure all seven of the calibration ions are observed while the instrument is acquiring; then stop the infusion.
 - 13. After the instrument has been given appropriate time to thermally stabilize (see **Note** above), close the TOF MS Driver program.
 - 14. Before going to the next step, restart the infusion of the tune mix from either the calibrant vial or syringe pump at 10µl/min and let it run for a few minutes before starting AutoTune to insure a stable calibrant flow.
 - 15. On your desktop, double-click on **AutoTune.exe**.

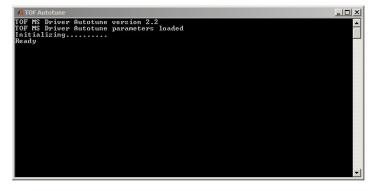


The AutoTune application starts and displays the **Select File to Open** dialog.

Look in:	J Autotune	- 🖛 🗈 💣 🎟-	
-	Name 🔺		
2	AutoRefine	8/14/2013 9:13 AM TOFSCR2 File	
Recent Places	DAUnormalize	8/14/2013 9:13 AM TOFSCR2 File	
	DetectorNormalize	8/14/2013 9:13 AM TOFSCR2 File	
	LensTune	8/14/2013 9:13 AM TOFSCR2 File	
Desktop	TrapPulse	8/14/2013 9:13 AM TOFSCR2 File	
	TuneMethod	8/14/2013 9:13 AM TOFSCR2 File	
Computer Computer Network			
	File name: TuneMethod	▼ Open	

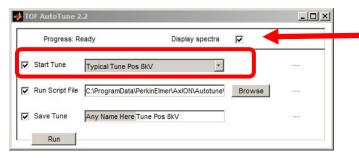
16. Select **TuneMethod** then click **Open**.

The following AutoTune screens appear.



Progress: R	eady Display sp	ectra 🔽	
Start Tune	Typical Tune Pos 8kV	¥	
Run Script File	C:\ProgramData\PerkinElmer\AxION\Au	totune Browse	
Save Tune	ATfrom_Typical Tune Pos 8kV		

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



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

Select File to C	pen				? 🗙
Look in:	Autotune_Build	62	•	+ 🗈 💣 💷 +	
My Recent Documents Desktop My Documents	DAUnormalize.to DetectorNormaliz DetectorNormaliz LensTune.tofscr2 Traperuse.torscr TraneMethod.tofs	e.tofscr2			
My Computer					
My Network Places	File name:	TuneMethod.tofscr2		-	Open
	Files of type:	(*.tofscr2)		•	Cancel

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

The following screens display.

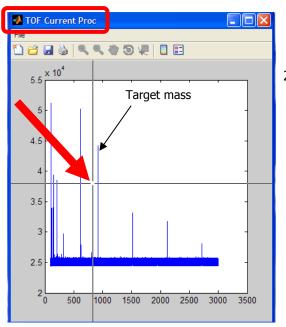
📣 TOF Autotune	<u>- 0 ×</u>
TOF MS Driver Autotune version 2.2 TOF MS Driver Autotune parameters loaded Initializing Ready	
	-

Progress: Ready			isplay spectra		
Star	t Tune	Typical Tune Pos 8kV			
Run	Script File	C:\ProgramData\PerkinElmer\/	AxION\Autotune)	Browse	
Sav	e Tune	ATfrom_Typical Tune Pos 8k	V		

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

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

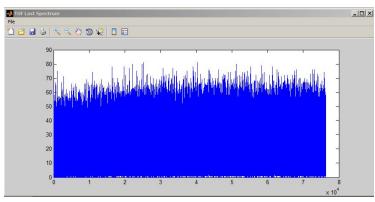
20. Click **Run** to start the AutoTune.



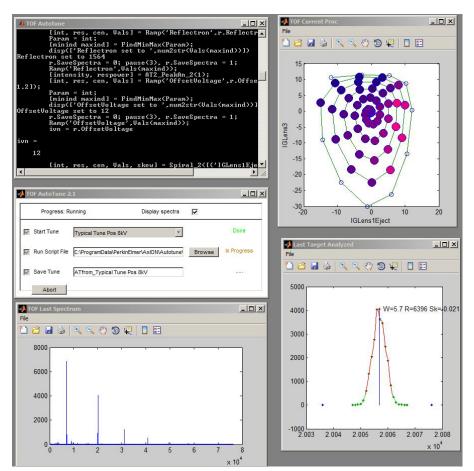
- 21. In the **TOF Current Proc** screen, as you move the mouse, the pointer turns to a cross hair.
- 22. First click on the left side of the target mass you want to tune on. Typically in positive ion mode select m/z 922 and in negative ion mode select m/z 1033.

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

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



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



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



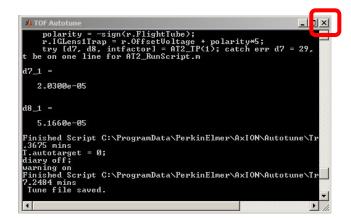
.... the instrument tunes on the next isotope. No action is required.

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

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

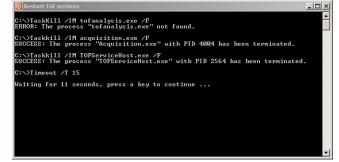
24. After AutoTune is complete close the AutoTune windows by clicking on the X in the upper-right of the TOF AutoTune screen as shown below and all of the other windows will close.

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25. Click the red button to restart TOF services





26. Open the TOF MS Driver.



27. Open the pulse Tune that AutoTune just created.

	Probe:	→ Polarity:	-		
,					
	User Name	△ Tune Name	Modified		
12	AutoTune	ATfrom_Typical Tune Pos 8kV	9/10/2013 12:04:44 pm		
13	AutoTune	AT from_AD5KVD SAdemofinal	8/14/2012 1:20:54 pm		
14	AutoTune	AT-PS-from_start_tune_positive_ion_8kV	8/1/2012 5:01:38 pm		
15	AutoTune	AT3from_start_tune_positive_ion_8kV	7/30/2012 4:40:13 pm		
16	AutoTune	AT from Typical Tune Pos 10kV by	11/21/2012 10:50:49 am		
17	AutoTune	ATfrom_AD5KVAug2012	8/8/2012 1:55:54 pm		
18	AutoTune	AT from Typical Tune Pos 10kV by TuneMethod Trap	11/19/2012 5:05:11 pm		
19	AutoTune	ATfrom_AD8KVPOs	8/8/2012 1:07:07 pm		
20	AutoTune	Reserpine Sensitivity TW ESI 11 KV	11/16/2012 11:20:17 am		
21	AutoTune	AT from AD8KVPOs by TuneMethod Trap	8/8/2012 1:07:06 pm		
22	AUTOTUNE	Typical Tune Pos 8kV	9/1/2013 10:00:00 pm		
23	AUTOTUNE	Typical Tune Neg 8kV	9/1/2013 10:00:00 pm		
24	AUTOTUNE	Typical Tune Neg 11kV	9/1/2013 10:00:00 pm		
25	AUTOTUNE	Typical Tune Pos 11kV	9/1/2013 10:00:00 pm		
26	AUTOTUNE	Typical Tune Neg 5kV	9/1/2013 10:00:00 pm		
	AUTOTUNE	Typical Tune Pos 5kV	9/1/2013 10:00:00 pm		
	FACTORY	Vent	9/1/2013 10:00:00 pm		

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

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

- > AutoTune was designed to automatically achieve very good resolution, but also to balance the need for sensitivity. Consequently, it may not deliver resolution that meets or exceeds the AxION 2 instrument specification of >12,000 at $m/z \sim 1000$ (using 11kV flight tube voltage).
- If the goal is proving that the instrument performance specification for resolution can be met, then typically some minor manual tuning will be required after an AutoTune to demonstrate the resolution specification.
- If AutoTune provides the desired performance required by the user, then proceed to the section on how to perform a default calibration (associated with the specific Tune).
- If the user requires even better performance than that which was delivered using Auto Tune, contact a PerkinElmer Product Specialist.

Calibration

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

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

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

About Samples

Tune Mix (PerkinElmer Part Number ZG2421A)

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

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

Calibrants

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

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

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

Calibrant Delivery by Syringe Pump

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

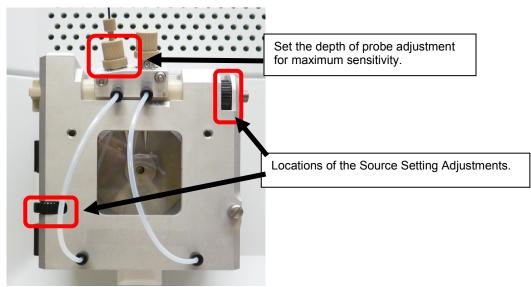
NOTE: Using a 500 µL Hamilton syringe, set the inner diameter to 3.26 mm with a flow rate of 5 to 10 µL/min.

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

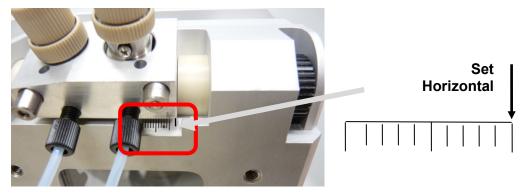
👔 Manual Tune - Typical Tune Pos 8kV	
Primary Variables	Data Acquisition
Spectra Per Sec.: 1	Spectra Acquired: 0
Acq. Function: Pulse	Saved Count: 0
Low m/z: 100	
High m/z: 3002	Spectra Saving is OFF
Ion Polarity: Positive	
	Acquisition is OFF
Calibrate 🎒 Apply	
Ion Source Optics Ramping Syringe Pump	Optics / Flight Tube DAU Trap Enhancement Comments
- Syringe	
Manufacturer: Hamilton - Microliter S	eries Gastight
Model: 500 µl 💌 Diar	meter: 3.26 mm
Liquid	
10,00 · Unit	ts: µl/min 💌
Pump	is OFF

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

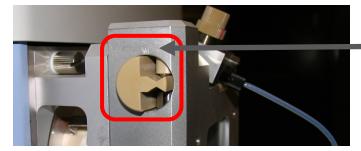
For the Dual ESI source, connect the infusion line to the left hand side of the dual sprayer. (The left sprayer is recommended since it must be used when the lockmass calibrant is infused simultaneously with the LC effluent.) The probe needle depth and the tilt positions should be the same as for the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



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



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



Set Tilt Position Here

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



3. From the File menu, open Manual Tune.



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

Probe: User Na AutoTun AutoTun AutoTun AutoTun AutoTun AutoTun AutoTun	e e	Polarity: Tune Name Alfinom_Typical Tune Pos B/V Alfinom_ADBX/USAdemotinal AT PS-from_start_tune_positive_ion_8k/V Alfinom_start_tune_positive_ion_8k/V	Modified 9/10/2013 12:04:44 pm 8/14/2012 1:20:54 pm 8/12/2012 5:01:38 pm
AutoTun AutoTun AutoTun AutoTun AutoTun AutoTun	e e	ATfrom_Typical Tune Pos 8kV ATfrom_AD5KVDSAdemofinal AT-PS-from_start_tune_positive_ion_8kV	9/10/2013 12:04:44 pm 8/14/2012 1:20:54 pm
AutoTun AutoTun AutoTun AutoTun AutoTun AutoTun	e e	ATfrom_Typical Tune Pos 8kV ATfrom_AD5KVDSAdemofinal AT-PS-from_start_tune_positive_ion_8kV	9/10/2013 12:04:44 pm 8/14/2012 1:20:54 pm
AutoTun AutoTun AutoTun AutoTun AutoTun	e . e .	ATfrom_AD5KVDSAdemofinal AT-PS-from_start_tune_positive_ion_8kV	8/14/2012 1:20:54 pm
AutoTun AutoTun AutoTun AutoTun	e . e .	AT-PS-from_start_tune_positive_ion_8kV	
i AutoTun AutoTun AutoTun	e .		8/1/2012 5:01:38 pm
AutoTun AutoTun		AT3from start tune positive ion 8kV	
AutoTun	e .		7/30/2012 4:40:13 pm
		ATfrom Typical Tune Pos 10kV by	11/21/2012 10:50:49 am
autoTur 8	e .	ATfrom_AD5KVAug2012	8/8/2012 1:55:54 pm
	e .	ATfrom Typical Tune Pos 10kV by TuneMethod Trap	11/19/2012 5:05:11 pm
AutoTun	е.	ATfrom_AD8KVPOs	8/8/2012 1:07:07 pm
AutoTun	e	Reserpine Sensitivity TW ESI 11 KV	11/16/2012 11:20:17 am
AutoTun	e .	ATfrom AD8KVPOs by TuneMethod Trap	8/8/2012 1:07:06 pm
AUTOTU	INE	Typical Tune Pos 8kV	9/1/2013 10:00:00 pm
AUTOTU	INE	Typical Tune Neg 8kV	9/1/2013 10:00:00 pm
AUTOTU	INE	Typical Tune Neg 11kV	9/1/2013 10:00:00 pm
AUTOTI	INE	Typical Tune Pos 11kV	9/1/2013 10:00:00 pm
AUTOTI	INE	Typical Tune Neg 5kV	9/1/2013 10:00:00 pm
LUTOT	IN IT	Typical Tune Pos 5kV	9/1/2013 10:00:00 pm
AUTOTU	JNE I		
AUTOTU AUTOTU AUTOTU	JNE JNE JNE	Typical Tune Neg 11kV Typical Tune Pos 11kV Typical Tune Neg 5kV	9/1/2013 10:00:00 pm 9/1/2013 10:00:00 pm 9/1/2013 10:00:00 pm

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

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

The instrument loads the Tune.

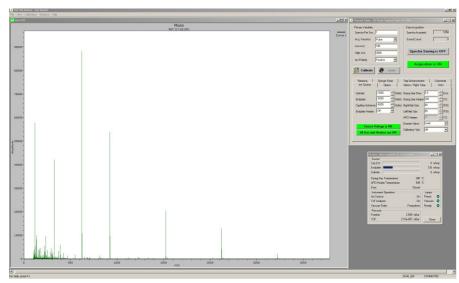
👔 Manual Tune - Al	from_Typical Tune	Pos 8kV	×
Primary Variables -		Data Acquisition	
Spectra Per Sec.:	1	Spectra Acquired:	0
Acq. Function:	Pulse 💌	Saved Count:	0
Low m/z:	100		
High m/z:	3002	Spectra Sav	ing is OFF
Ion Polarity:	Positive 🔹		
X Calibrate	Apply	Acquisitio	n is OFF
Ramping	Syringe Pump	Trap Enhancement	Comments
Ion Source	Optics	Optics / Flight Tube	DAU
Cylinder:	3500 · (Volts)	Drying Gas Flow: 8.0	· (/m)
Endplate:	-5000 - (Volts)	Drying Gas Heater: 300	
Capillary Entrance	-6000 - (Volts)	RightNeb Gas 80	(PSI)
Endplate Heater:	Off	LeftNeb Gas 0	(PSI)
		APCI Heater: 25	(°C)
Courses V	oltage is ON	Diverter Valve: Load	
	Heaters are ON	Calibration Vial: Off	•
	neaters ale UN		

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

Manual Tune - ATfrom_Typic	al Tune Pos 8kV	
Primary Variables	Data Acquisition	1
Spectra Per Sec.: 1	Spectra Acquire	ed: 1
Acq. Function: Pulse	Saved Count:	0
Low m/z: 100		
High m/z: 3002	Spectra	Saving is OFF
Ion Polarity: Positive		sition is ON
🖄 Calibrate 🛛 🦓 Ap	pply	
Ramping Syringe Pur	n Trap Enhancemen	t Comments
Ion Source Optics	Optics / Flight Tub	DAU DAU
Cylinder: -3500	(Volts) Drying Gas Flow:	8.0 · (l/m)
Endplate: -5000	(Volts) Drying Gas Heater	
Capillary Entrance: -6000	• (Volts) RightNeb Gas	80 · (PSI)
Endplate Heater: Off	 LeftNeb Gas 	0 (PSI)
	APCI Heater:	25 (°C)
	Diverter Valve:	25 · (°C) Load ·
Source Voltage is Of	Diverter Valve:	

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

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



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

	D.:	1712 100	1100 54-1	ToF May	ss Spectrum - Config	uuro Calibratio				×
I UF MS	Driver - UZ.	1712_100	_1100_5Hz_3	TOP Plas	s spectrum - coning	jure calibratic	JII			
File View	Calibration	Windows	Help	Calib	rants			Calibratio	on Settings —	
	Calibration				Compound Name 🛆	Mass 🛆	In Use	- Polarit	u	
Workbook	🗸 Manual		×	21		42.03380			-	
🖃 🖑 Dat	Automati	· -		22	Agilent 1	118.08625	V	• P	ositive	
	Automati	۰ I		23	Agilent 2	322.04812	V	0 N	egative	
÷	C			24	Agilent 3	622.02896				
	Configure	Э		25	Agilent 4	922.00979				
I '				26		1521.97147		No Spe	ctra to Averac	ie: 30
				27		2121.93315		into: ope		,
					Agilent 7	2721.89483		Minimun	s/N·	3
				29		Add			10/14.	
				30		Delete		Search	Span (amu):	2
				31		Edit				
				32		Validate Row		Polynom	nial Order:	2
				33						
					APCI L3 APCI L4	622.02896				
						922.00979			.oad	Save
				36	APCI L5	1221.99063	•		.uau	
					Display Both Masses		Clear All			
									ОК	Cancel

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

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

- 10. Use **30** for the **No. Spectra to Average** and use the value 3 for the **Minimum S/N**. The **Search Span** should be set to **2** amu; however if the masses are off more than **2** mass units you will have to open the span wider. The **Polynomial Order** should be **2** but if this does not work well you can set the order to 1 or 3 and rerun the calibration.
- 11. Click **OK** to set these values.
- 12. In the **Manual Tune** dialog, click on the **Calibrate** button and allow the calibration to complete.

🚺 Manual Tune - ATfrom_Typical Tune I	Pos 8kV
Primary Variables	Data Acquisition
Spectra Per Sec.: 1	Spectra Acquired: 475
Acq. Function: Pulse	Saved Count: 0
Low m/z: 100	
High m/z: 3000	Spectra Saving is OFF
Ion Polarity: Positive	Acquisition is ON
Calibrate Apply	
Ramping Syringe Pump Ion Source Optics	Trap Enhancement Comments Optics / Right Tube DAU
Cylinder: -3500 (Volts)	Drying Gas Flow: 8.0
Endplate: -5000 (Volts)	Drying Gas Heater 300
Capillary Entrance: -6000 (Volts)	RightNeb Gas 80 (PSI)
Endplate Heater: Off	LeftNeb Gas 0 (PSI)
	APCI Heater: 25 (°C)
Source Voltage is ON	Diverter Valve: Load 💌
All Gas and Heaters are ON	Calibration Vial: Right

When the calibration is finished the calibration window will appear.

Manual Ca Accept	Show Stats	Dat 1 ancel	Becalculate							Poly. Order:	
Eccel.			La Contra da		Ma	ee.				2-9	<u> </u>
45000-	Agilent 1 m/z 118.08625 Bin 22198.374	5	Agilent 3 m/z 622.028960 Bin 50657.56053:			Agilent 5 m/z 1521.971476 Bin 79113.071514				Agilent 7 m/z 2721.894830 Bin 105723.126017	
40000-											
35000-											
30000-											
25000- Hultinge 20000-	m/:	lent 2 z 322.048121 : 36513.002935		Agilent 4 m/z 922.009799 Bin 61625.892942			t i i i i i i i i i i i i i i i i i i i	Agilent 6 π/z 2121.933153 ðin 93373.230358			
15000-											
10000-											
5000-			tulu at		h				Ι.	 	
0	1 1 1 1 1 1 1	500	1 12.1	1000	150	0 m/z	2000		2500	3000	

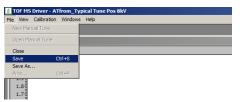
13. Click the **Show Stats** button.

The information appears on the bottom half of the screen.

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Manual Ca	alibratio (3) - T	oF Data1						
Accept	Hic <u>S</u> tats	Cancel	<u>B</u> ecalculate					Poly. Order: 2
				Ma	ass			
45000 40000 35000	Agilent 1 m/z 118.08 Bin 22198.3	6255 374107	Agilent 3 m/z 622.028960 Bin 50657.56053		Agilent 5 m/z 1521.971476 Bin 79113.071514		Agile: m/z 3 Bin 1	nt 7 2721.894830 05723.126017
30000 pp 25000 up 20000 15000		Agilent 2 m/z 322.048121 Bin 36513.00293		Agilent 4 m/z 922.009799 Bin 61625.892942		Agilent 6 m/z 2121.933153 Bin 93373.230358		
10000 5000 0 0	, <u></u> ,	500	<u> </u> ,	1000 15	00 2000 m/z	,	2500	
				Curve/Residual				
0.00050 0.00040 0.00030 0.00020 (Z) 0.00010		Ţ]			110000 - 100000 - 90000 - 80000 - 70000 m	Calculated Mass 118.06625 322.04812 622.02896 922.00980 1521.97148 2121.93315	-0.00049 0.00012 0.00018 0.00001
0.00010 0.00000 0.00000 -0.00020 -0.00020 -0.00030						-50000 -50000 -40000 -30000	2721.89483 Root Mean Square	0.00044
-0.00040		500	1000	1500 2000 m/z	2500	20000	Std. Dev Poly. Order	0.00035

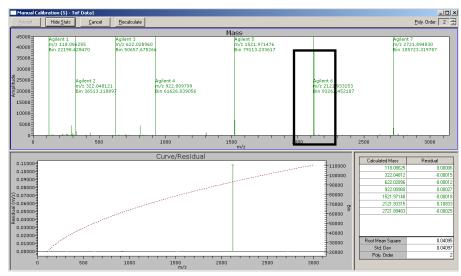
- 14. Make sure the Residuals are less than 0.000x as shown above (there should be three zeros for each of the Residuals). Click the **Accept** button.
- 15. If all of the Residuals are less than 0.000x as shown above, select **Save** from the **File** menu. This ties the calibration information to this Tune file.



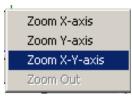
16. If any Residuals are too high, as shown in the following example, perform the following procedure to adjust the residuals to an acceptable level.

	Calculated Mass	Residual
	118.08625	0.00006
	322.04812	-0.00015
	622.02896	-0.00012
	922.00980	0.00027
	1501-071-40	0.00010
ĺ	2121.93315	0.10833
L	0701-00400	0.00005
	Root Mean Square	0.04095
	Std. Dev	0.04097
	Poly. Order	2

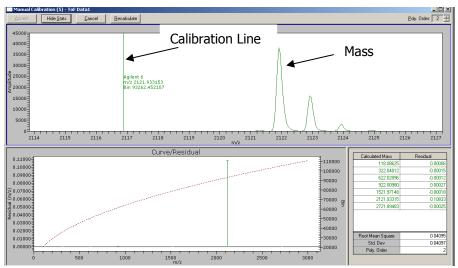
a. Left-click and drag the mouse to draw a box around the mass and the calibration line. The following example shows the mass and calibration line close to each another.



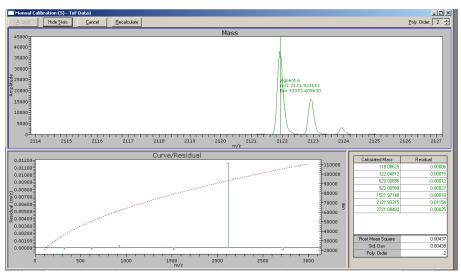
After drawing the box, release the mouse button and the Zoom pop-up box appears.



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

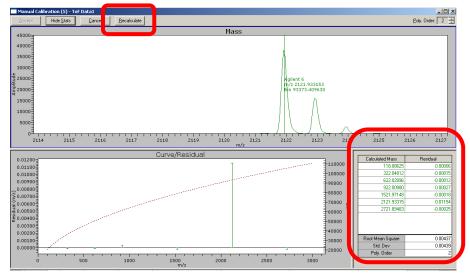


c. Left-click on the calibration line and drag it to the center of the mass peak as shown below.



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

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



- e. To complete the calibration procedure, click the **Accept** button. Then from the TOF MS Driver window, select **Save** from the **File** menu.
- 17. If this procedure fails, increase the concentration of your calibration mix.
 - ➢ For the Tune Mix in positive ion mode, change it from 100:1 to 50:1.
 - > In the negative ion mode, change concentration from 1000:1 to 100:1
 - > Then rerun the calibration procedure.

