Finnigan™ TSQ® Quantum Ultra™

Getting Started

70111-97122 Revision A March 2006

Analyze • Detect • Measure • Control™

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Finnigan TSQ Quantum Ultra
Getting Started

Revision A
70111-97122

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The manual contains all of the information I need.	1	2	3	4
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EN 61000-3-2	1995, A1; 1998, A2; 1998, A14; 2000	EN 61000-4-4	1995, A1; 2001, A2; 2001
EN 61000-3-3	1998	EN 61000-4-5	1995, A1; 2001
EN 61326-1	1998	EN 61000-4-6	1996, A1; 2001
EN 61000-4-2	2000	EN 61000-4-11	1994, A1; 2001
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Contents

Read This First

Welcome to the Thermo Electron, Finnigan™ TSQ Quantum Ultra™ system! The TSQ Quantum Ultra is a member of the TSQ® family of Finnigan mass spectrometers.

This **TSQ Quantum Ultra Getting Started** manual provides you with information on how to set up, calibrate, and tune the TSQ Quantum Ultra and how to acquire MS data. All of these procedures can be performed from Tune Master.

TSQ Quantum Ultra Getting Started includes the following chapters:

Chapter 1: Introduction answers typical questions about the TSQ Quantum Ultra and lists LC/MS instrument parameters for typical analyses.

Chapter 2: Setting Up the Ion Source for Tuning and Calibrating the Mass Spectrometer provides instructions for setting up the ESI source for automatic tuning and calibrating.

Chapter 3: Tuning and Calibrating the Mass Spectrometer in ESI/MS/MS Mode provides procedures for tuning and calibrating your TSQ Quantum Ultra.

Chapter 4: Optimizing the Mass Spectrometer with Your Compound in ESI/MS/MS Mode describes how to optimize instrument tuning in ESI mode using your compound of interest.

Chapter 5: Acquiring ESI/MS/MS Data with Tune Master describes how to set up the TSQ Quantum Ultra for acquiring SRM data and then describes a simple procedure for acquiring ESI/MS/MS data on your TSQ Quantum Ultra system.

Chapter 6: Setting Up the Ion Source for Acquiring Data in APCI/MS/MS Mode provides instructions for setting up the APCI source for acquiring APCI/MS/MS data.

Chapter 7: Optimizing the Mass Spectrometer with Your Compound in APCI/MS/MS Mode describes how to optimize instrument tuning in APCI mode using your compound of interest.

Chapter 8: Acquiring APCI/MS/MS Data with Tune Master describes how to set up the TSQ Quantum Ultra for acquiring SRM data and then describes a simple procedure for acquiring APCI/MS/MS data on your TSQ Quantum Ultra system.

Appendix A: Solution Formulations provides instructions for preparing solutions that you can use to acquire data with your TSQ Quantum Ultra.

Appendix B: Instrument Method Development Guidelines provides guidelines for developing instrument methods for your TSQ Quantum Ultra. These instructions can be used to set up scan parameters for Full MS, Full MS/MS, SIM MS, SIM MS/MS, or SRM scan types.

Appendix C: Microflow Operation provides operational guidelines for using the microflow ESI option.

Changes to the Manual and Online Help

To suggest changes to this manual or the online Help, please send your comments to:

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You are encouraged to report errors or omissions in the text or index. Thank you.

Abbreviations

The following abbreviations are used in this and other manuals and in the online Help.

A	ampere
ac	alternating current
ADC	analog-to-digital converter
ASCII	American Standard Code for Information Interchange
AU	absorbance unit
b	bit
B	byte (8 b)
baud rate	data transmission speed in events per second
°C	degrees Celsius
CD	compact disc
CD-ROM	compact disc read-only memory
cfm	cubic feet per minute
cm	centimeter
cm^3	cubic centimeter
CPU	central processing unit (of a computer)
<Ctrl>	control key on the terminal keyboard
d	depth
DAC	digital-to-analog converter
dc	direct current
<Enter>	enter key on the terminal keyboard
°F	degrees Fahrenheit
ft	foot
FTP	file transfer protocol
g	gram
G	giga (10^9)
GND	electrical ground
GPIB	general-purpose interface bus
h	hour
h	height
HPLC	high-performance liquid chromatograph
HV	high voltage
Hz	hertz (cycles per second)
ID	inside diameter

IEC	International Electrotechnical Commission
IEEE	Institute of Electrical and Electronics Engineers
in.	inch
k	kilo (10^3, 1000)
K	kilo (2^{10}, 1024)
kg	kilogram
l	length
L	liter
LAN	local area network
lb	pound
LC	liquid chromatograph; liquid chromatography
LC/MS	liquid chromatograph / mass spectrometer
LED	light-emitting diode
µ	micro (10^{-6})
m	meter
m	milli (10^{-3})
M	mega (10^6)
MB	megabyte (1048576 bytes)
min	minute
mL	milliliter
mm	millimeter
nm	nanometer
NIST	National Institute of Standards and Technology (USA)
OD	outside diameter
Ω	ohm
Pa	pascal
PCB	printed circuit board
P/N	part number
P/P	peak-to-peak voltage
ppm	parts per million
psi	pounds per square inch
RAM	random access memory
RF	radio frequency
RMS	root mean square
ROM	read-only memory

RS-232	industry standard for serial communications
s	second
TCP/IP	transmission control protocol / Internet protocol
Torr	torr
URL	uniform resource locator
V	volt
V ac	volts alternating current
V dc	volts direct current
vol	volume
w	width
W	watt
WWW	World Wide Web

Note Exponents are written as superscripts. In the corresponding online Help, exponents are sometimes written with a caret (^) or with *e* notation because of design constraints in the online Help. For example:
MS^n (in this manual) MS^n (in the online Help)
10^5 (in this manual) 10^5 (in the online Help) ▲

Typographical Conventions

Typographical conventions have been established for Thermo Electron San Jose manuals for the following:

- Data input
- Boxed information
- Topic headings

Data Input

Throughout this manual, the following conventions indicate data input and output by way of the computer:

- Messages displayed on the screen are represented by capitalizing the initial letter of each word and by italicizing each word.
- Input that you enter by keyboard is enclosed in double or single quotes.
- For brevity, expressions such as "choose **File > Directories**" are used rather than "pull down the File menu and choose Directories."
- Any command enclosed in angle brackets < > represents a single keystroke. For example, "press <F1>" means press the key labeled *F1*.
- Any command that requires pressing two or more keys simultaneously is shown with a plus sign connecting the keys. For example, "press **<Shift> + <F1>**" means press and hold the <Shift> key and then press the <F1> key.
- Any button that you click the screen is represented in boldface letters and a different font. For example, "click **Close**".

Boxed Information

Information that is important but not part of the main flow of text is displayed in a box such as the one below.

Note Boxes such as this are used to display information. ▲

Boxed information can be of the following types:

- **Note** – information that can affect the quality of your data. In addition, notes often contain information that you might need if you are having trouble.
- **Tip** – helpful information that can make a task easier.

- **Important** – critical information that can affect the quality of your data.
- **Caution** – information necessary to protect your instrument from damage.
- **CAUTION** – hazards to human beings. Each CAUTION is accompanied by a CAUTION symbol. Each hardware manual has a blue CAUTION sheet that lists the CAUTION symbols and their meanings.
- **DANGER** – laser-related hazards to human beings. It includes information specific to the class of laser involved. Each DANGER is accompanied by the international laser radiation symbol.

Topic Headings

The following headings are used to show the organization of topics within a chapter:

Chapter 1 Chapter Name

Second Level Topics

Third Level Topics

Fourth Level Topics

Reply Cards

Thermo Electron San Jose manuals contain one or two reply cards. All manuals contain a Customer Registration / Reader Survey card and some contain a Change of Location card. These cards are located at the front of each manual.

The Customer Registration / Reader Survey card has two functions. First, when you return the card, you are placed on the Thermo Electron San Jose mailing list. As a member of this list, you receive application reports and technical reports in your area of interest, and you are notified of events of interest, such as user meetings. Second, it allows you to tell us what you like and do not like about the manual.

The Change of Location card allows us to track the whereabouts of the instrument. Fill out and return the card if you move the instrument to another site within your company or if you sell the instrument. Occasionally, we need to notify owners of our products about safety or other issues.

Chapter 1 Introduction

The TSQ Quantum Ultra™ is a member of the TSQ® family of Finnigan™ mass spectrometers. The TSQ Quantum Ultra is a high performance triple stage quadrupole mass spectrometer. It includes a syringe pump, a divert/inject valve, an atmospheric pressure ionization (API) source, and the Xcalibur® data system. In a typical analysis, a sample can be introduced in any of the following ways:

- Using the syringe pump without the divert/inject valve or an LC system (direct injection). The syringe pump can be connected directly to the ion source to provide a steady state introduction of sample or tuning and calibration solution.
- Using the syringe pump and an LC system without the divert/inject valve (infusion into LC flow). The syringe pump can be used to infuse sample into the flow of mobile phase from an LC system.
- Using the inject valve fitted with a loop and an LC system (flow injection analysis). A syringe pump can be used to fill the loop (auto loop injection) or you can manually fill the loop (manual loop injection).
- Using the divert valve and an LC system fitted with an analytical column. The data system can be configured to divert the solvent flow to waste to avoid unnecessary contamination of the mass spectrometer with undesired sample materials.
- Using an LC system without the divert/inject valve. The LC system can be connected directly to the ion source to reduce LC system void volume.

In analysis by LC/MS, a sample is injected onto an LC column. The sample is then separated into its various components. The components elute from the LC column and pass into the mass spectrometer where they are analyzed. Analysis by direct infusion or flow injection provides no chromatographic separation of components in the sample before it passes into the mass spectrometer. The data from the mass spectrometer are then stored and processed by the Xcalibur data system.

This introduction answers the following questions:

- Is H-ESI or APCI better for analyzing my samples?
- How can I introduce my samples into the mass spectrometer?

- What types of buffers do I use?
- How do I set up the mass spectrometer for various LC flow rates?

Is H-ESI or APCI Better for Analyzing My Samples?

You can operate the mass spectrometer in either of two atmospheric pressure ionization modes:

- Heated-electrospray ionization (H-ESI)
- Atmospheric pressure chemical ionization (APCI)

Typically, polar compounds such as amines, peptides, and proteins are best analyzed by H-ESI, and non-polar compounds such as steroids are best analyzed by APCI.

Sample ions can carry a single charge or multiple charges. The number of charges carried by the sample ions depends on the structure of the analyte of interest, the mobile phase, and the ionization mode.

Using H-ESI/MS

The *H-ESI* mode generally produces mass spectra consisting of singly-charged ions, but it depends on the structure of the analyte and the solvent. When multiply-charged ions are produced, the resulting mass spectrum can be mathematically transformed to express the molecular weight of the sample.

The H-ESI mode transfers ions in solution into the gas phase. Many samples that previously were not suitable for mass analysis (for example, heat-labile compounds or high molecular weight compounds) can be analyzed by H-ESI. H-ESI can be used to analyze any polar compound that makes a preformed ion in solution. The term *preformed ion* can include adduct ions. For example, polyethylene glycols can be analyzed from a solution containing ammonium acetate because of adduct formation between the NH_4^+ ions in the solution and oxygen atoms in the polymer. With H-ESI, the TSQ Quantum Ultra can analyze compounds with molecular weights that are greater than 100,000 u because of multiple charging. H-ESI is especially useful for the mass analysis of polar compounds, which include biological polymers (for example, proteins, peptides, glycoproteins, and nucleotides); pharmaceuticals and their metabolites; and industrial polymers.

You can use the H-ESI mode in either positive or negative ion polarity mode. The ion polarity mode is determined by the polarity of the preformed ions in solution: Acidic molecules form negative ions in high pH solution, and basic molecules form positive ions in low pH solution. A positively-charged H-ESI needle is used to generate positive ions and a negatively-charged needle is used to generate negative ions.

You can vary the flow rate from the LC into the mass spectrometer from less than 1 µL/min (using the Nanospray ion source) to 1000 µL/min (using the standard H-ESI source). Refer to Table 1-2. (In H-ESI, the buffer and the buffer strength both have a noticeable effect on sensitivity. Therefore, it is important to choose these variables correctly.) In the case of higher molecular weight proteins or peptides, the resulting mass spectrum typically consists of a series of peaks corresponding to a distribution of multiply-charged analyte ions.

The H-ESI process is affected by droplet size, surface charge, liquid surface tension, solvent volatility, and ion solvation strength. Large droplets with high surface tension, low volatility, strong ion solvation, low surface charge, and high conductivity prevent good electrospray.

Mixed organic–aqueous solvent systems that include organic solvents such as methanol, acetonitrile, and isopropyl alcohol are superior to water alone for H-ESI. Volatile acids and bases are good, but salts above 10 mM are not recommended. Strong mineral acids and bases are extremely detrimental to the instrument.

Recommendations for generating stable electrospray are as follows:

- Refrain from using non-volatile salts and buffers in the solvent system. For example, avoid the use of salts containing sodium or potassium and avoid the use of phosphates. If necessary, use ammonium salts instead.
- Use organic–aqueous solvent systems.
- Use volatile acids and bases.
- If possible, optimize the pH of the solvent system for your analyte of interest. For example, if your analyte of interest contains a primary or secondary amine, your mobile phase should be acidic (pH 2 to 5). The acidic pH tends to keep positive ions in solution.

Using APCI/MS

Like H-ESI, ***APCI*** is a soft ionization technique. APCI provides molecular weight information for compounds of low polarity that are somewhat volatile. APCI is typically used to analyze small molecules with molecular weights up to about 1000 u.

APCI is a gas phase ionization technique. Therefore, the gas phase acidities and basicities of the analyte and solvent vapor play an important role in the APCI process.

The rate of solvent flowing from the LC into the mass spectrometer in APCI mode is in the range of 0.2 and 2 mL/min. Refer to Table 1-3.

You can use APCI in positive or negative ion polarity mode. Molecules with basic sites produce a strong ion current in positive ion mode. Molecules with acidic sites, such as carboxylic acids and acid alcohols, produce a strong ion current in negative ion mode.

Although, in general, fewer negative ions are produced than positive ions, the negative ion polarity mode can be more specific. This is because the negative ion polarity mode typically generates less chemical noise than does the positive mode. Thus, the signal-to-noise ratio might be better in the negative ion mode than in the positive ion mode.

How Can I Introduce My Samples into the Mass Spectrometer?

You can introduce your samples into the mass spectrometer in a variety of ways. Refer to Table 1-1.

Table 1-1. Techniques for introducing samples into the mass spectrometer

	Sample Introduction Technique	Analytical Technique	Figure Reference
Direct Injection	Direct injection (with syringe pump)*	H-ESI automatic tuning and calibrating H-ESI analysis of a pure analyte in solution	Figure 3-1
LC Flow Without Chromatographic Separation	Auto loop injection into LC flow (with syringe pump)	H-ESI or APCI automatic optimization with compound of interest H-ESI or APCI analysis of a pure analyte in solution	Figure 4-1 (H-ESI) Figure 7-1 (APCI)
	Manual loop injection into LC flow	H-ESI or APCI automatic optimization of tune with analyte of interest H-ESI or APCI analysis of a pure analyte in solution	Figure 5-1 (H-ESI) Figure 8-1 (APCI)
	Infusion into LC flow (with syringe pump)*	H-ESI or APCI analysis of a pure analyte in solution	"Chapter 12: Making Plumbing Connections to Run Samples on the TSQ Quantum Ultra" in *Finnigan TSQ Quantum Ultra Getting Connected*
	Autosampler injections into LC flow	H-ESI or APCI analysis of one or more solutions	
LC Flow With Chromatographic Separation	Autosampler injections onto a column via LC flow	H-ESI or APCI analysis of one or more mixtures	

* Provides steady state introduction of sample into mass spectrometer

Note To find additional plumbing diagrams for alternative ion source / sample introduction combinations, refer to "Chapter 12: Making Plumbing Connections to Run Samples on the TSQ Quantum Ultra" in *Finnigan TSQ Quantum Ultra Getting Connected.* ▲

Note Compound optimization solutions, such as the reserpine sample solution, can contaminate your system at high concentrations. Therefore, the recommended technique for introducing optimization solutions into the mass spectrometer is the auto loop injection into LC flow technique. ▲

The syringe pump is often used to introduce tuning and calibration solution for automatic tuning and calibrating in H-ESI mode. You can also use this technique to introduce a solution of pure analyte at a steady rate in H-ESI or APCI mode.

You can also use a tee union to direct samples from the syringe pump into an LC flow (either with or without a column), which then enters the mass spectrometer. This technique is used to introduce sample at a steady rate and at higher solvent flow rates. It is used especially for optimizing tune parameters in H-ESI or APCI mode on an analyte of interest. You can also use this technique to introduce a solution of pure analyte at a steady rate in H-ESI or APCI mode.

You can introduce samples from a syringe into the loop of the divert/inject valve. You can then use the divert/valve to introduce the sample into an LC flow, which then enters the mass spectrometer. This technique is used in H-ESI or APCI mode to introduce pure analytes into the mass spectrometer in a slug. It is useful when you have a limited quantity of pure analyte.

You can also use an LC autosampler to introduce samples into an LC flow. This technique is also used in H-ESI or APCI mode to introduce a solution of pure analyte into the mass spectrometer in a slug.

Finally, you can use an LC autosampler to introduce a mixture onto an LC column. This technique is used with H-ESI or APCI to separate the analytes before they are introduced sequentially into the mass spectrometer.

Refer to subsequent chapters in this manual and to *Finnigan TSQ Quantum Ultra Getting Connected* for plumbing diagrams for the various methods of sample introduction.

What Types of Buffers Do I Use?

We recommend the use of volatile buffers, when possible, to obtain the highest performance for your assays. Many volatile buffer solutions are available that can be used instead of nonvolatile ones. Volatile buffer solutions can include the following:

- Acetic acid
- Ammonium acetate
- Ammonium formate
- Ammonium hydroxide
- Triethylamine (TEA)
- Trifluoroacetic acid (TFA)

Some LC applications use nonvolatile buffers such as phosphate or borate buffers. However, the use of nonvolatile buffers can lead to a buildup of salt in the ion source and can cause a loss of sensitivity.

For LC applications that require nonvolatile buffers use the following guidelines for best performance:

- Optimize probe position.
- Install the optional ion sweep cone.
- Reduce the concentration of buffers to an absolute minimum.

Note You may need to increase the frequency of ion source maintenance when using nonvolatile buffers. ▲

How Do I Set Up the Mass Spectrometer for Various LC Flow Rates?

The H-ESI probe can generate ions from liquid flows of 1 µL/min to 1.0 mL/min. This flow rate range allows you to use a variety of separation techniques: capillary LC, microbore LC, and analytical LC. An optional nanospray ion source is available for allowing sub-microliter analysis.The APCI probe can generate ions from liquid flows as low as 50 µL/min, but typical flow rates are from 0.2 to 2.0 mL/min. Within this range of flow rates, you can use the following separation techniques: microbore LC, analytical LC, and semi-preparative LC.

As you change the rate of flow of solvents entering the mass spectrometer, you need to adjust several of the mass spectrometer parameters, as follows:

- For H-ESI, you need to adjust the temperatures of the H-ESI vaporizer and ion transfer tube and adjust the gas flow rates for the sheath gas and auxiliary gas.
- For APCI, you need to adjust the temperature of the ion transfer tube and the vaporizer and adjust the gas flow rates for the sheath gas and auxiliary gas.

In general, the higher the rate of liquid flowing into the mass spectrometer, the higher the temperature of the ion transfer tube (and vaporizer) and the higher the gas flows.

Table 1-2 provides guidelines for setting H-ESI operating parameters for various LC solvent flow rates. Table 1-3 provides guidelines for setting the operating parameters for APCI.

Table 1-2. Guidelines for setting operating parameters for LC/H-ESI/MS (compound dependent)

LC Flow Rate (µL/min)	Suggested Column ID Size (mm)	H-ESI Vaporizer Temperature (°C)	Spray Voltage (V)	Capillary Temperature (°C)	Sheath Gas (psi)	Auxiliary Gas (arbitrary units)
≤ 10	Capillary	0 (off) to 50	3000 (-2500*)	200 to 250	5 to 30	Off
50 to 100	1.0	50 to 200	3000 (-2500)	250 to 300	10 to 30	5 to 10
200 to 400	2.1 to 4.6	200 to 400	3000 (-2500)	300 to 350	20 to 40	10 to 20
≥ 400	4.6	300 to 450	3000 (-2500)	350	30 to 60	10 to 40

*negative ion mode

Table 1-3. Guidelines for setting operating parameters for LC/APCI/MS

LC Flow Rate (mL/min)	Capillary Temperature (°C)	APCI Vaporizer Temperature (°C)	Sheath Gas (psi)	Auxiliary Gas (arbitrary units)	Corona Discharge Current (µA)
0.2 to 2.0	200 to 350	400 to 600	30 to 40	0 to 5	+4 (-10*)

*negative ion mode

Chapter 2 Setting Up the Ion Source for Tuning and Calibrating the Mass Spectrometer

This chapter provides information on setting up the hardware for tuning and calibrating your TSQ Quantum Ultra. You tune and calibrate in H-ESI mode before you acquire data in either the H-ESI or the APCI mode.

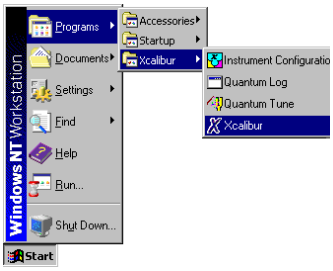
This chapter contains the following topics:

- Placing the LC/MS System in Standby
- Removing the APCI Probe
- Removing the Ion Max Ion Source Housing (optional)
- Installing the Ion Sweep Cone (optional)
- Installing the Ion Max Ion Source Housing
- Installing the H-ESI Probe

Placing the LC/MS System in Standby

The LC/MS system needs to be placed in Standby condition before you can remove the ion source. Use the following procedure to place the LC/MS system in Standby:

1. If necessary, stop the flow of solvent to the API source, as follows:



   a. If Xcalibur is not already open, choose **Start > Programs > Xcalibur > Xcalibur** from the Windows® taskbar to open the Xcalibur window.

   b. In the Xcalibur Home Page window – Roadmap view, choose **GoTo > Instrument Setup** to open the Instrument Setup window.

   c. Click on the Surveyor® MS Pump button on the view bar in the Instrument Setup window to display the Surveyor MS Pump view.

   d. Choose **Surveyor MS Pump > Direct Control** to open the Direct Control dialog box.

   e. In the Direct Control dialog box, click on the Stop button on the toolbar to stop the LC pump.

2. If Tune Master is not already open, choose **Start > Programs > Xcalibur > Quantum Tune** from the taskbar to open Tune Master. See Figure 2-1.

   You can determine the state of the mass spectrometer by observing the state of the On/Standby button on the Control / Scan Mode toolbar. (The three different states of the On/Standby button are shown at the left.)

3. If the mass spectrometer is On, click on the On/Standby button to place the mass spectrometer in Standby mode. When the mass spectrometer is in Standby, TSQ Quantum Ultra turns Off the ion source sheath gas, auxiliary gas, and high voltage.