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

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

👔 Manual Tune - ATfrom_Typical Tune	Pos 8kV
Primary Variables	Data Acquisition
Spectra Per Sec.: 1	Spectra Acquired: 962
Acq. Function:	Saved Count: 0
Low m/z: 100	
High m/z: 3000	Spectra Saving is OFF
Ion Polarity: Positive	
	Acquisition is ON
Calibrate 🌈 Apply	
Ion Source Optics	Optics / Flight Tube DAU
Ramping Syringe Pump	Trap Enhancement Comments
I Manual IG Exit Low, D	
rap/Pulse D	elay, D8: 70 (μs)
	High 1000
Enhancement Value	IE Graph
	- Max 0
Pulse Frequency: Automatic	(KHz)

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

🚺 Manual Tune - ATfrom_Typical Tune F	Pos 8kV
Primary Variables Spectra Per Sec.: 1	Data Acquisition Spectra Acquired: 1031
Acq. Function: Trap	Saved Count: 0
Low m/z: 100 High m/z: 3000	Spectra Saving is OFF
Ion Polarity: Positive	Acquisition is ON
Calibrate Apply	
Ion Source Optics Ramping Syringe Pump	Optics / Flight Tube DAU Trap Enhancement Comments
Manual IG Exit Low, D	7: <mark>63 (μs</mark>)
Trap/Pulse Del	High 1000
Min Pulse Frequency: Automatic	Max 1 Default

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

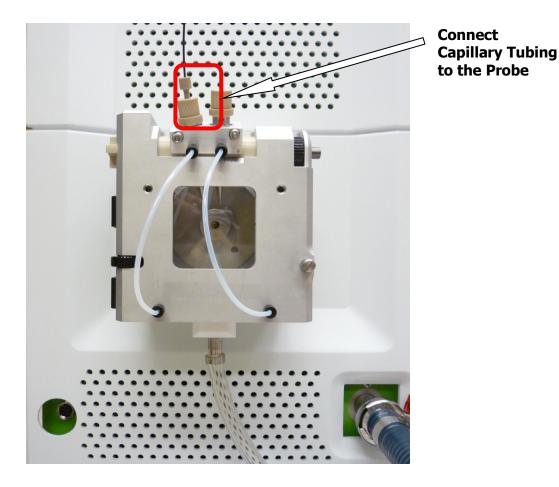
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<u>Ramping the AxION 2 TOF MS</u> <u>Capillary Exit</u>

Setting up a Sample Infusion

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

- 1. Fill a syringe with a \sim 50 pg/µl reserpine solution in LCMS grade methanol and water in a 75:25 ratio in 5 mM ammonium formate
- 2. Since this example demonstrates a dual-probe system, connect a Peek transfer line from the syringe needle to the left ESI sprayer. (Either sprayer may be used if infusing a solution.)
- 3. With the syringe prepared for infusion, the next part of the process covered in the next section involves ramping (to optimize) the capillary exit voltage.



Ramping Parameters - Optimizing the Capillary Exit Voltage

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

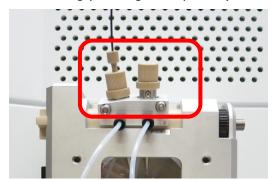
To Ramp the Capillary Exit voltage:

1. Open a Manual Tune.

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

Manual Tune	ATfrom_Typical	Tune Pe	os 8kV		
Primary Variables			- Data Acquisition	n	
Spectra Per Sec	a <mark>1</mark>		Spectra Acquir	ed:	72
Acq. Function:	Pulse	-	Saved Count:		0
Low m/z:	100				
High m/z:	3000		Spectra	Savin	g is OFF
Ion Polarity:	Positive	-			
5.4			Acqui	isition	is ON
💦 Calibrate	🕴 Appl	9			
Ramping	Syringe Pump		Trap Enhancemer	,	Comments
Ramping Ion Source	Syringe Pump		Trap Enhancemer Optics / Flight Tul	,	Comments DAU
	Optics	l' (,	
Ion Source	Optics	(Volts)	Optics / Flight Tul	be 8.0	DAU
Ion Source Cylinder:	-3500 + -5000 +	(Volts) (Volts)	Optics / Flight Tul Drying Gas Flow:	be 8.0	DAU
Ion Source Cylinder: Endplate:	Optics -3500 -50006000	(Volts) (Volts) (Volts)	Optics / Flight Tul Drying Gas Flow: Drying Gas Heate	be 8.0 r <mark>;</mark> 300	DAU
Ion Source Cylinder: Endplate: Capillary Entran	Optics -3500 -50006000	(Volts) (Volts) (Volts)	Optics / Flight Tul Drying Gas Flow: Drying Gas Heate RightNeb Gas	be 8.0 r.300 80	DAU • (l/m) • (°C) • (°C) • (PSI)
Ion Source Cylinder: Endplate: Capillary Entran Endplate Heate	Optics 3500 ■ -5000 ■ -6000 ■ r: Off ▼	(Volts) (Volts) (Volts)	Optics / Right Tul Drying Gas Row: Drying Gas Heate RightNeb Gas LeftNeb Gas	be 8.0 r:300 80 0	DAU • (/m) • (°C) • (PSI) • (PSI)
Ion Source Cylinder: Endplate: Capillary Entran Endplate Heate Source	Optics -3500 -50006000	(Volts) (Volts) (Volts)	Optics / Right Tul Drying Gas Row: Drying Gas Heate RightNeb Gas LeftNeb Gas APCI Heater:	be 8.0 (300 80 0 25	DAU • (/m) • (°C) • (PSI) • (PSI)

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



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

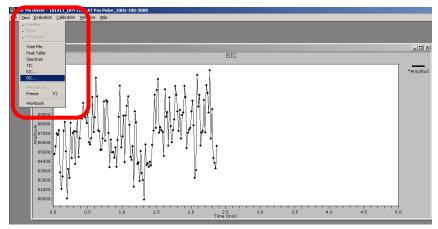
	_O×
BIC	
93000 92000 91000 99000 99000 99000 99000 99000 99000 99000 99000 9000 9000 90000 90000 90000 90000 90000 90000 90000 90000 90000 90000 90000 90000 90000 90000 90000 90000 90000 90000 90000 90000 90000 90000 90000	*Amplitud
M m/z (6)	
	<u>_ ×</u>
Reserpine peak	Curve 1

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

<mark>M</mark> m/z (6)		-DX
1743.517, 26082.578	Mass - Frozen RET (00.31900)	
80000 50000 50000 50000 20000 10000 10000 500		Curve 1
	m/z	
Cntrd m/z Max A pl m/z Cntrd m/z Max A pl m/z 609.2716 609.2719		Rel Amp 100
		F
Export To PeakList Close		

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

5. Click Close.



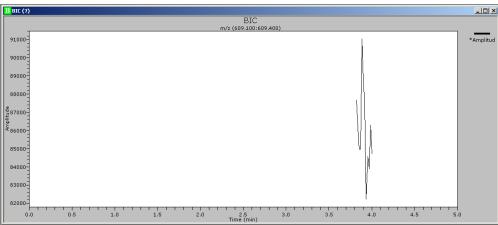
6. In the TOF MS Driver screen, select **BIC** from the **View** menu.

The Base Peak Preferences dialog displays.

Base Peak Preferences	×
Start m/ <u>z</u> : 609.1 End m/z: 609.4	Trace Selection ✓ Amplitude Resolution <u>m</u> /z
Display Replace Curves	Range Limits None
	0K Cancel

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

The BIC screen displays.



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

1		
Manual Tune -	ATfrom_Typical Tun	e Pos 8kV
Primary Variables		Data Acquisition
Spectra Per Sec	a: 1	Spectra Acquired: 235
Acq. Function:		Saved Count: 0
Acq. Function:	Pulse	Saved Counc J U
Low m/z:	100	
High m/z:	3000	Spectra Saving is OFF
-		
Ion Polarity:	Positive 💌	Ramping is OFF
Nº		
🔀 Calibrate	Apply	
Ion Source	Optics	Optics / Flight Tube DAU
Ramping	Syringe Pump	Trap Enhancement Comments
- Item to Contro	bl	
Page: Op	tics	•
Control: Ca	pillary Exit	T
Ramping Para	ameters	Steps
Start: 10		
Start: 10	Increment:	5 Spectra Per: 1
Start: 10 Stop: 25		5 Spectra Per: 1 Volts Number of:

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

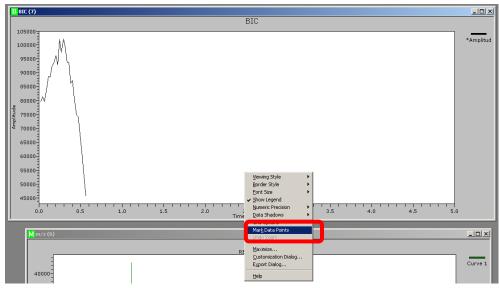
Function	Parameters	Values/Settings
Item to Control	Page	Optics
	Control	Capillary Exit
Ramping Parameters	Start	100
	Stop	250
	Increment	5
	Units	Volts
Steps	Spectra Per	1

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

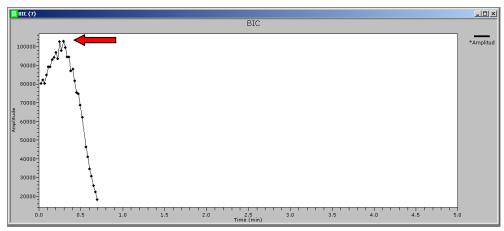
It turns green and changes to **Ramping is ON**

Ramping is ON

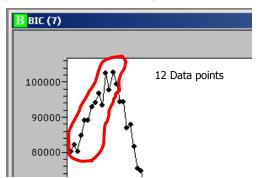
13. When the ramp completes, right-click in the **BIC** screen and select **Mark Data Points** from the popup menu.



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



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



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

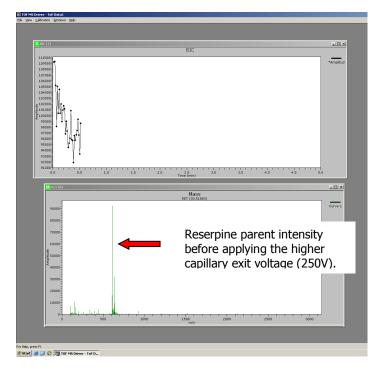
m/z: 100 nm/z: 3000 Polarity: Positive ▼ Calibrate Apply amping Syringe Pump Trap E on Source Optics Optics nn Transport Lenses (Vots) of	ed Count: 0 Spectra Saving is OFF Acquisition is OFF
n m/z: 3000 Polarity: Positive Calibrate Apply amping Syringe Pump Trap E on Source Optics Optics in Transport Lenses (Volts)	
Polarity: Positive Calibrate Apply amping Syringe Pump Trap E on Source Optics Optics in Transport Lenses (Volts)	
Calibrate Apply amping Syringe Pump Trap E on Source Optics Optics on Transport Lenses (Volts) lo	Acquisition is OFF
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on Source Optics Optics on Transport Lenses (Volts) lo	
on Source Optics Optics on Transport Lenses (Volts) lo	
	hancement Comments / Flight Tube DAU
Capillary Exit: 160 🖨 🛛 F	iuide
	requency:
okimmer: J ²⁰ 💌 RF	/oltage: 500 🚔
Offs	et Voltage: 10.8 🌻

- 17. Select **Save Tune** from the **TOF MS Driver File** menu.
- 18. Turn the acquisition on and view you spectrum in the Mass screen.

Ramp Again to Optimize the Signal Intensity of a Fragment Ion

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

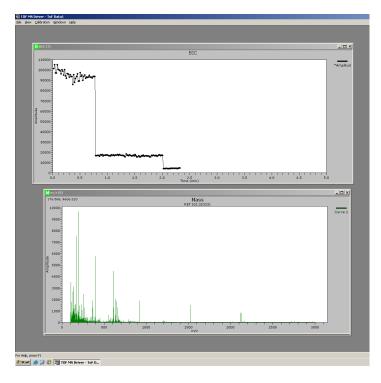
The following screen shows reserpine optimized at 160V.



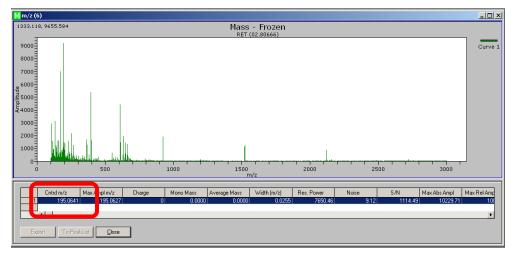
1. To view the fragment, increase the **Capillary Exit** value (in this example we set it to 250V) and click **Apply**.

		_10
01C (7)	BIC	
10000 100000 100000 10000 10000 10000 10000 10000 10000 10000		*Ampli
	Main fragment ion at 250V. Right click on the apex of the most intense fragment ion.	Gurve

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

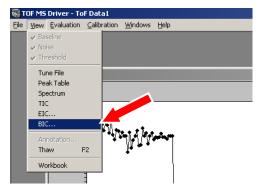


3. In the *m/z* screen, right-click on the apex of the most intense fragment ion(s) observed and write down the observed values.



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

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



The BIC Base Peak Preferences dialog displays.

Base Peak Preferences	×
Start m/ <u>z</u> : [195 End m/z: [195.1]	Trace Selection ✓ Amplitude □ Resolution □ <u>m</u> /z
Display Replace Curves	OK Cancel

- 5. Type a **Start** *m*/*z* value to the left of your target peak and an **End** *m*/*z* value to the right your target peak. Select **Amplitude** for the **Trace Selection** and **Replace Curves** for the **Display**.
- 6. Click **OK**.
- 7. Stop acquiring by clicking the green **Acquisition is ON** button. The button changes to **Acquisition is OFF**.
- 8. In the Manual Tune dialog, select the Ramping tab.

	ATfrom_Typical Tur		
Tianual Tune -	Altrom_Typical Tur	ie Pos 8kv	
Primary Variables		Data Acquisition	
Spectra Per Sec	.: 1	Spectra Acquired:	129
Acq. Function:	Pulse 💌	Saved Count:	0
Low m/z:	100		
High m/z:	3000	Spectra Savi	ng is OFF
Ion Polarity:	Positive 💌		
🖄 Calibrate	💣 Apply	Ramping i	
Ion Source	Optics	Optics / Flight Tube	DAU
Ramping	Syringe Pump	Trap Enhancement	Comments
Item to Contro	l		
Page: Opt	ics	•	
Control: Cap	villary Exit	-	
- Ramping Para	meters	Steps	
Start: 160) Increment:	5 Spectra Pe	er: 1
Stop: 300	Units:	Volts Number of	19

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

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

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

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

- 11. Right-click in the BIC screen and select **Mark Data Points** from the pop-up menu.
- After the ramp is complete as shown in the previous example, start counting the data points from the left side of the BIC screen to the apex of the displayed peak.
 Each data point is 5V; this is the value you entered as an **Increment** in the Ramping parameters.

Ramping is ON

- 13. Multiply the counted number of data points by the voltage per data point (5V).
- 14. Click on the **Optics** tab. Enter the above calculated value as the **Capillary Exit** value.
- 15. Select Save Tune from the TOF MS Driver File menu.
- 16. Turn the acquisition back on and view you spectrum in the Mass screen.

Creating Methods and Sequences

Creating an MS Method

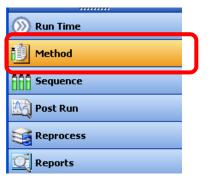
The foundation for an optimal MS Method is starting with a good mass calibrated Tune. Using the previous calibration instructions, ensure the tune is proven to be optimized, using Calibration, before proceeding.

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

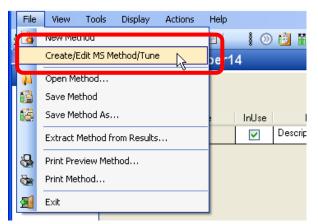
IMPORTANT: If quantification of a compound using Chromera is desired, then the EIC masses of the ions of interest must be predefined in the TOF MS Driver Method in order to be available to Chromera for processing. EICs added post-acquisition are invalid for reprocessing.

To create an MS method:

1. Click Method to open the Chromera Method screen.



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



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

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Usete Image: Periods Usete Image: Periods Usete Image: Periods Image: Periods Periods Image			
Image: constrained of the second of the s			uration (minutes): 5.00
Tune 110.2013 TW Neg. 8kV Browse Tunes Use Tune Parameters from: C Database C Method File Mass Range: (50, 1101) Spectral Rate: 5.00 Cap Exit: -100 Time Period Settings Save spectra to disk Syringe 0 Jurini Cal Vial Off Diverter Load		Red Line	Evaluation Load DEICs Calbration Default
Save spectra to disk Syringe 0 Law Vial 0ff Diverter Load		Reason Time Use Tune Parameters from: Image: Discourse of the parameters from the p	Spectral Rate: 5.00
Syringe 0 µU/min 💌 Cal Vial 0ff 💌 Diventer Load		-	
		Syringe 0 µU/min 💌	
		Diveter Load Period 1 4 Delete	

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

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

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

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

TOF MS Driver	×
ie Method Windows Help	
Hethod Editor - DemoMethod.tofmethod2	
Fund Settings 4 5007 5006 5007 5008 5009 5009 5009 5009 5009 5009 5009 5009 5009 <	
O do 0 s 10 15 20 25 30 3.5 4.0 4.5 s0 Tune ATtrom_Typical Tune Pos & V Image: growse Tunes Image: growse Tunes from: Tune Parameters from: Spectral Rate: 1.00 Mass Range: (100, 3002) Spectral Rate: 1.00 Cap Exit: 1.20	
Time Period Settings	
I ⊆ave spectra to disk	
Syringe 0 µL/min 💌	
CalVial Off 💌	
Diverter Load 💌	
Period 2 4 Delete	
r Help, press F1 DUAL_ESI CONVECTED	//.

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

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

- **NOTE:** If, during acquisition, a method is configured to use a tune stored in the database but that tune name is not found (renamed or deleted), the system will automatically use the tune parameters in the method file. Also, if a method file is set to read only then the functionality to pull the tune from the database is not functional.
 - 6. The peripheral device settings in the lower part of the screen are method *and* time period specific. These specific settings in the method will be used over the tune settings during acquisition and when the TOF Driver method is loaded into Chromera to get to Operate mode.
 - a. Only those parameters shown below can be changed at the method level. Consequently, be very careful with the **Method File** option for fetching the Tune Parameters. With this selected, the peripheral values that cannot be set within the method are also static. For example, the syringe TYPE. If a method uses the **Method File** tune parameters and the syringe TYPE is changed in the tune, that change is not adopted during method-controlled acquisition. The **Database** option for fetching tune parameters will adopt such changes.

Time Period Sett	
✓ Save spece	stra to disk
Syringe	0 µl/min 💌
Cal Vial	Right 💌
Diverter	Load
Period	

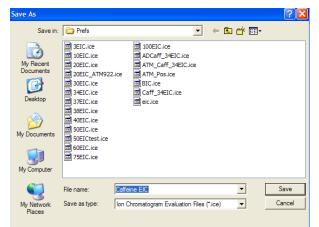
IMPORTANT: For processing in conjunction with Chromera, ALWAYS have "Save spectra to disk" selected.

 Define EICs: In the **Evaluation** section of the Method Editor screen, click the **Load** button. The following dialog displays.

Tune Period Settings	Method Windows Help	od2	
	Tune Period Setting:	Trace	alion 0 EICs 0 O EICS

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

- 8. Click the **Insert** key on your keyboard and enter the EIC value in *m*/*z*; with this example it is 195.0877 for protonated caffeine.
- 9. The Tolerance (+/-) of the EIC can be selected in parts per million (ppm), millimass units (mmu), or m/z as in the **Selection** section; here, **mmu** was used for the EIC tolerance with a value of 10 millimass units. Make sure to press the Enter key on the keyboard to update the method with this value.
- **NOTE:** The resolving power and exact mass measurement capabilities of the AxION 2 TOF provides a tremendous amount of selectivity that should be utilized to the user's advantage. Entering an EIC mass to 4 decimal place accuracy and specifying a tolerance of a few mmu (or ppm) will significantly increase the selectivity of detection for the specified analyte.
 - Click the Save button (to save the EIC for future use). The following Save As dialog displays.



11. Type a File name.

In this example, the name is **Caffeine EIC**.

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

TOF MS Driver	J ×
File Method Windows Help	
Hethod Editor - DemoMethod.tofmethod2	4
Run Settingt Duration (minutes) 5.00 Store Duration (minutes) 5.00 Store Disk Space (KB) 247305.20 Valuation Load Lead Store Disk Space (KB) 247305.20 Valuation Load Lead Store Disk Space (KB) 247305.20 Valuation Load Lead Store Disk Space (KB) 247305.20 Valuation Load Load Disk Disk Space (KB) 247305.20 Valuation Load Load Disk Disk Space (KB) 247305.20 Water Disk Space (KB) Disk Space (KB) Disk Disk Space (KB) 247305.20 Disk Disk Space (KB) Disk Space (KB) Disk Disk Space (KB) Disk Space (KB) Disk Disk Space (KB) Disk Space (KB) Disk Disk Space (KB)	
Tune ATfrom Typical Tune Pos 8kV Browne Tunes C Database Special Rate: 100 C Method File Cap Exit: 120	I
Time Period Setting:	
Save spectra to disk	
Syringe 0 Jul/min 💌	
Cal Vial Right	
Diveter Load	
Period 1 • Delete	
For Help, press F1 DUAL_ESI CONNECTED	٦,

In this example we selected the **Default** Calibration setting which uses the calibration that was updated within the selected Tune (by utilizing the **Calibrate** button on the Tune page). Alternatively, two Lockmass values can be assigned to be used for mass correction during acquisition. Parameter sets can be saved for future use. Refer to the next section for additional information.

14. To continue, click **Save** from the main **File** menu on the **TOF MS Driver** window. The **Save As** dialog appears:

TOF MS Driver	_					
The tend of all up to provide the data of the tend of	II Save As	Disk Space (KB) E-colution Load Calcutor Load Calcutor Com	247305.20 TEICs ass T Lockmans			×
Tune AThom_Typical Tune Pos 8kV	Computer + Lo	Local Disk (C:) • ProgramData • PerkinElmer • AxIC	IN - Method	- 🛄	Search Method	2
	Organize 👻 New folder					1 1 • 😧
The Peed Selling: Description of the selling of th	Konstanti Konstanti Constanti	Construction Understand Construction Constructin Construction Construction Construction Construc	Date modified 91/20/2013 LISB PM 91/20/2013 LISB PM 91/20/2013 SIGB M 91/20/2013 SIGB PM 91/22/2013 SIGB PM	Type Top MS Driver Dool TOP MS Driver Dool	Size 412 413 413 718 615 513 513 513 1218 1218 1218 1218 1218 1218 1218 12	v V X Carcel
For Help, press F1					DUAL_EST	CONNECTED

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

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

Using Lockmass in Real-Time

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

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

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

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

- 1. From the **File** menu on the TOF MS driver window, main menu, select **New Acquisition Method**.
- 2. Select a TOF Tune with the desired scan range and rate that contains a recent calibration. The **Method Editor** window will look similar to the following:

TOF MS Driver File TOF Status Tune Windows Help	×
Method Editor - DemoMethod.tofmethod2	
Retention Time	Disk Space (K8) 247306.20 Load TEICs Load TEICs Calibration Calibration Mass 1: 180558 Mass 2: 32.0097
Save spectra to disk	Search Span 50 mmu
Syringe 0 µl/min 💌	OK Cancel Load Save
Diverter Load	
Period T • Delete	
For Help, press F1	DUAL_ESI CONNECTED //

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

- 3. In the **Calibration** section of the screen, select **Lockmass** from the drop-down, then click on the **Enter Lockmass** button.
- 4. Type in the desired mass values in the **Lockmass Calibration** window; these can be saved for future reference by clicking the **Save** button. Selecting **OK** will associate the configuration (saved or not) to the method.

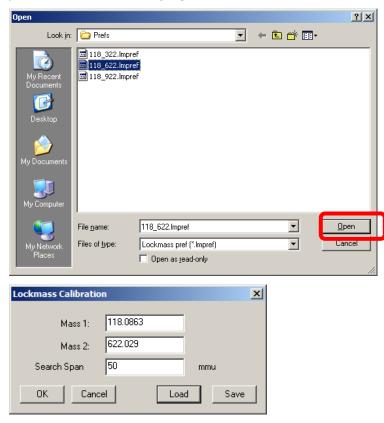
Loc	kmace Calibratio	-			×
	Mass 1:	118.0863			
	Mass 2:	922.0097			
	Search Span	50		mmu	
	OK Canc	el	Load	d Sa	ve

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

File <u>n</u> ame:	118_922.Impref	•	<u>S</u> ave
Save as <u>t</u> ype:	Lockmass pref (*.Impref)	•	Cancel

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



7. Click **OK** to update the method.

The screen displays with lock masses as 118.086 and 922.010.

TOF MS Driver File ToF Status Tune Windows Help	×
Method Editor - DemoMethod.tofmethod2	
Run Settings	
4.878 Time Periods	Duration (minutes): 5.00
100	Disk Space (KB): 247306.20
80	Evaluation
70	Load 1 EICs
80 - 50 -	<u><u>C</u>lear</u>
§ 40-	
	Calibration Lockmass
20	Enter Lockmass
	Masses: 118.0863, 922.0097 Search Span (mmu) 50
Retenton Time	
Use Tune Parameters from: Use Tune Parameters from: O Database	Mass Range: (100, 3002)
O Method File	Spectral Rate: 1.00 Cap Exit: 120
Time Period Settings	
Save spectra to disk	
Syringe 0 µl/min 💌	
Cal Vial Right 💌	
Diverter Load V	
Period 1 Delete	
For Help, press F1	UAL_ESI CONNECTED
rentricity provint x	UAL_ESI CONNECTED

- 8. Save the above method. This setup can be used for performing on-the-fly lockmass.
- **NOTE:** If a method is configured to apply lockmass calibration but the system does not find the peaks, the system will revert to using the default calibration in the tune. Batch Convert can be used to apply different lockmass parameters in the case, for example, the wrong mass was originally specified.
 - 9. Close the **TOF MS Driver** window.

Creating a Chromera Method

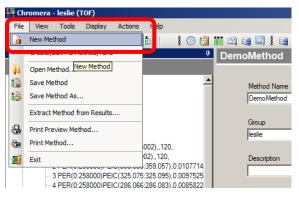
After creating the MS method in the TOF MS Driver application, open Chromera to create a Chromera method. The Chromera method will define all the operating requirements for all the other components in the Chromera configuration.

To create a Chromera method:

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

»

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



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

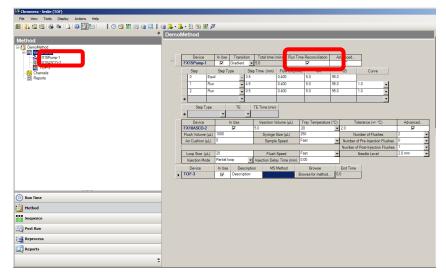
E Chromera - leslie (TOF)			_02
File Wew Tools Display Actions Help			
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E 1 DemoMethod		Direct Control	
E Instruments	Method Name DemoMethod		
FX15Pump-1 FX10ASCO-2	[Centrineirou	Start LC Pump Change Tray	
TOF-3	Group	Stop MS Equil	
- Reports	lesie 💌	St Vent MS	
	Description	K Tune Control	
	Manual annotation		
	Notes		
		L	
		Status Panel	
		Sequence Status	End Plate Current (nA)
			0.0 nA
		Pump Pressure	Corona Current (nA)
		0 psi	0.0 nA
		Vacuum State Pumped Down	AS Status Ready
()) Run Time			Drying Gas Temperat.
Method		Current Vial	299.0 °C
Sequence		MS Analysis Status	Pump Step Time
Post Run		Not Acquiring Pump Status	0.0 min APCI Vaporizer Tem.
Reprocess		Shutdown	549 °C
C Reports		Capillary Entrance C	Source Door
		10.0 nA	Closed
-		Instrument Properties	J

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

- 4. Select **Save Method** from the **File** menu.
- 5. Enter your instrument parameters by clicking on each instrument.

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

IMPORTANT: Be sure Run Time Reconciliation is checked.



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

Chromera - leslie (TOF)												
File View Tools Display Actions Help												
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Method			_	_	_	_		_	_		_	
E 1 DemoMethod		Device FX15Pump-1	In Use	Transition Gradient	Total time (m	in) Run Tin	ne Reconciliation	Ac	tvanced]		
TOF-3	-	<u> </u>						1				
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- m reports		1 Ru		4.5		0.400	5.0	95.0	1	0 =1		
		2 Ru		- 0.5		0.400	5.0	95.0	1			
		*										
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		Step Type	_	TE	TE Time (min)							
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Sequence												

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

Chromera - leslie (TOF)												
File View Tools Display Actions Help												
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E 1 DemoMethod												
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TOF-3	Step		p Time (min) Flow (ml		%B	Curve						
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	1 R		0.400			1.0 🚽						
	2 R		0.400			1.0						
	*		0.400			····						
	Step Type		TE Time (min)									
	*	<u> </u>										
	Device	In Use	Injection Volume (µL)			lerance (+/- °C)	Advanced					
	FX10ASCO-2 Flush Volume (µL		5.0 Syringe Size (µL)	20	▼ 2.0	mber of Flushes	2					
	Air Cushion (µL)		Sample Size (µL)	Fast		Pre-Injection Flushes	0					
	Air Cushion (pc)	•	Sample Speed	1.001		Post-Injection Flushes	1	÷				
	Loop Size (µL)	20	Flush Speed	Fast		Needle Level	2.0 mm	-				
	Injection Mode	Partial loop 👻	Injection Delay Time (mi		-			_				
	Device	In Use Descriptio	on MS Method	Browse	End Time							
	TOF-3	Description		Browse for method	0.0	1						
						-						
()) Run Time												
<u> </u>												
Method												
Sequence												
Post Run												

8. Click Browse for Method.

The **Select Method** dialog displays.

Select Method					X
🕞 🕞 🕨 - Computer - Local I	Disk (C:) • MSData • Method	-	Search Method		2
Organize 👻 New folder)= • 🔟	0
🔆 Favorites	Name	Date modified ~	Туре	Size	<u> </u>
🧮 Desktop	DemoMethod.tofmethod2	9/10/2013 3:17 PM	TOF MS Driver Docu	10 KB	
Downloads	Caffeine Analysis.tofmethod2	9/10/2013 11:08 AM	TOF MS Driver Docu	4 KB	
📃 Recent Places	090313-c60.tofmethod2	9/5/2013 5:25 PM	TOF MS Driver Docu	7 KB	
📜 Libraries	🗇 test144.tofmethod2	9/4/2013 3:49 PM	TOF MS Driver Docu	6 KB	
Documents	CalOFF.tofmethod2	8/27/2013 10:31 AM	TOF MS Driver Docu	5 KB	
J Music	EICTest_2timeperiod.tofmethod2	8/27/2013 10:26 AM	TOF MS Driver Docu	12 KB	
E Pictures	EICTest_4timeperiod.tofmethod2	8/27/2013 9:35 AM	TOF MS Driver Docu	12 KB	
🔠 Videos	EICTest_3timeperiod.tofmethod2	8/27/2013 9:31 AM	TOF MS Driver Docu	11 KB	
	EICTest_1timeperiod.tofmethod2	8/26/2013 5:37 PM	TOF MS Driver Docu	11 KB	
Computer Local Disk (C:)	15minute_SoloTest.tofmethod2	8/23/2013 2:40 PM	TOF MS Driver Docu	11 KB	
public (\bfdf001) (Z:)	082213-benzos_plasma-80.tofmethod2	8/22/2013 7:35 PM	TOF MS Driver Docu	14 KB	
	082213-benzos_plasma-30.tofmethod2	8/22/2013 5:28 PM	TOF MS Driver Docu	14 KB	
👊 Network	082013-benzos_plasma.tofmethod2	8/22/2013 1:10 PM	TOF MS Driver Docu	11 KB	
	DBvsFILETest_081913.tofmethod2	8/19/2013 4:05 PM	TOF MS Driver Docu	5 KB	
	SWTest_Saving_081913.tofmethod2	8/19/2013 3:51 PM	TOF MS Driver Docu	10 KB	-
File name: D	emoMethod.tofmethod2		 method files (*.t 	ofmethod2)	•
			Open 👻	Cancel	

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

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

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

5 Chromera - leslie (TOF)														
File View Tools Display Actions Help														
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4	leslie :	DemoMe	thod											
Method							_	_			_			_
E 🔂 DemoMethod														
E Instruments	_				101			0				-		
FX15Pump-1 FX10ASCO-2	-	Device FX15Pump-1	in U			Total time (r ▼ 5.0	nin) H	Run Time	Reconciliation	A	dvanced	-		
TOF-3			-	·	1				1	1				
D 🕅 Channels		Step 0	Ste Equil	р Туре	3.5	tep Time (min)	Flow (0.400	(mL/min)	%A 5.0	95.0	%B	Curve		
E TO FER(0:258000)PSCAN(100:3002),,120,			Run	*	4.5		0.400		90.0	10.0		10 -		
1 PER(0.20000)PSCAN(100:3002), 120,		2	Run	-	4.0		0.400		90.0	10.0		1.0 -		
2 PER(0.258000)PEIC(359.035:359.057).0.0107714.120.		2	Run		0.5		0.400		90.0	10.0		1.0		
3 PER(0:258000)PEIC(325.075:325.095).0.00975255,120, 4 PER(0:258000)PEIC(286.066:286.083).0.00858223,120,		*		-								-		
5 PER(0:258000)PEIC(284.111:284.128).0.00852358,120,		Step T	ype	TE		TE Time (min)								
6 PER(0:258000)PEIC(271.055:271.071).0.0081319.120.		*		•	•									
7 PER(0:258000)PEIC(326.076:326.095).0.00978257,120, 8 PER(0:258000)PEIC(321.01:321.029).0.00963058.120.		Device		In Use		Injection Vo	olume (µ	μL) 1	ray Temperature	(°C)	To	lerance (+/- °C)	Advanced	i
9 PER(0.258000)PEIC(316.039:316.058).0.00948145,120.		FX10ASCO-2		2		5.0		2			2.0		v	
10 PER(0:258000)PEIC(314.084:314.103).0.00942281,120.		Flush Volume)		Syringe S				_		mber of Flushes	2	-
11 PER(0:258000)PEIC(309.081:309.099).0.00927271.120. 12 PER(0:258000)PEIC(301.065:301.083).0.00903221,120.		Air Cushion (μL) 5		_	Sample	Speed	Fi	ast	•		f Pre-Injection Flushes	0	-
13 PER(0:258000)PEIC(287.05:287.067),0.00861175.120,			1) 20					10	ast	_		Post-Injection Flushes	1 2.0 mm	<u> </u>
14 PER(0:258000)PEIC(290.102:290.119),0.00870331,120, 15 PER(0:258000)PEIC(388.155:388.162),0.00388159.120,		Loop Size (µ Injection Mo		ial loop		Flush	Speed	(min) (151	-		Needle Level	2.0 mm	-
15 PER(0:258000)PEIC(388.155:368.162).0.00388153,120, 16 PER(0:258000)PEIC(285.07:285.087).0.00855237.120.	_													
		Device TOF-3			script		Method ata\Meth	De L De	Browse wse for method	4	End Time	-		
□ TOP-5 → 0 PER(0:258000)PSCAN(100:3002)120.	.0	101-3	F	Descri	puon	MoDi	atavinetr	no Bro	wse for method	4.	<u> </u>	_ _ _		
- 1 PER(0.238000)PSCAN(100.3002), 120,														
🚫 Run Time														
A Method		-			_			Δc	siane	h.	TOF	Method		
		Impo	orte	d EI(S	5			-					
Sequence								tin	ne bet	foi	re re	econcilia	tion	
Post Run													0.011	
Reprocess														
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•														
, view of the second se														

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

	lesi	ie :	: DemoMe	thoo	d								
			Device		In Use	Transitio		me (min) F	Due Tiere (Reconciliation	0.	ivanced	
	-		FX15Pump-1			Gradient	→ 5.0	me (min)		V	A	Jvanced	
Run Time Reconciliation		-	Step	_	Step Typ		Step Time (n	in) Elmu	(mL/min)	%A	-	%B Curve	
				Equil			3.5	0.400			95.0	AD Curve	
The pump run time exceeds the end time for the following device: The				Run			1.5	0.400		90.0	10.0	10	
detector TOF-3 end time is 4.3.Do you want the end time of the above to be extended to be the same as the pump run time 5.0?				Run			1.5	0.400		90.0	10.0	1.0	
extended to be the same as the pany fair time stor			*										
Yes No			Step T	/pe	_	TE	TE Time	(min)					
			*		-	-	•						
			Device			i Use		ion Volume (µ		ay Temperature		Tolerance (+/- °C)	Advanced
			FX10ASCO-2			~	5.0		20		-	2.0	-
			Flush Volume					inge Size (µL)) 250 Fat			Number of Flushes	2 •
		2	Air Cushion (μL)	5		Sa	imple Speed	Fas	st	•	Number of Pre-Injection Flushes	U -
			Loop Size (L	1.	20		-	lush Speed	Fas	**		Number of Post-Injection Flushes Needle Level	2.0 mm 👻
			Injection Mo		Partial loc	00	✓ Injection	iush opeed	1 40		_	Needle Level	2.01111
		-					_		1		1		
			Device TOF-3	_	In Use	Descriptio		MS Method MSData\Meth		Browse vse for method	5.	End Time	
			101-5		M	Descriptio		modalametri	Drov	vse for method	. J.		
						Δ	ssian	ed Ti	OF	Metho	hd	T	
						tir	me a	fter r	reco	ncilia	tic	n	

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.

🛞 Run Time	
Method	
Sequence	
Maj Post Run	
Reprocess	
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	» •

The Sequence screen opens with the last run sequence displayed.

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

-	🖣 Chi	romera - leslie (TOF)										
[File	View Tools Display	Actions	Help								
	à	New Sequence		o 🔁	iii 🖄	3	1	1	3	- 🛃 - 🚦	1	S 🕷 🐖
	Т	Import Sample List	New Segu	ence		ą	De	mo	Se	equence	Ð	
2	1	Open Sequence	inchi bequ	crice								Name
à	1	Save Sequence										
	(Save Sequence As					-		De	moSequenc	e	
	8	Print Preview Sequence								Sample Type		Samp Nam
	Ğ.	Print Sequence					r	+	1	Sample	-	DemoBlan
	<u>E</u>	Exit						- 	2	Sample	-	Demo1
Ľ								<u>+</u>	2	Sample	-	Demot
							6	•	3	Sample	•	Demo2

A blank sequence screen displays.

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

			N	lame		0	escription	Group		Author	Edit	or S	ample Tray Type	Activate				
	Den	noSequenc	e					leslie	▼ TOF		TOF	10	0-Position Tra 👻	V				
		Sample Type		Sample Name	Vial			Method		Star	dard	Injections	Dilution Factor	Sample Description	Injection Volume (µL)	IS Amounts	Details	AxION SOLO
٠	1	Sample	-	DemoBlank	37 (2 mL)	-	DemoMethod					1	1		5.0			
٠	2	Sample	-	Demo1	9 (2 mL)	Ŧ	DemoMethod					3	1		5.0			
٠	3	Sample	-	Demo2	10 (2 mL)	-	DemoMethod					3	1		5.0			
	ſ		-												1			

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

Method

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

			1	Name		C	Description	Group		Author	Edit	or S	ample Tray Type	Activate Injection Info				
. [Dem	noSequen	e					leslie 🗸	TOF		TOF	10	0-Position Tra 👻					
		Sample Type		Sample Name	Vial			Method		Stand	dard	Injections	Dilution Factor	Sample Description	Injection Volume (μL)	IS Amounts	Details	AxION SOLO
÷.	1	Sample	-	DemoBlank	37 (2 mL)	-	DemoMethod]		1	1		5.0			
	2	Sample	-	Demo1	9 (2 mL)	Ŧ	DemoMethod					3	1		5.0			
÷-	3	Sample	•	Demo2	10 (2 mL)	•	DemoMethod]		3	1		5.0			
	Γ		•]								

- 5. Select the **Method** for this sequence by clicking the button **Important Provide International Security** in the **Method** field.
- 6. The **Data Selector Single Method** dialog displays. Click the plus sign **→** to expand the appropriate **Method Group**

-		cor - Single Method	_		Show Searc	<u>ם –</u> א <u>(1</u>)
Ор	en 📜	Organize • Actions •				Delete 🗙
-l M	lethod Gro	up : leslie (10 items)	1	1	1	
ME	lethod Gro Select	up : leslie (10 items) Method Name	Created Date/Time ▼	Last Edited Date/Time	Author	Editor
M L			Created Date/Time ▼ 9/10/2013 3:45:03 PM	Last Edited Date/Time 9/10/2013 4:07:28 PM	Author TOF	Editor
M L	Select	Method Name	9/10/2013 3:45:03 PM			

- 7. Click in the **Select** box to select the method. This example shows **DemoMethod** is selected.
- 8. Click **Open** to insert this method in the sequence.
- 9. Click the plus sign + to display additional run parameters.

Den	Name Description Group							Edi	itor	Sample Tray Type	Activate Injection Info				_
	DemoSequence				leslie	▼ TOF		TOF		100-Position Tra	- 🔽				
ſ	Sample Type	Sample Name	Vial		Method		Stan	dard	Injection	s Dilution Factor	Sample Description	Injection Volume (μL)	IS Amounts	Details	AxION SOLO
	1 Sample -	DemoBlank	37 (2 mL)	- DemoMethod]		1	1		5.0			
1	Desision	Deliet Turce	Desision Med	e leest	Nama Tarra	Time	Cile Turce	1	Outrue To	nat Davies	Accession				
	1	-		-		-		-			Г				

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

- 10. Additional runs added to the Sequence will inherit the values of the run before it.
- 11. Select Save Sequence As... from the File menu.

Ch	romera - leslie (TOF)	
File	View Tools Display	Actions Help
	New Sequence	1 💿 📋 🎬 🖄 📦
	Import Sample List	1
71	Open Sequence	
	Save Sequence	
6	Save Sequence As	
8	Print Preview Sequence	
(1)	Print Sequence	Save Sequence As
<u>51</u>	Exit	

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



⇔

13. Click **OK**.

Starting Data Acquisition

Preparing for an Analysis

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

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

Equilibrate the System

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

To equilibrate the system:

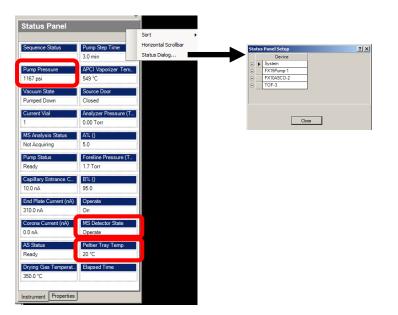
1. In Chromera click **Run Time** then click **Manual Control** for the **Control Mode**.

File View Tools Display Help	
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	leslie
Run Time	PumpPressureData: 0 : : 0
Control Mode	
Manual Control	-
○ Single Run ○ Sequence	
- Sequence	160) 40
	a. 20-1 2 -
Plots	
⊡- Plot 1	0
	Time (min)
Reference Plots	Manual Control
Manual Control Devices	2
Manual Control Devices	
	Monitor Baseline Method Method Name
	Start Browse for method None
	Pump Settings Flow (mL/min) %A () %B ()
	Apply 1.000 5.0 95.0
	Purge Pump Flow (mL/min) 100% A () 100% B ()
	Apply 1.000
	Flush Autosampler Flush Volume (μL) Number of Flush Cycles
	Арру
	Peltier Tray Temperature (*C) Tolerance (+/- *C) Apply 20 2.0
	Vent MS Apply
🕥 Run Time	Standby
Method	Apply
	Operate Method file name Browse
Sequence	Apply DemoMethod Browse for metho
Post Run	
Reprocess	
Reports	
»	

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

Select Operate Method			×
Computer - Local	sk (C:) • MSData • Method • 🛃	Search Method	2
Organize 👻 New folder		III 🔹 🚺	•
★ Favorites	Name Date modified - Typ	pe Size	Ŀ
🛄 Desktop	DemoMethod.tofmethod2 9/10/2013 4:35 PM TO	F MS Driver Docu 12 KB	
bownloads	Caffeine Analysis.tofmethod2 9/10/2013 11:08 AM TO	F MS Driver Docu 4 KB	
🔛 Recent Places	090313-c60.tofmethod2 9/5/2013 5:25 PM TO	F MS Driver Docu 7 KB	
📜 Libraries	II test 144. tofmethod 2 9/4/2013 3:49 PM TO	F MS Driver Docu 6 KB	
Documents	CalOFF.tofmethod2 8/27/2013 10:31 AM TO	F MS Driver Docu 5 KB	
J Music	EICTest_2timeperiod.tofmethod2 8/27/2013 10:26 AM TO	F MS Driver Docu 12 KB	
Pictures	EICTest_4timeperiod.tofmethod2 8/27/2013 9:35 AM TO	F MS Driver Docu 12 KB	
Videos	EICTest_3timeperiod.tofmethod2 8/27/2013 9:31 AM TO	F MS Driver Docu 11 KB	
Computer	EICTest_1timeperiod.tofmethod2 8/26/2013 5:37 PM TO	F MS Driver Docu 11 KB	
Local Disk (C:)	15minute_SoloTest.tofmethod2 8/23/2013 2:40 PM TO	F MS Driver Docu 11 KB	
	© 082213-benzos_plasma-80.tofmethod2 8/22/2013 7:35 PM TO	F MS Driver Docu 14 KB	
	082213-benzos_plasma-30.tofmethod2 8/22/2013 5:28 PM TO	F MS Driver Docu 14 KB	-
File name:	moMethod.tofmethod2	method files (*.tofmethod2)	•
		Open 👻 Cancel	

- 3. Select the method (in this example **DemoMethod.tofmethod2**) and click **Open**. The selected method displays in the **Method file name** field.
- 4. In the **Operate** row click **Apply**. The settings for the initial time period of the TOF method will be loaded.
- **NOTE:** The mass accuracy of a TOF MS (running <u>without</u> lockmass) is very much dependent on the thermal stability (i.e., temperature equilibrium) of the system. If ion polarity has not been switched, the TOF should be allowed to pulse over the desired mass range for at least 30 minutes prior to calibration (the best results are obtained with 60-120 minute equilibration time). After calibration, the analyses to be performed should be run as soon as possible to avoid "cooling off" of the flight tube and electronics. If the samples cannot be run immediately after a calibration, the TOF should be left pulsing to keep it thermally stable. If the analyzer's polarity is switched, the instrument should pulse for 2 hours before running a calibration.
 - 5. Make sure the chromatographic tubing is connected between the LC system and the AxION 2 TOF MS detector.
 - 6. Enter **Pump Settings** and click **Apply** to start the pump. In this example, enter a 1.0 mL Flow, 5% A, and 95% B.
 - Set the autosampler **Peltier Tray** temperature, if applicable. Enter the target temperature and tolerance and click **Apply** to ramp to target.
 In this example, enter 20°C Target with a ± 2.0 °C Tolerance.
 - 8. Monitor the parameters in the **Status Panel**. Right clicking on the Status Panel Header will bring up a menu. Status Dialog can be selected and additional parameters added or removed from the panel.



Running a Sequence

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

To run a sequence:

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



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

Ŀ	Ch	romera -	leslie (1	TOF)						
	File	View	Tools	Display	Action	5	Help	D		
		New Seq	uence		1 0	8	\odot	ŧ9	101	3
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	1 11	Save Sec	quence	Open	Sequenc	_				
		Import S	ample List		Sequenc					
Ľ	i	Save Me	thod							
		Print Pre	view	•						
		Print		•						
	<u>5</u>	Exit								
	s pl	ots			-					

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

		2 1				Show Search 🔃
pen	1	Organize • Action	15 *			Delete 🗙
Beque	ence G	roup : leslie (9 items)				
Se	lect	Sequence Name	Author	Created Date/Time 🗸	Editor	Last Edited Date/Time
	•	DemoSequence	TOF	9/10/2013 4:08:42 PM		9/10/2013 4:08:42 PM
		EICTimePeriodTest-3and4	TOF	8/27/2013 9:29:43 AM	TOF	8/27/2013 9:30:33 AM
		EICTimePeriodTest-only2	TOF	8/27/2013 9:00:42 AM	TOF	8/27/2013 10:25:37 AM
		EICTimePeriodTest	TOF	8/26/2013 5:26:31 PM	TOF	8/26/2013 5:36:39 PM
		DBvsFile	TOF	8/19/2013 4:04:05 PM		8/19/2013 4:04:05 PM
		SWTest-Spectra	TOF	8/19/2013 3:46:26 PM		8/19/2013 3:46:26 PM
		15minSolo-3	TOF	8/19/2013 1:33:46 PM	TOF	8/23/2013 1:39:24 PM
		15minSolo-2	TOF	8/19/2013 11:20:09 A		8/19/2013 11:20:09 AM
	Π	15minSolo	TOF	8/19/2013 10:04:23 A		8/19/2013 10:04:23 AM

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

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

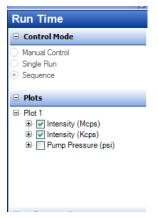
The sequence displays and is ready to run, indicated by the green **Start** button.