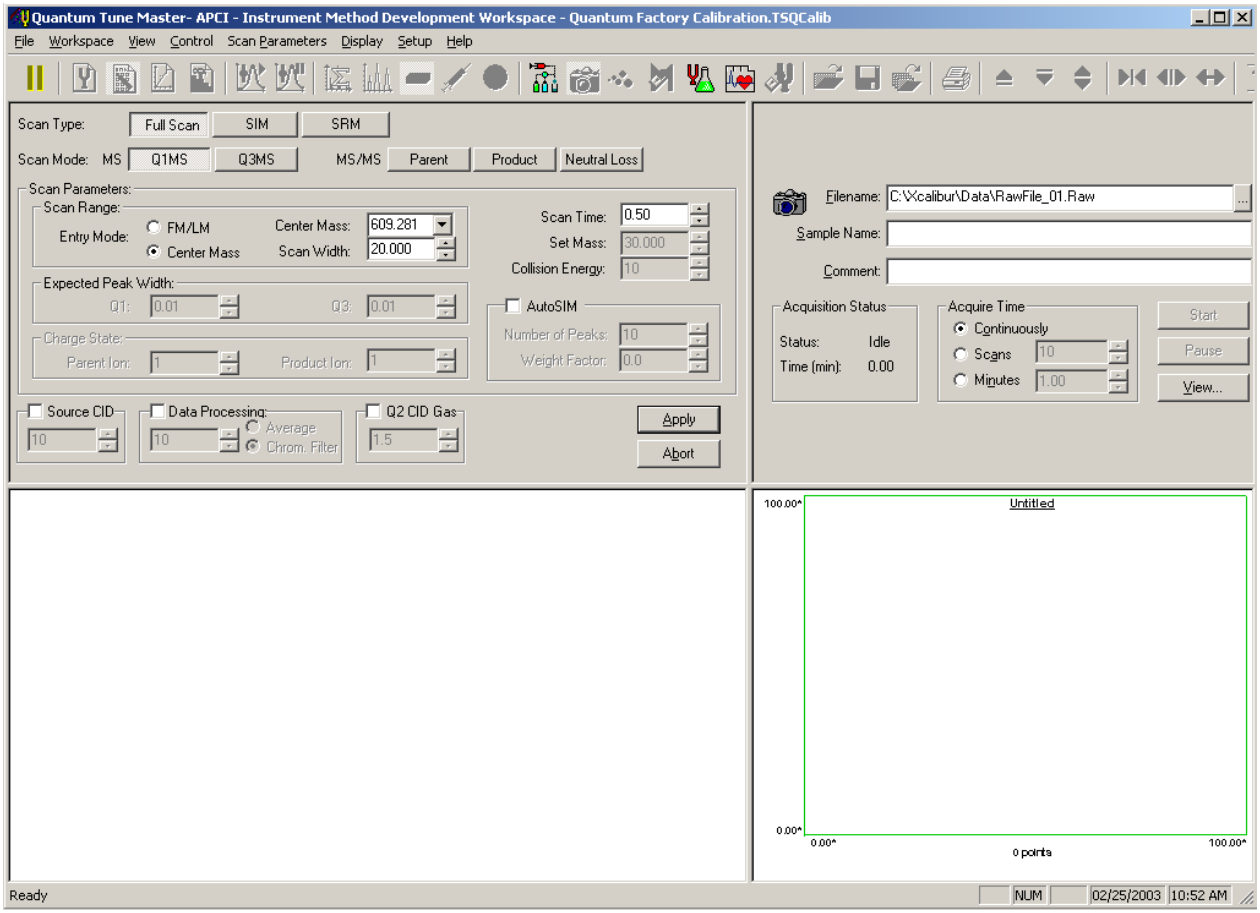


Figure 2-1. Tune Master window – Instrument Method Development workspace, showing the mass spectrometer in the Standby mode

The LC/MS system is now in Standby, and it is safe to remove the ion source.

Go to the next topic: “Removing the APCI Probe”.

Removing the APCI Probe

This topic describes how to remove the APCI probe.

Note The following procedures assume that you are familiar with your instrument and software. If you need additional guidance, refer to TSQ Quantum Ultra online Help, *Finnigan TSQ Quantum Ultra Getting Connected*, *Finnigan Ion Max API Source Hardware Manual*, or the *Finnigan H-ESI Probe Operator's Manual*. ▲

Remove the APCI probe as follows:

1. Unplug the vaporizer heater cable from the vaporizer heater cable socket on the APCI probe. See Figure 2-2.

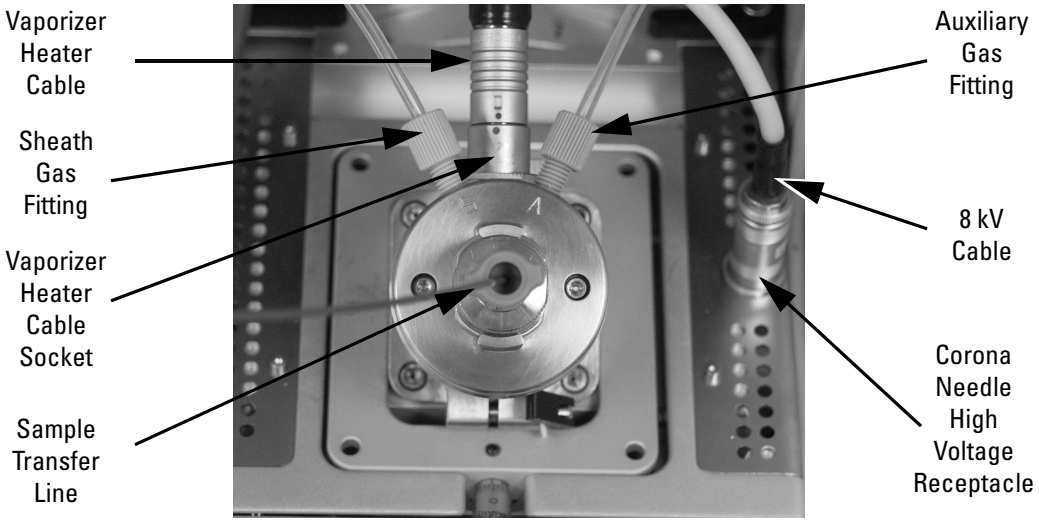


Figure 2-2. Ion Max with APCI probe installed

2. Connect the vaporizer heater cable to the ESI interlock socket on the ion source housing. See Figure 2-3.

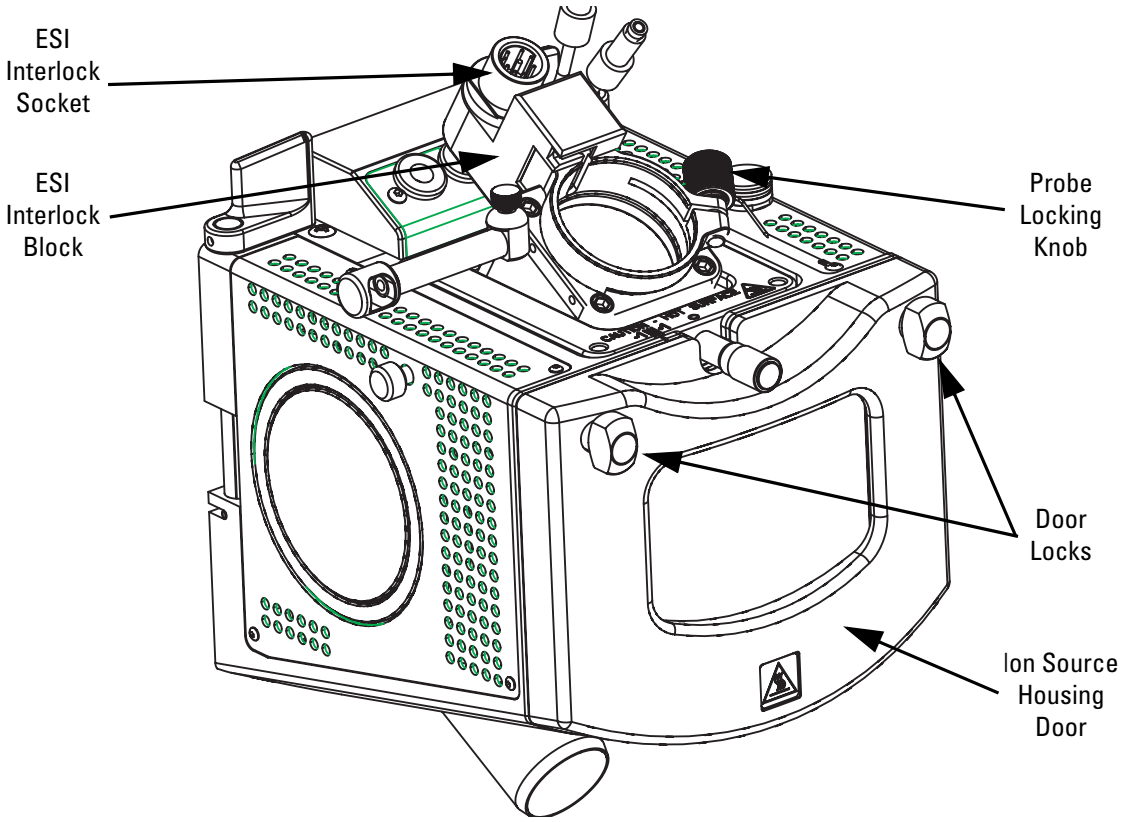


Figure 2-3. Ion Max, detail of components

3. Disconnect the sample transfer line from the APCI probe. See Figure 2-2.

4. Remove the Auxiliary gas line (green colored fitting) from the APCI probe.

5. Remove the Sheath gas line (blue colored fitting) from the APCI probe.

CAUTION AVOID BURNS. At operating temperatures, the APCI vaporizer can severely burn you! The APCI vaporizer typically operates between 400 and 600 ºC. **Always allow the heated vaporizer to cool to room temperature (for approximately 20 min) before you touch or remove this component.** ▲

6. Remove the APCI probe as follows:

    a. Loosen the probe locking ring by turning the probe locking knob counterclockwise.

    b. Carefully pull the probe straight back in the port in the housing until it meets with the slot in the ESI interlock block. The guide pin on the probe manifold will prevent you from twisting the probe until the pin is aligned with the slot in the ESI interlock block. Once the probe is all the way back and aligned with the slot, turn the probe 45 degrees counter-clockwise to free the probe from the alignment notch. Be careful not to break the fused-silica sample tube or PEEK safety sleeve.

    c. Pull the probe straight out to remove it from the ion source housing.

    d. Store the APCI probe in its original shipping container.

7. Remove the 8 kV cable from the corona needle high voltage receptacle as follows:

    a. Unlock the cable by twisting the locking ring counter-clockwise.

    b. Unplug the 8 kV cable from the corona needle high voltage receptacle.

CAUTION AVOID INJURY. The corona discharge needle is very sharp and can puncture your skin. Handle it with care. ▲

8. Remove the corona needle as follows:

    a. Unlock the ion source housing door by turning the locks 90 degrees so that the knobs are horizontal.

    b. Open the ion source housing door.

    c. Using pliers, grasp the needle by the gold plated contact and pull the needle straight out of the socket. See Figure 2-4.

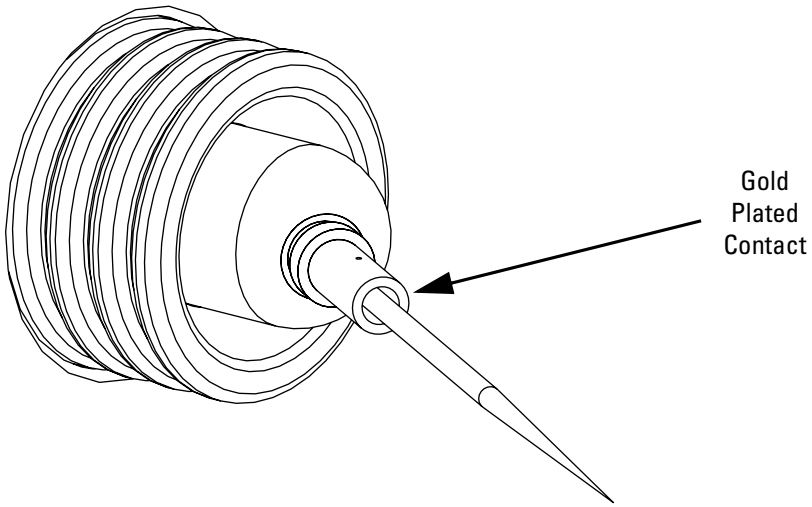


Figure 2-4. Corona needle, view from rear

d. Close and lock the ion source housing door.

9. Store the corona needle in its original shipping container.

If you want to install the optional ion sweep cone, go to the next topic: "Removing the Ion Max Ion Source Housing".

If you do not want to install the ion sweep cone, go to the topic: "Installing the H-ESI Probe".

Removing the Ion Max Ion Source Housing

You need to remove the Ion Max ion source housing to access the ion sweep cone.

Note If an ion source probe is still installed in the ion source housing, the external liquid lines should first be disconnected before removing the ion source housing. ▲

Remove the ion source housing as follows:

1. Remove the drain tube from the ion source housing drain. See Figure 2-5.

2. Rotate the ion source housing locking levers 90 degrees to release the ion source housing from the ion source mount assembly.

3. Remove the ion source housing by pulling straight off of the ion source mount assembly, and place the housing in a safe location for temporary storage.

Go to the next topic: “Installing the Ion Sweep Cone”.

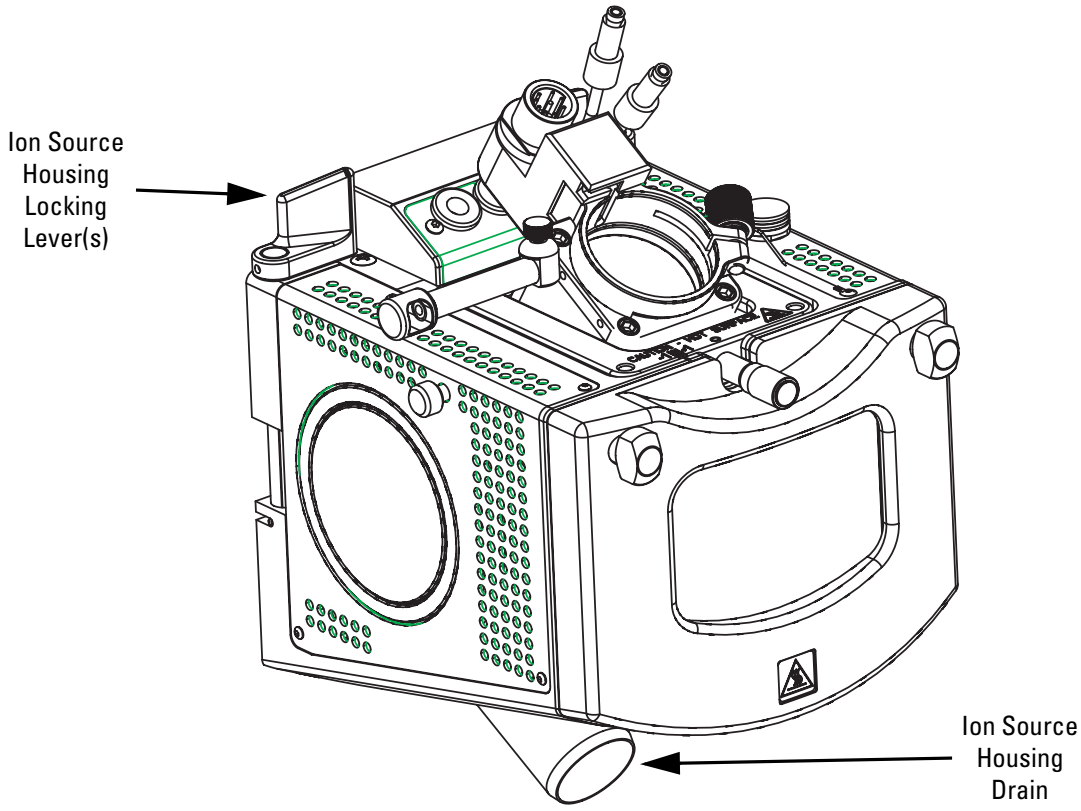


Figure 2-5. Ion Max, detail of components

Installing the Ion Sweep Cone

The ***ion sweep cone*** is a metallic cone over the capillary. The ion sweep cone channels the sweep gas towards the entrance of the capillary. This helps to keep the entrance of the ion transfer tube free of contaminants. The net result is a significant increase in the number of samples that can be analyzed without a loss of signal intensity. In addition, keeping the capillary entrance cleaner reduces the need for ion source maintenance.

CAUTION AVOID BURNS. At operating temperatures, the ion transfer tube can severely burn you! The ion transfer tube typically operates between 200 and 400 °C. **Always allow the ion transfer tube to cool to room temperature (for approximately 20 min) before you attempt to install the ion sweep cone.** Always be careful not to touch the entrance end of the ion transfer tube when it is exposed. ▲

Install the ion sweep cone as follows:

1. Remove the ion sweep cone from its storage container. Inspect and clean it if necessary.

2. Note the location of the sweep gas supply port in the API cone seal. The gas inlet on the ion sweep cone is placed in this port. See Figure 2-6 and Figure 2-7.

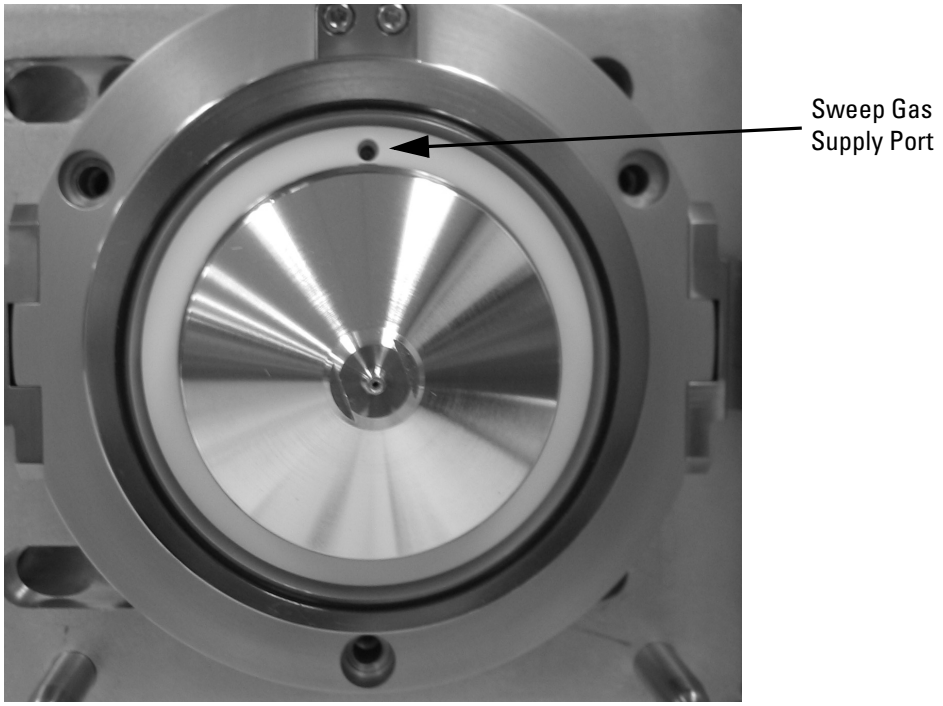


Figure 2-6. Capillary heater cage, showing the sweep gas supply port in the API cone seal

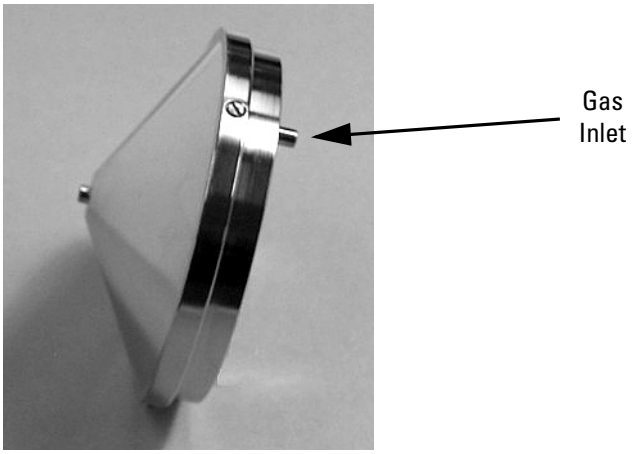


Figure 2-7. Ion sweep cone, showing the gas inlet

CAUTION AVOID BURNS. At operating temperatures, the ion transfer tube can severely burn you! The ion transfer tube typically operates between 200 and 400 °C. **Always allow the ion transfer tube to cool to room temperature (for approximately 20 min) before you install the ion sweep cone.** Always be careful not to touch the entrance end of the ion transfer tube when it is exposed. ▲

3. After the ion transfer tube has cooled to room temperature, carefully align the gas inlet on the ion sweep cone with the sweep gas supply port on the ion source mount. Firmly press the ion sweep cone into position.

The ion sweep cone is now properly installed on the mass spectrometer.

Go to the next topic: “Installing the Ion Max Ion Source Housing”.

Installing the Ion Max Ion Source Housing

Reinstall the Ion Max ion source housing as follows:

1. Carefully align the two guide pin holes on the rear of the ion source housing with the ion source housing guide pins on the mass spectrometer, and carefully press the ion source housing onto the ion source mount. See Figure 2-8 and Figure 2-9.