File View Tools Display Actions Help																	
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Run Time		sile															
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ve sequence	Pressun	30 - 20 -															
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Reference Plots		Time (min)									-						
Method		- yun		DemoSequ													
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			Name	Description		Grou	ip AL	uthor	Editor	r Sample	Tray Ty	/pe A	ctivate stion Info				000
			DemoSequence	1	leslie		- TOF		TOF	100-Pos	ition Tra						Coting Looks
			Sample Type	Sample Name	Vi	al	Method		Standard	Injection	s D F	lilution actor	Sample Description	Injection Volume (µL)	IS Amounts	Details	
			1 Sample 👻	DemoBlank	1 (2 mL	.) -	DemoMethod			1	1			5			
			2 Sample 💌	Demo1	2 (2 mL)	 DemoMethod 			3	1			5			
			3 Sample 💌	Demo2	3 (2 mL) -	 DemoMethod 			3	1			5			
🛞 Run Time			·														T
Method								Sample F									
Sequence			Sample Type	Frequenc		R	leport Template	Outp	ut Target	File Type	_		Output Name				
	_	-		Report per Samp	_				*								
Not Run					Sam	ple Na	ming Template										
Reprocess			Prefix	Nu	mber		Suffix	Vial Start	Vial Increment	Apply To							
C Reports									1	-							
	»																
																	•

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

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

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



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

Running a Chromera Internal Calibration

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

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

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

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

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

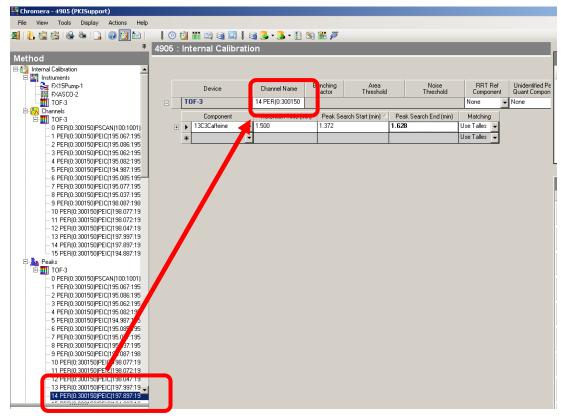
1. Start by creating a Chromera method.

In this example, the method is named **Internal Standard** and the Group is named **Calibration**.

👆 Chromera - 4905 (PKISupport)	
File View Tools Display Actions Help	
🗐 📙 🏥 🔮 🤮 🛸 🛄 🗐 🛅 🗎 👘	i 🕐 👜 🛗 🖄 🥃 🖾 i 🧕 s 🥵 s 🏥 🖄 🕷 🐖
	Internal Calibration
Method	
Instrume Calibration Image: Calibration → FrASDPump1 → France → TOF 3 → TOF 3 → TOF 3 → TOF 3 → TEFRIQ 300150PE(1155, 002150) → TEFRIQ 300150PE(1155, 002150) → TEFRIQ 300150PE(1155, 002150)	Method Name Internal Calibration Group Calibration Calibration
5 PER(0.300150)PEIC(134.987:195	Notes
9 PER(0:300150)PEIC(198.087:198	
- 12 PER(0:300150)PEIC(198.047:19	
13 PER(0:300150)PEIC(197.997:19	
14 PER(0:300150)PEIC(197.897:19	
🖻 🚣 Peaks	
⊡	
0 PEB/0 300150(PSCAN(100-1001)	

2. In the **Peaks** tree select a channel for your internal standard.

In this example, we selected the channel **14 PER(0:300150)PEIC(197:897...** and appears in the **Channel Name** field.



- 3. Type a **Component** name. In this example, **13C3Caffeine**.
- Click the plus sign to the left of Caffeine to display additional fields. The **Type** and **Reference Peak** fields display.

4905	5:	Internal Calibratio	on								
		Device	Channel Name		unching Factor	Area Threshold		Noise Threshold	BBT B Compon		Unidentified Pe Quant Compon
=		TOF-3	15 PER(0:300150						None	-	None
		Component	Retention Time (m	in)	Peak Se	arch Start (min) 🛆	Pea	ak Search End (min)	Matching		
	-	Caffeine 👻	1.500		1.372		1.628	3	Use Talles	-	
		Тур	e		eference	Peak					
		2	-			•					
		Uses an Internal Sta Uses a Retention Ti		in)	Peak Se	arch Start (min) 🛆	Pea	ak Search End (min)	Matching		
		*							Use Talles	-	

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

490	5:	Internal Calibrati	on						
		Device	Channel Name	Bunchi Facto			Noise Threshold	RRT Ref Component	Unidentified Pe Quant Compon
-		TOF-3	15 PER(0:300150					None	 None
		Component	Retention Time (m	nin) Pea	ak Search Start (min) 🔺	Peal	< Search End (min)	Matching	
	-	Caffeine 🗣	1.500	1.37	2	1.628		Use Talles 🖵	
		Typ	De	Refer	rence Peak				
		🔰 Uses an Internal St	andard 🗨		-				
	1303	3Caffeine (TOF-3/14 PER(I	D:300150)PEIC(197.89	97:198.297	7),0.2,100,)				
		Component	Retention Time (m	nin) Pea	ak Search Start (min) 🛆	Peal	< Search End (min)	Matching	
		*	•					Use Talles 🖵	

7. In the **Calibration** tree select a channel for your calibration data.

In this example, we selected the channel **14 PER(0:300150)PEIC(197:897...**

.	Interna	ıl Calib	ration						
Method	Summary	Detail	Set Up Sta	ndards					
- 1 PER(0:300150)PEIC(195.067:195 -									
- 2 PER(0:300150)PEIC(195.086:195									
- 3 PER(0:300150)PEIC(195.062:195									
- 4 PER(0:300150)PEIC(195.082:195	Dev	rice	C	nannel Name	Outlier Limit (%)				
- 5 PER(0:300150)PEIC(194.987:195	TOF-3		14 PI	ER(0:300150)P	15.0				
6 PER(0:300150)PEIC(195.085:195 7 PER(0:300150)PEIC(195.077:195								1	
- 7 PER(0:300150)PEIC(195.077:195 - 8 PER(0:300150)PEIC(195.037:195		nponent		pration Type	Origin Treatment	Quantify Using	Scaling Factor	Weighting Factor	R-Squar
- 9 PER(0:300150)PEIC(198.037:198	13C3Ca	affeine	👻 Linea		Ignore 🔹	Use Area	None		*
- 10 PER(0:300150)PEIC(198.077:19	*		•			· ·	·	-	+
- 11 PER(0:300150)PEIC(198.072:19			_						_
- 12 PER(0:300150)PEIC(198.047:19									
- 13 PER(0:300150)PEIC(197.997:19									
- 14 PER(0:300150)PEIC(197.897:19									
- 15 PER(0:300150)PEIC(194.887:19									
🕀 🏒 Calibration	1								
🗄 🎹 TOF-3	1								
0 PER(0:300150)PSCAN(100:1001)									
- 1 PER(0:300150)PEIC(195.067:195									
- 2 PER(0:300150)PEIC(195.086:195									
- 3 PER(0:300150)PEIC(195.062:195									
- 4 PER(0:300150)PEIC(195.082:195	1								
- 5 PER(0:300150)PEIC(194.987:195									
- 6 PER(0:300150)PEIC(195.085:195									
- 7 PER(0:300150)PEIC(195.077:195									
- 8 PER(0:300150)PEIC(195.037:195									
- 9 PER(0:300150)PEIC(198.087:198									
- 10 PER(0:300150)PEIC(198.077:19									
- 11 PER(0:300150)PEIC(198.072:19									
- 12 PER(0:300150)PEIC(198.047:19									
-14 PER(0:300150)PEIC(197.897.19									
- 15 PER(0:300150)PEIC(194.887:19									

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

ummary Detail Set	Up Standards	
Device	ChannenName	To Add a Standard
TOF-3	0 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
TOF-3	1 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
TOF-3	2 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
TOF-3	3 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
TOF-3	4 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
TOF-3	5 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
TOF-3	6 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
TOF-3	7 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
TOF-3	8 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
TOF-3	9 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
TOF-3	10 PER(0:30015	To create a new standard column, right-click and select "Add a Standard"
TOF-3	11 PER(0:30015	To create a new standard column, right-click and select "Add a Standard"
TOF-3	12 PER(0:30015	To create a new standard column, right-click and select "Add a Standard"
TOF-3	13 PER(0:30015	To create a new standard column, right-click and select "Add a Standard"
TOF-3	14 PER(0:30015	To create a new standard column, right-click and select "Add a Standard"
Component	∇ Units	Calibration Type Origin Treatment
13C3Caffeine		▼ Linear ▼ Ignore ▼
 Device	Channel Name	To Add a Standard

- 1.3 PEH(0:30015... To create a new standard column, right-click and select. Add a standard 14 PER(0:30015... To create a new standard column, right-click and select. "Add a Standard" TOF-3 TOF-3 Component

 I3C3Caffeine
 Units Calibration Type Origin Treatment - Ignore ppt Device To Add a Standard TOF-3 ew standard column, right-click and select "Add a Standard" opb te a pg/mL Component Cali ration Type Origin Treatment ng/mL ng/L Caffeine Linea ▼ Ignore • ng/g ng/dl
- 9. Click in the Units field and select ppm from the drop-down list.

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

	nary Detail Set U	p standards	1					
	Device	Device	Channel Name		Mass		erLimit	To Add a Standar
۲		QMS	0 PER(0:300150			15		To create a new standard column, right-click and se
	TOF-3	QMS	1 PER(0:300150	0		15		To create a new standard column, right-click and s
	TOF-3	QMS	2 PER(0:300150	0		15		To create a new standard column, right-click and st
	TOF-3	QMS	3 PER(0:300150	0		15		To create a new standard column, right-click and se
	TOF-3	QMS	4 PER(0:300150	0		15		To create a new standard column, right-click and s
	TOF-3	QMS	5 PER(0:300150	0		15		To create a new standard column, right-click and se
	TOF-3	QMS	6 PER(0:300150	0		15		To create a new standard column, right-click and se
	TOF-3	QMS	7 PER(0:300150	0		15		To create a new standard column, right-click and se
	TOF-3	QMS	8 PER(0:300150	0		15		To create a new standard column, right-click and se
	TOF-3	QMS	9 PER(0:300150	0		15		To create a new standard column, right-click and se
	TOF-3	QMS	10 PER(0:30015	0		15		To create a new standard column, right-click and se
	TOF-3	QMS	11 PER(0:30015	0		15		To create a new standard column, right-click and se
	TOF-3	QMS	12 PER(0:30015	0		15		To create a new standard column, right-click and se
	TOF-3	QMS	13 PER(0:30015	0		15		To create a new standard column, right-click and se
	TOF-3	QMS	14 PER(0:30015	0		15		To create a new standard column, right-click and se
	Component	0.2	Unit		Calibratio	on Type	Origin	nTreatment
	13C3Caffeine			-	Linear	-	Ignore	-
	Device		anannel Name		Mass	Outli	erLimit	To Add a Standar
	TOF-3	QMS	15 PER(0:30015	0		15		To create a new standard column, right-click and s
	Component	0.2	Unit		Calibratio	n Type	Origin	nTreatment
	Caffeine			-	Linear		Ignore	

11. Type a standard name.

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

		Up Standards		1		1		
	Device	Device	Channel Name	Mass	OutlierLimit			To Add a Standard
►	TOF-3	QMS	0 PER(0:300150		15			mn, right-click and sel
	TOF-3	QMS	1 PER(0:300150		15			imn, right-click and sel
	TOF-3	QMS	2 PER(0:300150	0	15	To create a	a new standard colu	imn, right-click and se
	TOF-3	QMS	3 PER(0:300150	0	15	To create a	a new standard colu	imn, right-click and se
	TOF-3	QMS	4 PER(0:300150	0	15	To create a	a new standard colu	imn, right-click and se
	TOF-3	QMS	5 PER(0:300150	0	15	To create a	a new standard colu	imn, right-click and sel
	TOF-3	QMS	6 PER(0:300150	0	15	To create a	a new standard colu	imn, right-click and se
	TOF-3	QMS	7 PER(0:300150	0	15	To create a	a new standard colu	imn, right-click and se
	TOF-3	QMS	8 PER(0:300150	0	15	To create a	a new standard colu	imn, right-click and se
	TOF-3	QMS	9 PER(0:300150	0	15	To create a	a new standard colu	mn, right-click and se
	TOF-3	QMS	10 PER(0:30015	0	15	To create a	a new standard colu	imn, right-click and se
	TOF-3	QMS	11 PER(0:30015	0	15	To create a	a new standard colu	imn, right-click and se
	TOF-3	QMS	12 PER(0:30015	0	15	To create a	a new standard colu	imn, right-click and se
	TOF-3	QMS	13 PER(0:30015	0	15	To create a	a new standard colu	imn, right-click and se
	TOF-3	OMS	14 PEB(0:30015		15	To create a	a new standard colu	mn_right-click_and_se
	Component	0.2	.5	1.0	5.0	10	Unit	Calibration Ty
	13C3Caffeine							🖵 Linear
	Davias	Device	Channel Marrie	Marca	Outlined insit			To dd a Standard
•	TOF-3	QMS	15 PER(0:30015	0	15	To create a	a new standard colu	mn, right-click and se
	Component	0.2	.5	1.0	5.0	10	Unit	Calibration Ty
	Caffeine							✓ Linear

Sum	mary Detail Set l	Jp Standards						
	Device 🗠	Device	Channel Name	Mass	OutlierLimit			To Add a Standard
	TOF-3	QMS	0 PER(0:300150) 0	15	To create	a new standard colu	mn, right-click and se
	TOF-3	QMS	1 PER(0:300150) 0	15	To create	a new standard colu	mn, right-click and se
	TOF-3	QMS	2 PER(0:300150) 0	15	To create	a new standard colu	mn, right-click and se
	TOF-3	QMS	3 PER(0:300150) 0	15	To create	a new standard colu	mn, right-click and se
	TOF-3	QMS	4 PER(0:300150) 0	15	To create	a new standard colu	mn, right-click and se
	TOF-3	QMS	5 PER(0:300150) 0	15	To create	a new standard colu	mn, right-click and se
	TOF-3	QMS	6 PER(0:300150) 0	15	To create	a new standard colur	mn, right-click and se
	TOF-3	QMS	7 PER(0:300150) 0	15	To create	a new standard colur	mn, right-click and se
	TOF-3	QMS	8 PER(0:300150) 0	15	To create	a new standard colu	mn, right-click and se
	TOF-3	QMS	9 PER(0:300150) 0	15	To create	a new standard colu	mn, right-click and se
	TOF-3	QMS	10 PER(0:30015	i 0	15	To create	a new standard colur	mn, right-click and se
	TOF-3	QMS	11 PER(0:30015	i O	15	To create	a new standard colu	mn, right-click and se
	TOF-3	QMS	12 PER(0:30015	5 O	15	To create	a new standard colu	mn, right-click and se
	TOF-3	QMS	13 PER(0:30015	i O	15	To create	a new standard colu	mn, right-click and se
•		0.110	11 FER(0.00010		10	70 0.000	a non standard oola.	right-click and se
	Component	0.2	.5	1.0	5.0	10	Unit	Calibration Ty
	13C3Caffeine	2	2	2	2 2.	000000	ppm	Linear
	Device 🗠	Device	Channel Name	Mass	OutlierLimit			To Add a Standard
•	TOF-3	QMS	15 PER(0:30015	5 0	15	To create	a new standard colu	mn, right-click and se
	Component	0.2	.5	1.0	5.0	10	Unit	Calibration Ty
	Caffeine							↓ Linear

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

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

	Device 🛆	Device	Channel Nan	ne Mass	s OutlierLimi	t l		To Add a Standar
-		QMS	0 PER(0:3001		15		a new standard colur	
÷	TOF-3	QMS	1 PER(0:3001	50 0	15	To create	a new standard colur	nn, right-click and se
	TOF-3	QMS	2 PER(0:3001	50 0	15	To create	a new standard colur	nn, right-click and se
	TOF-3	QMS	3 PER(0:3001	50 0	15	To create	a new standard colur	nn, right-click and se
	TOF-3	QMS	4 PER(0:3001	50 0	15	To create	a new standard colur	nn, right-click and se
	TOF-3	QMS	5 PER(0:3001	50 0	15	To create	a new standard colur	nn, right-click and se
	TOF-3	QMS	6 PER(0:3001	50 0	15	To create	a new standard colur	nn, right-click and se
	TOF-3	QMS	7 PER(0:3001	50 0	15	To create	a new standard colur	nn, right-click and se
	TOF-3	QMS	8 PER(0:3001	50 0	15	To create	a new standard colur	nn, right-click and se
	TOF-3	QMS	9 PER(0:3001	50 0	15	To create	a new standard colur	nn, right-click and se
	TOF-3	QMS	10 PER(0:300	15 0	15	To create	a new standard colur	nn, right-click and se
	TOF-3	QMS	11 PER(0:300	15 0	15	To create	a new standard colur	nn, right-click and se
	TOF-3	QMS	12 PER(0:300	15 0	15	To create	a new standard colur	nn, right-click and se
	TOF-3	QMS	13 PER(0:300	15 0	15	To create	a new standard colur	nn, right-click and se
]	TOF-3	QMS	14 PER(0:300	15 0	15	To create	a new standard colur	nn, right-click and se
	Component	0.2	.5	1.0	5.0	10	Unit	Calibration Ty
	13C3Caffeine	2	2	2	2	2.000000	ppm	
	Device 🛆	Device	Channel Nan	ne Mass	s OutlierLimi	t		To Add a Standar
1	105.0	que	15 DED(0.000	15.0	15			ht-click and se
	Component	0.2	.5	1.0	5.0	10	Unit	Calibration Ty
	Caffeine	0.2	0.5	1	5	10.000000	ppm	↓ L near

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

omera -	4905 (KISuppor	t)						
View	Tools	Display	Actions						
New Method									
Create/Edit MS Method/Tune									
Open Method									
Save Method									
Save Method As									
Extract I	Method fr	om Results							
	View New Met Create/E Open Me Save Me Save Me	View Tools New Method Create/Edit MS M Open Method Save Method Save Method As	New Method Create/Edit MS Method/Tune Open Method Save Method						

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

File View Tools Display Actions He		
	🛓 🛃 + 🛃 + 🖹 🖎 🗰 💭	
🐖 Exit	Internal Calibration	
Reprocess	Reprocess	
	Blank Peak Detection Peak ID Calibration Quantitation	Reporting
	A	
	Process these Channels:	
	Make new batch – copy all samples	
	Sequence Results	1
	Name Description Group Author Editor	<u> </u>
	InternalCalibOct1 4905 VKJSupport PKJSupport	

The Data Selector displays.

16. Select the batch that you want to analyze.

In this example we selected **Internal Calibration**.

3		2					Show Search 🔍		
Op	ien 길	Organize 🔹	Actions *		Delete 🗙				
	Select	Batch Name	Batch Group	Batch Description	Created Date/Time	•	Reprocessed By	Repro	
3- - -		102011_Test	4905	Missing_data_pts	10/20/2011 11:48 AM				
3		10_18_Exceptio_	4905	LMTest	10/19/2011 11:04 AM				
		10_18_Exceptio	4905	LMTest	10/19/2011 11:01 AM				
3		10_18_Exceptio	4905	LMTest	10/18/2011 11:35 AM				
-		101211_LM	4905	LMTest	10/12/2011 3:58 PM				
-		TOTETT_EM	4303	EMILOS	10/12/2011 0.001 M	1			
3		InternalCalibOct	4905		10/12/2011 10:50 AM		PKISupport	10/20/2	
-		A 1 B 11	<u>^</u>	A	10/11/0011 10:00 414		TOP	10/11/0	
- E		092911_16hr	4905	4905	10/5/2011 9:31 AM				
3		092911_16hr	4905	4905	10/5/2011 9:06 AM				
3-		092911_16hr	4905	4905	10/5/2011 9:02 AM				
]		092911_16hr	4905	4905	10/5/2011 7:59 AM				
3	Π	092911_16hr	4905	4905	10/5/2011 7:52 AM				

17. Click the **Open** button.

The **Batch Copy Selection** box appears requesting if you want to create a new batch.

Batch Copy Selection
A new batch must be created when changes are made to the batch or sample information.
Do you want to keep the current settings and create a new batch or revert to the original batch?
Create New Batch Revert to Original

- In this example we clicked Create a New Batch. The new batch displays.
- 19. Click in **Sample Type** field and select **Calib. Replace**. You are replacing to reset your calibration curve.

	InternalCalibOct12NoUV (Idle) Reprocess													
	В	ank		Peak Detect	tion	Peak ID Calibra			ation	Quanti	tation	Reporting		
	4	<u>}</u>]		0 0]		0	Y		
	Process these Channels:													
		🕨 🔲 🚳 🔯 Use Latest Stored Version of Method 🛛 🝷 Make new batch – copy all samples										•		
Sequence Results													1	
			Name	Descript	ion	Group		Author		Editor			-	
	= <u>.</u>	Inte	malCalibOct			4905		✓ PKISupport		PKISupport				
		R	eprocess	Sample Type		Sample Name	Method		Standard		Injections	Dilution Factor		
		1		Sample 🗸	Blank	(1	1.00		
	+	2	V	oampio	- "e	ine0.2ppma					1	1.00		
	+	3	V	Calib: Replace Calib: Average	Caff	ine0.2ppmb					1	1.00		
	+ 4 🔽		V	Sample Matrix	Caff	ine0.5ppma					1	1.00		
	+			fine0.5ppmb					1	1.00				
	+	6	V	Wash	Caff	ine1ppma					1	1.00		
	+	7	•	Sampic	carte	ine1ppmb					1	1.00		

20. Select Calib. Ave.

One run is a replace and the other run is an average.