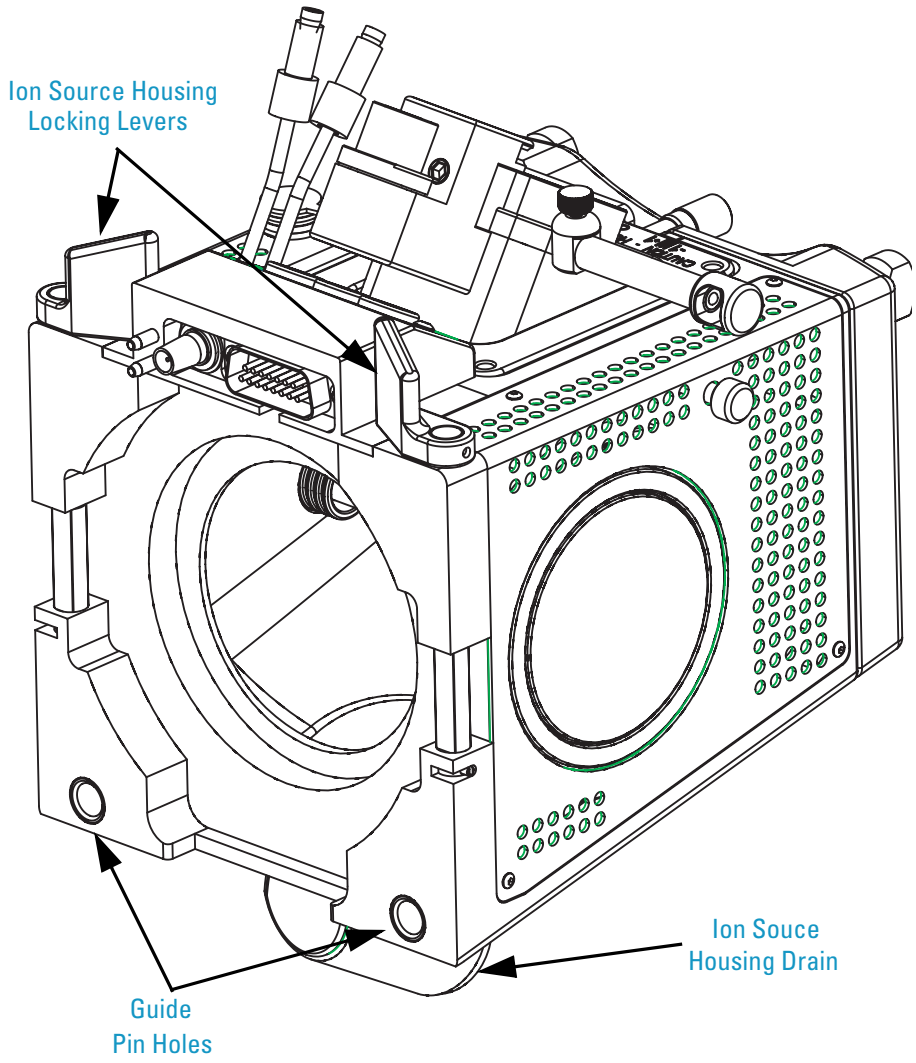


Figure 2-8. Rear view of the Ion Max ion source housing

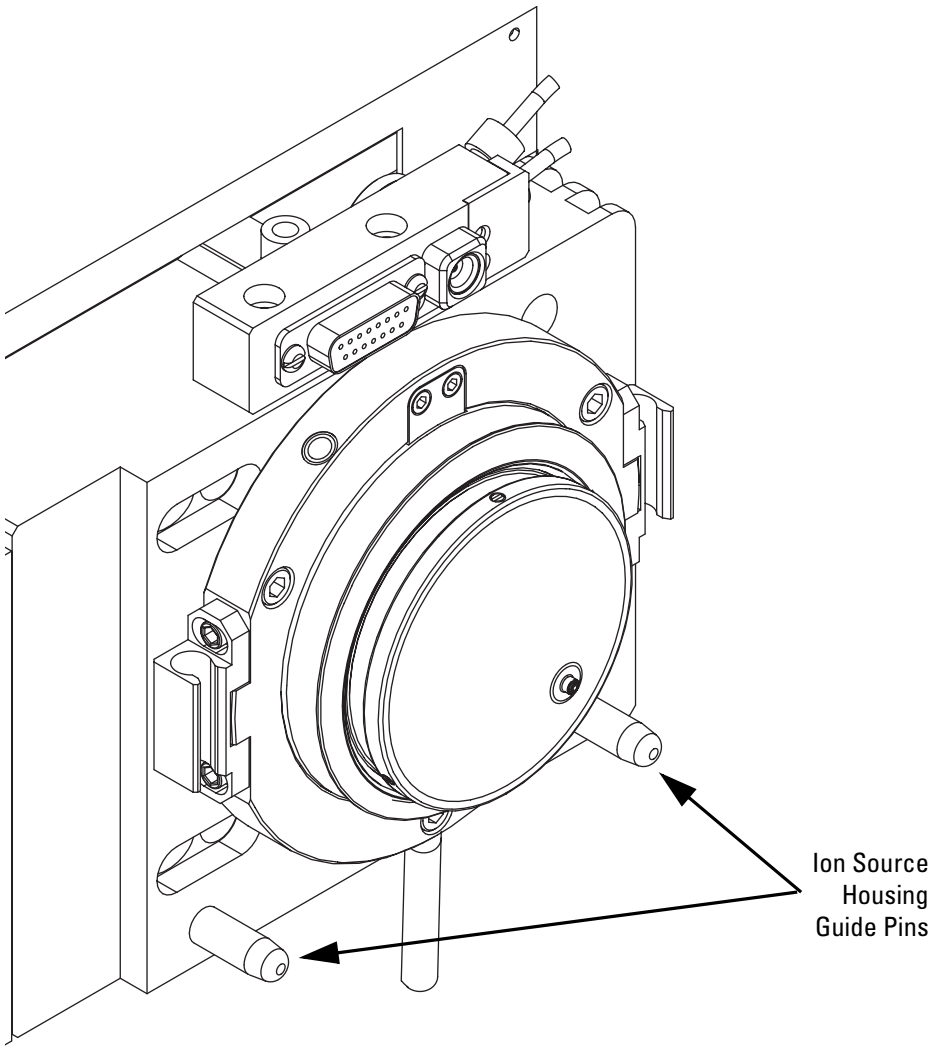


Figure 2-9. Ion source mount showing ion source housing guide pins

2. Rotate the ion source housing locking levers 90 degrees to lock the ion source housing onto the ion source mount assembly.

Caution Prevent solvent waste from backing up into the ion source and mass spectrometer. Always ensure that liquid in the drain tube is able to drain to a waste container. ▲

3. Reinstall the ion source drain tube as follows:

Caution Do **not** vent the API source drain tube (or any vent tubing connected to the waste container) to the same fume exhaust system to which you have connected the forepumps. The analyzer optics can become contaminated if the API source drain tube and the (blue) forepump exhaust tubing are connected to the same fume exhaust system.

Your laboratory must be equipped with at least two fume exhaust systems. Route the (blue) forepump exhaust tubing to a dedicated fume exhaust system. Route the drain tube from the API source to a waste container. Vent the waste container to a dedicated fume exhaust system. ▲

   a. Connect the 1-in. ID Tygon® tubing (P/N 00301-22922) to the ion source housing drain fitting.

   b. Attach the free end of the hose to a dedicated drain system. Ideally, the drain system should be vented to a fume exhaust system.

The Ion Max is now properly installed on the mass spectrometer.

Go to the next topic: “Installing the H-ESI Probe”.

Installing the H-ESI Probe

Install the H-ESI probe as follows:

1. Remove the H-ESI probe from its storage container. Inspect and clean it if necessary.

Note If your H-ESI probe does not already have a sample tube (fused-silica capillary) and safety sleeve attached, you need to follow the procedure for installing a sample tube and PEEK safety sleeve in the topic "Installing a New Fused-Silica Sample Tube and PEEK Safety Sleeve" on page 4-6 of the *Finnigan H-ESI Probe Operator's Manual*.▲

2. Ensure that the probe locking ring is opened to its widest position. See Figure 2-10.

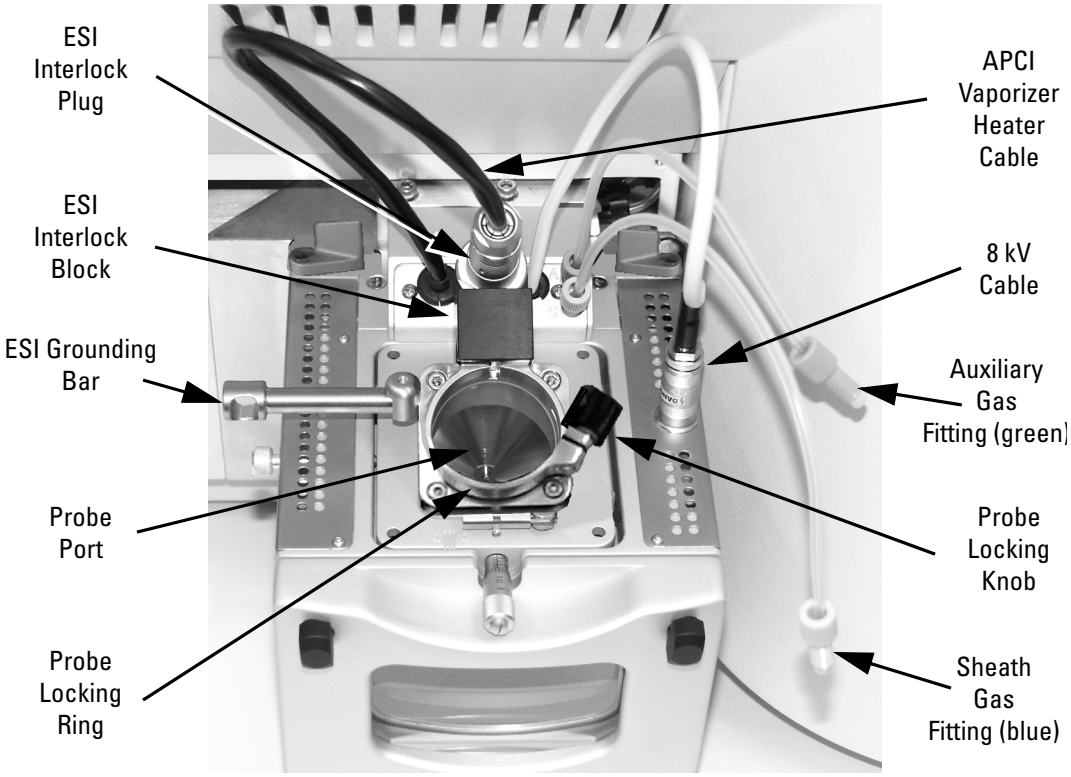


Figure 2-10. Ion Max ion source housing, probe locking lever open

3. Insert the H-ESI probe into the port in the ion source housing, align the guide pin (see Figure 2-11) on the probe body at a minus 45 degree angle from the ESI interlock block.

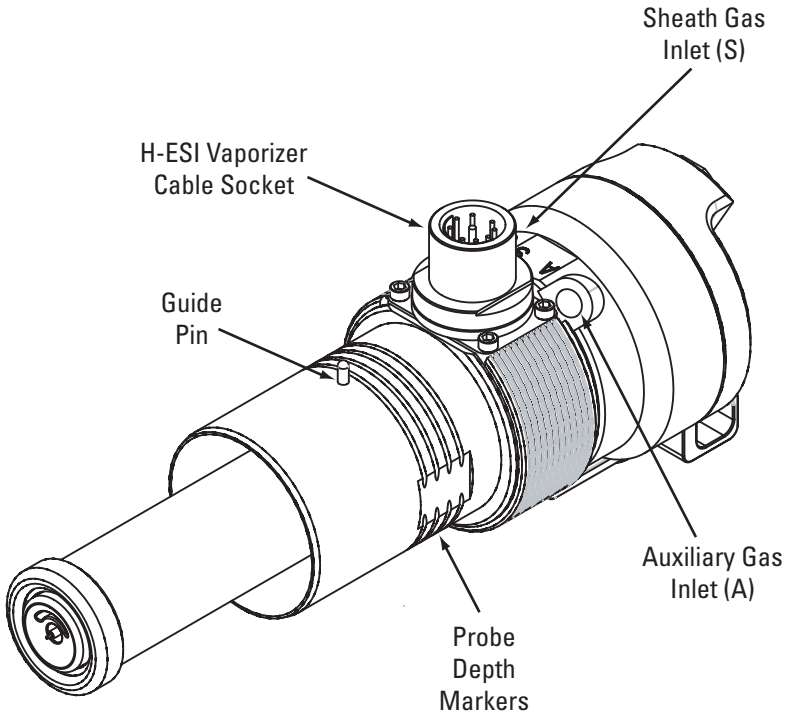


Figure 2-11. H-ESI probe, front view

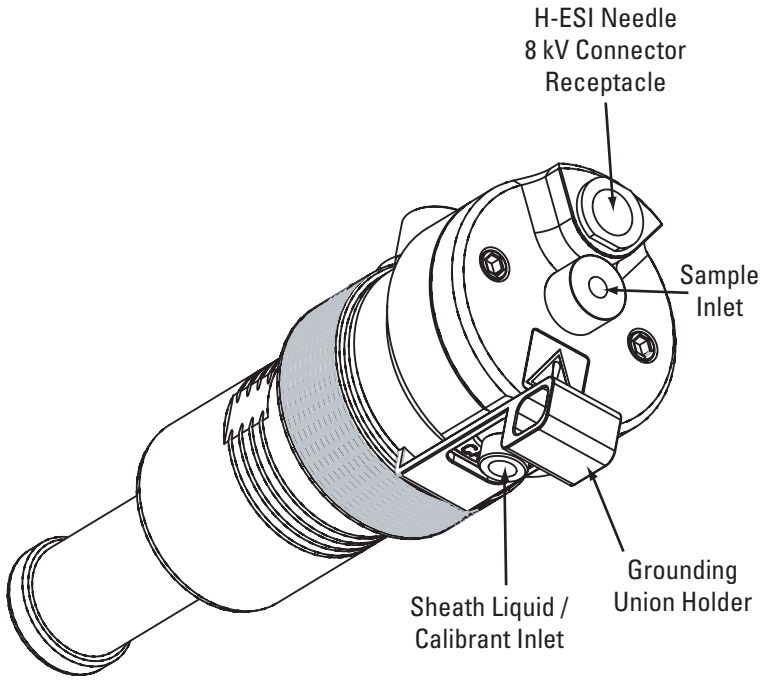


Figure 2-12. H-ESI probe, rear view

4. Push the probe into the port until the guide pin meets with the locking ring on the ion source housing.

5. Turn the probe 45 degrees clockwise and align the guide pin with the slot in the ESI interlock block (you may need to pull the probe towards you slightly to properly align the pin with the notch). Once you have turned the probe far enough to align the pin with the alignment notch at the rear of the port, push the probe straight in until the guide pin stops at the bottom of the alignment notch.

6. Lock the probe in place by turning the probe locking knob clockwise.

7. Ensure that the grounding union (stainless steel ZDV fitting) is seated in the grounding union holder on the H-ESI probe. See Figure 2-12.

8. Connect the sheath gas fitting (blue) to the sheath gas inlet (S) on the probe manifold. See Figure 2-11 and Figure 2-13.

9. Connect the auxiliary gas fitting (green) to the auxiliary gas inlet (A) on the probe manifold. See Figure 2-11 and Figure 2-13.

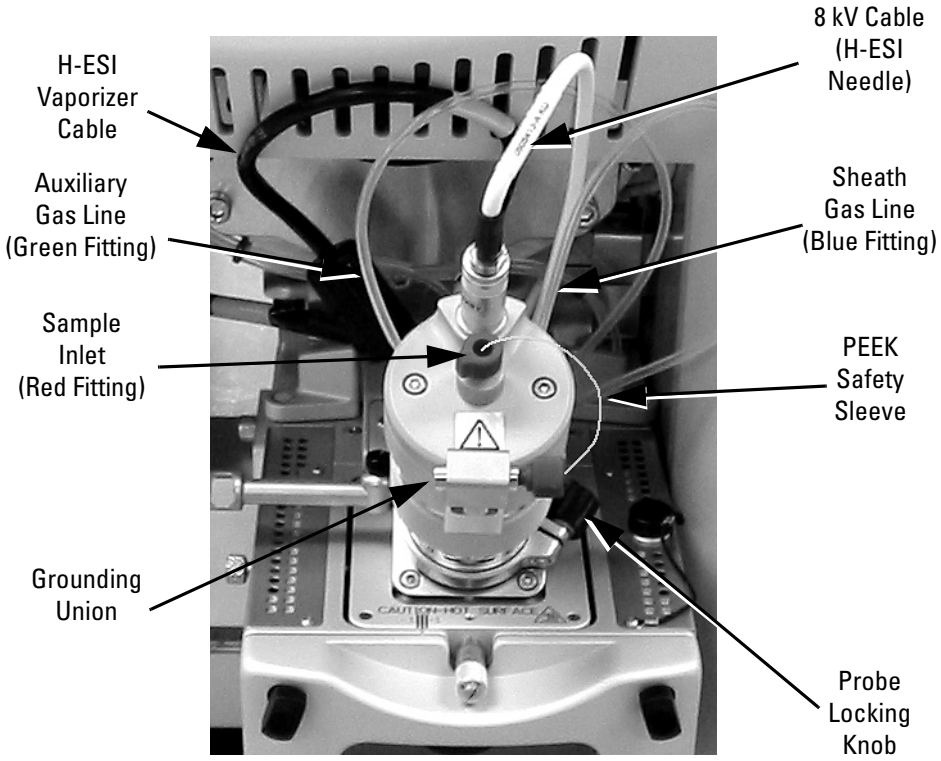


Figure 2-13. H-ESI probe installed in the Ion Max housing

10. Unplug the H-ESI vaporizer cable from the ESI interlock socket.

11. Connect the H-ESI vaporizer cable to the vaporizer cable socket on the H-ESI probe. See Figure 2-11.

12. Connect the 8 kV cable to the H-ESI needle high voltage receptacle on the H-ESI probe. See Figure 2-12. Tighten the locking ring on the 8 kV connector.

13. Connect the sample transfer tubing (LC line) to the grounding union.

The H-ESI source is now properly installed on the mass spectrometer.

Note Before you analyze samples with the H-ESI source, you need to change to H-ESI source mode in Quantum Tune Master by choosing **Setup > Change Ion Source > HESI**. ▲

Leave the LC/MS system in Standby and go to the next chapter: “Tuning and Calibrating the Mass Spectrometer in H-ESI/MS Mode”.

Chapter 3 Tuning and Calibrating the Mass Spectrometer in H-ESI/MS Mode

This chapter describes the procedure for tuning and calibrating the mass spectrometer. These procedures use a tuning and calibration solution, which is introduced directly into the mass spectrometer in low flow mode. You need to tune and calibrate every one to three months for optimum performance over the entire mass range of the detector.

To tune and calibrate your mass spectrometer, you do the following:

- Infuse a low concentration tuning and calibration solution containing polytyrosine directly into the H-ESI source by using the syringe pump.
- Test the efficiency and stability of the spray of the tuning and calibration solution into the mass spectrometer. You can observe the following singly-charged, positive ions for the polytyrosine monomer, trimer, and hexamer: *m/z* 182, *m/z* 508, and *m/z* 997, respectively.
- Start the automatic tuning and calibration procedure.

This chapter contains the following topics:

Setting Up to Introduce Sample by Direct Injection

The sample introduction device that you use for the H-ESI tuning and calibration procedure is a syringe pump. The syringe pump allows you to infuse the tuning and calibration solution directly into the H-ESI source for extended periods.

The syringe and the syringe pump are located on the front panel of your TSQ Quantum Ultra mass spectrometer. The plumbing connections for H-ESI/MS sample introduction from the syringe pump are shown in Figure 3-1.

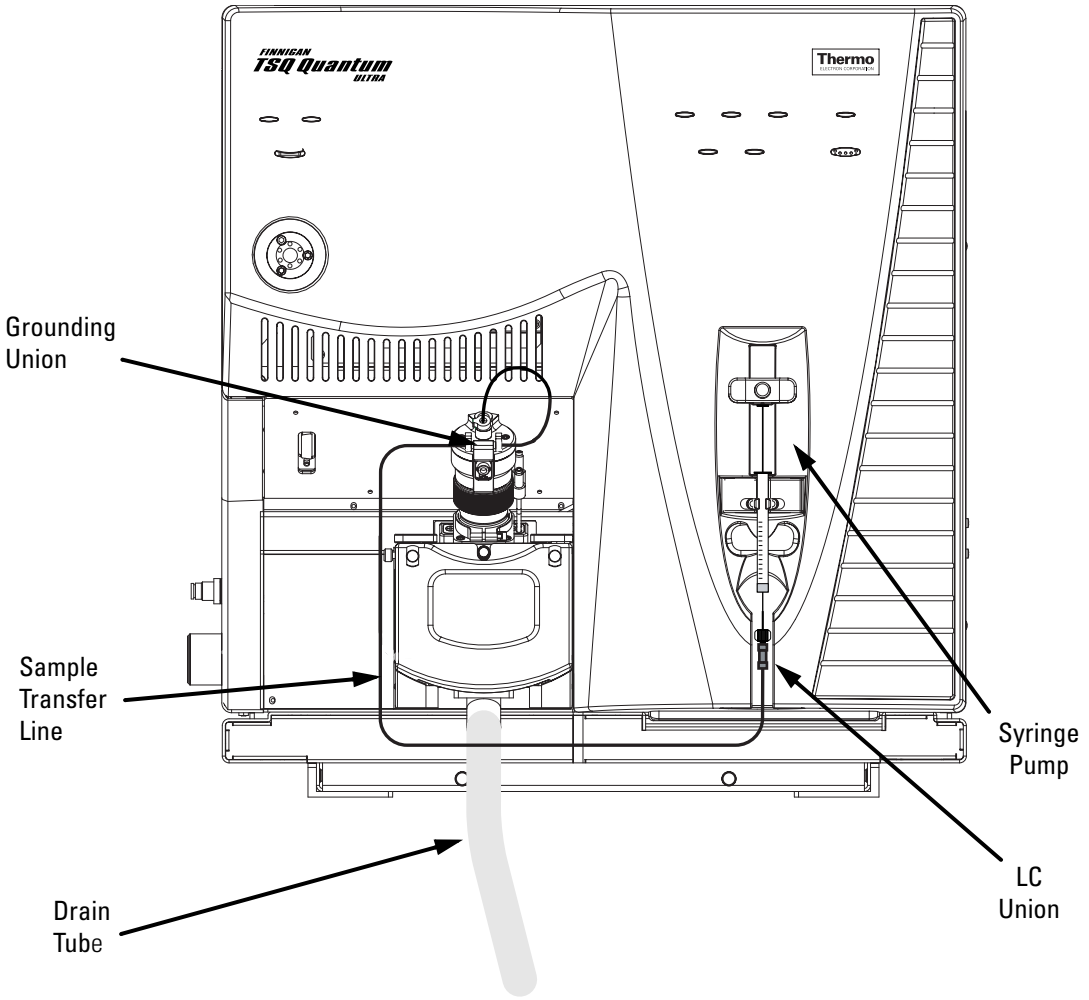


Figure 3-1. H-ESI/MS plumbing connections for sample introduction by syringe pump direct injection

To introduce solutions for tuning and calibrating, you need to install onto the syringe pump a syringe containing the tuning and calibration solution.

Your LC/MS system needs to be in standby, as described in the topic "Placing the LC/MS System in Standby" in Chapter 2, before starting this procedure.

Use the following procedure to set up the syringe pump for introducing tuning and calibration solution into the H-ESI source:

1. Install a sample transfer line between the LC union on the syringe adapter assembly and the grounding union on the H-ESI probe. See Figure 3-1.

Note To minimize the possibility of cross-contamination, use a different syringe and a different sample transfer line for your tuning and calibration solution than you do for your samples and compound optimization solution. ▲

2. Load a clean, 500-µL Unimetrics® syringe with 420 µL of the polytyrosine – 1, 3, 6 tuning and calibration solution. (Refer to "Appendix A: Solution Formulations" for the procedure for preparing the tuning and calibration solution.)

Note Be sure to wipe off the tip of the needle with a clean, lint-free tissue before reinserting it into the syringe adapter assembly to minimize the possibility of cross-contamination of the assembly. ▲

3. While holding the plunger of the syringe in place, carefully insert the tip of the syringe needle into the end of the Teflon® tube on the syringe adapter assembly. See Figure 3-2.

4. Place the syringe into the syringe holders of the syringe pump.

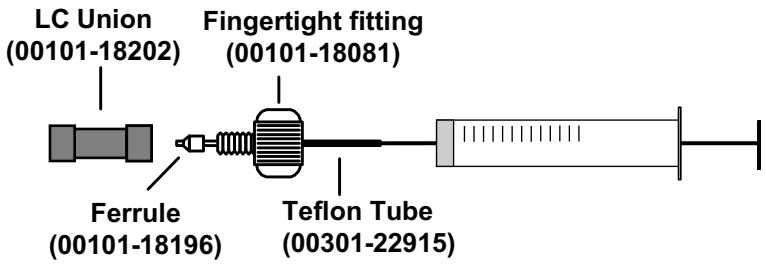


Figure 3-2. Syringe and syringe adapter assembly

5. While squeezing the black release button on the syringe pump handle, push the handle down until it just contacts the syringe plunger.

Go to the next topic: “Setting Up for Tuning and Calibrating”.

Setting Up for Tuning and Calibrating

To ensure optimum performance of the automatic tuning and calibration procedure, you need to properly set up the instrument.

Note You can perform the following automatic tuning and calibration procedure using an electrospray ionization (ESI) probe. ▲

CAUTION Before you begin normal operation each day, ensure that you have sufficient nitrogen supply for your API source. The presence of oxygen in the ion source when the mass spectrometer is On could be unsafe. ▲

Set up the mass spectrometer for tuning and calibrating as follows:

1. Click on the On/Standby button on the Control / Scan Mode toolbar to turn On the mass spectrometer. (The three different states of the On/Standby button are shown at the left.) When you turn On the mass spectrometer, you initiate the following events:

   - The mass spectrometer begins scanning.
   - Nitrogen begins to flow through the H-ESI probe.
   - A high voltage is applied to the H-ESI probe.
   - Tune Master shows a real-time display in the Spectrum view.

2. Tune Master must be placed in the H-ESI source mode before analyzing samples with the H-ESI source. The ion source mode is indicated at the top of the title bar as shown in Figure 3-3. Choose **Setup > Change Ion Source > HESI** to place Tune Master in the H-ESI source mode.

3. Click on the Compound Optimization Workspace button on the Control / Scan Mode toolbar to display the Compound Optimization workspace. See Figure 3-3.

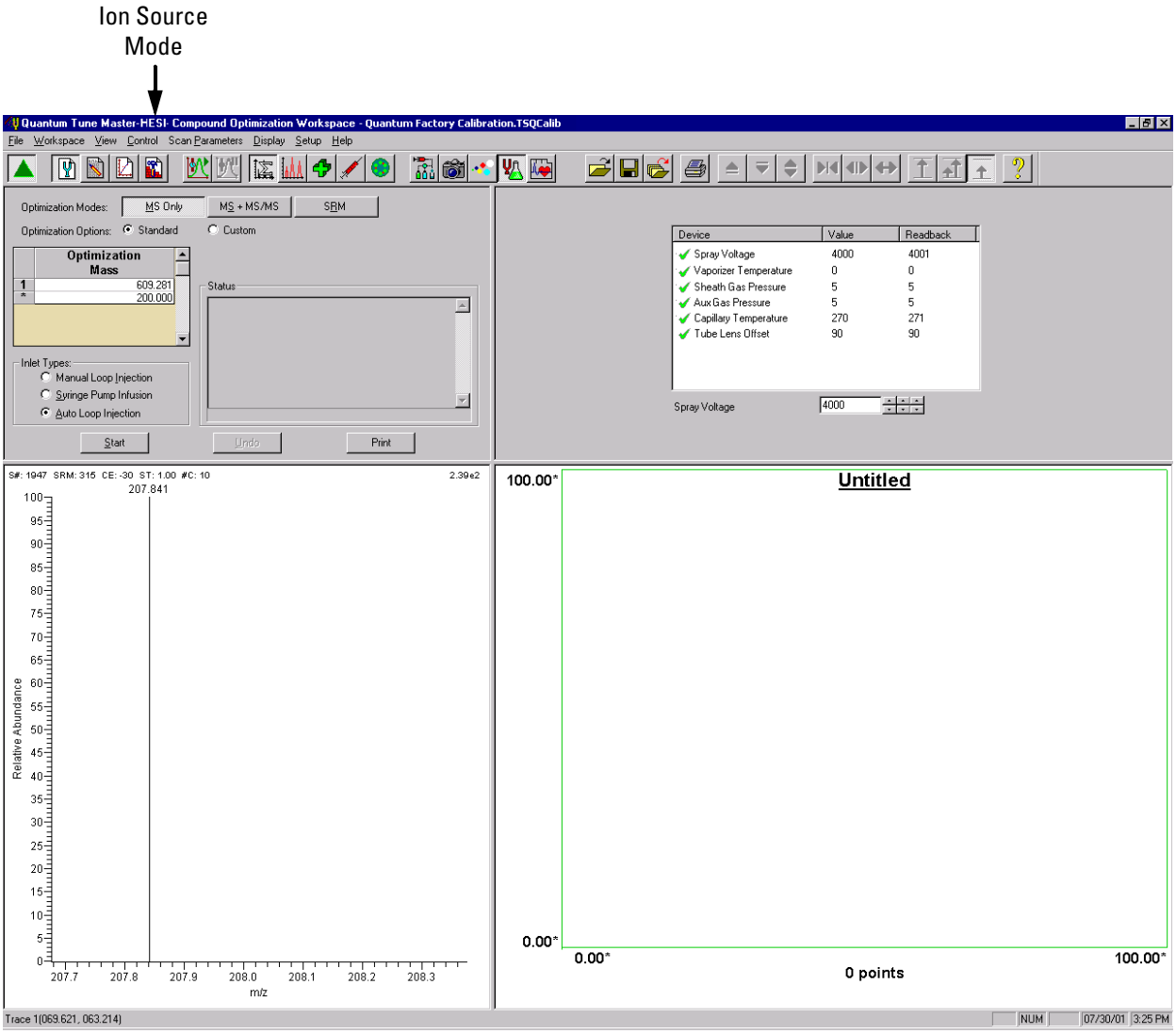


Figure 3-3. Compound Optimization workspace, showing the mass spectrometer in H-ESI mode

4. Set the values for several of the compound dependent devices as follows:

   a. Change the pressure of the sheath gas to 5 psi as follows:

      i. In the Device Display box in the Optimize Compound Dependent Devices view, click on Sheath Gas Pressure. See Figure 3-4.

      ii. Enter **5** in the Device spin box to set the sheath gas pressure.

   b. Set the H-ESI vaporizer temperature to 0 °C as follows:

      i. In the Device Display box, click on Vaporizer Temperature.

      ii. In the Device spin box enter **0** to set the vaporizer temperature.

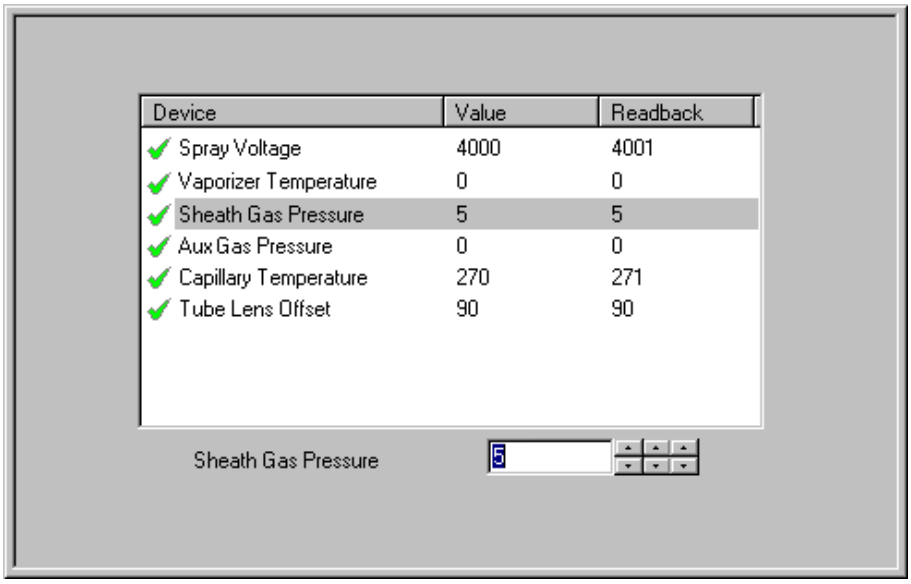


Figure 3-4. Optimize Compound Dependent Devices view

c. Change the pressure of the auxiliary gas to 0 as follows:

   i. Click on Aux Gas Pressure in the Device Display box.

   ii. Enter **0** in the Device spin box to 0. (Auxiliary gas pressure should be adjusted within the range of 0 to 5 to achieve the best ion beam intensity and stability.)

d. Change the temperature of the ion transfer capillary to 270 °C as follows:

   i. Click on Capillary Temperature in the Device Display box.

   ii. Enter **270** in the Device spin box to set the capillary temperature. (You might need to wait for a few minutes for the capillary temperature to stabilize at the set value.)

5. Configure the syringe pump to inject the polytyrosine – 1, 3, 6 tuning and calibration solution and start the syringe pump as follows:

   a. Choose **Setup > Syringe Pump & Sample Loop** to display the Syringe Pump and Sample Loop view in the top right corner of the workspace. See Figure 3-5.

   b. In the Syringe Flow Control group box, select the On option button to make active the Flow Rate spin box.

   c. Enter **5.00** in the Flow Rate spin box to set a flow rate of 5.00 µL/min. (The flow rate can be adjusted within the range of 1.00 to 15.00 µL/min to achieve the best ion beam intensity and stability.)

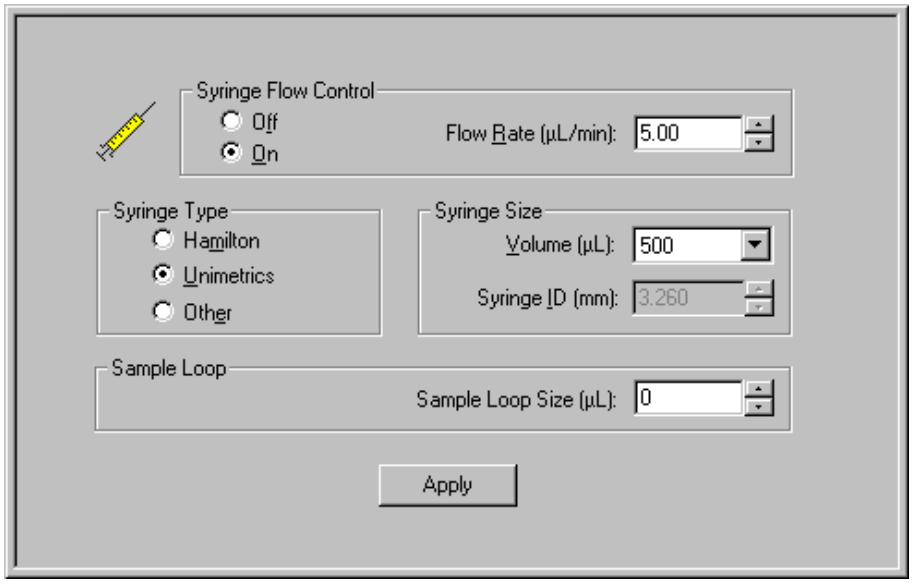


Figure 3-5. Syringe Pump and Sample Loop view

- If you are using either a Unimetrics or Hamilton syringe, go to step 5.d.
- If you are *not* using either a Unimetrics or Hamilton syringe, go to step 5.f.

d. In the Syringe Type group box, select the Unimetrics (or Hamilton) option button to specify a Unimetrics (or Hamilton) syringe.

e. In the Syringe Size group box, select *500* (or the size of your syringe) from the Volume list box to specify that the volume of your syringe is 500 µL.

When you specify the syringe type and syringe volume, Tune Master automatically sets the proper syringe ID value. Go to step 5.g.

f. If you are using a make of syringe other than Unimetrics or Hamilton, you need to manually specify the syringe ID by doing the following:

i. In the Syringe Type group box, select the Other option button to specify that you are using a syringe other than a Unimetrics or Hamilton syringe and to enable the Syringe ID spin box.

ii. In the Syringe Size group box, select the volume of your syringe from the Volume list box.

iii. Enter the inner diameter of your syringe in the Syringe ID spin box.

g. Click on **Apply** to apply these settings and to start the syringe pump. Polytyrosine – 1, 3, 6 tuning and calibration solution should now flow into the ion source.

The mass spectrometer is now set up for tuning and calibration.

You now need to establish a stable ion beam as described in the next topic: "Establishing a Stable Ion Beam with the H-ESI Source".

Establishing a Stable Ion Beam with the H-ESI Source

You need to establish a stable ion beam before you start the tuning and calibration procedure. The intensity and stability of the ion beam largely depends on the performance of the ion source. You adjust the sheath gas pressure to optimize the ion beam intensity and stability.

To establish a stable ion beam with the H-ESI source, do the following:

1. Set the scan parameters in preparation for observing the intensity and stability of the ion beam as follows:

   a. On the Control / Scan Mode toolbar, click on the Instrument Method Development Workspace button to display the Instrument Method Development workspace. See Figure 3-6.

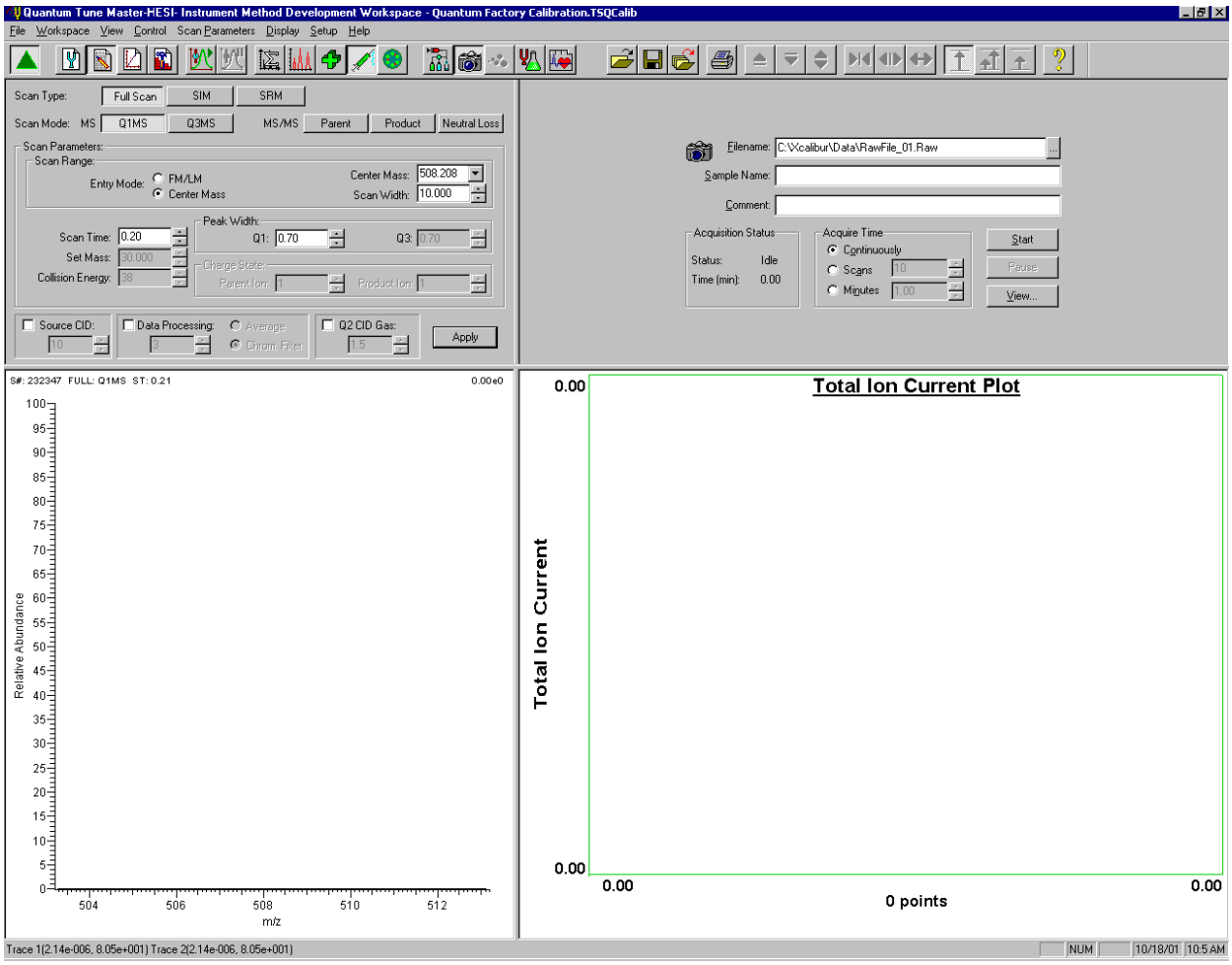


Figure 3-6. Instrument Method Development workspace

   b. In the Define Scan view in the top left corner of the workspace, select the Scan Type: Full Scan option button to display the Scan Parameters group box. See Figure 3-7.

c. Select the Scan Mode: Q1MS button to select the Q1MS scan mode.

d. In the Scan Parameters group box, in the Scan Range group box, select the Entry Mode: Center Mass option button to display the Center Mass and Scan Width spin boxes.

e. In the Center Mass spin box, enter **508.208** to set the center of the scan range to 508.208 u.

f. In the Scan Width spin box, enter **10.000** to set the scan width to 10.000 u.

g. In the Scan Time spin box, enter **0.20** to set the scan time to 0.20 s.

h. In the Peak Width group box, in the Q1 spin box, enter **0.70** to set the peak width to 0.70 u.

i. Confirm that the Source CID, Data Processing, and Q2 CID Gas check boxes are not selected to ensure that these options are Off.

j. Click on **Apply** to apply these scan parameters.

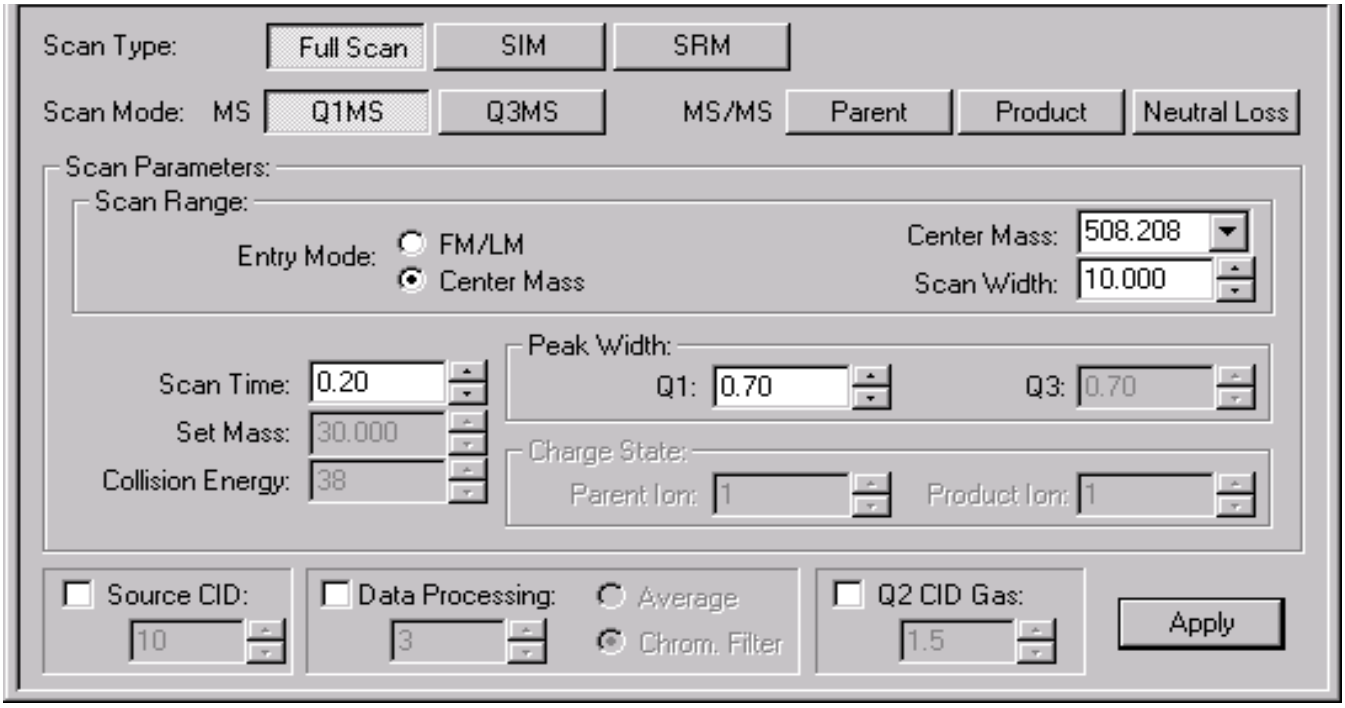


Figure 3-7. Define Scan view, showing typical settings for establishing a stable ion current


Display TIC

2. On the Control / Scan Mode toolbar, click on the Display TIC button to begin an ion current trace in the Graph view in the bottom right corner of the workspace.


Profile


Centroid

3. Ensure that the Profile/Centroid button is in the profile state to display profile type data. If the Profile/Centroid button is in the centroid state (as shown at the left), click on the Profile/Centroid button to toggle the data type to profile.


Positive Polarity


Negative Polarity

The mass spectrometer needs to be in the positive ion polarity mode to complete this procedure. You can determine the ion polarity mode of the mass spectrometer by observing the state of the Polarity button (as shown at the left). Ensure that the Polarity button is in the positive polarity state.

- If the mass spectrometer is already in the positive ion polarity mode, go to step 5.
- If you need to change the ion polarity mode of the mass spectrometer, go to step 4.

4. Place the mass spectrometer in the positive ion polarity mode as follows:

   a. Turn Off the spray voltage as follows:

      i. Click on the Optimize Compound Dependent Devices button to display the Optimize Compound Dependent Devices view in the top right corner of the workspace. See Figure 3-8.

      ii. In the Device spin box, enter **0** to set the spray voltage to 0 V.

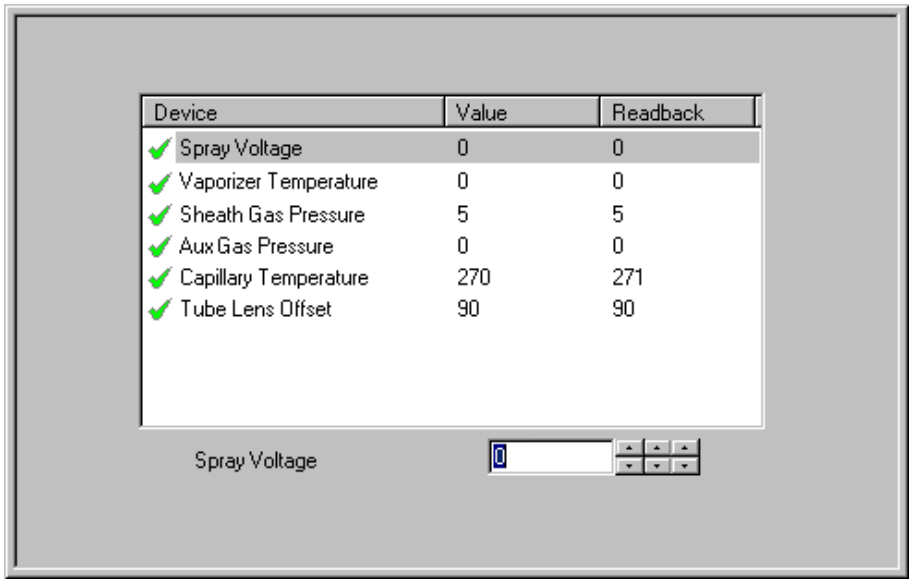


Figure 3-8. Optimize Compound Dependent Devices view

b. On the Control / Scan Mode toolbar, click on the Polarity button to toggle the mass spectrometer to the positive ion polarity mode.

c. Turn On the spray voltage again: In the Optimize Compound Dependent Devices view, in the Device spin box, enter **4000** to set the spray voltage to 4000 V.

5. Ensure that the H-ESI probe is positioned correctly:

a. Shine a flashlight through the front window of the Ion Max source and observe the electrospray through the left window.

b. Use the micrometer on the top front of the Ion Max source to adjust the H-ESI probe position so that the outer edge of the spay barely flows past the entrance of the ion transfer tube. Make sure that the bulk of the spray does not physically hit the nose of the ion transfer tube. See Figure 3-9.

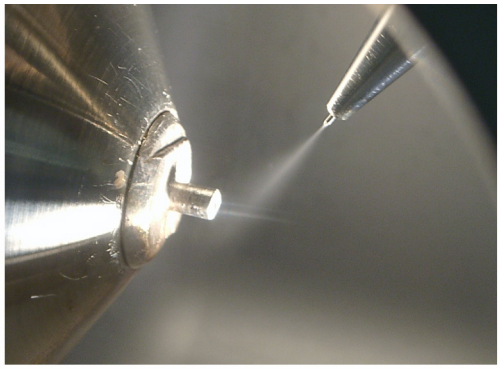


Figure 3-9. Ion transfer tube (capillary), spray nozzle, and electrospray

6. Determine if you have a stable ion beam as follows:

a. In the Spectrum view in the bottom left corner of the workspace, observe the mass spectra of the ion at *m/z* 508.208.

b. Click anywhere in the Spectrum view to enable the Display buttons on the File / Display toolbar.

c. Click on the Creep toolbar button to invoke the creep Y-axis normalization mode. This allows you to observe the relative intensity of the ion at *m/z* 508.208. See Figure 3-10.