InternalCalibOct12NoUV (Idle) Reprocess													
	lank		Peak Dete	ection	Peak	ID	Calibra	tion	Quantit	ation	Reporting		
	A	_		_]]	Y						
Proc	Process these Channels:												
Make new batch – copy all samples													
Sequence Results Name Description Group Author Editor													
		Name malCalibOct1		iption	Grou 4905	ιp Ψ	Author PKISupport		Editor PKISupport	-			
	R	eprocess	Sample Type		Sample Name		lethod		Standard	Injections	Dilution Factor		
±	1		Sample	👻 Blank	llank					1	1.00		
+	2	V	Calib: Rep		ine0.2ppma				•	1			
+	3		Sample		ine0.2ppmb					1	1.00		
÷.	4		Calib: Replac Calib: Averag	e Cale	ine0.5ppma					1	1.00		
ŧ.	Somela				ine0.5ppmb					1	1.00		
				Cafe	ine1ppma				1		1.00		
										1	1.00		
	+ 8 🔽 Sample 🗸 Caffeine5ppma									1	1.00		
	9	~	Sample		ine5ppmb					1	1.00	-	

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

Interna Reprocess		libNoU∖	oct.12b									
В	lank		Peak Dete	ectio	on Peak	ID Ca	librati	on Quar	ntitation	Reporting		
	\wedge	_]		0		-0-		0	Y		
Process these Channels: 0 PER(0:300150)PSCAN(100:1001),,100,, 1 PER(0:300150)PEIC 💌												
Image: Stored Version of Method Make new batch – copy all samples												
Sequend	e Re	esults										
	Re	eprocess	Sample Type		Sample Name	Method		Standard	Injections	Dilution Factor		
	5	~	Calib: Ave	-	Caffeine0.5ppmb	Internal Calibratio	Fill Selected					
+	6	◄	Calib: Rep	•	Caffeine1ppma	InternalCalibNoU	Fill Do		1			
.	7	7	Calib: Ave	•	Caffeine1ppmb	InternalCalibNoU	Fill All					
+	8	•	Calib: Rep	•	Caffeine5ppma	InternalCalibNoU	Card \	/iew				
+	9	◄	Calib: Ave	•	Caffeine5ppmb	InternalCalibNoU	Select	Columns	1			
+	÷ 10 🔽		Calib: Rep	•	Caffeine10ppma	InternalCalibNoU E		id All	1			
+	÷ 11 🔽		Calib: Ave	e 🖵 Caffeine10ppmb		InternalCalibNoL Co		Collapse All				
+	12		Calib: Rep	-	Caffeine20ppma	InternalCalibNoU						

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

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

lt	InternalCalibNoUVoct.12b													
F	Reproces	s												
	E	Blank	: 1	Peak Detec	tion	Peak	ID	Calibra	ation	Quanti	tation	g		
		A			_	0]		0	Y		
		4												
	Proc	cess th	iese Channels	s: O PE	R(0:300)150)PSCAN(1	00:1001),,;	100,, 1 PER(0):30015	50)PEIC 💌				
			🕰 🦻 I u	lse Latest Store	d Versi	on of Method	- P	4ake new batcl	h – cop	y all samples		•		
	Sequen	Sequence Results												
			Name	Descrip	tion	Gro	чр	Autho	r	Editor			-	
		Inte	rnalCalibOct1			4905	-	PKISupport		PKISupport				
		R	eprocess	Sample Type	Sample Sample Type Name		Þ	Method		Standard	Injections	Dilution Factor		
		1		Sample _	Blan	<	Internal (Calibratio			1	1.00		
	.	2	~	Calib: Rep	Caffe	eine0.2ppma	Internal Calibratio			•	1			
	÷	3	V	Calib: Ave	Caffe	eine0.2ppmb	Internal Calibratio		0.2 .5		1			
	٠	4	2	Calib: Rep	Caffe	Caffeine0.5ppma		Calibratio	1.0 5.0		1			
	÷	5	•	Calib: Ave		eine0.5ppmb	Internal Calibratio		10		1		_	
	÷.	6	V	Calib: Rep 🗖		eine1ppma	Internal Calibratio			•			_	
		7	~	Calib: Ave		eine1ppmb	Internal Calibratio			•			_	
		8	•	Calib: Rep 🖣		eine5ppma		Calibratio		-				
	÷.	9	2	Calib: Ave	-	eine5ppmb		Calibratio		•			_	
		10	•	Calib: Rep		eine10ppma	Internal (•			_	
		11	V	Calib: Ave	_	eine10ppmb		Calibratio		-		1.00	_	
		12		Sample -	-	eine20ppma		Calibratio	<u> </u>		1	1.00	_	
	±.	13		Sample -		eine20ppmb		Calibratio			1	1.00	_	
	÷	14		Sample -	Unkr			Calibratio	1		1	1.00	_	
		15		Sample -	_		Internal (-		1	1.00	-	
		16	<u> </u>	Sample Calib: Ave		ine0.2ppmc	Internal (_		1.00	_	
1		17	<u>र</u>	Calib: Ave	_	ine0.5ppmc		Calibratio	<u> </u>				_	
		18	<u>v</u>	Calib: Ave	-	inel.oppmc			<u> </u>	•			_	
	÷	19	V	CallD. Ave		ane rppinc	Internal Calibratio			•	<u> '</u>			

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

process	:											
в	lank		Peak Dete	ection	Peak	ID	Calibra	tion	Quantit	Reporting		
	A									1		
4												
Process these Channels: 0 PER(0.300150/PSCAN(100:1001),.100,, 1 PER(0.300150/PEIC 💌												
Make new batch - copy all samples												
Sequence Re uts												
ame Description Group Author Editor												
-	Inter	nalCalibOct	1		4905	-	PKISupport		PKISupport			
	Reprocess		Sample Type			м	ethod		Standard	Injections	Dilution Factor	
	1		Sample	🛨 Blank		Internal C	Internal Calibratio			1	1.00	
<u>ب</u>	2	7	Calib: Rep	y	пео.2ррта	Internar calibratio				1		
+	3	V	Calib: Ave	Caffe	ine0.2ppmb	Internal C	alibratio	0.2				
	4	V	Calib: Rep		ine0.5ppma	Internal C	alibratio	.5 💌		1		
+	5	V	Calib: Ave		ine0.5ppmb	Internal C	alibratio	.5	•	1		
	6	7	Calib: Rep		ine1ppma	Internal C	alibratio	1.0	•	1		
+	7	V	Calib: Ave	👻 Caffe	ine1ppmb	Internal C	alibratio	1.0	•	1		
+	8	V	Calib: Rep		ine5ppma	Internal C	alibratio	5.0		1		
	9	7	Calib: Ave		ine5ppmb	Internal C	alibratio	5.0	•	1		
÷	10	•	Calib: Rep		ine10ppma	Internal C	alibratio	10	•	1		
	11	V	Calib: Ave	- Caffe	ine10ppmb	Internal C	alibratio	10	•	1		
+	12	Г	Sample	Caffe	ine20ppma	Internal C	alibratio				1.00	-

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

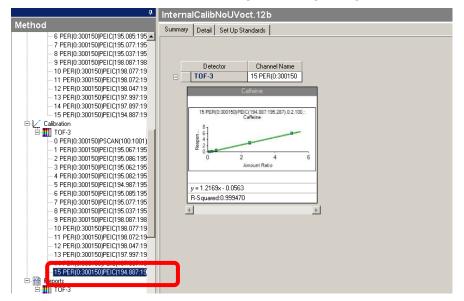
B	lank		Peak Dete	ction	Peak ID Cal			bration Quantita		ation	orting				
0				cuon	i car		Galibi		Quar						
	\wedge		U		U	_					J		1		
, Dut	ess the	se Channe	ls: DP	EB(0:30	0150)PSCAN(1	00.1001) 1	00 1 PEBI	D:3001	50)PEIC				_		
			,									-			
_			Use Latest Sto	ed versi	on or Method		lake new bati		oy all samples						
enc	e Re	sults ame	Descr		Gro		Auth	-	Editor					•	al
		ane nalCalibOct		ipuon	4905		PKISupport			_				Ē	
Γ	L		_					1		-		Dilution		-	
	Re	process	Sample Type		Sample Name	M	tethod		Standard		Injections	Factor		_	
•	1		Sample	➡ Blar	k	Internal C	Calibratio]		[1	1.00			
•	2	2	Calib: Rep	▼ Caff	eine0.2ppma	Internal C	Calibratio	0.2		•	1				
•	3	~	Calib: Ave		eine0.2ppmb	Internal C	Calibratio	0.2		Ŧ	1				
•	4		Calib: Rep	🚽 Caff	eine0.5ppma	Internal C	Calibratio	.5		•	1				K —
•	5	K	Calib: Ave		eine0.5ppmb	Internal C	Calibratio	.5		•	1				
•	6	>	Calib: Rep	▼ Caff	eine1ppma	Internal C	Calibratio	1.0		Ŧ	1				
•	7	7	Calib: Ave	▼ Caff	eine1ppmb	Internal C	Calibratio	1.0		•	1				
•	8	V	Calib: Rep	✓ Caff	eine5ppma	Internal C	Calibratio	5.0		Ŧ	1				
•	9	2	Calib: Ave		eine5ppmb	Internal C	Calibratio	5.0		•	1				
•	10	7	Calib: Rep		eine10ppma	Internal C	_	10		•	1				
•	11	V	Calib: Ave	_	eine10ppmb	Internal C	_	10		-	1				
•	12		Sample		eine20ppma	Internal C	_				1	1.00			
•	13		Sample		eine20ppmb	Internal C					1	1.00			
•	14	7	Sample		nown	Internal C	_				1	1.00		_	
•	15	V	Sample		nown	Internal C	_				1	1.00		_	
+}	16	V	Sample		nown	Internal C	_				1	1.00			
	17	~	Calib: Ave	 Caff 	eine0.2ppmc	Internal C	Calibratio	0.2		-	1				

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

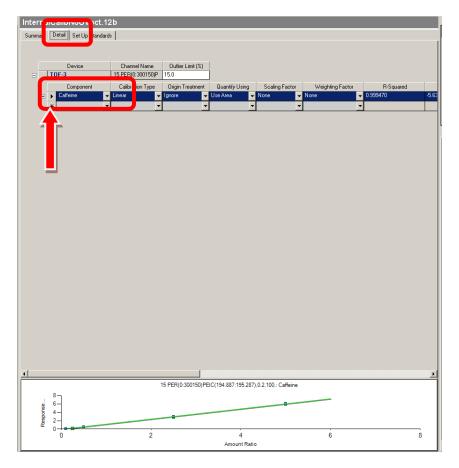
25. When complete, click the **Method** button in the navigation pane then click the **Summary** tab.

Interne	CalibNoUVo	ct.12b	
Summary	Detail Set Up Sta	andards	
	Detector	Channel Name	
Ξ	TOF-3	14 PER(0:300150	
	1303	3Caffeine	
	14 PER(0:300150)PEIC 13C	C(197.897:198.297),0.2,100,: 3Caffeine	
	12000		
	100000 - 80000 - 60000 - 92 20000 -	E	
	28888		
	0 0.5	1 1.5 2 2.5	
		Amount(µg/mL)	
	y = 0x + 0		-
-	R-Squared:NaN		
			▶ _

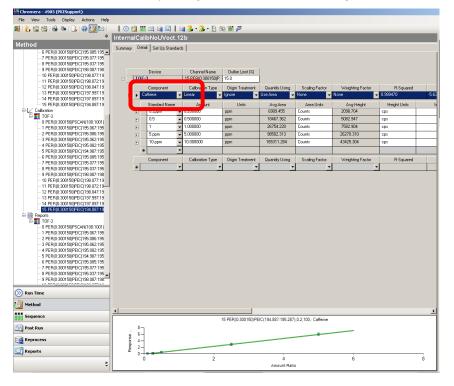
26. In the Calibration tree, select 15 PER(0:300150)PEIC(194:887...



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



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



Running a Chromera External Calibration

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

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

- Start by creating a Chromera method. In this example, the method is named **External Calibration** and the Group is named **Calibration**.
- 2. Click on **Peaks** and select a channel to define your **Component** and **Retention Time**.

Method								
E 🚼 ExtremalCalibration								
🕀 🛄 Instruments								
- TOF-3			Device		Channel Name	Bunching Factor	Area Threshold	N
-Sa FX15Pump-1			TOE-3		0 PER(0:300150)	1	121599.5460	24319.909
FXASCD-2	÷	·				1	121033.0460	24313.303
E 🚱 Channels		- I	TOF-3		1 PER(0:300150)			
🖻 🎹 TOF-3	Ŧ		TOF-3		2 PER(0:300150)			
- 0 PER(0.300150)PSCAN(100:1001),,100,			TOF-3		3 PER(0:300150)			
	÷	·			,			
	÷	3	TOF-3		4 PER(0:300150)			
3 PER(0:300150)PEIC(195.082:195.092).0.005.10 4 PER(0:300150)PEIC(195.077:195.097).0.01.100	Ŧ		TOF-3		5 PER(0:300150)			
	Ŧ		TOF-3		6 PER(0:300150)			
- 6 PER(0:300150)PEIC(195.062:195.112).0.025.10			TOF-3		7 PER(0:300150)			
7 PERI0:300150/PEIC(195.032:195.137).0.05.100	E		105-3		0 DED(0.000150)			
8 PERI0:300150/PEIC(194.987;195.187).0.1.100.	. /		105.3		0.000000000000			
9 PER(0:300150)PEIC(194,887-105,007,007,100,	≁[TOF-3		9 PER(0:300150)			
E A Peaks								
E TOF-3			Component	A	Retention Time (r		Search Start (min)	Peak Search E
0 PERI0:300150IPSCAN(100:1001)100.		٠	Caffeine	-	2.524	2.443		2.634
- 1 PER(0:300150)PEIC(195.086:195.088),0.001,10			*	-				
- 2 PER(0:300150)PEIC(195.085:195.089),0.002,10				_				·
- 4 PER(0:300150)PEIC(195.077:195.097),0.01,100								
5 PER(0:300150)PEIC(195.067:195.107).0.02,100								
- 6 PER(0:300150)PEIC(195.062:195.112),0.025,10								
- 7 PER(0:300150)PEIC(195.037:195.137).0.05,100								
8 PER(0:300150)PEIC(194.987:195.187),0.1,100,								
9 PER(0:300150)PEIC(194.887:195.287).0.2,100,								

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

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

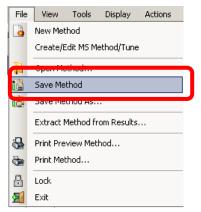
4. Click on **Set Up Standards** tab then click in the **Units** field, and select **ppm** from the drop-down list.

Sumr	mary Detail Set I	Jp Standards	
	Device 🕒	Channel Man	To Add a Standard
	TOF-3	0 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
	TOF-3	1 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
	TOF-3	2 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
	TOF-3	3 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
	TOF-3	4 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
	TOF-3	5 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
	TOF-3	6 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
	TOF-3	7 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
	TOF-3	8 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
•	TOF-3	9 PER(0:300150	To create a new standard column, right-click and select "Add a Standard"
	Component	Units	alibration Type Origin Treatment
	Caffeine		
		opt	
		pm	
		ppb	
		pg/mL	
		ng/mL	
		ng/L ng/g	
		ng/dL	v

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

Summary	D	etai	Set Up Stand	ard	s			
			Device		Channel Name	Outlier Limit (%)		
Ξ	T)F-3	3		9 PER(0:300150)P	15.0		
			Component		Calibration Type	Origin Treatment	Quantify Using	Scaling Factor
Ξ		Ca	ffeine	Ŧ	Linear 🗸 👻	Ignore 👻	Use Area 🛛 👻	None 🗸 M
			Standard Name		Amount	Units	Avg Area	Area Units
	+		S1	•	0.500000			
	+		S2	-	1.000000			
	+		S3	-	5.000000			
	+	۲	S4	•	20.000000			
		*		•				
			Component		Calibration Type	Origin Treatment	Quantify Using	Scaling Factor
	*			•	-	•	•	-
				_				

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



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



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

File	View	Tools	Disp
1	Open Da	ata	
₿	Lock		
2	Exit		

The **Data Selector** displays.

		0 2				Show Search 🛃	
Ope	en 📜	Organize 🔹	Actions •			Delet	e 🗙
S	Select	Batch Name	Batch Group	Batch Description	Created Date/Time 👻	Reprocessed By	Reproc
ŧ.		102011_Test	4905	Missing_data_pts	10/20/2011 11:48 AM		
÷.		10_18_Exceptio	4905	LMTest	10/19/2011 11:04 AM		
		10_18_Exceptio	4905	LMTest	10/19/2011 11:01 AM		
÷.		10_18_Exceptio	4905	LMTest	10/18/2011 11:35 AM		
+		101211_LM	4905	LMTest	10/12/2011 3:58 PM		
÷.		101211_LM	4905	LMTest	10/12/2011 3:58 PM		
+		InternalCalibOct	4905		10/12/2011 10:50 AM	PKISupport	10/20/20
ŧ.		Internaticaliboot	4900		10/12/2011 10:50 AM	ENIOUPPOR	10/20/20
+		CustomBatch	Group	Created From Batc	10/11/2011 10:33 AM	TOF	10/11/20
		000011_101	1005	1005	10/5/0011 0 01 111		
+		092911_16hr	4905	4905	10/5/2011 9:06 AM		
•]		092911_16hr	4905	4905	10/5/2011 9:02 AM		
+		092911_16hr	4905	4905	10/5/2011 7:59 AM		

9. Select you data from the list and click **Open**.

In this example we selected **CustomBatch.**

The **Batch Copy Selection** box appears requesting if you want to create a new batch.

Batch Copy Selection	
A new batch must be created wi batch or sample information.	hen changes are made to the
Do you want to keep the curren batch or revert to the original bai	
Create New Batch	Revert to Original

10. In this example we clicked **Create a New Batch**.

The new batch displays. In this example it is named **CustomBatch**.

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

1	Blanl	k	Peak	Detection	Peak	ID	Calibratio	n G	uantitation	Re	porting
	R			-0	(0					
Process these Chan											
			,		-				-		
			Use Latest Sto	ored Version of Meth	od • Up	date current	batch – no new copy	/	•		
lenc		eculto					//_				
		lame			âroup	Author		r			
	Cust	omBatch	Creal d I		-	PKISupport	PKISupport				
			Sar ple Type	Sample Name	Me	ethod	Standard	Injections	Dilution Factor	Sample Description	Injectio Volume
•	1	•	Sample	Caffeine0.5ppm	a 🗌			1	1.00		1
•	2	~	Sample	Caffeine0.5ppm	b			1	1.00		1
•	3	V	Sample	▼ Caffeine1ppma				1	1.00		1
÷-	4	~	Sample	 Caffeine1ppmb 	ine1ppmb		1		1.00		1
•	5	V	Sample	▼ Caffeine5ppma				1	1.00		1
•	6	◄	Sample	 Caffeine5ppmb 				1	1.00		1
÷	7	V	Sample	Caffeine20ppma	1			1	1.00		1
•	8	V	Sample	 Caffeine20ppmt 				1	1.00		1
•	9	V	Sample	 Caffeineunknov 	'n			1	1.00		1
÷	10	V	Sample	 Caffeineunknov 	'n			1	1.00		1
						Per Sar	mple Report				
	Sa	ample Type	e Fi	Frequency Report To		port Template Output Target		File Type		Output Name	

The Data Selector opens.

🔏 Da	ta Select	or - Single Method					_ 🗆 >
3		@ 1			She	ow Search 💫	
Ope	en 📜	Organize 🔹	Actions *			Delet	e 🗙
±м	ethod Gro	up : 4905 (4 items)					
-l M	ethod Gro	up : Calibration (3 item:	s)				
	Select	Method Name		Created Date/Time	Last Edited Date/Time 🗸	Author	Editor
		ExtremalCalibration		10/10/2011 1:59 PM	10/25/2011 2:19 PM	TOF	PKISupp
		External Calibration		10/25/2011 1:44 PM	10/25/2011 1:44 PM	PKISupport	
		Internal Calibration		10/12/2011 10:43 AM	10/20/2011 2:59 PM	TOF	PKISupp

- 12. Select the method from the list displayed in the Method Group. In this example it is **External Calibration**.
- 13. Right-click on the method and select **Fill Down** from the pop up box.

1	Blank	(Peak	k De	etection	Pea	k ID	Calibration		Q	Quantitation		Reporting	
	R			_	0]				0		<u> </u>	
	4	7												
Proc	ess the	ese Channel	s: 0	PEF	R(0:300150)PSCA	N(100:1001),	,100,, 1 F	PER(0:30015	50)PEIC(195.086	195.088),0.00	1,100, 💌			
		🗳 🖗 🛛	Jse Latest S	tored	Version of Method	• M	ake new b	atch – copy	all samples		-			
quenc	e Re	sults												
	N	ame	Description		ion Gi	oup	Au	thor	Editor					
	CustomBatch - C		Created	Fron	n Bat Group	PKISuppo		ort PKISupport						
	Re	process	Sampl Type	e	Sample Name	M	lethod		Standard	Injections	Dilution Factor	Sample Description	Injection Volume (μL	
	1	7	Sample	Ŧ	Caffeine0.5ppma	Extremal	Calibratio			1 1.00		1		
	2	2	Sample	•	Caffeine0.5ppmb			Fill Selected Fill Down		1	1.00		1	
	3	>	Sample	•	Caffeine1ppma		Fill All		All	1	1.00		1	
	4	◄	Sample	•	Caffeine1ppmb			Card View		1	1.00		1	
.	5	2	Sample	•	Caffeine5ppma			Select Co	lumns	1	1.00		1	
•	6	V	Sample	•	Caffeine5ppmb			Expand A	11	1	1.00		1	
	7	•	Sample	•	Caffeine20ppma			Collapse /	411	1	1.00		1	
•	8	V	Sample	•	Caffeine20ppmb						1.00		1	
.	9	V	Sample	•	Caffeineunknown					1	1.00		1	
•	10	>	Sample	•	Caffeineunknown					1	1.00		1	
Per Sample Report														

The method is associated with all rows to reprocess.

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

In this example, one run is a replace and the other run is an average.

Sequ	enci	e F	lesults								
			Name	Desc	ripti	on		Grou	ıp.	Author	
		Cu	stomBatch - C	Created F	ron	n B.	at	Group	•	PKISupport	
		F	eprocess}	Sample Type				Sample Name	Method		
	+ 1 V			Calib: Rep	•	Q	affei	ne0.5ppma	Extremal	Calibratio	
	2 🔽			Calib: Ave	•	C	affei	ne0.5ppmb	Extremal	Calibratio	
	+)	3 🔽		Calib: Rep 🖵		Caffei		ne1ppma	ExtremalCalibratio		
6	+)	4	V			C			ExtremalCalibratio ExtremalCalibratio		
E	+)	5	V			C					
	+)	6		Calib: Ave	Ŧ	G	affei	ne5ppmb	Extremal	Calibratio	
	+)	7	V	Calib: Rep	Ŧ	C	affei	ne20ppma	Extremal	Calibratio	
	+)	8	V	Calib: Ave	Ŧ	9	affei	ne20ppmb	Extremal	Calibratio	
	+)	9	V	Sample	Ŧ	C	affei	neunknown	ExtremalCalibratio		
	.	10	V	Sample	Ŧ	С	affeineunknown		Extremal	Calibratio	

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

	Blan	k	Peak D	etecti	on	Peak ID		Calibration		Quantitation		Reporting		
	A	<u> </u>		0		0		0]			
Proc	Process these Channels:			R(0:30	0150)PSCAN((100:1001)	"100" 1 PEF	1(0:30	10150)PEIC(195.08	6:195	.088),0.01	01,100, 💌		
🕨 🔲 😤 🖏 🌍 🛛 Use Latest			Jse Latest Store	atest Stored Version of Method Make new batch – copy all samples										
equenc	>e R	esults												
		Name	Descrip	tion	Gro	ир	Autho	ı	Editor					
:)	Cus	tomBatch - C	Created Fro	m Bat	Group	-	PKISupport		PKISupport					
	R	eprocess	Sample Type		Sample Name	N	fethod		Standard	l	ections	Dilution Factor	Sample Description	Injection Volume (µ
	1	~	Calib: Rep	Caffe	eine0.5ppma	Extremal	Calibratio	S1		- 1]		1
	2	•	Calib: Ave	Caffe	eine0.5ppmb	Extremal	Calibratio	S1		- 1]		1
•	3	>	Calib: Rep	Caffe	eine1ppma	Extremal	Calibratio	S2		- 1]		1
	4	7	Calib: Ave	Caffe	eine1ppmb	Extremal	Calibratio	S2		- 1]		1
.	5	V	Calib: Rep	Caffe	eine5ppma	Extremal	Calibratio	S3		- 1]		1
•	6	V	Calib: Ave	Caffe	eine5ppmb	Extremal	Calibratio	S3	-	- 1]		1
	7	V	Calib: Rep	Caffe	eine20ppma	Extremal	Calibratio	S4		- 1				1
+	8	>	Calib: Ave	Caffe	eine20ppmb	Extremal	Calibratio	S4	ŀ	- 1]		1
•	9	v	Sample	Caffe	eineunknown	Extremal	Calibratio	L		1		1.00		1
+	10	V	Sample	Caffe	eineunknown	Extremal	Calibratio	L		1		1.00		1
							Per Sa	1111 - L	Poport					
	S	ample Type	Free	quency	F	Report Ter	nplate	Out	put Target	Fi	le Type		Output Name	

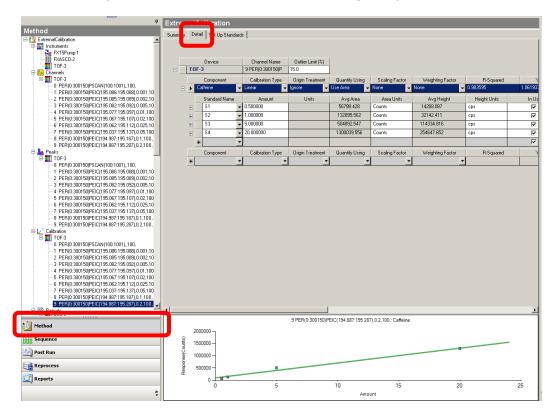
17. Click on the green **Start** button to start reprocessing.