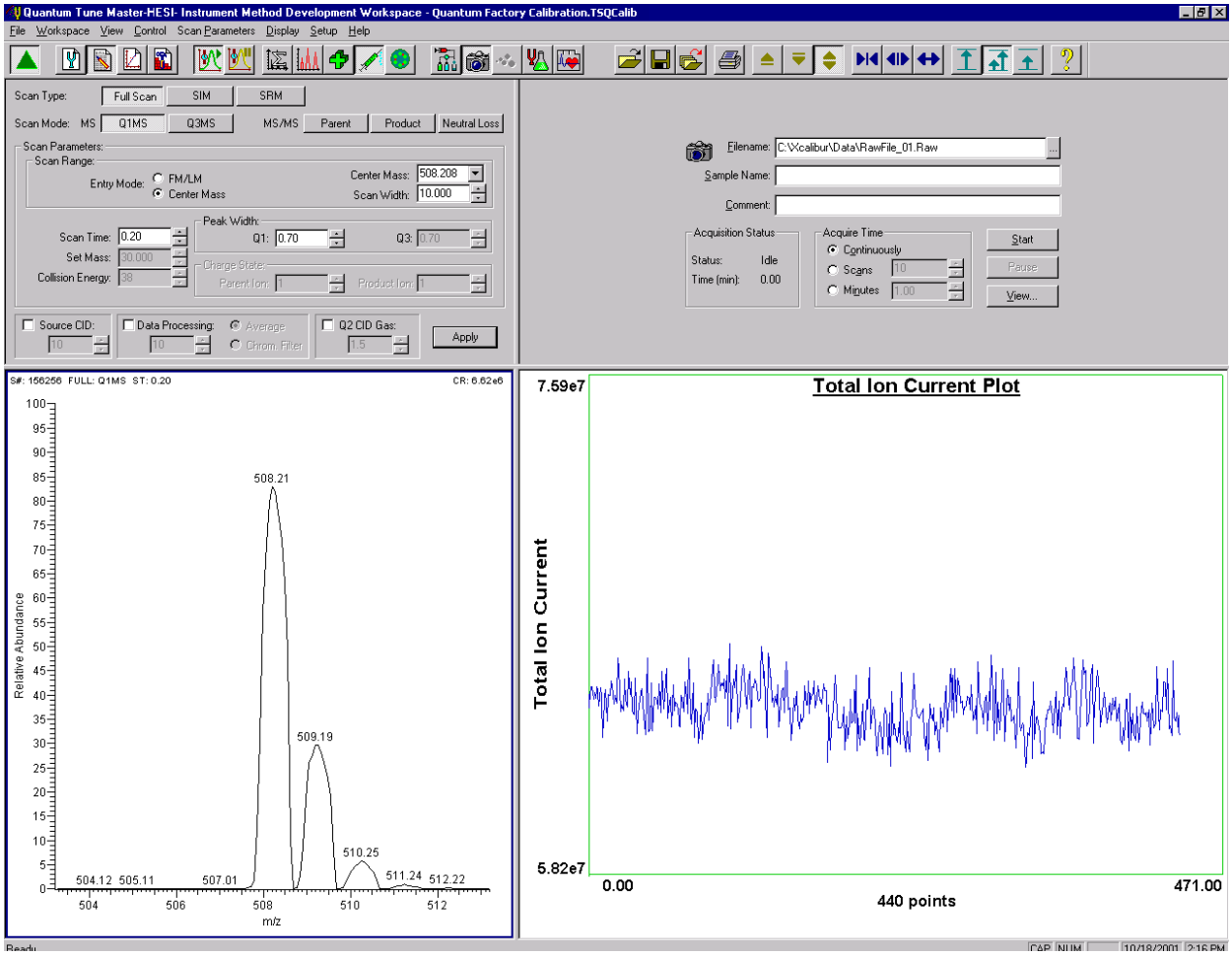


Figure 3-10. Instrument Method Development workspace, showing stable ion beam

Note The sheath gas pressure can be adjusted within the range of 1 to 15 psi. You adjust the sheath gas pressure to establish a stable ion beam. Too low a sheath gas pressure can cause a loss of signal stability, whereas too high a pressure can cause a loss of peak intensity. ▲

d. Observe the height of the peak at *m/z* 508.208 in the Spectrum view.

- If the top of the peak is steady, you have a stable ion beam and you do not need to adjust the sheath gas pressure. The peak height should not vary by more than about 30% from scan to scan.
- If the top of the peak is unsteady, you need to adjust the pressure of the sheath gas to establish a stable ion beam. The ion current fluctuation can also be observed in the Graph view in the bottom right corner of the workspace.

Once you have established a stable ion beam, you adjust the scan parameters to observe the other polytyrosine tuning peaks in the Spectrum view, as described in the next topic: “Verifying Operation in the H-ESI/MS Mode”.

Verifying Operation in the H-ESI/MS Mode

You are now ready to verify the proper operation of the mass spectrometer. To do this, you inject the polytyrosine tuning and calibration solution directly into the H-ESI source and monitor the mass spectrum of the solution.

To monitor the mass spectrum of the tuning and calibration solution, do the following:

1. Set the scan parameters for verifying the operation of the mass spectrometer in Q1 as follows:

    a. In the Define Scan view in the top left corner of the workspace, in the Scan Parameters group box, in the Scan Range group box, select the Entry Mode: FM/LM option button. This displays the First Mass and Last Mass spin boxes. See Figure 3-11.

    b. In the First Mass spin box, enter **150.000** to set the low endpoint of the scan range to 150.000 u.

    c. In the Last Mass spin box, enter **1050.000** to set the high endpoint of the scan range to 1050.000 u.

    d. In the Scan Parameters group box, in the Scan Time spin box, enter **1.20** to set the scan time to 1.20 s.

    e. Select the Data Processing check box to enable the Data Processing spin box and the Average option button.

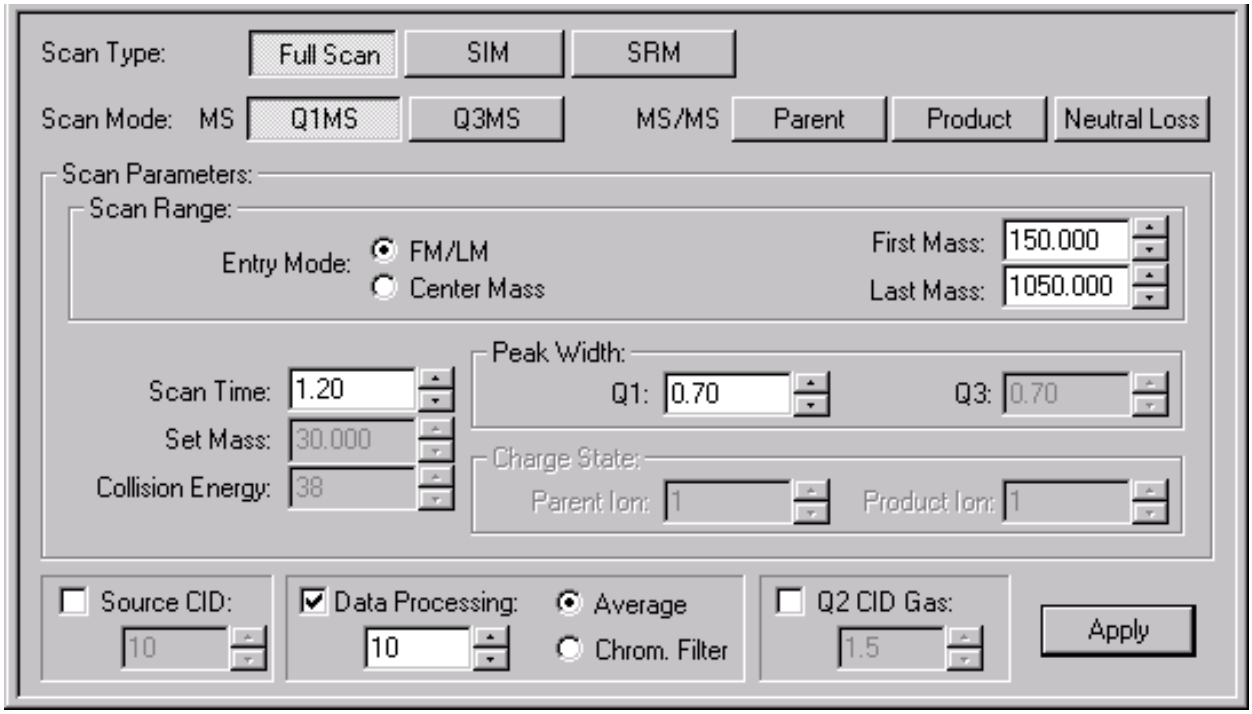


Figure 3-11. Define Scan view, showing typical settings for testing the operation of the mass spectrometer in the Q1MS scan mode

f. Select the Average option button and enter **10** in the Data Processing spin box to turn on the 10 scan averaging filter.

g. Click on **Apply** to apply these scan parameters.

2. Monitor the tuning and calibration solution in Q1 as follows:

a. Click anywhere in the Spectrum view in the bottom left corner of the workspace to enable the Display buttons on the File / Display toolbar (shown at the left).

b. Select the Normalize button to normalize the spectrum.

c. In the Spectrum view, observe the mass spectrum of the singly-charged ions of the tuning and calibration solution. The ions are as follows:

- Tyrosine monomer: *m/z* 182.082
- Polytyrosine trimer: *m/z* 508.208
- Polytyrosine hexamer: *m/z* 997.398

Observe the values of the normalized ion current signal in the Spectrum view. See Figure 3-12.

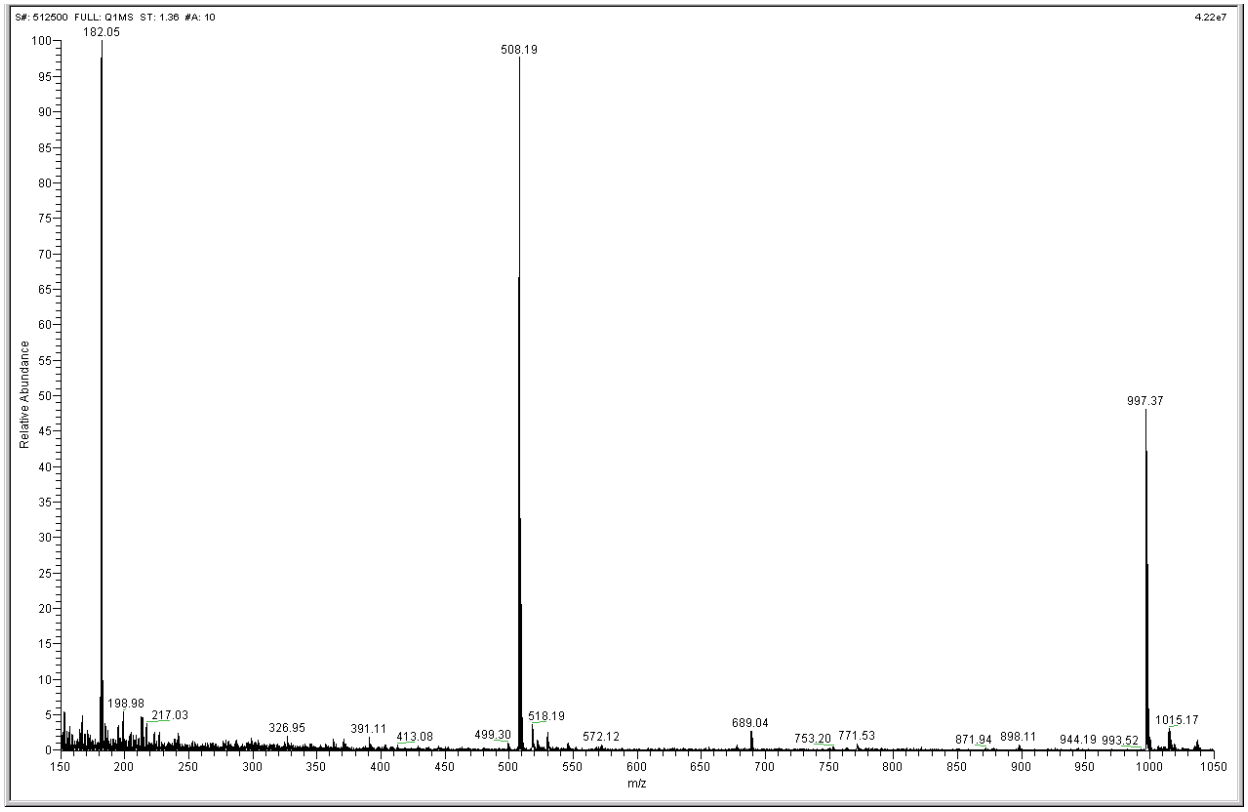


Figure 3-12. Spectrum view, showing a real-time spectrum of polytyrosine tuning and calibration solution

3. As tuning and calibration solution is detected and the readback values fluctuate, ask yourself the following questions about the normalized ion current signal:

   - Are the three polytyrosine ion peaks the predominant peaks?
   - Are the heights of the tyrosine polymers within one order of magnitude of each other?
   - Are the polytyrosine peak heights in the range of high 10^6 or low 10^7 counts?
   - Is the signal stable, varying by less than about 15% from scan to scan?
   - Are the peaks for the tuning and calibration solution symmetrical, well resolved, and unsplit?

   If you answered “yes” to these questions, then your mass spectrometer is operating properly in the Q1MS mode.

Note Tuning parameters can be adjusted to establish a good polytyrosine tuning and calibration signal. Sheath gas pressure can be adjusted within the range of 1 to 15 psi, auxiliary gas pressure can be adjusted within the range of 0 to 5 (arbitrary units), and the tuning and calibration solution flow rate can be adjusted within the range of 1 to 15 µL/min. ▲

If you answered "no" to any of these questions, try the following troubleshooting measures and then perform the operational inspection again:

- Adjust the sheath or auxiliary gas pressure settings, or adjust the tuning and calibration solution flow rate.
- Ensure that the fused-silica sample tube does not extend beyond the tip of the H-ESI needle. Refer to the *Finnigan H-ESI Probe Operator's Manual*.
- Ensure that the entrance of the ion transfer capillary is clean and is not covered with a piece of septum.
- Ensure that the H-ESI probe is position properly.
- Ensure that the solution entering the probe is free of air bubbles and that the tubing and connectors are free of leaks.

4. Set the scan parameters for verifying the operation of the mass spectrometer in Q3 as follows:

   a. In the Define Scan view, select the Mode: Q3MS option button to enable Q3 scanning. See Figure 3-13.

   b. Verify that the scan parameters have not changed from those set for Q1.

   c. Click on **Apply** to apply these scan settings.

5. Again, observe the values of the normalized ion current signal in the Spectrum view. If the spectrum meets the requirements of step 3 above, then your mass spectrometer is operating properly in the Q3 MS mode.

You are now ready to tune and calibrate the mass spectrometer. Leave your TSQ Quantum Ultra as it is, and go to the next topic: "Tuning and Calibrating Automatically in the H-ESI/MS, Positive Ion Mode".

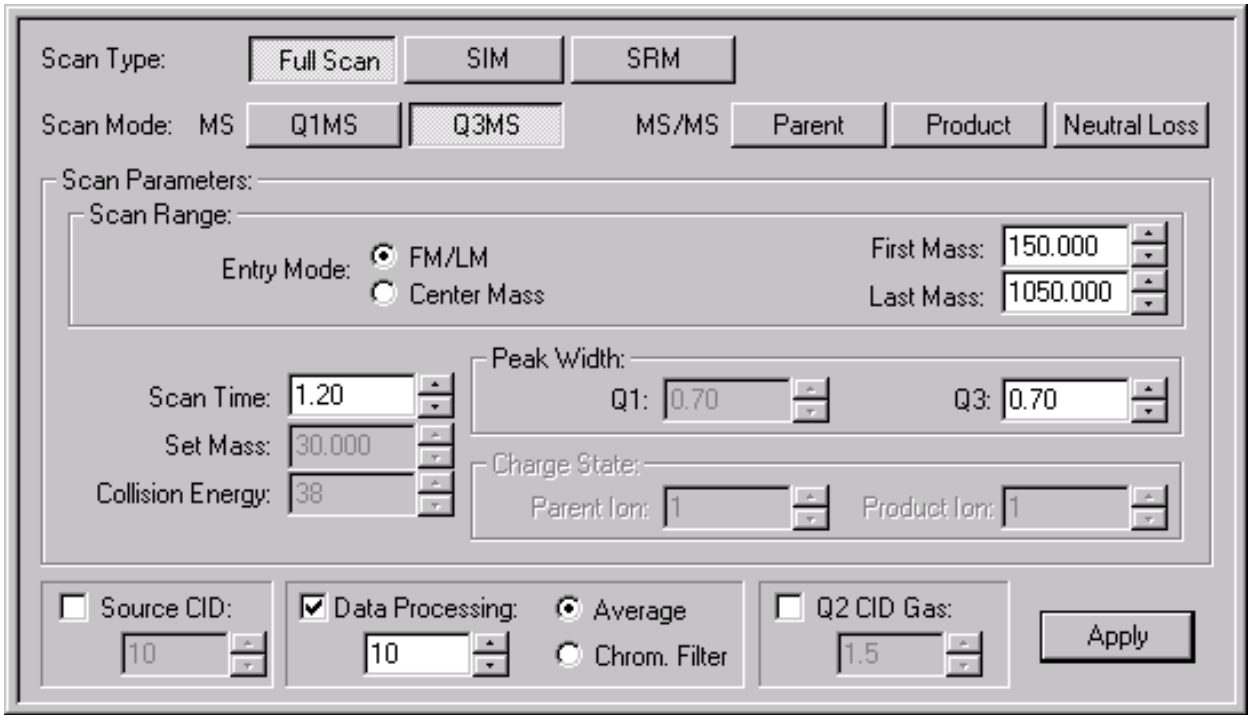


Figure 3-13. Define Scan view, showing typical settings for testing the operation of the mass spectrometer in the Q3MS scan mode

Tuning and Calibrating Automatically in the H-ESI/MS, Positive Ion Mode

You are now ready to tune and calibrate the mass spectrometer for H-ESI operation. The automatic tuning and calibration procedure first tunes the instrument with tuning and calibration solution to establish a stable spray of solution and to ensure that enough ions are detected to perform a calibration of the mass spectrometer. It then calibrates the mass spectrometer automatically.

Perform the tuning and calibration procedure periodically (every one to three months) to ensure optimum performance of the mass spectrometer.

To tune and calibrate your mass spectrometer automatically in the H-ESI/MS, positive ion mode, you do the following:

1. Click on the System Tune and Calibration Workspace button to display the System Tune and Calibration workspace. See Figure 3-14.

Note You can password protect the secure workspaces in Tune Master. The workspaces you can protect are as follows: System Tune and Calibration, Full Instrument Control, and Diagnostics.

There are three levels of protection possible, as follows: no protection, automatic protection, and custom password protection. No protection means that all operators can access all workspaces. Automatic protection means that Tune Master uses the default password, *lctsq*, to protect the secure workspaces. Custom password protection means that the Key Operator (or Laboratory Administrator or Manager) can select a password to protect the secure workspaces.

If your TSQ Quantum Ultra has been password protected, you need to obtain the password before you can access the secure workspaces (including the System Tune and Calibration workspace). If the instrument password is lost, you need to reinstall the TSQ Quantum Ultra software to reset the default password (*lctsq*). ▲

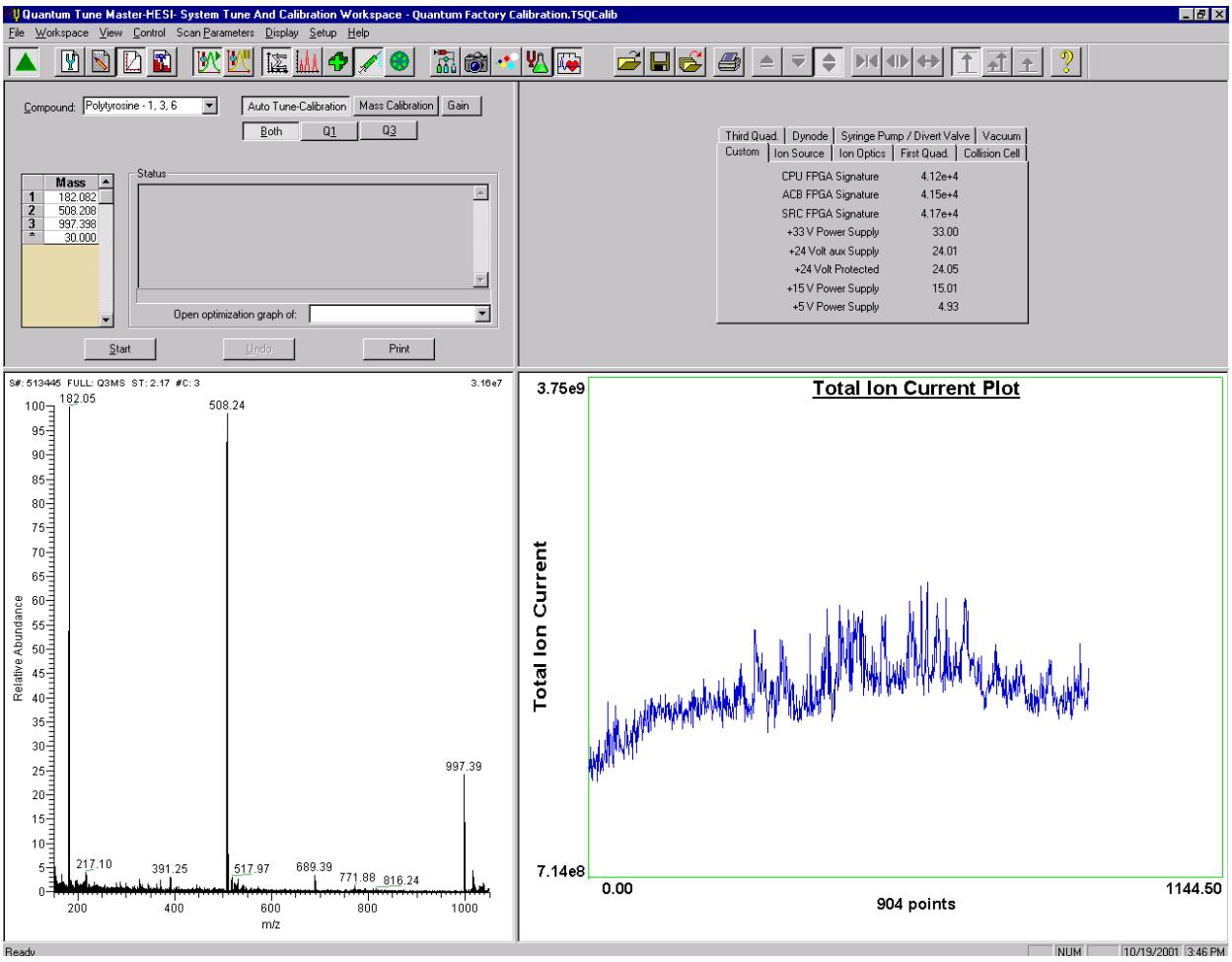


Figure 3-14. System Tune and Calibration workspace, showing polytyrosine signal

2. In the System Tune and Calibration view in the top left corner of the workspace, select *Polytyrosine – 1, 3, 6* from the Compound list box. This automatically selects the three positively-charged polytyrosine ions to be used for automatic tuning and calibrating. See Figure 3-15.

3. Select **Auto Tune-Calibration** to specify a full tune and calibration.

4. Select **Both** to tune and calibrate in both the first and third quadrupoles.

5. Click on **Start** to start the automatic tuning and calibration procedure.

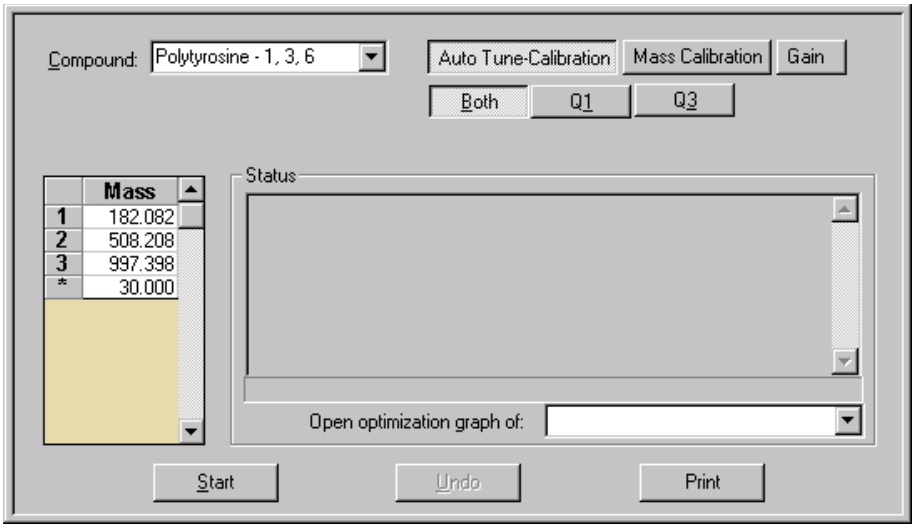


Figure 3-15. System Tune and Calibration view

The Status box displays real-time messages about the system tune and calibration so that you can monitor the progress of each sub-procedure. Once a sub-procedure is complete, the result is reported (for example, whether it passed or failed). At the end of the entire procedure, it displays a summary. See Figure 3-16.

- If errors occur during the automatic tuning and calibration procedure, go to step 6.
- If the automatic tuning and calibration procedure finishes without errors, go to step 7.

6. If errors occur during the automatic tuning and calibration procedure, restore the previous mass spectrometer device settings and perform the tuning and calibration procedure again by completing the following steps:

   a. Click on **Undo** to restore the prior tuning and calibration settings.

   b. Click on **Accept** to reload the prior tuning and calibration settings to the mass spectrometer.

   c. Troubleshoot and correct the problem that caused the tuning and calibration procedure to fail.

   d. Go to step 5 and restart the tuning and calibration procedure.

7. Click on **Accept** to accept the results of the tuning and calibration procedure.

After you accept the results of the tuning and calibration procedure, a message box asks whether you want to copy the positive ion tuning and calibration settings to the negative ion mode. See Figure 3-17.

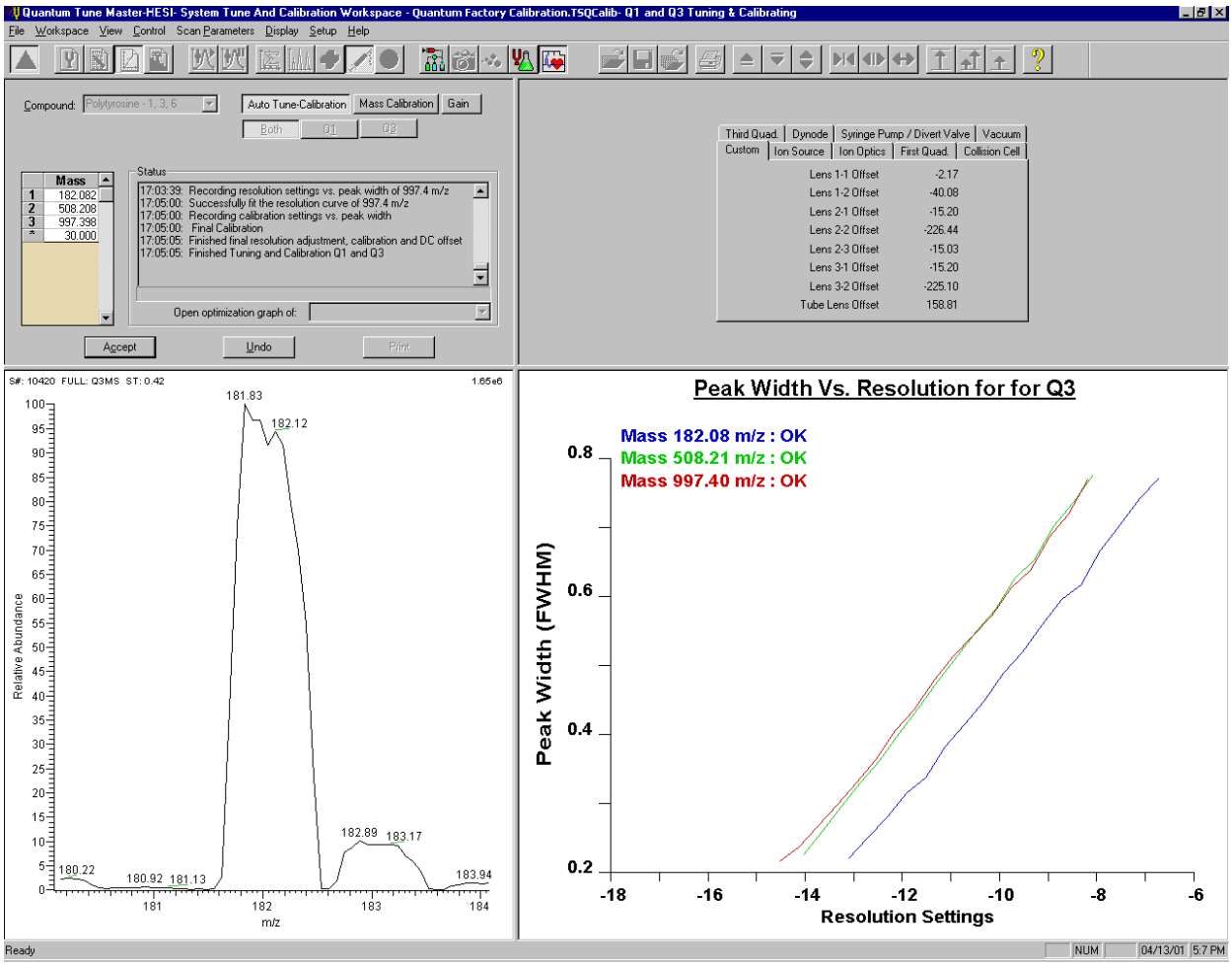


Figure 3-16. System Tune and Calibration workspace during an automatic tune and calibration

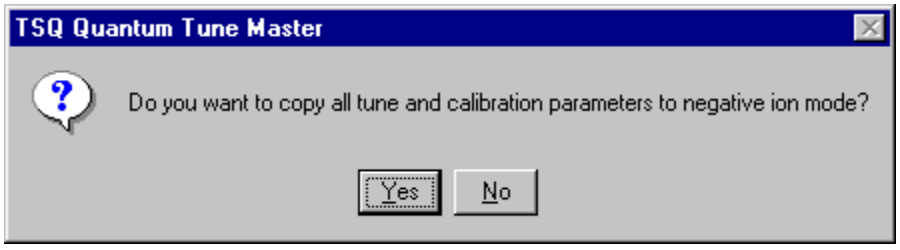


Figure 3-17. Message box prompting you to save the tuning and calibration parameters to negative ion mode

- If you already tuned and calibrated the instrument successfully in the negative ion mode, click on **No**. (Do not copy the positive ion mode parameters to the negative ion mode.)

- If you have not tuned and calibrated the instrument in the negative ion mode, click on **Yes**.

Note If you intend to perform high sensitivity negative ion mass spectrum analysis, it is recommended that you also perform a full tune and calibration of the instrument in the negative ion mode. This procedure is found in the next topic: "Tuning and Calibrating Automatically in the H-ESI/MS, Negative Ion Mode". ▲

8. Save the calibration file by doing the following:

   a. Click on **Save Calib. As** to open the Save Calibration File dialog box. See Figure 3-18.

   b. In the File Name text box, enter a name for your calibration file.

   c. Click on **Save** to save the calibration file.

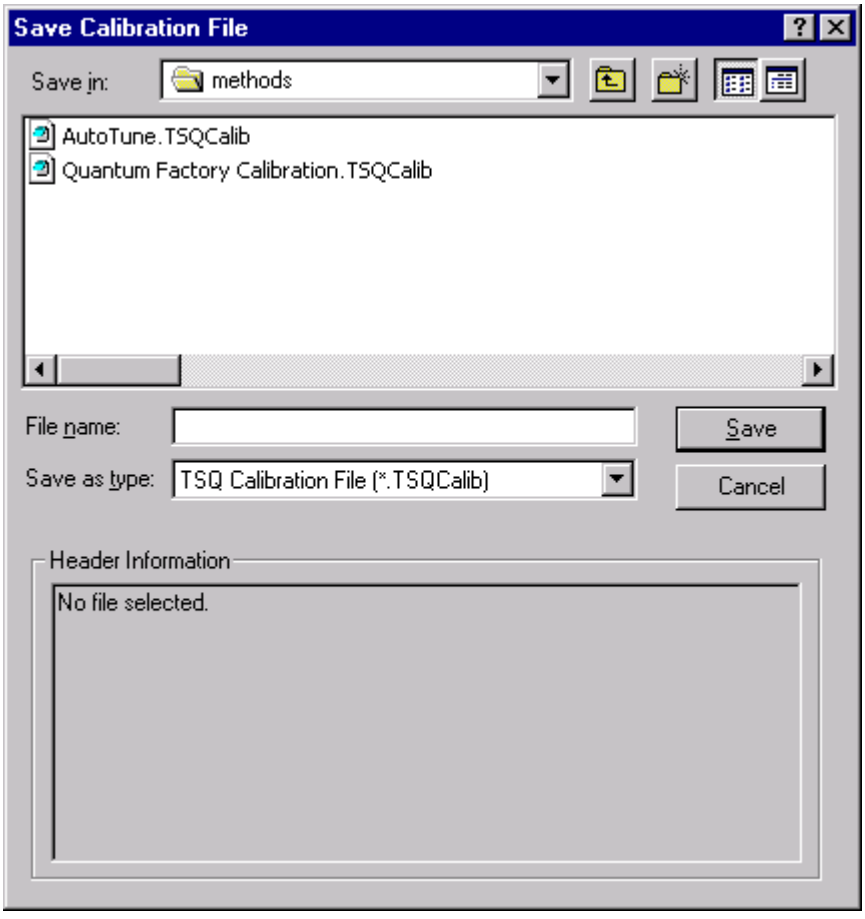


Figure 3-18. Save Calibration File dialog box.

The Save As dialog box is now displayed. See Figure 3-19.

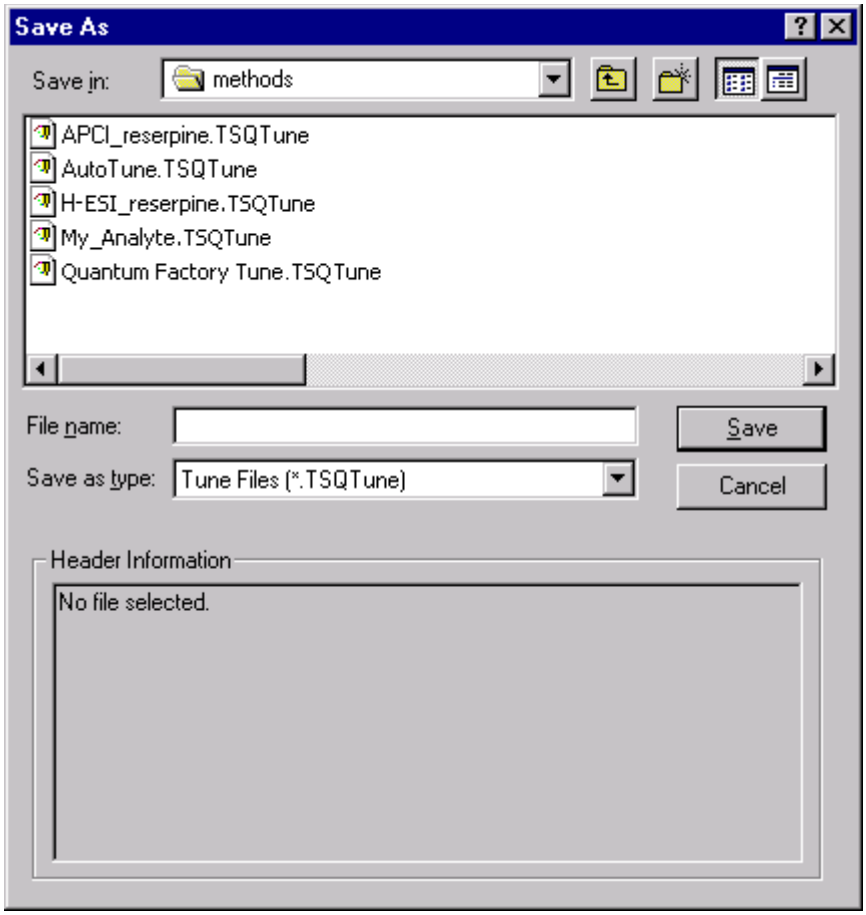


Figure 3-19. Tune Method Save As dialog box

9. Save the Tune Method file as follows:

    a. In the File Name text box, enter a name for your Tune Method file.

    b. Click on **Save** to save the Tune Method file.

The mass spectrometer is now tuned and calibrated in the positive ion mode.

If you want to tune and calibrate the mass spectrometer in the negative ion mode, go to the next topic: “Tuning and Calibrating Automatically in the H-ESI/MS, Negative Ion Mode”.

If you do not intend to perform high sensitivity negative ion mass spectrum analysis, you can skip the negative ion tuning and calibration procedure. Go to the topic: “Flushing the System after Tuning and Calibrating” on page 3-34.

Tuning and Calibrating Automatically in the H-ESI/MS, Negative Ion Mode

Once you have tuned and calibrated the instrument in the positive ion mode, you have the option of tuning and calibrating the instrument in the negative ion mode.

- If you already copied the positive ion tuning and calibration parameters to the negative ion mode and you do *not* intend to perform high sensitivity negative ion mass spectrum analysis, skip this topic. Instead, go to the topic "Flushing the System after Tuning and Calibrating".
- If you have *not* copied the positive ion tuning and calibration parameters to the negative ion mode or you intend to perform high sensitivity negative ion mass spectrum analysis, tune and calibrate the mass spectrometer in the negative ion mode as described in this topic.

Tune and calibrate the mass spectrometer automatically in the H-ESI/MS, negative ion mode, as follows:

1. Change the mass spectrometer to negative ion polarity mode:

   a. Turn Off the spray voltage:

      i. Click on the Optimize Compound Dependent Devices button to display the Optimize Compound Dependent Devices view in the top right corner of the workspace. See Figure 3-20.

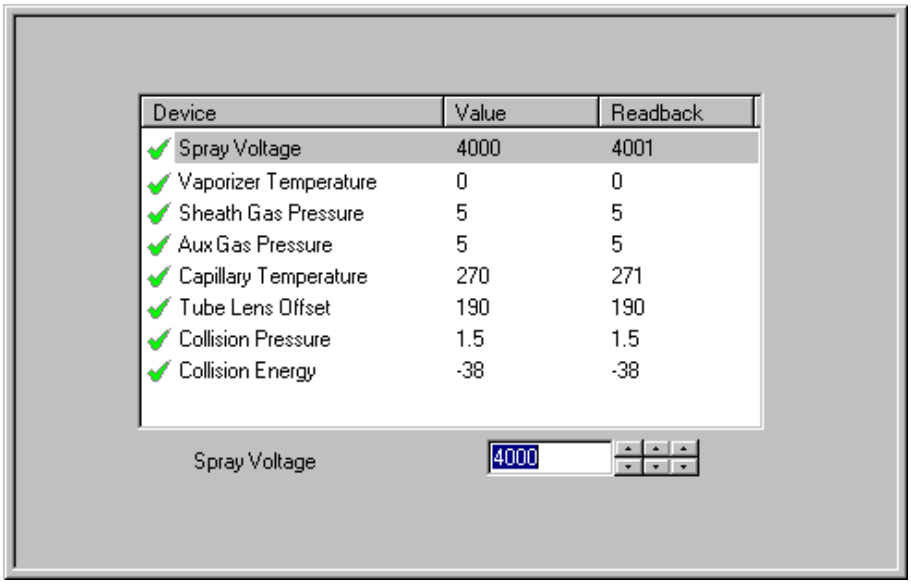


Figure 3-20. Optimize Compound Dependent Devices view.


Positive Polarity


Negative Polarity

      ii. In the Device spin box, enter **0** to set the spray voltage to 0 V.

   b. On the Control / Scan Mode toolbar, click on the Polarity button to toggle the detector to the negative ion polarity mode.

c. Turn On the spray voltage again: In the Optimize Compound Dependent Devices view, in the Device spin box, enter **3000** to change the spray voltage to 3000 V.

d. Change the sheath gas pressure as follows:

i. In the Device Display box, click on Sheath Gas Pressure to change the label of the Device spin box to *Sheath Gas*.

ii. In the Device spin box, enter **15** to change the sheath gas pressure to 15 psi.

2. In the System Tune and Calibration view in the top left corner of the workspace, select *Polytyrosine – Neg* from the Compound list box. This selects the three negatively-charged polytyrosine ions to be used for automatic tuning and calibration. See Figure 3-21.

3. Select **Auto Tune-Calibration** to specify a full tune and calibration.

4. Select **Both** to tune and calibrate in both the first and third quadrupoles.

5. Click on **Start** to start the automatic tuning and calibration procedure.

   You can monitor the progress of the system tune and calibration by observing the Status box. At the end of the procedure, it displays a summary. See Figure 3-22.

- If errors occur during the automatic tuning and calibration procedure, go to step 6.
- If the automatic tuning and calibration procedure finishes without errors, go to step 7.

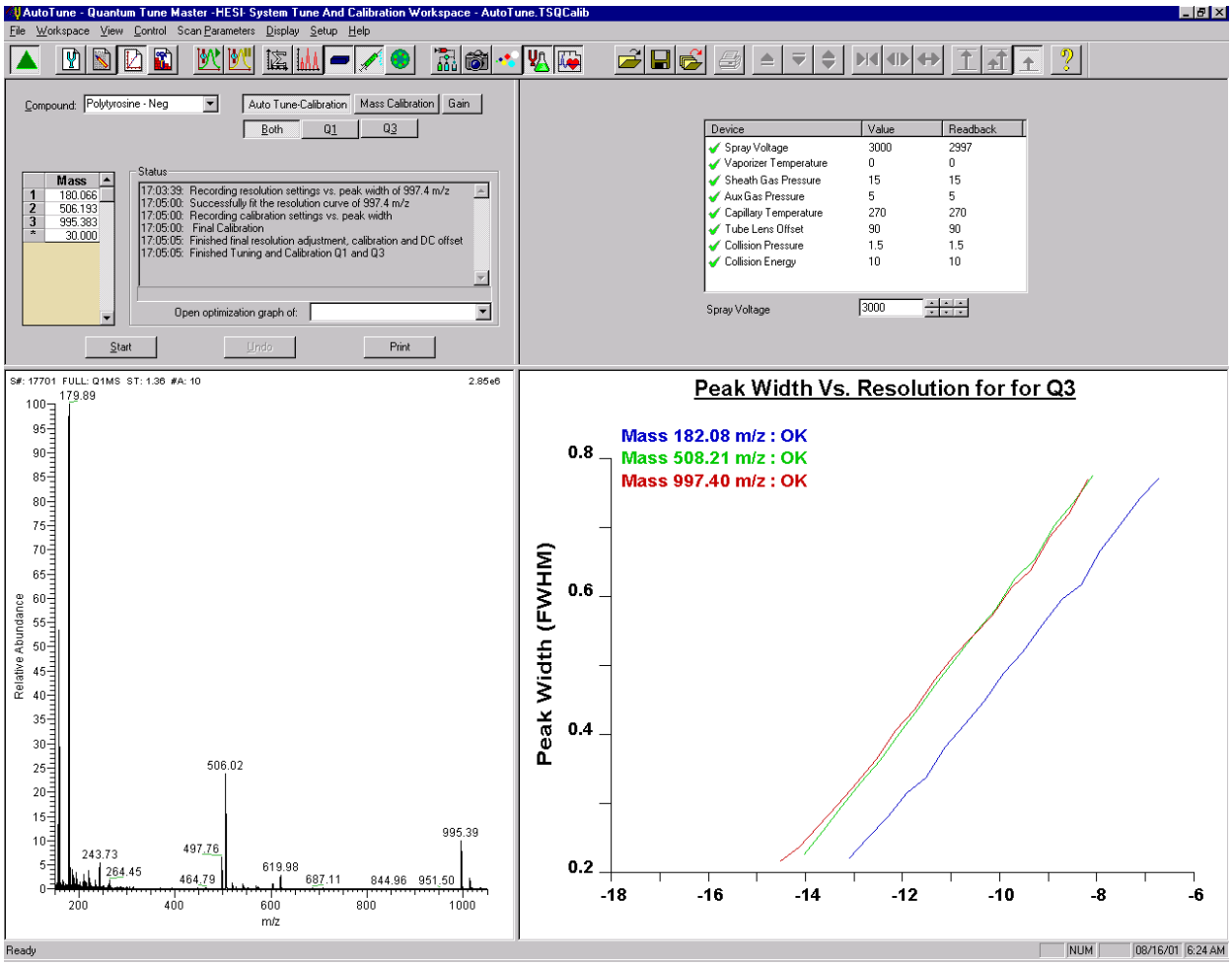


Figure 3-21. System Tune and Calibration workspace, showing preparation for negative ion mode tuning and calibration

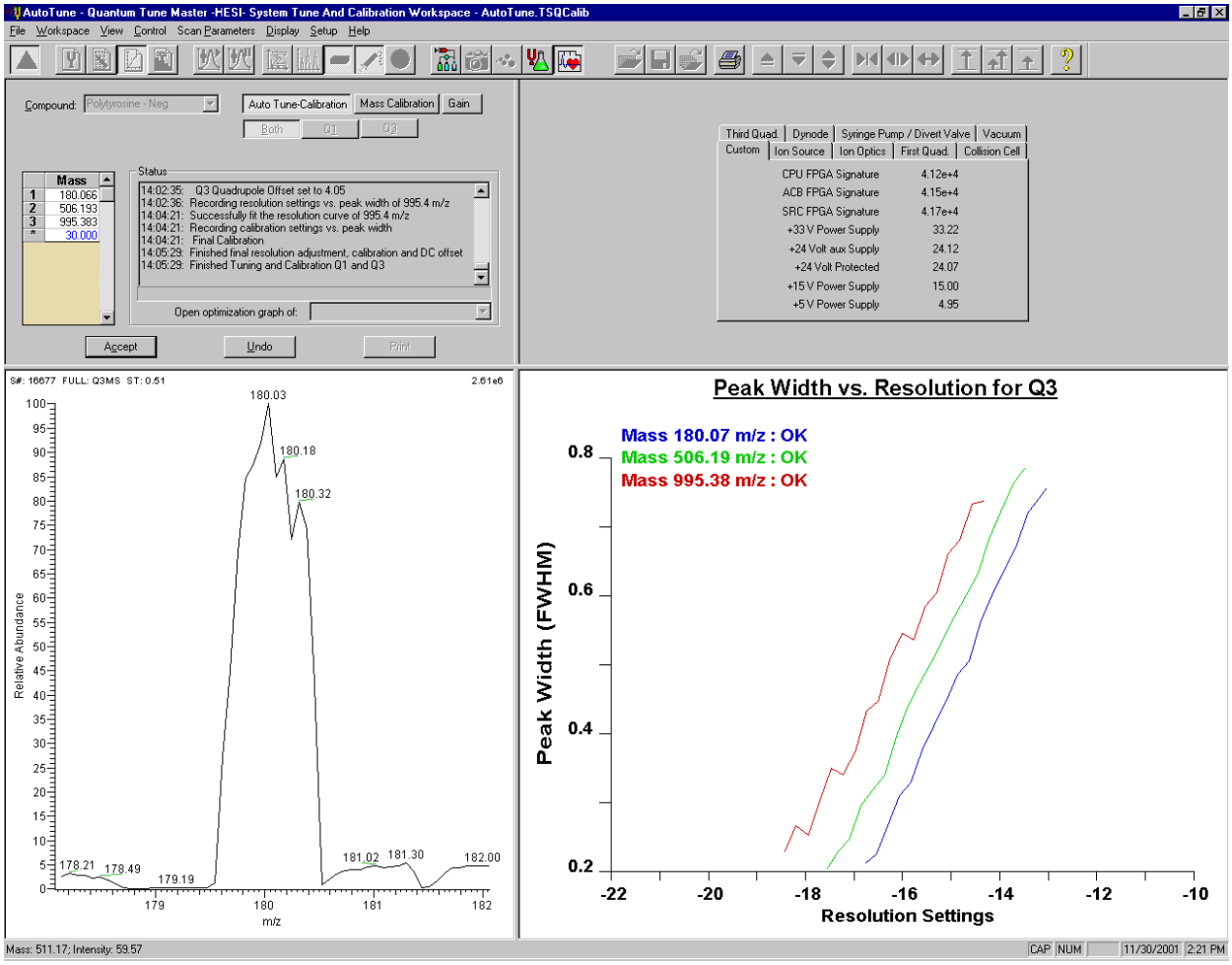


Figure 3-22. System Tune and Calibration workspace during an automatic tune and calibration

6. If errors occur during the automatic tuning and calibration procedure, restore the previous mass spectrometer device settings and perform the tuning and calibration procedure again by completing the following steps:

   a. Click on **Undo** to restore the prior tuning and calibration settings.

   b. Click on **Accept** to reload the prior tuning and calibration settings to the mass spectrometer.

   c. Troubleshoot and correct the problem that caused the tuning and calibration procedure to fail.

   d. Go to step 5 and restart the tuning and calibration procedure.