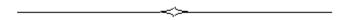
	mBatch (Idle	,																		
proces	::																			
	Blank	Peak Dete	ction	Peak ID	Calibration	Q	uantitation	Rep	oorting											
λ																				
	hese Channels:	0 PER(0:	: 300150)PSCAN(10	00:1001),,100,, 1 PER(0	300150)PEIC(195.086:1	95.088),0.001	1,100, 💌		vese Channels: 0 PER(0:300150)PSCAN(100:1001),,100,, 1 PER(0:300150)PEIC(195.088:195.088),0.001,100,											
🚬 📄 🐔 🖏 🖏 Use Latest Stored Version of Method 🔹 Make new batch – copy all samples 🔹																				
	1 🐮 🖾 🖓 Us	se Latest Stored Ve	ersion of Method	 Make new batch - 	copy all samples		•													
		se Latest Stored Ver	ersion of Method	 Make new batch - 	copy all samples		•													
		e Latest Stored Ver			copy all samples		•]													
	esults		Group				•]													
	Name	Description	Group	Author	Editor	Injections	▼ Dilution Factor	Sample Description	Injection Volume (μL)											
	Name CustomBatch - C Reprocess	Description Created From Ba Sample Type	Group at Group Sample Name	Author	Editor PKISupport Standard	Injections 1	Dilution	Sample Description	Injection Volume (μL)											

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

Custo	mBa	itch - C	opy 10-2	5-2011 14-23	8-21 (Processir	ig)				
Reproce	\$\$									
	Blan	k	Peak D	etection	Peak ID	Calibration	alibration C		Rep	porting
	Я		0							
	4			u .	ŭ	u u		ŭ		
Pro	Process these Channels:									
00		🗅 🖗 u	lse Latest Store	d Version of Method	 Make new ba 	tch – copy all samples		Ŧ		
Sequer	nce R	esults								
		Name	Descrip		oup Auth					
	Cus	tomBatch · C	Created Fro	m Bat Group	 PKISuppo 	rt PKISupport				
	R	eprocess	Sample Type	Sample Name	Method	Standard	Injections	Dilution Factor	Sample Description	Injection Volume (μL)
÷	1	V	Calib: Rep	Caffeine0.5ppma	ExtremalCalibratio	S1	v 1]		1
	2	▼	Calib: Ave	Caffeine0.5ppmb	ExtremalCalibratio	S1	• 1			1
÷	3	V	Calib: Rep	Caffeine1ppma	ExtremalCalibratio	\$2	- 1]		1
÷	4	V	Calib: Ave	Caffeine1ppmb	ExtremalCalibratio	\$2	↓ 1			1
	5	V	Calib: Rep	Caffeine5ppma	ExtremalCalibratio	\$3	→ 1			1
ŧ	6	7	Calib: Ave	Caffeine5ppmb	ExtremalCalibratio	\$3	→ 1			1
	7		Calib: Ben 🛛	Caffeine20nnma	ExtremalCalibratio	S4	u 1			11

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





Analyze Results in Post Run

Viewing the Results in Post Run

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

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

To view results in Post Run:

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

🚺 Method	
Sequence	
Nost Run	
Reprocess	
C Reports	
	»

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

Sec.	nromera - UHPLC_TOFMS - *Data Only* (TOF)
File	View Tools Display Actions Help
1	Open Data
	Add Data Open Data
-	Close Batch
8	Print Preview Report
<u></u>	Print Report
<u>51</u>	Exit
	- Matrix Overlay

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

	98	2 2					Show Search 🕘	
Ope	en 📜	Organize 🔻	Actions •				Delet	e 🗙
-		Batch Name	Batch Group	Batch Description	Created Date/Time	•	Reprocessed By	Reprocess
• ·		Caffeine Injection	TOF Group		9/23/2011 2:07 PM			
•		Caffeine Injection	TOF Group		9/23/2011 1:55 PM			
		Caffeine Injection	TOF Group		9/23/2011 1:48 PM			
3-		092211_16hr run	4900	8kV, 4900	9/22/2011 5:25 PM			
+		092111_16hr run	4900	8kV, 4900	9/21/2011 6:34 PM			

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

Post Run Caffeine Injection General Vews	Chromera - LCM4900 (ImageID) File View Tools Display Actions Help								
Control Batch: Caffeine Injection Control Caffeine : 0 PER(0:300200)PSCAN(100:1001),,120, : 1 Control Caffeine : 0 PER(0:300200)PSCAN(100:1001), 120, : 1 Caffeine : 0 PER(0:300200)PSCAN(100:1001), 120, : 1 Caffeine : 0 PER(0:300200)PSCAN(100:1001), 120, : 1 Caffeine : 0 PER(0:300200)PSCAN(100:1001), 120, : 1 Caffeine : 0 PER(0:300200)PSCAN(100:1001), 120, : 1 Caffeine : 0 PER(0:300200)PSCAN(100:1001), 120, : 1 Caffeine : 0 PER(0:300200)PSCAN(100:1001), 120, : 1 Caffeine : 0 PER(0:300200)PSCAN(100:1001), 120, : 1 Caffeine : 1 Control : 0 PER(0:300200)PSCAN(100:1001), 120, : 1 Caffeine : 1 Control : 0 PER(0:300200)PSCAN(100:1001), 120, : 1 Caffeine : 1 Control : 0 PER(0:300200)PSCAN(100:1001), 120, : 1 Caffeine : 1 Control : 0 PER(0:300200)PSCAN(100:1001), 120, : 1 Caffeine : 1 Control : 0 PER(0:300200)P : 1046 Control : 12431135 I I I Stand Amount : 1 Caffeine : 0 PER(0:300200)P : 1046 Control : 12431135 I I Stand Amount : 1 Caffeine : 0 PER(0:3002		M S 🖸 I S 🗷 - 🔼 -	E) 🔊 🕷 🥭	144.6		Scale: All C	harts		
Post Run Caffeine : 0 PER(0:300200)PSCAN(100:1001),,120, : 1 Matrix Overlay Sacked Plots Sacked Plots Overlay Sacked Plots Sacked Plots Overlay Results Data eine Injection Claffeine Sample Name Sample Description Injection Number Matrix Overlay Caffeine 1 Caffeine 1 Matrix Overlay Caffeine Sample Name Sample Description Injection Number Matrix Overlay OPER(0:300200)/PEIC(190:1200:1),5:120. Caffeine 1 Caffeine 1 Operation Science (Claffeine Caffeine 1 Caffeine 1 Caffeine 1 Operation Science (Claffeine Claffeine 1 Caffeine 1 1 Operation Science (Claffeine Channel Ret Time Component Name Area Height Final Amount Final Amount Unit Operation Science (Classocoop) 1046 308384.62 6706.72 1 1038859.33 2 2 1038859.33 2 2 1038859.33 2 1038859.33 2 1038859.33 1038859.33						-			_
Mews Caffeine : 0 PER(0:300200)PSCAN(100:1001),,120, : 1 General Views									_
Specked Role Specked Role Overlay 0.5 0.5 0.6	🗆 Views	C	Caffeine : 0	PER(0:300200)PSCAN(1	00:1001),	,120, : 1		
Sample Name Sample Description Inime (min) Sample Name Sample Description Inime (min) Inime (<mark>Single Plot</mark> Stacked Plots Matrix								
Caffeire Sample Name Sample Description Injection Number Image: Sample Name Caffeine 1 Image: Sample Name Area Height Final Amount Final Amount Unit Image: OPER(0.300200)P 0.0908 5202.056 35649.60 Image: OPER(0.300200)P 1.046 30639.52 260497.08		0.0 0.5	1.0 1.5			3.5	4.0 4	1.5 5.0	
Organ Sample Name Sample Description Injection Number OFFR0300200/PSCAN(100.1001), 120. Caffeine 1 OFFR0300200/PSCAN(100.1001), 5120. Caffeine 1 OFFR0300200/PSCAN(100.1001), 5120. OFER(0.300200)PSCAN(100.1001), 5120. Final Amount Vinit OFFR0300200/PSCAN(100.1001), 5120. OFER(0.300200)P 0.175 124311.95 111873.69 OFFR0300200/PSCAN(100.1001), 5120. OFER(0.300200)P 0.098 52020.55 35649.60 0 OFER(0.300200)P 1046 305334.62 67905.72 1 1	₽ Inj1	Results							
Caffeine 1 Channel Ret. Time Component Name Area Height Final Amount Final Amount <t< td=""><td></td><td>Sample Name</td><td>Sample</td><td>Description</td><td>Injection Number</td><td></td><td></td><td></td><td></td></t<>		Sample Name	Sample	Description	Injection Number				
Channel Ret. Time Component Name Area Height Final Amount Final Amount Initiation • 0 PER(0.300200)PEIC(190.1:200.1),5.120. • 0 PER(0.300200)P 1.15 111873.69 • • 0 PER(0.300200)P 0.398 52020.56 35649.60 • • 0 PER(0.300200)P 1046 305834.62 • • • 1 PER(0.300200)P 1044 1908593.33 260497.08 •	TOF-3	E- Caffeine		1					
0 0 PER(0:300200)P 0.908 52020.56 35649.60 0 0 PER(0:300200)P 1.046 305834.62 67906.72 1 1 PER(0:300200)P 1.044 1908859.33 260497.08		Channel	Ret. Time	Component Name	Area	Height	Final Amount	Final Amount Units	
0 PER(0:300200)P 1.046 305834.62 67906.72 1 PER(0:300200)P 1.044 1908859.33 260497.08		0 PER(0:300200)P	0.175		124311.95	111873.69			
1 PER(0.300200)P 1.044 1908859.33 260497.08		0 PER(0:300200)P	0.908		52020.56	35649.60			1
]
1 PER(0:300200)P 1.864 4138.53 2305.21		1 PER(0:300200)P	1.864		4138.53	2305.21]

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

3. Click on Scan.

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

Chromera - LCM4900 (ImageID)		
File View Tools Display Actions Help		
📙 🗟 🗞 🗶 🖳 😡 🔛 🖉 👘	🖄 😂 🖸 🖡 🤮 🛃 + 🛃 + 🛐 🖏 🎆 🗭 🛛 🚟 🐴 🎼 🥩 🖄 🖄 🕼 🖉 A Alles	
	Batch: Caffeine Injection	9
Post Run	Caffeine : 0 PER(0:300200)PSCAN(100:1001),,120, : 1	Control Panel
Views		Direct Control
General Views	0.00	Stop LC Pump
Stacked Plots		🜮 Change Tray
Matrix Overlay	0.58	St Vent TOF
Ovenay	0.56 Undo Zoom	Tune Control
	Plot Information	=
	Reference State St	
Data	Add User Label	
eine Injection	0.50 Annotations	ø
Caffeine	0.48 Plot Style	Status Panel
Ø Inj1 ▼Sar BPump-1	Save Plot Image	
Pump Pressure	0.90 0.95 1.00 1.0 Print Preview 1.20 1.25	Sequence Status
TOF-3	Print	Completed
1 PER(0:300200)PEIC(190.1:200.1).5.120.	Results Copy Image to Clipboard	Pump Pressure
	Sample Name Sample Description Copy Data to Clipboard	380 psi
	Caffeine Export Current Curve to Excel	Vacuum State
	Channel Ret. Time Componen Sa Rescale 1005 Amount Final Amount Units	PumpdownTolerance
	0 PER(0:300200)P 0.175 Examine Mass Spectra	Current Vial
	0 PER(0:300200)P 0.908 52020.56 35649.60	
	▶ 0 PER(0:300200)P 1.046	MS Analysis Status Not Acquiring
	1 PER(0:300200)P 1.044 1908859.33 260497.08 1 PER(0:300200)P 1.864 4138.53 2305.21	5
	Trangeodecopy (1991	Pump Status Ready
		ii.
		Capillary Entrance Current (nA) 31.6 nA
		2
<		End Plate Current (nA)

- 4. Move the mouse pointer to the apex of the peak (it turns to a hand) at retention time ~1.0 min. and then right-click.
- 5. Select **Examine Mass Spectra** from the menu.

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

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

皆 Chromera - chromera 3753 (ImagelD)										
File	View	Tools	Display	Acti	ons	He	lp			_
8.3	\$	de 1 🛃	2	<u>88</u>)	Star	ndar	d Post Run	Display		3 -
				2	Pea	k Ide	entification I	Review		1
Post	Run	1		8 .00	-			a di mata a		
🗆 Vie	WS			щų	Exa	mine	• Mass Spec	tra		sto
	neral Vie Single I									

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

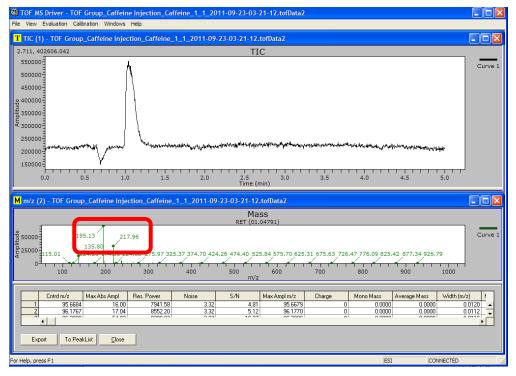
The **TOF MS driver** window displays a Total Ion Chromatogram **(TIC)** in the upper half of the window. If the mouse was right-clicked in the Chromera chromatogram (as in the first example above), the spectrum from that retention time will be displayed. If no point in the Chromera chromatogram is selected,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

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

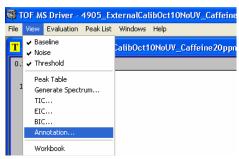
However, "all ions" are only those that were within the sampled mass range, which was determined by the Method used for the data acquisition and the Tune contained within that Method.

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

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



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



The Chromatogram Annotation dialog displays.

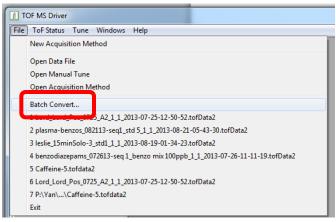
Chromatogram Annotation	<u> </u>
Туре	Threshold
Peak retention time	All peaks
🔲 Peak top time	C % Full scale 50
🔲 Peak area	C Amplitude 1000000
🔲 Peak height	,
Peak relative height	
🔲 Spectrum base peak time	
Decimal places: 5	OK Cancel

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

Applying Calibration in Post Run Analysis

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

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



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

- a. **Existing:** Retrieves the Calibration configuration used for the initial acquisition and reexecutes calibration using those parameters. The tofcal2 file generated for the run is required. This can be used if adjustments to only the Baseline, Noise or Peak Detect settings are desired while maintaining the existing calibration.
- b. Default: Utilizes the calibration coefficients found during calibration of the tune parameters.
- c. **Lockmass:** Utilizes specific peaks to calibrate the remaining data, like internal calibration, but the calibration is applied to the entire run.

Calibration Preferences Calibration Mode: Lock Mass		Calbration Settings: Masses(118.0863, 922.0098) Search(50 mmu)	Â
Data File Conversion Preferences	Lockmass Calibration	Preferences	
Baseline Noise	Lock Mass 1:	118.08625	
NOTE: Evaluation preferences use	Lock Mass 2:	922.00979 Save	
Data File Selection	Search Span:	50 mmu	E
Files to Convert:	Lockmass Evaluation	Noise Peak Detect	^
	NOTE: Evaluation	preferences used for Lockmass calibration only!	
		OK Cancel	
Select Files	,	Convert Files	Ŧ

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

Batch Convert		×	
Calibration Preferences Calibration Mode: External Modify Settings		albraton Setting:	
Data File Conversion Preference Evaluation Preferences Baseline	Select Calibration File) Local Disk (C;) + MSData + 2013-08-20 + 4+ Sternch 2013-08-20	× -
Data File Selection	E Desktop Downloads Dropbox Recent Places	Name plasma-benzos (082013-dilutionSeries; blank, 1,1,2013-08-20-11-28-49.tofca20 plasma-benzos (082013-dilutionSeries; blank, 2,1,2013-08-20-11-39-10.tofca20 plasma-benzos (082013-dilutionSeries; blank, 2,1,2013-08-20-11-48-40.tofca20 plasma-benzos (082013-dilutionSeries; blank, 2,1,2013-08-20-11-48-40.tofca20 plasma-benzos (082013-dilutionSeries; blank, 2,1,2013-08-20-11-48-40.tofca20	
	ibraries Documents → Music = Pictures Wideos	p plasma-benero, 000031-diskutordenie, auf. 2, 1, 2013-08-20-12-09-51.tofex10 plasma-benero, 000031-diskutordenie, st. 0, 1, 2, 0013-08-20-12-28-18.tofex10 plasma-benero, 000031-diskutordenie, st. 0, 2, 1, 2013-08-20-12-28-18.tofex10 plasma-benero, 000031-diskutordenie, st. 0, 2, 1, 2013-08-20-12-28-18.tofex10	
Select F	Computer DataTest (\\BFDF003) (B Local Disk (C:) SullivLN (\\BFDF001\Sta public (\\BFDF001) (P:)	plasma-benzos_082013-dilutionSeries_std3_3_1_2013-08-20-01-12-39.tofcal2 plasma-benzos_082013-dilutionSeries_std4_1_1_2013-08-20-01-21-49.tofcal2	•
	File nam	me: Calibration Files (*.tofcal2) Open Cancel	

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

Calbration Preferences				
Calibration Mode: Internal	Calibration Settings:			
Modify Settings	Start Time(2), End Time(3) PolyOrder(1), Search(0.05 amu), Min CalPoints(622.02896, 1521.971476)	5/N(3)		
Data File Conversion Preferences				
Evaluation Preferences	Time Range to Convert:			
Baseline Internal Calibration Preference	15	0		
NOTE: Evaluat Calibration Time	Calibration Calibrants	0		
	nin Calbrants			
Data File Selection End Time: 3	nin			
Files to Convert:				
		ToF Mass Spectrum - Config	gure Calibration	×
	OK Cancel	Calbranto		Calibration Settings
		Compound Name /	Mass / In Use	Polarite
		1 H20]2H+	37.02840	C Positive
		2 [H20]3H+	55.03890	
		3 (H20)4H+ 4 (H20)5H+	73.04953	C Negative
	v	4 (H2U)H+ 5 (H20)H+	19.01780	
< >		6 ACN +H	42,03380	
		7 Agilent 1	118.09625	No. Spectra to Average: 1
Select Files	Convert Files	8 Agilent 2	322.04812	Minimum S/N: 3
		9 Agient 3	622.02896	Philipina Street
		10 Agilent 4	322.00979	Search Span (anu) 0.05
		11 Aglent 5 12 Aglent 6	1521.97147 F 2121.93315	
		13 Agient 7	2721.89483	Polynomial Order: 1
		14 APCI L1	121.05087	
		15 APCI L2	322.04812	
		16 APCI L3	622.02896	Load Save
		🖂 Display Both Masses	Clear All	
				OK. Cancel

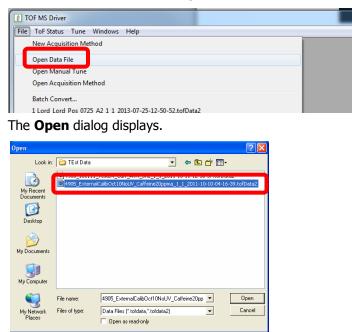
 Select the desired calibration type and make any necessary updates to parameters. Choose the Select Files button and select the tofdata2 files to be processed. Then select Convert Files. The right-side display will show progress.

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

1 Batch Convert	×
Calibration Preferences	
Calibration Mode: Lock Mass	Calibration Settings:
	Masses(118.0863, 922.0098) Search(50 mmu)
Modify Settings	search(30 mmu)
Data File Conversion Preferences	
Evaluation Preferences	Time Range to Convert:
Baseline Noise Peak Detect .	
NOTE: Evaluation preferences used for file conversion only	Full End Time: 0
Data File Selection Files to Convert: C:\MSData\lesle_15miSolo_blank_1_1_2013-08-19 C:\MSData\lesle_15miSolo_25td1_1_2013-08-19 C:\MSData\lesle_15miSolo_25td1_1_2013-08-1 C:\MSData\lesle_15miSolo_25td1_2_1_2013-08-1 C:\MSData\lesle_15miSolo_35td2_1_1_2013-08-1 C:\MSData\lesle_15miSolo_35td2_1_2_013-08-1 C:\MSData\lesle_15miSolo_35td2_1_2_013-08-1 C:\MSData\lesle_15miSolo_3td2_2_1_013-08-1 C:\MSData\lesle_15miSolo_3td2_2_1_013-08-1 C:\MSData\lesle_15miSolo_3td2_1_2_013-08-1 C:\MSData\lesle_15miSolo_3td2_1_2_013-08-1 C:\MSData\lesle_15miSolo_3td2_2_1_013-08-1 C:\MSData\lesle_15miSolo_3td2_108-1 C:\MSData\lesle_15miSol08-1 C:\MSData\le	Data File Conversion Conversion Status: FIE: Ieale_1SminSolo_blank_1_1_2013-08-19-10- - Opened Califorated. - Coopened -
	Close

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

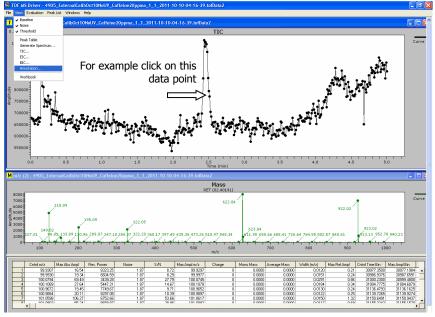
3. In the TOF MS Driver, select **Open Data File** from the **File** menu.



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

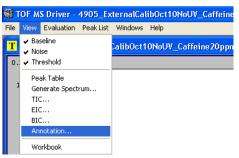
4905_ExternalCalibOct10NoUV_Caffeine20ppma_1_12011-10-10-04-16-39.tofData2

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



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

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



The Chromatogram Annotation dialog displays.

Chromatogram Annotation	
Туре	Threshold
Peak retention time	 All peaks
Peak top time	C % Full scale 50
🔲 Peak area	C Amplitude 1000000
Peak height	,
Peak relative height	
Spectrum base peak time	
Decimal places: 5	OK Cancel

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

Observe that the masses now have five decimal places.

595.222, 7402.353 Mass RET (02.40191)													
8000 7000 6000 5000 4000	• ¹	18.08947					622.03739	1			922.01898	1	
3000		- f	95.09195	322.052				623.042				923.01999	
000	5721 100.0 100	07938 156.0498 1 4 498 200	35 211.99156 26	,,	373 380.28055 400	436.30543 492.	.34270 548.337:	, 1 .,,,,,	668.08325 724.	57324 781.6073 800	6 837.79176 900	913.13027 9 10	71.80773
1000 0 	100	200	30	10	400	++	m/z 600	-,1-,,,,,,,,,,,,	700	800	900		, , , , , , , , , , , , , , , , , , ,
	100 d m/z	200 Max Abs Ampl	Res. Power	00 Noise	400 S/N	Max Ampl m/z		Mono Mass	700 Average Mass	800 Width (m/z)	900 Max Rel Ampl	Cntrd Time Bin /	000 Max Ampl Bin
	d m/z 1 99.9307	200 Max Abs Ampl 16.54	30 Res. Power 8323.25	Noise 1.87	400 S/N 8.72	500 Max Ampl m/z 99.9297	m/z 600	Mono Mass 0.0000	700 Average Mass 0.0000	800 Width (m/z) 0.0120	900 Max Rel Ampl 0.21	Cntrd Time Bin / 30977.3500	Max Ampl Bin 30977.198-
	d m/z 1 99.9307 99.9930	200 Max Abs Ampl 16.54 19.34	Res. Power 8323.25 6604.59	Noise 1.87 1.87	400 5/N 8.72 8.29	500 Max Ampl m/z 99,9297 99,93977	m/z 600	Mono Mass 0.0000 0.0000	Average Mass 0.0000 0.0000	800 Width (m/z) 0.0120 0.0151	900 Max Rel Ampl 0.21 0.24	Cntrd Time Bin / 30977.3500 30986.9376	Max Ampl Bin 30977.198 30987.659
	d m/z 1 99.9307 99.9300 100.0794	200 Max Abs Ampl 16.54 19.34 69.49	Res. Power 8323.25 6604.59 3439.28	Noise 1.87 1.87 1.87	400 S/N 8.72 8.29 27.79	Max Ampl m/z 99.9297 99.9977 100.0745	m/z 600	Mono Mass 0.0000 0.0000 0.0000	Average Mass 0.0000 0.0000 0.0000	Width (m/z) 0.0120 0.0151 0.0291	Max Rel Ampl 0.21 0.24 0.86	Cntrd Time Bin / 30977.3500 30986.9376 31000.2300	Max Ampl Bin 30977.198- 30987.659 30999.480
1000 0 	d m/z 1 99,9307 99,9300 100.0794 100.1089	200 Max Abs Ampl 16.54 19.34 69.49 27.64	Res. Power 8323.25 6604.59 3439.28 5447.21	Noise 1.87 1.87 1.87 1.87 1.87 1.87	400 S/N 8.72 8.29 27.79 14.67	Max Ampl m/z 500 Max Ampl m/z 99.9297 99.9297 100.0745 100.1078	m/z 600	Mono Mass 0.0000 0.0000 0.0000 0.0000	Average Mass 0.0000 0.0000 0.0000 0.0000	Width (m/z) 0.0120 0.0151 0.0291 0.0184	Max Rel Ampl 0.21 0.24 0.86 0.34	Cntrd Time Bin / 30977.3500 30986.9376 31000.2300 31004.7775	Max Ampl Bin 30977.198 30987.659 30999.4800 31004.607
1000 0 	d m/z 1 99.9307 99.9307 100.0794 100.1089 100.9672	200 Max Abs Ampl 16.54 19.34 69.49 27.64 19.45	8323.25 6604.59 3433.28 5447.21 7743.67	Noise 1.87 1.87 1.87 1.87 1.87 1.87 1.87 1.87 1.87	400 S/N 8.72 8.29 27.79 14.67 9.71	Max Ampl m/z 99.9297 99.9977 100.0745 100.1078 100.652	m/z 600	Mono Mass 0.0000 0.0000 0.0000 0.0000 0.0000	Average Mass 0.0000 0.0000 0.0000 0.0000 0.0000	Width (m/z) 0.0120 0.0151 0.0291 0.0184 0.0130	Max Rel Ampl 0.21 0.24 0.34 0.34 0.24	Critrd Time Bin / 30977.3500 30986.9376 31000.2300 31004.2775 31136.4793	Max Ampl Bin 30977.138 30987.559 30999.480 31004.607 31136.162
1000 0 	d m/z 1 99,9307 99,9300 100.0794 100.1089	200 Max Abs Ampl 16.54 19.34 69.49 27.64	Res. Power 8323.25 6604.59 3439.28 5447.21	Noise 1.87 1.87 1.87 1.87 1.87 1.87	400 S/N 8.72 8.29 27.79 14.67	Max Ampl m/z 99.9297 99.9977 100.0745 100.1078 100.9652 100.9852	m/z 600	Mono Mass 0.0000 0.0000 0.0000 0.0000	Average Mass 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000	Width (m/z) 0.0120 0.0151 0.0291 0.0184	Max Rel Ampl 0.21 0.24 0.86 0.34	Cntrd Time Bin / 30977.3500 30986.9376 31000.2300 31004.7775	Max Ampl Bin 30977.138 30987.559 30999.480 31004.607 31136.162

8. Select Lockmass from the Calibration menu.

😽 TOF MS Driver - 4905_ExternalCalibOct10NoUV_0					
File View Evaluation	View Evaluation Calibration Windows Help				
T TIC (1) - 4905	Manual Automatic	10NoUV_Caffein			
0.403, 1025004.8	3) Lockmass				
1000000	External Default				
950000-	Configure				

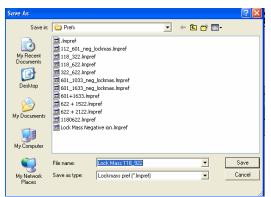
The Lockmass Calibration dialog displays.