7. Click on **Accept** to accept the results of the tuning and calibration procedure.

After you accept the results of the tuning and calibration procedure, a message box asks whether you want to copy the negative ion tuning and calibration settings to the positive ion mode. See Figure 3-23.

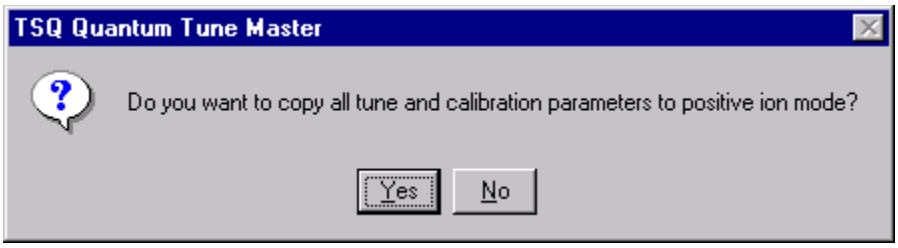


Figure 3-23. Message box prompting you to save tuning and calibration parameters to positive ion mode

- If you already tuned and calibrated the instrument successfully in the positive ion mode, click on **No**. (Do not copy the negative ion mode parameters to the positive ion mode.)
- If you have not tuned and calibrated the instrument in the positive ion mode, click on **Yes**.

Note If you intend to perform high sensitivity positive ion mass spectrum analysis, it is recommended that you also perform a full tune and calibration of the instrument in the positive ion mode. ▲

8. You can now save the calibration file by doing the following:

   a. Click on **Save Calib. As** to open the Save Calibration File dialog box. See Figure 3-24.

   b. In the File Name text box, enter a name for your calibration file.

   c. Click on **Save** to save the calibration file.

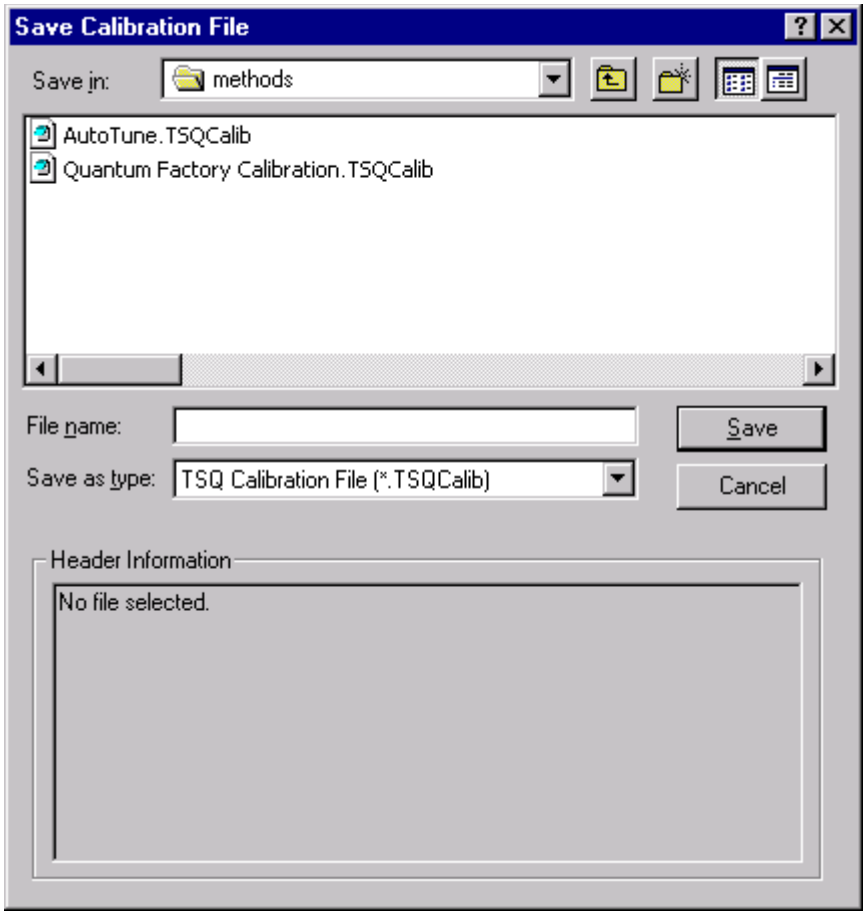


Figure 3-24. Save Calibration File dialog box

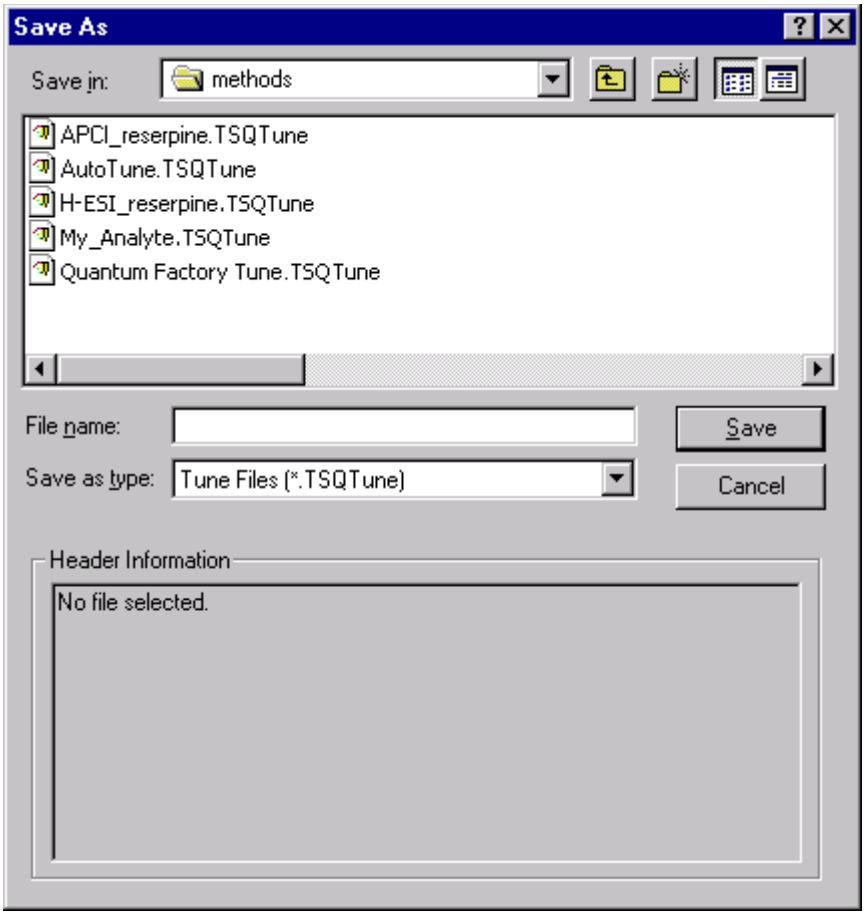


Figure 3-25. Tune Method Save As dialog box

9. Save the Tune Method file as follows:

    a. In the File Name text box, enter a name for your Tune Method file.

    b. Click on **Save** to save the Tune Method file.

The mass spectrometer is now tuned and calibrated in the negative ion mode.

You need to clean the system before optimizing the mass spectrometer with your compound. To clean the system, go to the next topic: “Flushing the System after Tuning and Calibrating”.

Flushing the System after Tuning and Calibrating

This topic describes how to clean your mass spectrometer after performing the tuning and calibration procedure. It is recommended that you clean the mass spectrometer before acquiring data on your analyte of interest.

Clean the mass spectrometer as follows:

1. Turn Off the flow of liquid from the syringe pump as follows:

   a. Select **Setup > Syringe Pump & Sample Loop** to display the Syringe Pump and Sample Loop view in the top right corner of the workspace. See Figure 3-26.

   b. In the Syringe Flow Control group box, select the Off option button and then click on **Apply** to stop the syringe pump.

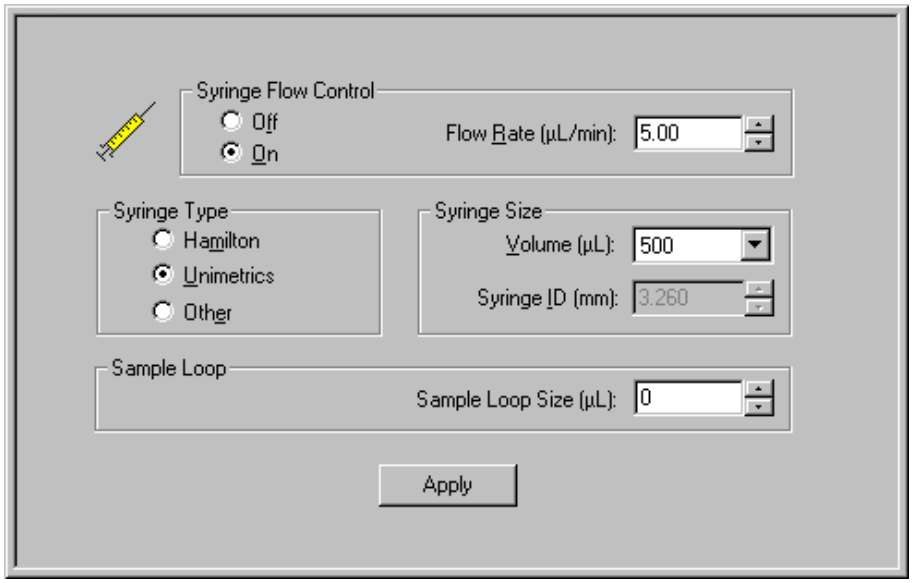


Figure 3-26. Syringe Pump and Sample Loop view, showing pump On


On


Standby

2. If necessary, click on the On/Standby button on the Control / Scan Mode toolbar to place the mass spectrometer in Standby.

3. Remove the syringe from the syringe pump holder as follows:

   a. Lift the handle off the syringe while depressing the black release button on the syringe pump handle.

   b. Remove the syringe.

   c. Remove the tip of the syringe needle from the end of the Teflon tube on the syringe adapter assembly.

4. Clean the syringe thoroughly with a solution of 50:50 methanol–water.

CAUTION Ensure that the TSQ Quantum Ultra is in Standby mode (or Off) before you expose the H-ESI source to atmospheric oxygen. The presence of oxygen in the ion source when the mass spectrometer is On could be unsafe. ▲

CAUTION AVOID BURNS. At operating temperatures, the ion transfer capillary can severely burn you! The ion transfer capillary typically operates between 200 and 400 °C. **Always allow the ion transfer capillary and ion sweep cone to cool to room temperature (for approximately 20 min) before you touch or remove either component.** Always be careful not to touch the entrance end of the ion transfer capillary when it is exposed. ▲

5. Flush the sample transfer line, sample tube, and H-ESI probe as follows:

Note The solvent that you use to flush the syringe, sample transfer line, sample tube, and H-ESI probe assembly depends on the solvent system you use to dissolve your samples. For example, if you are using a buffered solution of a high concentration, an acidic solution is appropriate. ▲

   a. Fill the cleaned syringe with a solution of 50:50 methanol–water (or with another appropriate solvent).

   b. While holding the plunger of the syringe in place, carefully insert the needle of the syringe into the end of the Teflon tube on the syringe adapter.

   c. Flush the sample transfer line, sample tube, and H-ESI probe with the solution by slowly depressing the syringe plunger. Visually check that the solution is exiting the tip of the H-ESI probe on the inside of the probe assembly. Use a lint-free tissue to gently remove the excess solution as it exits the probe.

   d. Remove the needle of the syringe from the syringe adapter.

You have now completed flushing the system. To optimize the tune with your compound, go to the next chapter: “Optimizing the Mass Spectrometer with Your Compound in H-ESI/MS/MS Mode”.

Chapter 4 Optimizing the Mass Spectrometer With Your Compound in H-ESI/MS/MS Mode

This chapter provides information on fine tuning the mass spectrometer in the H-ESI/MS/MS mode using your analyte as the tuning compound. You optimize the sensitivity of the mass spectrometer for your analyte with an automatic tuning procedure.

The Tune Methods that are provided with your TSQ Quantum Ultra are useful for a wide range of applications. They can often be used without further tuning of your mass spectrometer. However, for certain applications you might need to optimize several mass spectrometer parameters. For instance, the parameters that affect H-ESI performance and signal quality are as follows:

- Spray voltage
- H-ESI Vaporizer temperature
- Sheath gas pressure
- Auxiliary gas pressure
- Capillary (ion transfer tube) temperature
- Tube lens offset voltage

The optimum settings for these parameters depend on the solvent flow rate and on the structure of your analyte. In general, you need to fine tune the mass spectrometer parameters whenever you change the solvent flow rate conditions of your particular application. When you optimize the mass spectrometer parameters using the automatic tuning procedure, the procedure adjusts all the parameters listed above and the voltages applied to the ion optics until the ion transmission of your analyte is maximized.

The ion transfer tube is heated to maximize the ion transmission to the mass spectrometer. For H-ESI only, you set the capillary (ion transfer tube) temperature so that it is proportional to the flow rate of your solution. Refer to Table 1-2 for guidelines for setting parameters for H-ESI/MS operation. For this procedure, the capillary temperature is set to 350 °C.

Note Ensure that you have performed the TSQ Quantum Ultra tuning and calibration procedure within the previous three months before you optimize the tune for your compound. If you need to tune and calibrate the system, refer to the procedure in the "Chapter 3: Tuning and Calibrating the Mass Spectrometer in H-ESI/MS Mode". ▲

In the example presented in this chapter, you optimize the mass spectrometer in the H-ESI/MS/MS mode for the reserpine transition from *m/z* 609.281 to *m/z* 195.066.

To optimize the mass spectrometer for reserpine or your compound in the H-ESI/MS/MS mode, you do the following:

- Set up the syringe pump and the divert/injection valve for auto loop injection.
- Set up the mass spectrometer for your specific compound from Tune Master.
- Run the automatic compound optimization procedure to fine tune the mass spectrometer parameters that are compound dependent.
- Save the new Tune Method.

This chapter contains the following topics:

- Setting Up to Introduce Sample by Auto Loop Injection in H-ESI Mode
- Setting Up to Optimize in H-ESI/MS/MS Mode with Your Compound
- Optimizing in H-ESI/MS/MS Mode Automatically with Your Compound

Setting Up to Introduce Sample by Auto Loop Injection in H-ESI Mode

The following procedure describes how to introduce your compound by auto loop injection. The plumbing connections for H-ESI/MS sample introduction from the syringe pump into the solvent flow from an LC are shown in Figure 4-1.

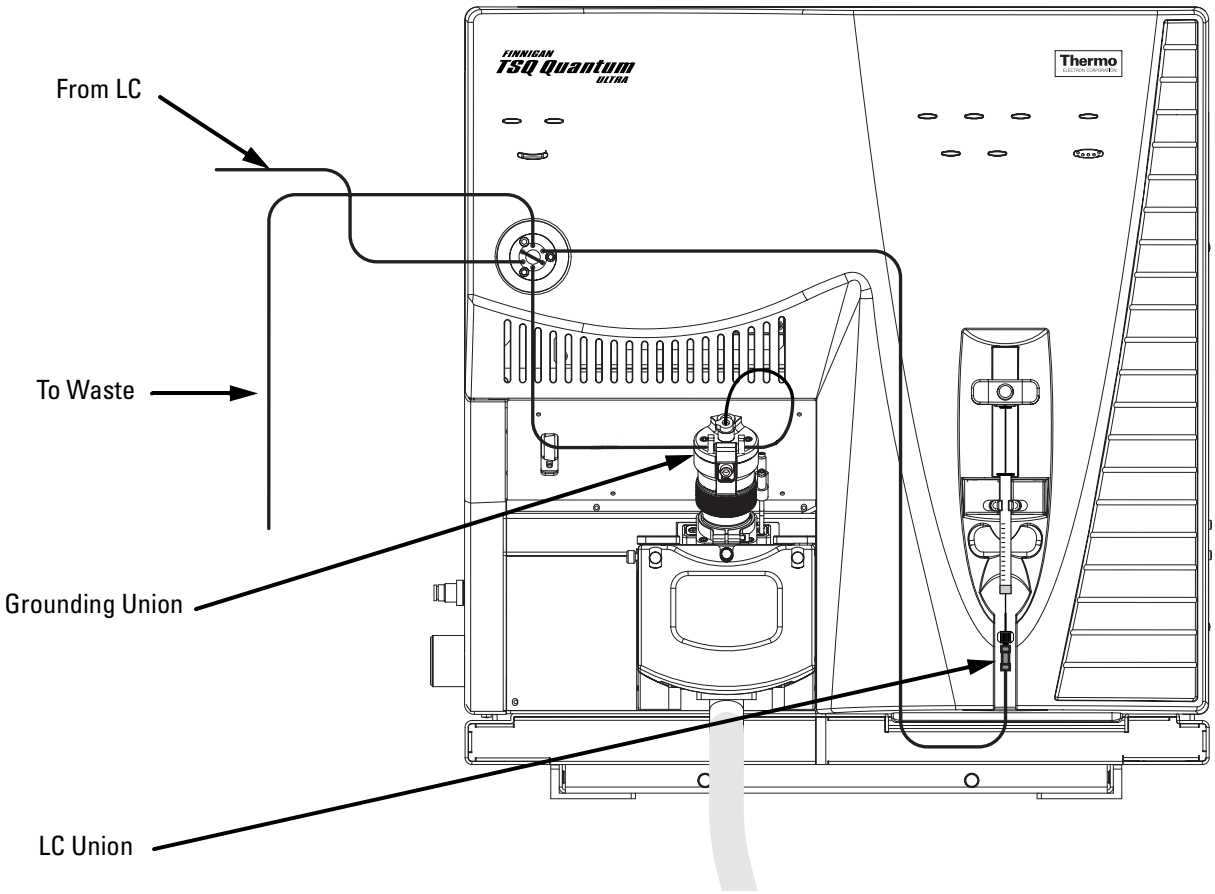


Figure 4-1. H-ESI/MS plumbing connections for sample introduction by auto loop injection into the solvent flow from an LC

Note You can use the reserpine sample solution described in "Appendix A: Solution Formulations", or you can use your compound of interest.▲

Note The following procedures assume that you are familiar with your TSQ Quantum Ultra instrument and with Tune Master. If you need additional guidance, refer to TSQ Quantum Ultra online Help, *Finnigan TSQ Quantum Ultra Getting Connected*, *Finnigan H-ESI Probe Operator's Manual,* or the *Finnigan TSQ Quantum Ultra Hardware Manual.* ▲

Make the plumbing connections for H-ESI/MS sample introduction from the syringe pump into the solvent flow from an LC, as follows:

1. Remove the syringe from the syringe pump holder as follows:

    a. Lift the handle off the syringe while depressing the black release button on the syringe pump handle.

    b. Remove the syringe.

    c. Remove the tip of the syringe needle from the end of the Teflon tube on the syringe adapter assembly. See Figure 4-2.

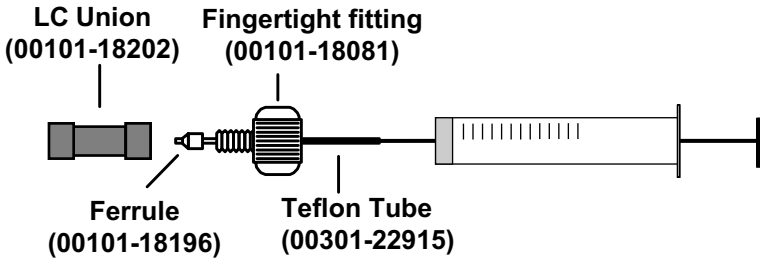


Figure 4-2. Syringe and syringe adapter assembly

2. Remove the sample transfer line installed between the syringe adapter assembly and the grounding union on the H-ESI probe.

3. Install a sample transfer line between the syringe adapter assembly and the divert/inject valve as follows:

    a. Connect an appropriate length of tubing to the LC union on the syringe adapter assembly.

    b. Connect the other end of the tubing fitted with a nut and a ferrule to port 5 of the divert/inject valve. See Figure 4-3.

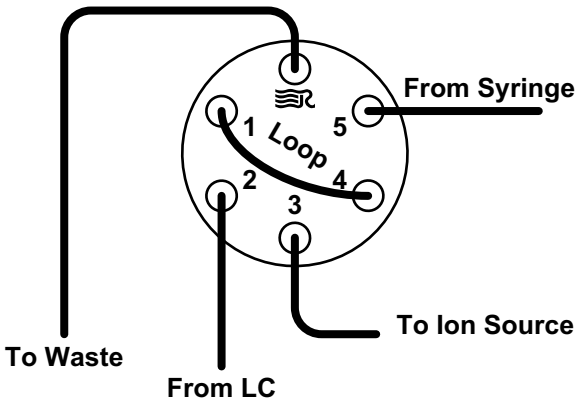


Figure 4-3. Divert/inject valve, showing plumbing for auto loop injection

Note To minimize the possibility of cross-contamination, use a different syringe and a different sample transfer line for your tuning and calibration solution than you do for your samples and compound optimization solution. ▲

4. Load a clean, 500-µL Unimetrics syringe with 420 µL of the 2 pg/µL reserpine sample solution. (Refer to the topic "Appendix A: Solution Formulations" for the procedure for preparing the reserpine solution.)

Note Be sure to wipe off the tip of the needle with a clean, lint-free tissue before reinserting it into the syringe adapter assembly to minimize the possibility of cross-contamination of the assembly. ▲

5. While holding the plunger of the syringe in place, carefully reinsert the tip of the syringe needle into the end of the Teflon tube on the syringe adapter assembly. See Figure 4-2.

6. Place the syringe into the syringe holders of the syringe pump.

7. While squeezing the black release button on the syringe pump handle, push the handle down until it just contacts the syringe plunger.

8. Install a sample transfer line between the divert/inject valve and the grounding union on the ion source as follows:

   a. Gather the necessary fittings for installing a sample transfer line. See Figure 4-4.