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

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

Lockmass Calibration			
Mass:	118.08625		
Mass:	922.00979		
OK Can	cel	Load	Save

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

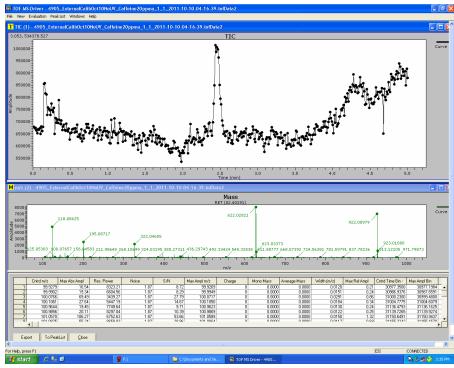


11. Type a **File name**.

Notice that the file name has the extension **.Impref** and it is saved in the same directory as your data.

Lockmass Calibration	×
Mass 1: 118.0	0862
Mass 2: 922.0	0097
Search Span 50	mmu
OK Cancel	Load Save

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



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

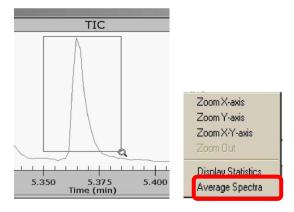
After the Lockmass run is complete you can select any data point and observe that the two Lockmasses do not change. The target mass should be accurate to within three decimal places anywhere on the TIC where the target mass resides.

Creating an Average Mass Spectrum in the TOF Driver

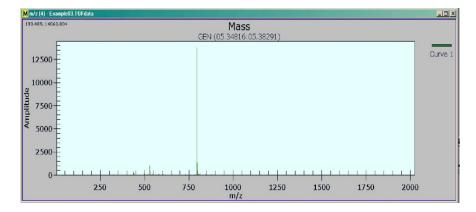
Below is an example of how to analyze the TIC peak.

Using the Left Mouse Button Command

- 1. Left-click and drag a box around the peak of interest.
- 2. Release the button and select the left mouse button command, Average Spectra.



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



Using the Generate Mass Spectrum Dialog

- 1. To create an average spectrum, activate the TIC spectrum and select **Generate Spectrum** from the **View** menu.
- 2. In the **Range Selection** section, select whether to use **Time Range** or **Spectra Range** to define the average spectrum.
- 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.

	te Mass Spe	ctrum	×
Range	e Selection		Curve Definition
A) B C D	Start Time 1.90000 1.60000 lear All	End Time 2.30000 1.70000 Time Range Spectra Range	Spectra Ranges (+ is add, - is sub) B A-B A-B Clear All
	rum m/z Limits ull Range		Display Separate Windows
Om	/z Range		
	Start m/z:	5	Tune Limits
	End m/z:	3000	Reset None
		ОК	Cancel

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

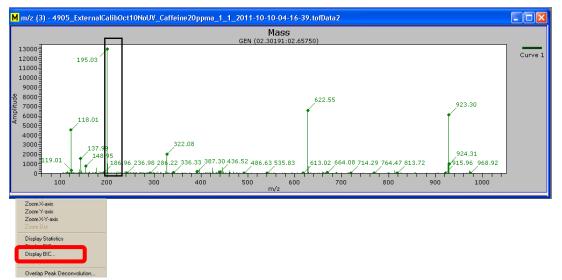
6. Click **OK**.

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

Creating an EIC and BIC from a Mass Spectrum

To create an EIC:

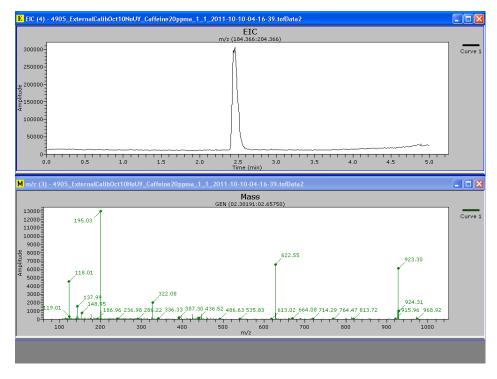
1. Left-click and drag a narrow box around a peak of interest in the Mass spectrum. The width of the box will be the set m/z range.



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

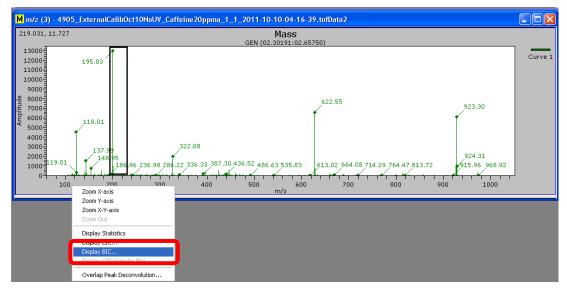
3. Click **OK** and the **EIC** is displayed.

The EIC displays where in the chromatogram mass peaks occur with m/z values within the set m/z range.



To create a BIC:

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

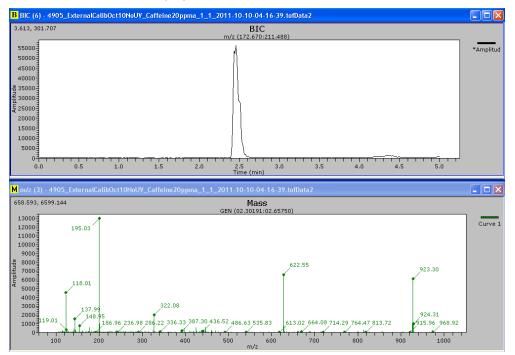


- Release the mouse button and select **Display BIC** from the menu. The **Base Peak Preferences** dialog displays.
- 3. In the **Trace Selection** section only select **Amplitude**.

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Base Peak Preferences				
Start m/z: End m/z:	172.67 211.488	Trace Selection ✓ Amplitude ☐ Resolution ☐ m/z		
Display New Window		Range Limits Reset None		
OK Cancel				

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



Processing of Mass Spectra in the TOF Driver Window

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

Freezing and Thawing Mass Spectra

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

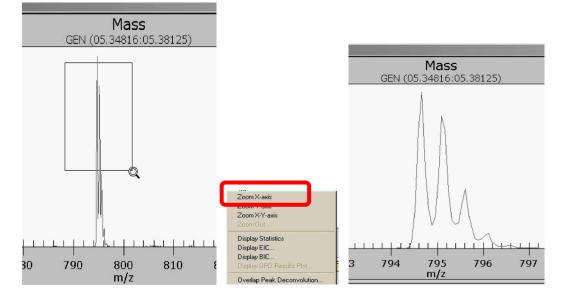
To freeze and thaw Mass Spectra:

- 1. When a mass spectrum is created using the "hand" the mass spectrum can be frozen by activating the spectrum window and selecting **Freeze** from the **Spectrum View** menu
- 2. Then, when a new spectrum is created it will be displayed in a new window. The previous spectrum is still available.
- 3. To thaw a frozen mass spectrum, activate the spectrum and select **Thaw** from the **View** menu.

Zooming In

To zoom in:

- 1. Left-click and drag a box around the area of interest.
- 2. Release the button and select Zoom X-axis.



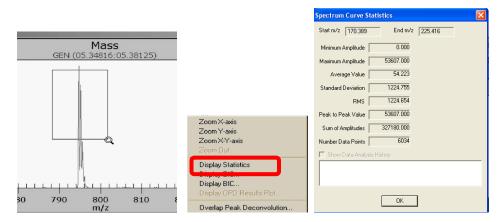
- 3. Left-click and drag a box somewhere in the spectrum. Release button and select Zoom Out.
- 4. Use the left-mouse button command **Undo Zoom**.

Displaying Statistics

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

To display statistics:

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



Using Right Mouse Click Menus

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

Baseline Calculations

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

To calculate a baseline:

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

Mass Spectrum Baseline 🛛 🔀				
· Auto				
Function: 0 - V-F Baseline	•			
Baseline segment size (points):	250			
Baseline noise window (points):	100			
Pick 1 point from every (points):	5			
Minimum peak width (points):	3			
C Manual				
Baseline:	0			
ОК	Cancel			

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

- 3. Enter shortest Baseline Segment Size, and Baseline Noise Window.
- **NOTE:** An increased Baseline segment value will flatten the baseline. A decreased value may lead to a baseline which interferes with the peaks.

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

- 4. Select **Manual** to create a **Manual** baseline by entering a value resulting in a straight line as a baseline.
- 5. The baseline can be hidden or displayed with the **Baseline** from the **View** menu.
- 6. The baseline can be subtracted from the chromatogram by selecting **Subtract Baseline** from the **Evaluation** menu.

Setting Spectrum Noise Calculation Preferences

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

To set mass spectrum noise calculation preferences:

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

Mass Spectrum Noise 🛛 🗙				
Auto				
Function:	0 - V-F Noise	•		
Noise segment size	(points):	250		
Median segment size	e (points):	0		
Pick 1 point from ev	ery (points):	5		
Minimum peak width	n (points):	3		
C Manual				
Function:	Function: 0 - Peak to peak			
Start (m/z): 5 End (m/z): 20000				
	OK	Cancel		

- 2. To detect the noise manually, select **Manual** and enter a mass range where there are not any peaks.
- 3. Select a Function: Peak to peak or 1-6 Times SD.
- 4. In the default version of the signal to noise calculation the following is done:
 - If the noise is **Peak to peak** then the amplitude of the peak top or the centroid is subtracted by the low value of the noise and divided by the peak-to-peak difference.
 - If the noise is **1-6 Times SD** then the amplitude of the peak top or the centroid is subtracted by the mean of the noise and divided by six times the standard deviation of the noise.
- 5. Click **OK**.

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

Setting the Mass Spectrum Threshold

To set the mass spectrum threshold:

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

Mass Spectrum	Threshold 🔀
<u>T</u> hreshold Value:	۵
ОК	Cancel

- Enter a threshold value and decide if the mass spectrum will be subtracted with this value.
 The threshold can be hidden or displayed with the **Threshold** from the **Chromatogram View** menu.
- **NOTE:** If a new chromatogram has not been created, the "undo" function is not available. To return to the original chromatogram, the data file has to be closed and opened again.

Mass Spectrum Smoothing

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

To smooth mass spectra:

- 1. Activate a mass spectrum and select **Smooth** from the **Evaluation** menu.
- 2. Enter the number of smooths (1-10), window size (0.01-10) and select the function by clicking on the drop-down list.

Mass Spectrum Sr	nooth 🛛 🔀
Function: Savitzk	y-Golay 🗾 💌
Order: 2	•
Passes: 1	
Steps:	
m/z	Size
5	5 🔺
0	0
0	0
0	0
0	0
Ö	0 -
Step transitions:	
C Staggered	
staggered	
OK	Cancel

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

Mass Spectrum Peak Detection

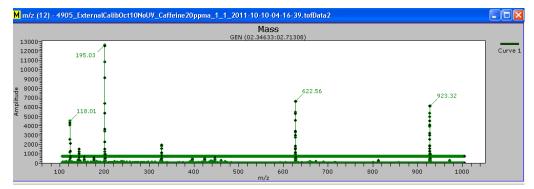
You can set mass spectrum peak detection on a spectrum, EIC, TIC, or BIC.

Manual Peak Detection

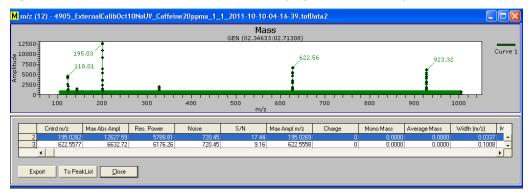
1. To display data points, select the right mouse button command **Mark Data Points**. This makes it easier to see the individual data points in the spectrum.

	Viewing Style	►
	Border Style	⊁
	Font Size	⊁
v	Show Legend	
	Numeric Precision	⊁
	Plotting Method	٠
	Data Shadows	٠
	Grid Options	٠
	Mark Data Points	
	Zoom In	
	Zoom Out	
	Zoom Out All	
	Maximize	
	Customization Dialog	
	Export Dialog	
	Help	

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



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



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

Automatic Peak detection

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

Mass Spectrum Peak Detect
Search method Image: Full Spectrum Image: Partial Spectrum Start (m/z): 5
Selection Criteria
S/N greater than:
E Abs. Ampl. greater than threshold
Rel. Ampl. greater than (% of max peak):
Abs. Area greater than:
Rel. Area greater than (% of max peak): 0
FwHM greater than (m/z): 0.01
FwHM less than (m/z): 0.01
No. Highest Peaks:
Max Valley (% above baseline): 75
m/z Assignment
C Peak Top
Centroid of the Top (% peak ampl): 75
Display
🔽 Display Results
OK Cancel

- 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**.

Chromatogram Peak Detect							
Search Method							
Full Chromatogram							
C Partial Chromatogram							
Start (min): 00.00000 End (min):	00.01666						
Selection Criteria							
S/N greater than:	4						
Abs. Ampl. greater than threshold							
🔲 Rel. Ampl. greater than (% of max peak):	0						
🔲 Abs. Area greater than:	0						
🔲 Rel. Area greater than (% of max peak):	0						
FWHM greater than (min):	00.00833						
FWHM less than (min):	00.01666						
No. Highest Peaks:	1						
Max Valley (% above baseline):	75						
Retention Time							
🔿 Peak Top							
Centroid of the Top (% peak ampl):	75						
Display							
✓ Display Results							
OK	Cancel						

7. Click **OK**.

М	M m/z (13) - 4905_ExternalCalibOct10NoUV_Caffeine20ppma_1_1_2011+10-10-04+16-39.tofData2											
5	571.724, 1605.531 Mass											
						GEN (02	.31300:02.7352	5)				
	110004		1									Curve 1
	90004	1	.95.03									CONTEL
	8000 -							622.56			923.32	
Amplitude	7000 = 6000 =		18.01				Ť				1	
ja Bil	50004		10.01									
Ę	4000	T									1	
	3000 a											
	1000											
	0		i la di seri d	· · · · · · · · · · · · · · · · · · ·	- In the stand of	· · · · · · · · ·		····				
		100	200	300	400	500	600	700	800	900	1000	
							m/z					
			I									
		Cntrd m/z	Max Abs Ampl	Res. Power	Noise	S/N	Max Ampl m/z	Charge	Mono Mass	Average Mass	Width (m/z)	Max Rel Ampl
	2	118.010			675.64 675.64	6.70	118.0119 195.0267	0	0.0000	0.0000	0.0219	41.23
	3	622.557	6584.89	6176.33	675.64	9.70	622.5553	Ő	0.0000	0.0000	0.1008	59.64
	4	923.3210	6098.70	5483.69	675.64	8.83	923.3266	0	0.0000	0.0000	0.1684	55.24
												•
	Expo	ort To Pe	akList Close									

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

The Mass Spectrum Annotation dialog displays.

Mass Spectrum Annotation	
Туре	Threshold
Peak centroid (m/z)	All peaks
Peak top (m/z)	C % Full scale 50
🗖 Peak area	C Amplitude 1000
Peak height	
Peak relative height	
🔲 Base peak (m/z)	
🗖 Series info	
Decimal places: 2	OK Cancel

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- 9. To close the peak table, click the **Close** button below the table.
- 10. To export the peak table, click the **Export** button below the table. The complete table will be copied to the clipboard.
- 11. Open Microsoft Excel (or other spreadsheet software) and **Paste** in an empty data sheet.
- 12. To print the table from Microsoft Excel, select **Print** from the **File** menu.

	A	В	C	D	E	F	G	Н	I	J	K	L	M	N	0	P	Q	R	S	
		Max Ampl		Mono	Average	Width	Res.			Max Abs	Max Rel	Cntrd	Max Ampl			Abs Cntrd		Peak End		Pea
1	Cntrd m/z	m/z	Charge	Mass	Mass	(m/z)	Power	Noise	S/N	Ampl	Ampl	Time Bin	Bin	Area	Ampl	Ampl	Start m/z	m/z	Start Bin	Bin
2	171.6405	171.6425	() () (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	
З	172.6243	172.6243	() () (0.0452	3823.09	0.39	15.24	6	0.65	10324	10324	7.54	0.65	6	172.5565	172.6921	10322	
4	179.4146	179.4091	() () (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	() () (0.0551	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	() () (0.0482	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	() () (0.048	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	() () (0.0567	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	194.7169	194.7173	() () (0.0469	4148.07	1.52	4.62	7	0.76	10955.99	10956	8.67	0.76	7	194.6453	194.7893	10954	
10	194.9434	194.939	() () (0.0664	2936.34	1.52	5.33	8.18	0.88	10962.28	10962.15	14.66	0.88	8.08	194.8254	195.0415	10959	
11	204.6533	204.6473	() () (0.0727	2814.42	1.61	8.66	14.15	1.53	11228.49	11228.33	26.49	1.51	13.91	204.5612	204.7458	11226	
12	205.4877	205.4865	() () (0.0502	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	() () (0.0744	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	() () (0.0565	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	() () (0.0629	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	ſ	n r	i r	0 0772	2878.41	2 14	6.76	16 71	18	11692.62	11692.76	32.03	1 79	16.47	222 0/152	222 226	11690	

<u>Additional Features and</u> <u>Functions</u>

AXION EC ID (Elemental Composition Identification)

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

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

New Features

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

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

Program start and default DB search

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

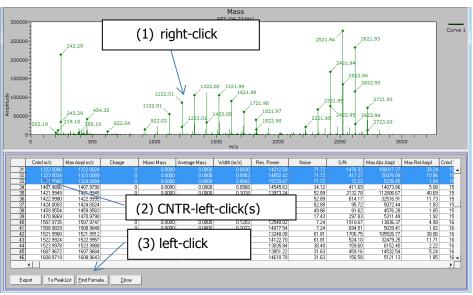


Figure 1. ECID tool is invoked by pressing "Find Formula..." button on the bottom of the peaklist panel of the Mass Spectrum window.

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

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

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

Neutral Loss analysis

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

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

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

AxION EC ID 3.1.1.	
Monoisotopic peak, (m/z) 132	Search in Compound (PC) N-loss P->F S22.0006 Candidates Score ppM-error Details
Charge carrier H+ (1.0) PpM error cutoff (1.5)	00728 Selected DataBase does not exist
lso cum-sigma, % 10	(2) tighton sutoff
A+1 299	9938.149
A+2 518 Search DB A+3 0	(3) left-click
Defaults A+4 0 A+5 0	
Elements A+6 0 Generator A+7 0	

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

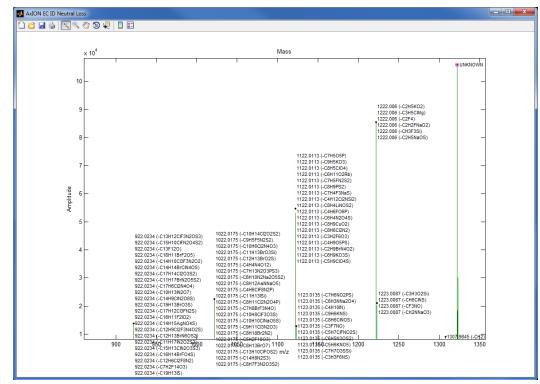


Figure 3b. parent-to-fragments neutral loss display for an unknown parent ion

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

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

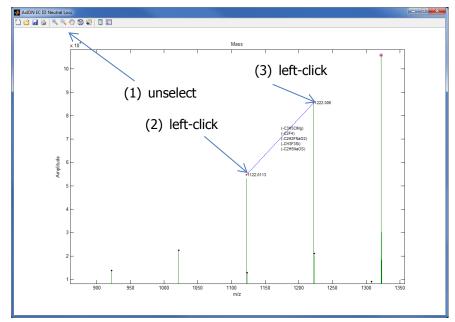


Figure 4. User interactive neutral loss listing.

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

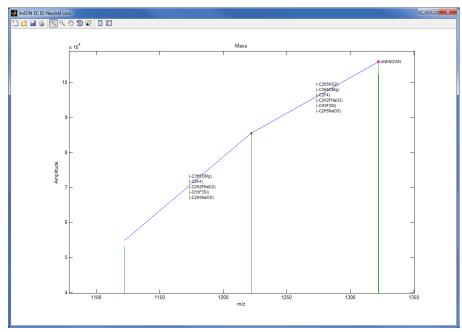


Figure 5. "sequential" neutral loss reveals polymer repeating unit

Polymer and Formula Generator

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

M AXION EC ID 3.1.1.	Mass Ge 😐 🗉 🔀
Search in Compound (PC) N-loss P->F	Lookup
Monoisotopic peak, (m/z) 1322.0006 Candidates Score ppM-error Details	Check
Charge carrier H+ I.00728 Selected DataBase does not exist.	
ppM error cutoff	
	Generator
	Target Mass 1320.9933 Run
Isotope abundances A 105895.83	Rep CH2 CH2
A+1 29938.149	End OH OH V
A+2 5187.9729	Element Min Max TypMax
Search DB A+3 0	▼ C 0 89 89
Defaults A+4 0	▼ F 0 9 9
A+5 0	✓ H 0 265 265
Elements A+6 0	▼ N 0 18 18
	▼ 0 0 18 18
	▼ S 0 9 9
	▼ CI 0 9 9
(1) left-click	✓ Br 0 9 9
	▼ 0 9 9
	▼ P 0 5 5 ▼ 0 5 5 15
(opens window)	✓ Si 0 45 45 ■ Na 0 0 5
	Na 0 0 5

Figure 6. Activation of the Lookup and Generator window

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

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

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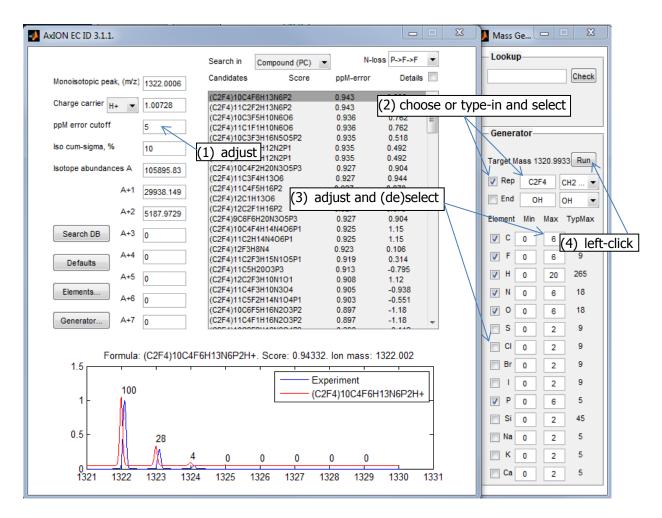


Figure 7. Polymer-like formula generation.

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

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

Formula Lookup

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

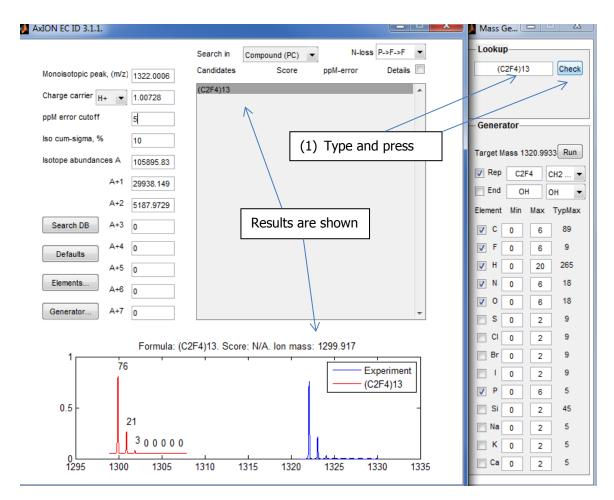


Figure 9. Using Formula Lookup feature

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

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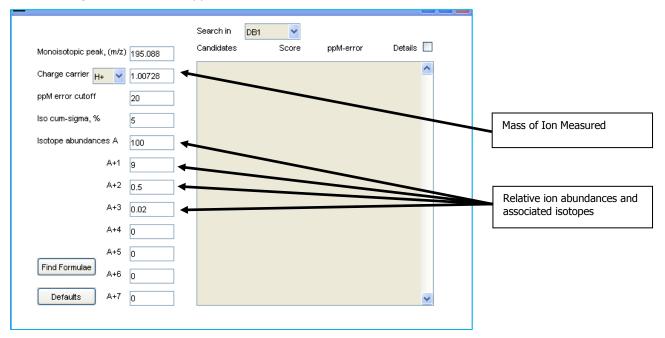
Running AxION EC ID

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

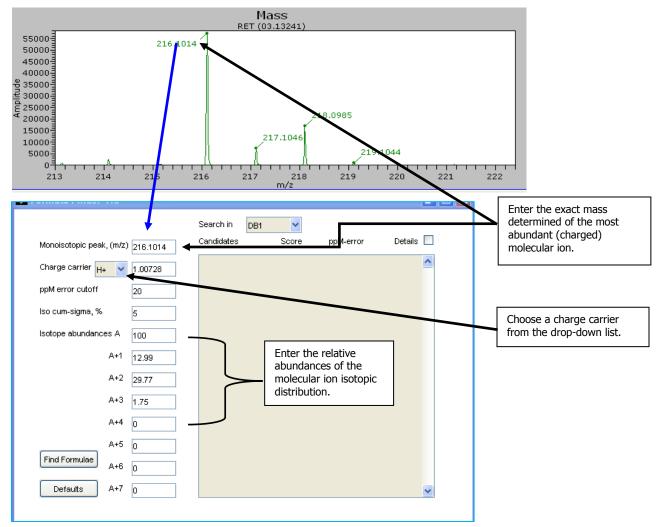
1. Open AxION EC ID by clicking on this icon on your desktop.



The following default window appears:



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



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

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

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

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

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

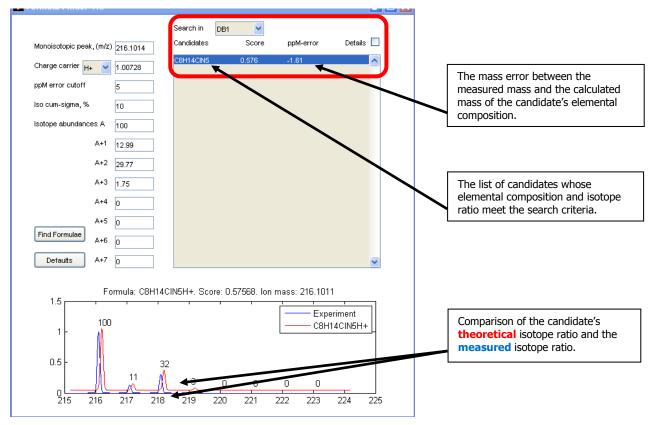
Typical values for the AxION 2 TOF are about **5 ppM mass error** and 10% **Iso cum-sigma**, % using a default (external) calibration. This type of mass accuracy can be obtained on a temperature equilibrated system that had been pulsing over the acquisition mass range for at least 1-2 hours. However,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

	Search in DB1	*		_
Monoisotopic peak, (m/z) 216.1014	Candidates	Score pr	pM-error De	tails
Charge carrier H+ 🖌 1.00728				
ppM error cutoff 5				
lso cum-sigma, % 10 🔹				
Isotope abundances A 100				
A+1 12.99				
A+2 29.77				
A+3 1.75				
A+4 0				
+5 0				
Find Formulae +6 0				
Defaults A+7 0				~

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

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

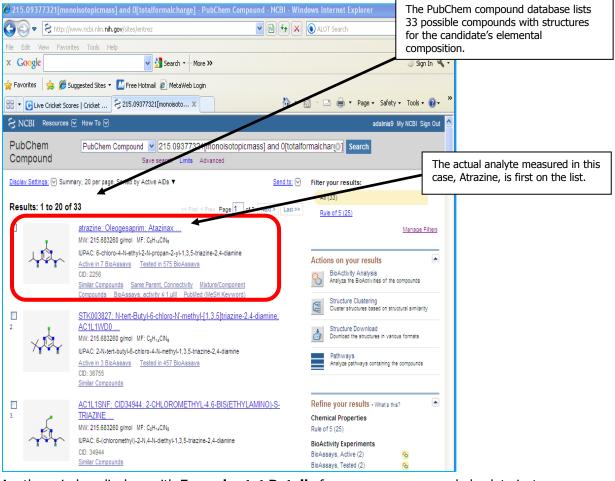
The window shows for each candidate the **ppM error** (between the measured mass and the expected mass) and the **Score** (from 0 to 1.000) for the given elemental composition. The higher the score, the better the match of the candidate to the unknown.



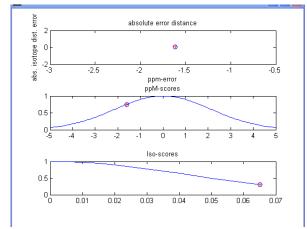
6. To get structures for a given elemental composition, click in the **Details** checkbox, highlight the elemental composition, and click again on it. This opens a new window from the internet browser

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

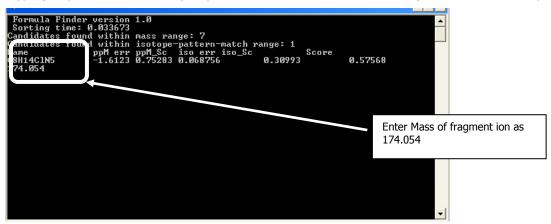
The following example shows a list of 33 compounds having the elemental composition **C8H14CIN5**. The compound analyzed in this case, atrazine, appears first on the list.



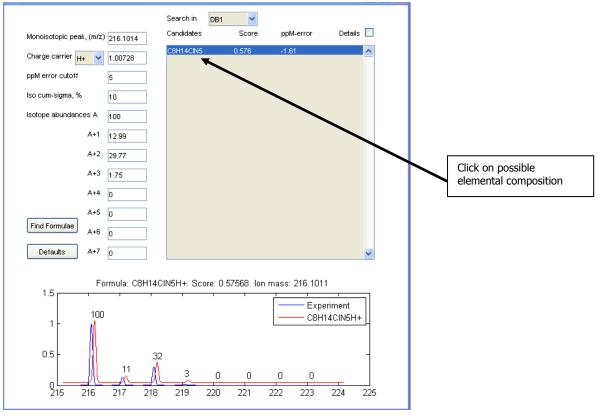
Another window displays with **Formula stat Details** for ppm mass error and absolute isotope distribution error, ppM scores and isoscores.



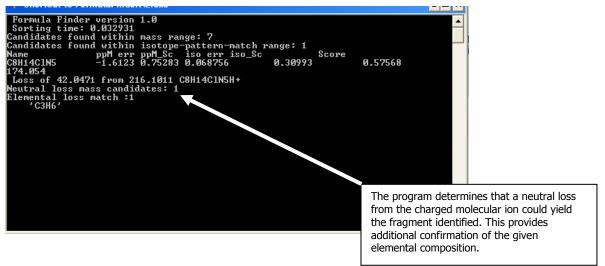
7. If the exact mass of a fragment ion is known, then you can enter it into following window. After typing it, press the **Enter** key to provide further confirmation of the given elemental composition.



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



The following window will appear.



Charge Carrier Options

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

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

Charge Carrier	Mass/Dalton
H ⁺	1.00728
Na ⁺	22.9892
K ⁺	38.9632
+	0.0005
NH ₄ ⁺	18.0338
-H ⁺	-1.00728
Cl	34.9694
Blank	Input mass for adduct such as formate, lithium or mass of an electron for radical anion

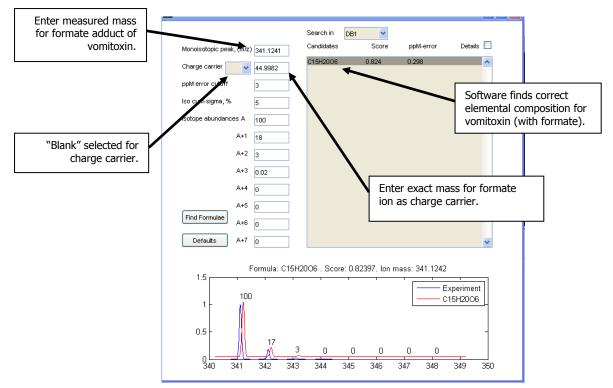
The example below shows the use of a formate ion as a charge carrier during the analysis of vomitoxin by LCMS.

- 1. Select the "blank" option from the charge carrier drop down menu.
- 2. Enter **44.99820** u for the mass of a formate ion in the charge carrier input cell.

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

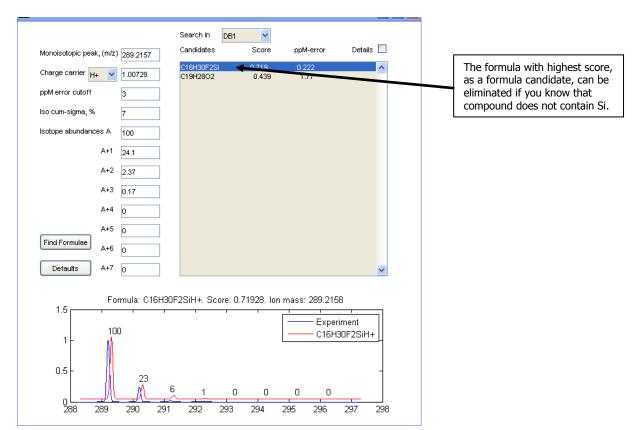
3. Click the Find Formulae button.

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

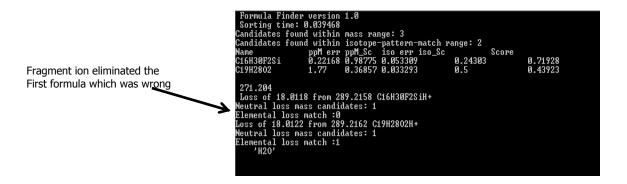


Depending on the measured mass, the ppM error and the sum of errors of relative isotope ratios, the software might identify more than one possible elemental composition. To reduce the number of potential choices, you can use the exact mass of a fragment ion, as demonstrated in the previous example. If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

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



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



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

Importing Chromera Data and Methods

To import the data into Chromera:

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

Тоо	ls Display Help	
5	Export •	- 🗈 🖄 🗰 🕖 🕴 🕮 🐴 🏑 🎚
4	Import 🕨	🛃 TotalChrom Data
	Preferences	🤹 Chromera Results
٤	Report Format Wizard	🥳 Chromera Methods
***	Sequence Wizard	🛃 Chromera Sequences
ş	Device Connections	强 Chromera 1.2 Data
	Error Log	
5	Dictionary Editor	
S	Reprocess	

The **Import Results** dialog appears:

Select batches to	import		
Name	Description	Created	Modified
Server		Import to result datab-	558
Server localhost\SQLEx	press	Import to result datab ChromeraBatchResu	
	press		
localhost\SQLEx	gress		k

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

The **Open** dialog appears:

Open						? 🛛
Look jn:	C ImportExport		*	G 🦻	• 🖭 对	
My Recent Documents						
Desktop						
My Documents						
My Computer						
	File <u>n</u> ame:				~	<u>O</u> pen
My Network	Files of type:	chxb files (*.chxb)			~	Cancel

- 5. Navigate to the directory containing your data.
- 6. Select the **data file** then click **Open**.

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

mport Results			
Source file name	C:\Program Files\Pe	erkinElmer\Chromera\Exar	mple Data\External Stan
Select batches to	import		
Name	Description	Created	Modified
External stds-UT	M-2	3/3/2009 2:14:58 PM	3/3/2009 2:15:25 PM
Server		Import to result	
localhost\SQLEx	press	ChromeraBatc	hResult
Import			Cancel
Messages			
			Close

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

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

port Results		
Source file name	C:\Program Files\Pe	rrkinElmer\Chromera\Example Data\External Stan
Select batches to	import	
Name External stds-UT	Description	Created Modified 3/3/2009 2:14:58 PM 3/3/2009 2:15:25 PM
Server		Import to result database
localhost\SQLEx	DIASS	ChromeraBatchResult
	picss	Cancel
Messages		
Import of results	successful.	Close

8. Click the **Close** button.

Setting the Calibration Vial and the Diverter Valve

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

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

🚺 Manual Tune - ATfrom_Typical Tune F	Pos 8kV
Primary Variables Spectra Per Sec.: 1	Data Acquisition Spectra Acquired: 72
Acq. Function: Pulse	Saved Count: 0
Low m/z: 100	
High m/z: 3000	Spectra Saving is OFF
Ion Polarity: Positive	Acquisition is ON
Calibrate 🎒 Apply	
Ion Source Optics	Trap Enhancement Comments Optics / Flight Tube DAU
Cylinder: -3500 (Volts)	Drying Gas Flow: 8.0
	Drying Gas Heater: 300 📩 (°C)
Capillary Entrance: -6000 (Volts)	RightNeb Gas 80 + (PSI)
Endplate Heater: Off	LeftNeb Gas 0 (PSI)
	APCI Heater: 25
Source Voltage is ON	Diverter Valve: Load
All Gas and Heaters are ON	Calibration Vial: Right

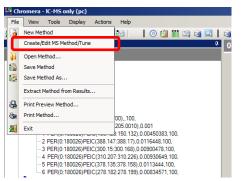
QuickStart Guide for LC-AxION 2 TOF MS Analysis Using "*on the fly" Lockmass*

- 1. Prepare Calibration Mix Reagent with Caffeine as described on page 24.
- 2. Launch the appropriate instrument configuration, then the application, in Chromera Manager.
- 3. Select the **Method** section. Then Select **Tune Control** in the right-side Control Panel. This allows TOF MS Driver software to take control of AxION 2 TOF.

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

Chromera - IC-MS only (pc)			
File View Tools Display Actions Help			
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*	051313-meth1-EXTRA		*
Method			Control Panel
C C Islameth -EXTRA			Direct Control
B T Instruments	Method Name	-	
FX15Pump-1 FXASCD-2 TOF-3	051313-meth1-EXTRA		Start LC Pump
TOF-3			Change Tray
E DA Channels	Group training		Stop MS Equil
B TOF-3	training 💌		View MC
 	Description	· · · · · · · · · · · · · · · · · · ·	C Tune Control
-1 PER(0.180026)PEIC(150.123.150.132),0.00450383,100,	Delcripson		AxION 2
— 2 PER(0.180026)PEIC(388.147.388.17).0.0116448.100.			Calor L
— 3 PER(0:180026)PEIC(300.15:300.168).0.00900478.100. — 4 PER(0:180026)PEIC(310.207:310.226).0.00930649.100.	Notes		
- 5 PER(0.180026)PEIC(310.207.310.226),0.0330645,100,	Twites		
6 PER(0:180026)PEIC(278.182:278.199).0.00834571.100.			
🖯 🛵 Peaks			
E TOF-3 - 0 PERIO:180026/PSCAN(100-700)_100.			
PER(0.180026)/PEIC(204.9990.205.0010).0.001			
1 PER(0:180026)PEIC(150.123:150.132),0.00450383,100,			4
2 PER(0:180026)PEIC(388.147:388.17).0.0116448.100. 3 PER(0:180026)PEIC(300.15:300.168).0.00900478.100.			Status Panel
 – 3 PER(0:180026)PEIC(300.15:300.163),0.00900478,100, – 4 PER(0:180026)PEIC(310.207.310.226),0.00930649,100, 			
-5 PER(0:180026)PEIC(378.135:378.158).0.0113444.100.			Sequence Status Source Door
6 PER(0:180026)PEIC(278.182.278.199).0.00834571.100.			Closed
Calibration			Pump Pressure (Torr)
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- PER(0:180026)PEIC(204.9990.205.0010).0.001			·
— 1 PER(0:180026)PEIC(150.123.150.132),0.00450383,100, — 2 PER(0:180026)PEIC(388.147:388.17),0.0116448,100.			Vacuum State A% ()
- 2 PER(0:160026)PEIC(368.147/368.17).0.0116446.100 - 3 PER(0:180026)PEIC(300.15:300.168).0.00900478.100.			Pumped Down 0.0
4 PER(0:180026)PEIC(310.207:310.226),0.00930649,100,			Current Vial B% ()
- 5 PER(0:180026)PEIC(378.135.378.158).0.0113444.100.			5 0.0
 6 PER(0:180026)PEIC(278:182:278:199).0.00834571,100. B Reports 			MS Analysis Status Operate
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— PER(0:180026)PEIC(204:3590,205:0010),0:001 — 1 PER(0:180026)PEIC(150:123:150.132).0:00450383:100.			Pump Status MS Detector State Shutdown Idle
-2 PER(0:180026)PEIC(388.147.388.17).0.0116448.100.			
- 3 PER(0:180026)PEIC(300.15:300.168),0.00900478,100,			Capillary Entrance Current (nA) Elapsed Time
4 PER(0:180026)PEIC(310.207:310.225).0.00930649,100, 5 PER(0:180026)PEIC(378.135:378.158).0.0113444,100.			0.0 nA
6 PER(0:180026)PEIC(278.182.278.199).0.00834571.100.			End Plate Current (nA) Injection Number
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Sequence			Pump Step Time Pump Elapsed Time
Post Run			0.0 min 0.0 min
~~			APCI Vaporizer Temperature (*C)
Reprocess			549 °C
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4. In Chromera select **File**, then **Create/Edit MS method/Tune**. This opens the TOF MS Driver application.



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

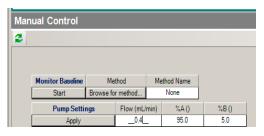
The Application automatically displays the Method Editor with Acquisition Method COM loaded. Close this method without saving.

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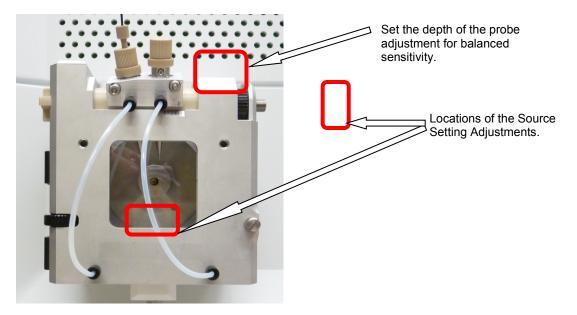
- Select File then Open Manual Tune. Select then Apply the appropriate Tune for the work being done. Check the Mass Range and ensure the Acquisition Rate (labeled Spectra/Sec) is between 3-5. A 3 GHz acquisition rate is more than sufficient as the UHPLC peaks are wide enough (3-4 secs) that sufficient data points are collected over the peak.
- 7. In the Manual Tune window, turn on the Calibrant Vial or Syringe and also turn on Acquisition.
- 8. Once the signal appears steady, select **Calibration** then **Configure** from the main menu. Select the desired calibration points (at least 4) and calibration settings. Execute Calibration and save it if acceptable. See section **Default Calibration** on page 36 for detailed instructions.
- 9. In the manual tune make the following updates for lockmass and save the tune. These changes adjust for HPLC flow of 0.4 mL/min to aid in de-clustering and desolvating the molecules:
 - Increase the Drying Gas Heater to 350-400 °C
 - Change the **Drying Gas Flow** to between 12-14 L/min
- **NOTE**: It is not necessary to set these values back to previous to recalibrate. This is a one-time adjustment to the tune.
 - 10. Move the calibrant inlet to the left probe of the Dual ESI ion source and connect the LC effluent to the right probe of the source.



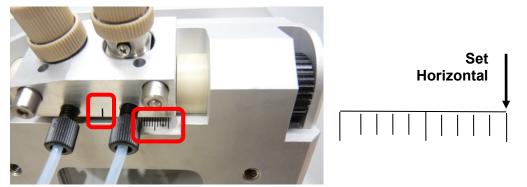
11. Without closing the TOF MS Driver, navigate back to Chromera and start the LC pump. Flow rate should be set to between 0.4 and 0.5 mL/min.



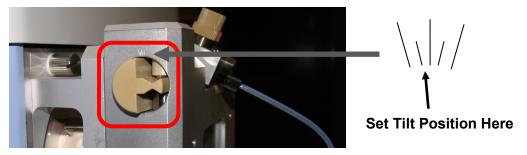
- *IMPORTANT:* Please do not exceed 0.4-0.5 mL/min especially when using a highly aqueous solvent. Greater than recommended flow will result in condensation on end plate. Even using the highest setting on drying gas and source temp the condensation can be quite high. Higher flow rates also diluted the calibrant solution and may make it impossible to get sufficient counts in each spectrum for successful lockmass.
 - 12. Without closing Chromera, navigate back to TOF MS Driver and confirm the lockmass calibrant ions are of sufficient signal counts with the HPLC flow also active.
 - 13. Adjust the positions of the dual sprayers to get the best signal of the analyte and the lockmass calibrants.
 - a. The calibrant ions 195.0876 (from caffeine) and 622.0290 are used as lockmass ions for this particular example. 118.08625 and 922.00979 can also be used as lock mass calibrants instead, however.
 - b. The goal of these adjustments is to ensure the counts on the lock mass calibrants are at least 3000 or greater (as high as 10-20,000 is acceptable). The peak shape is also important i.e. no peak splitting should be seen on top of peak so that centroid of the peak is correctly assigned. Peak splitting usually occurs if the counts are too low.



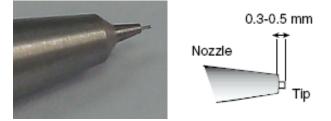
c. The "x" position refers to the horizontal movement of the probes. The position of the probes relative to each other are fixed hence adjusting one probe adjusts the other probe automatically in the "x" direction. The mark on the moving block (on the right side of the right probe) should be roughly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



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



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



- *NOTE:* The process of adjusting the "z" position may have to repeat this a couple times to establish the best position. Once this is done you do not have adjust again unless the signal for the lockmass ions is poor even after x and y positions have been adjusted.
 - 14. Perform a final check of the Lockmass ions for sufficient counts and acceptable peak shape. Ensure the tune file is saved and then close it.
 - 15. From the main TOF MS Driver menu select **New Acquisition Method**.
 - 16. Configure and save the method, associating the tune that was just used in the previous steps. Refer to the section titled **Creating an MS Method**, starting on page 60. Be sure to include the lockmass parameters in the method. The lockmass ions entered are to the accurate 4th decimal place with a search span of 50 mmu.
 - 17. Close TOF MS Driver and navigate back to Chromera.
 - Create a new Chromera Method. Refer to section Creating a Chromera Method, starting on page 68. Ensure the LC run time configured matches the *desired* total time in the TOF acquisition method and that Run Time Reconciliation is checked.

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- 19. Save the Chromera method with a unique name and associate it to a group. Reconcile the TOF method time when prompted.
- 20. Update the settings in Chromera so centroiding is automatically performed on each data file after it is collected.
 - a. Under Tools select Preferences.

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- b. Enable the centroiding function in the AxION2 subsection by selecting **Yes** for **Automatically Centroid**.
- c. Optionally, the incorporation of a substance list in the data files (tofline) for use in AxION SOLO can be enabled. Select **Yes** for **Enable AxION Solo/XPO**. The path for locating the substance list also needs to be specified in **Substance Data Path**. The actual substance list applied is defined per run.

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

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- Optional: Embed a target list in data files for analysis by AxION SOLO. For each run definition, select the AxION SOLO button and navigate to the directory where the substance list xml file is saved. Open the substance list and save. Fill down the column for rest of the samples. This sequence can be saved and then analyzed. The centroided data files embedded with the xml target list when opened in solo will have the target list associated with them.
- 22. Open the sequence to run. Refer to the section titled , starting on page 79. Start the run by selecting the green "play" button.

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