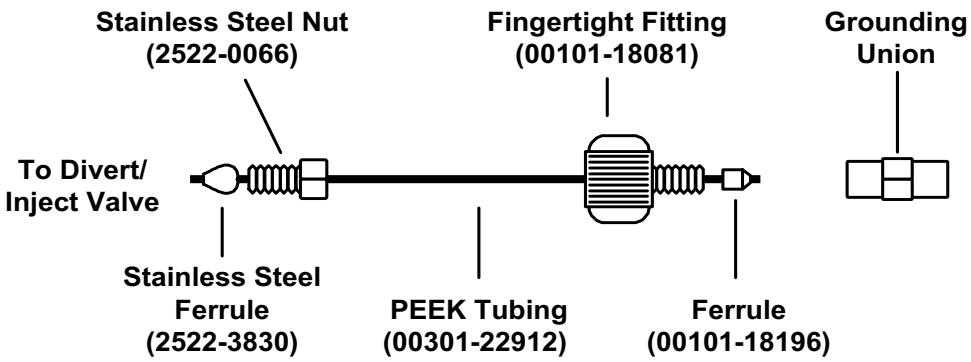


Figure 4-4. Sample transfer line, installed between the divert/inject valve and the grounding union

b. Connect an appropriate length of tubing fitted with a nut and a ferrule to port 3 of the divert/inject valve. See Figure 4-3.

c. Connect the other end of the tubing with a fingertight fitting and a ferrule to the grounding union on the ion source. See Figure 4-1.

9. Install a 5 µL sample loop with nuts and ferrules between ports 1 and 4 of the divert/inject valve.

10. Install a solvent line between the LC system and the divert/inject valve as follows:

    a. Connect an appropriate length of tubing with a proper fitting and a ferrule to the outlet of the LC system.

    b. Connect the other end of the tubing with a nut and ferrule to port 2 of the divert/inject valve.

11. Install a waste line on the divert/inject valve and direct the outlet to a waste container as follows:

    a. Connect an appropriate length of tubing with a nut and ferrule to port 6 of the divert/inject valve (port 6 is labeled with the Rheodyne® logo ®).

    b. Insert the other end of the tubing into the waste container.

You have completed setting up to introduce your compound by auto loop injection. Go to the next topic: “Setting Up to Optimize in H-ESI/MS/MS Mode with Your Compound”.

Setting Up to Optimize in H-ESI/MS/MS Mode with Your Compound

Use the following procedure to set up the mass spectrometer to optimize the compound dependent devices for your compound in H-ESI/MS/MS mode.


On


Standby

1. Click on the On/Standby button on the Control / Scan Mode toolbar to turn On the mass spectrometer.

2. If necessary, change the ion polarity mode to positive ion polarity as follows:

   a. Turn Off the spray voltage as follows:

      i. Click on the Optimize Compound Dependent Devices button to display the Optimize Compound Dependent Devices view in the top right corner of the workspace. See Figure 4-5.

      ii. In the Device spin box, enter **0** to set the spray voltage to 0 V.


Positive Polarity

   b. On the Control / Scan Mode toolbar, click on the Polarity button to toggle the ion polarity mode of the mass spectrometer.


Negative Polarity

- If you want to optimize the currently displayed Tune Method, go to step 4.
- If you want to optimize a different Tune Method than the one currently displayed, you first need to open the desired Tune Method as described in step 3.

3. Open the Tune Method file that stores reserpine tune settings, or the settings for your analyte, as follows:

   a. On the File / Display toolbar, click on the Open File button to display the Open dialog box. See Figure 4-5.

   b. Confirm that the folder *C:\Xcalibur\methods* is displayed. Select the file *H-ESI_reserpine.TSQTune* (or your Tune Method).

   c. Click on **Open** to open the file. Tune Master downloads the Tune Method parameters to the mass spectrometer.

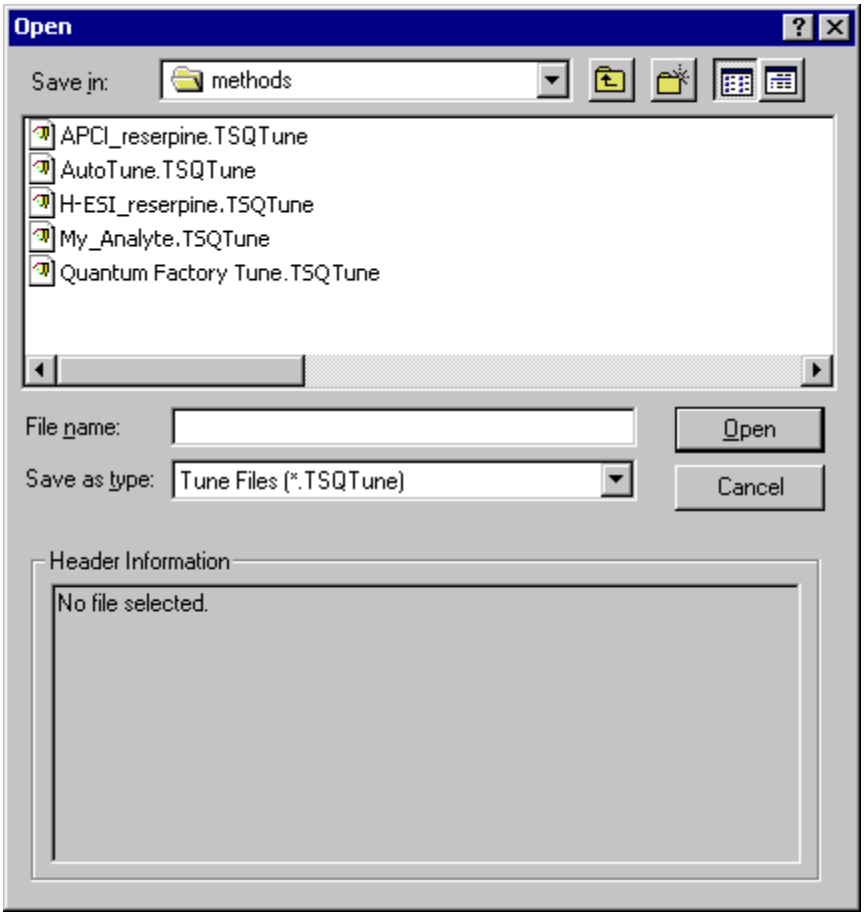


Figure 4-5. Open Tune Method dialog box

4. Click on the Optimize Compound Dependent Devices button on the Control / Scan Mode toolbar or choose **Display > Compound Dependent Devices** to display the Optimize Compound Dependent Devices view in the top right corner of the workspace. See Figure 4-6.

5. Set the values for the compound dependent devices as follows:

   a. Set the spray voltage to 4000 V as follows:

      i. In the Device Display box, click on Spray Voltage. This changes the Device spin box label to *Spray Voltage* and enables you to change the spray voltage.

      ii. In the Device spin box enter **4000** to set the spray voltage.

   b. Set the H-ESI vaporizer temperature to 300 °C as follows:

      i. In the Device Display box, click on Vaporizer Temperature.

ii. In the Device spin box enter **300** to set the vaporizer temperature.

c. Set the pressure of the sheath gas to 40 psi as follows:

i. In the Device Display box, click on Sheath Gas Pressure.

ii. In the Device spin box enter **40** to set the sheath gas pressure.

d. Set the pressure of the auxiliary gas to 15 units as follows:

i. Click on Aux Gas Pressure in the Device Display box.

ii. Enter **15** in the Device spin box to set the auxiliary gas pressure.

e. Set the temperature of the capillary (ion transfer tube) to 350 °C as follows:

i. Click on Capillary Temperature in the Device Display box.

ii. Enter **350** in the Device spin box to set the capillary temperature.

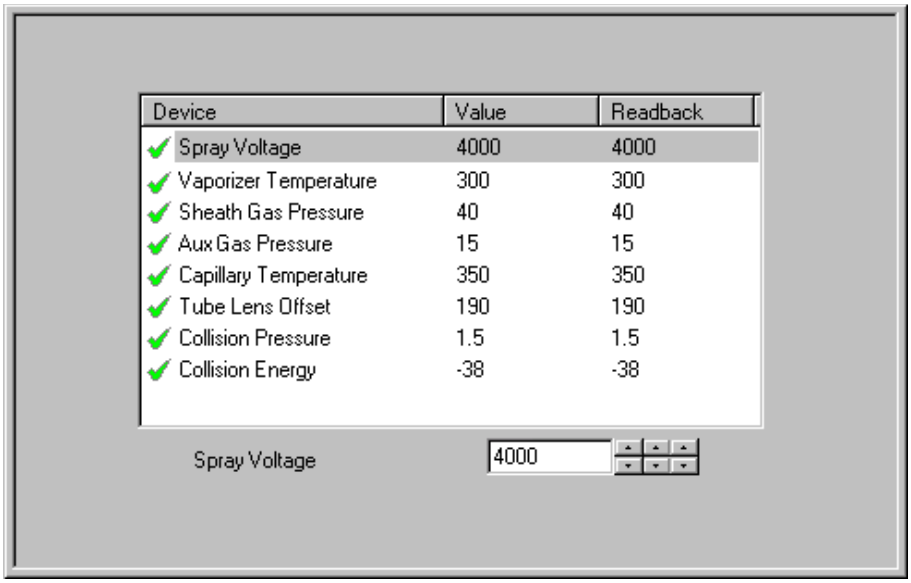


Figure 4-6. Optimize Compound Dependent Devices view, positive ion polarity settings

f. Set the collision pressure to 1.5 mTorr as follows:

i. Click on Collision Pressure in the Device Display box.

ii. Enter **1.5** in the Device spin box to set the collision pressure to 1.5 mTorr.

g. Set the collision energy to -38 eV as follows:

i. Click on Collision Energy in the Device Display box.

ii. Enter **-38** in the Device spin box to set the collision energy to -38 eV.

Ensure that the readbacks in the Device Display box are approximately equal to the set values. (You might need to wait for a few minutes for the H-ESI vaporizer and capillary temperatures to stabilize at the set value.)

6. Configure the Syringe Pump to automatically inject the reserpine sample solution into the sample loop as follows:

   a. Choose **Setup > Syringe Pump & Sample Loop** to display the Syringe Pump and Sample Loop view in the top right corner of the workspace. See Figure 4-7.

   b. Select the Off option button in the Syringe Flow Control group box to turn Off the syringe pump.

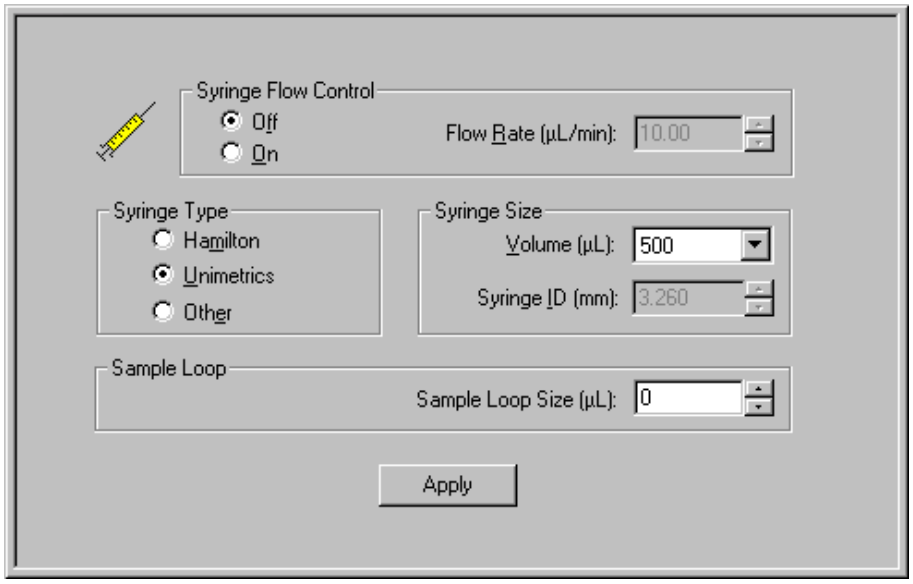


Figure 4-7. Syringe Pump and Sample Loop view, showing auto loop injection setup

- If you are using either a Unimetrics or Hamilton syringe, go to step 6.c.
- If you are *not* using either a Unimetrics or Hamilton syringe, go to step 6.e.

   c. In the Syringe Type group box, select the Unimetrics (or Hamilton) option button to specify a Unimetrics (or Hamilton) syringe.

   d. In the Syringe Size group box, select *500* (or the size of your syringe) from the Volume list box to specify that the volume of your syringe is 500 µL.

When you specify the syringe type and syringe volume, Tune Master automatically sets the proper syringe ID value. Go to step 6.f.

e. If you are using a make of syringe other than Unimetrics or Hamilton, you need to manually specify the syringe ID by doing the following:

   i. Select the Other option button in the Syringe Type group box to specify that you are using a syringe other than Unimetrics or Hamilton syringe and to enable the Syringe ID spin box.

   ii. In the Syringe Size group box, select the volume of your syringe from the Volume list box.

   iii. Enter the inner diameter of your syringe in the Syringe ID spin box.

f. In the Sample Loop group box, enter **5** in the Sample Loop Size spin box to specify a loop size of 5 µL.

g. Click on **Apply** to apply these settings. The syringe pump is now configured to fill the sample loop with the appropriate amount of sample.

7. Start the flow of solvent as follows:

a. On the Control / Scan Mode toolbar, click on the AS/LC Direct Control button to display the Inlet Direct Control view in the top right corner of the workspace. See Figure 4-8.

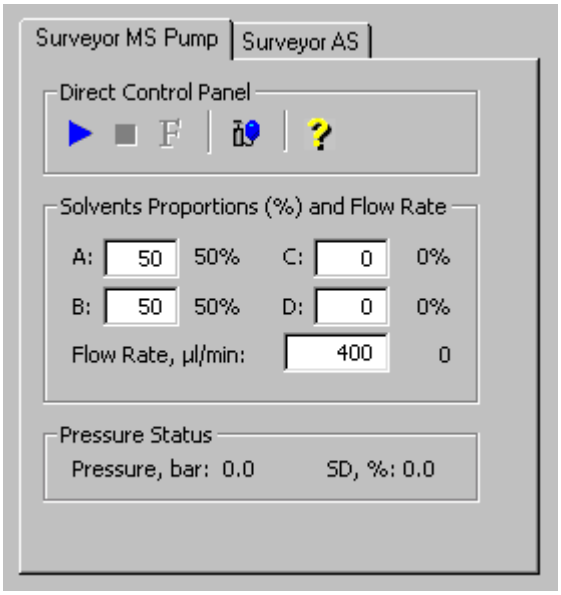


Figure 4-8. Inlet Direct Control view, showing pump Off

Note The following procedure assumes that isopropyl alcohol and HPLC grade water are in the solvent bottles labeled A and B, respectively. ▲

b. Set up the Surveyor MS Pump to deliver a solution of 50:50 isopropyl alcohol–water at 400 µL/min, as follows:

   i. In the Inlet Direct Control view, in the Solvents Proportions (%) and Flow Rate group box, enter **50** in the text box labeled *A* to specify a delivery proportion of 50% solvent A.

   ii. Enter **50** in the text box labeled *B* to specify a delivery proportion of 50% solvent B.

   iii. In the Flow Rate text box, enter **400** to set a flow rate of 400 µL/min.

c. In the Direct Control Panel group box, click on the Start button to start the Surveyor MS pump.

The system is now set up to automatically deliver reserpine to the ion source for optimizing the mass spectrometer with your compound.

Next you will optimize the compound dependent devices for the reserpine sample solution (or your compound) in H-ESI/MS/MS mode. Go to the next topic: “Optimizing in H-ESI/MS/MS Mode Automatically with Your Compound”.

Optimizing in H-ESI/MS/MS Mode Automatically with Your Compound

You optimize the mass spectrometer to maximize the ion transmission of your compound. Optimization is performed to fine tune compound dependent parameters such as spray voltage, capillary temperature, and tube lens offset. It is recommended that you optimize the mass spectrometer only after you have successfully tuned and calibrated the instrument.

Use the following procedure to automatically optimize the mass spectrometer in the H-ESI/MS/MS mode for the reserpine transition from *m/z* 609.281 to *m/z* 195.066.

1. On the Control / Scan Mode toolbar, click on the Compound Optimization Workspace button to display the Compound Optimization workspace. See Figure 4-9.

2. Set the optimization parameters for monitoring the reserpine transition from *m/z* 609.281 to *m/z* 195.066 as follows:

   a. In the Compound Optimization view in the top left corner of the workspace, select **Optimization Modes: SRM** to enable you to optimize a selected reaction. See Figure 4-10.

   b. Select the Optimization Options: Standard option button to tune the default selection of devices.

Note Tube lens offset and collision energy are the default compound sensitive devices that are optimized when you choose Optimization Options: Standard. To optimize the spray voltage, H-ESI vaporizer temperature, sheath gas pressure, auxiliary gas flow, capillary temperature, tube lens offset, source CID, collision energy, or collision pressure, choose Optimization Options: Custom. ▲

(Tube lens offset and collision energy are the default compound sensitive devices that are optimized in this configuration.)

   c. In the Optimization table, enter the parent mass **609.281** to set the parent mass of the SRM reaction to the ion at *m/z* 609.281.

   d. Enter the product mass **195.066** to set the product mass of the SRM reaction to the ion at *m/z* 195.066.

Note You need to select the inlet type option button appropriate to the inlet mode you use to introduce your sample into the mass spectrometer. This procedure uses the Auto Loop Injection option. ▲

   e. In the Inlet Types group box, select the Auto Loop Injection option button to have the TSQ Quantum Ultra automatically inject the optimization solution.

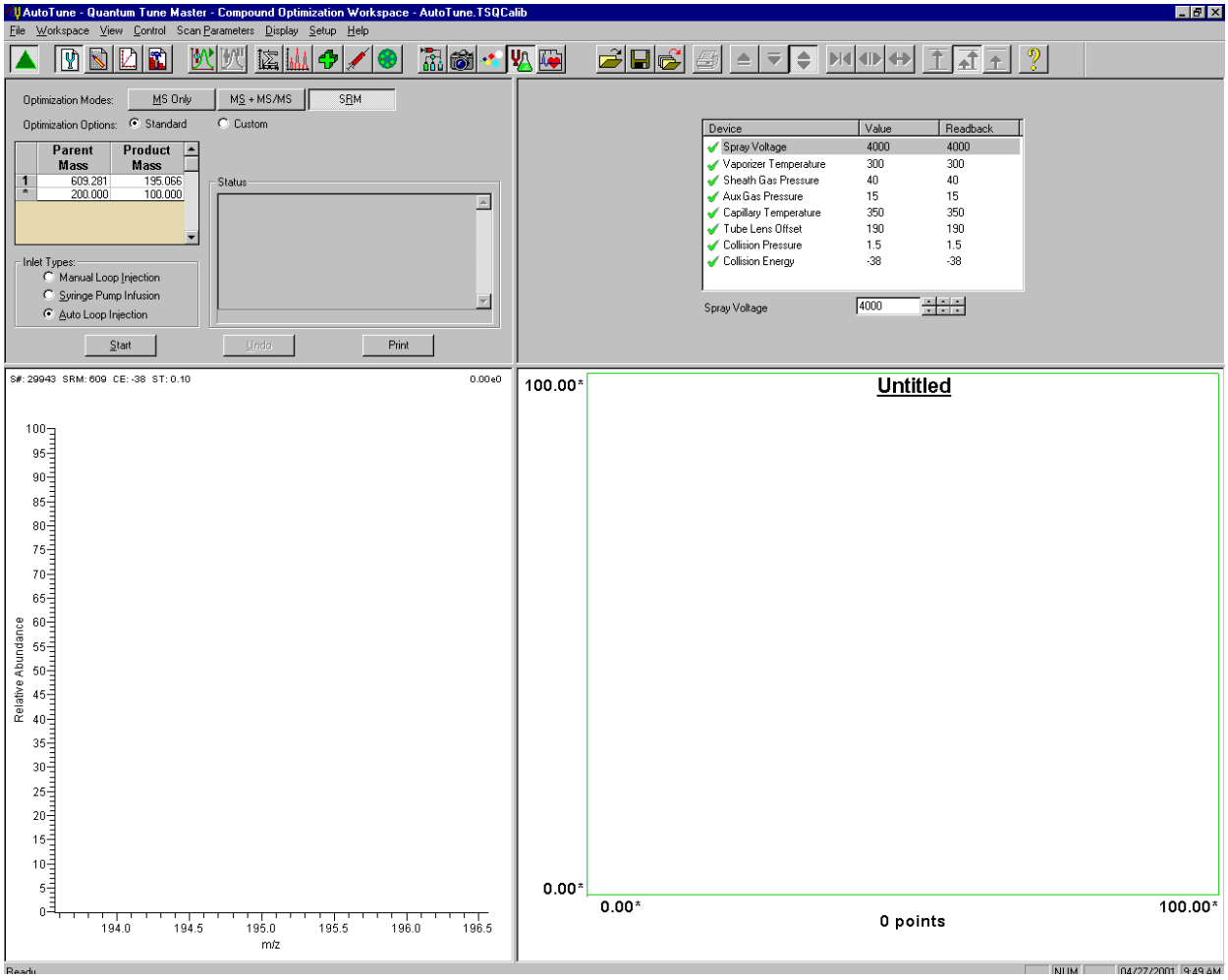


Figure 4-9. Compound Optimization workspace

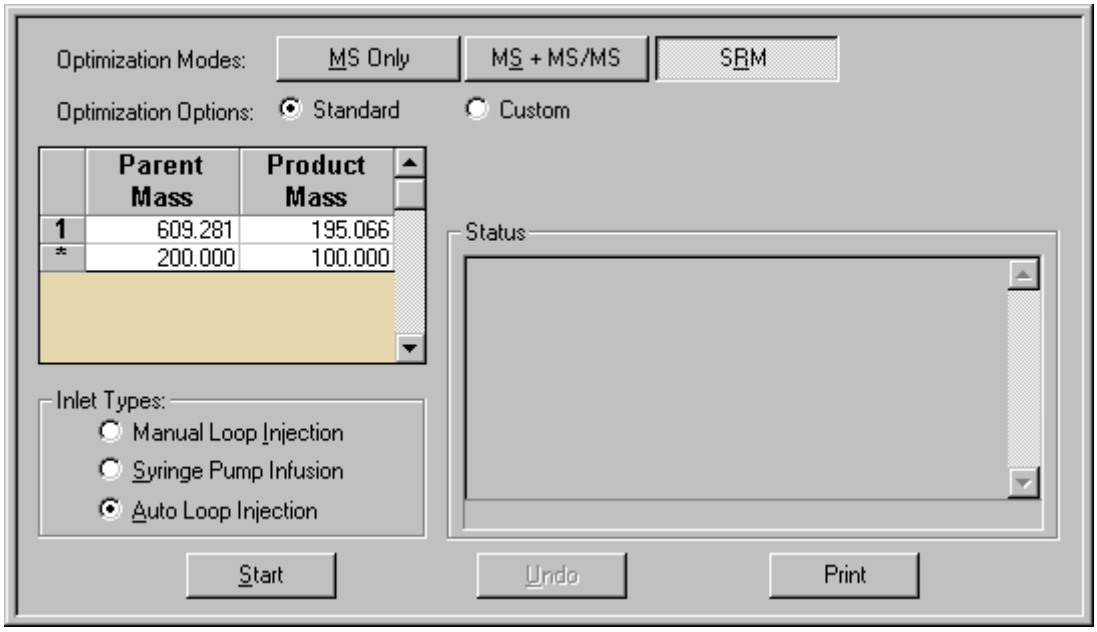


Figure 4-10. Compound Optimization view

3. Click on **Start** to start the automatic tuning procedure.

Note If the syringe runs out of sample during the compound optimization procedure, the instrument pauses the automatic tuning and displays the message: *Syringe out of sample, Reload and click OK*. If this occurs, reload the syringe and click on **OK** to continue the optimization. ▲

The message *Finish compound optimization* is displayed in the Status box in the Compound Optimization view when the compound optimization has completed successfully. See Figure 4-11.

- If the compound optimization procedure finishes without errors and the breakdown curve of the 195.066 fragment is Gaussian-shaped (as in Figure 4-12) or is a smooth, positive-sloped curve, go to step 5.
- If errors occur during the compound optimization procedure or the breakdown curve of the 195.066 fragment oscillates, contains multiple peaks, or is excessively noisy, go to step 4.

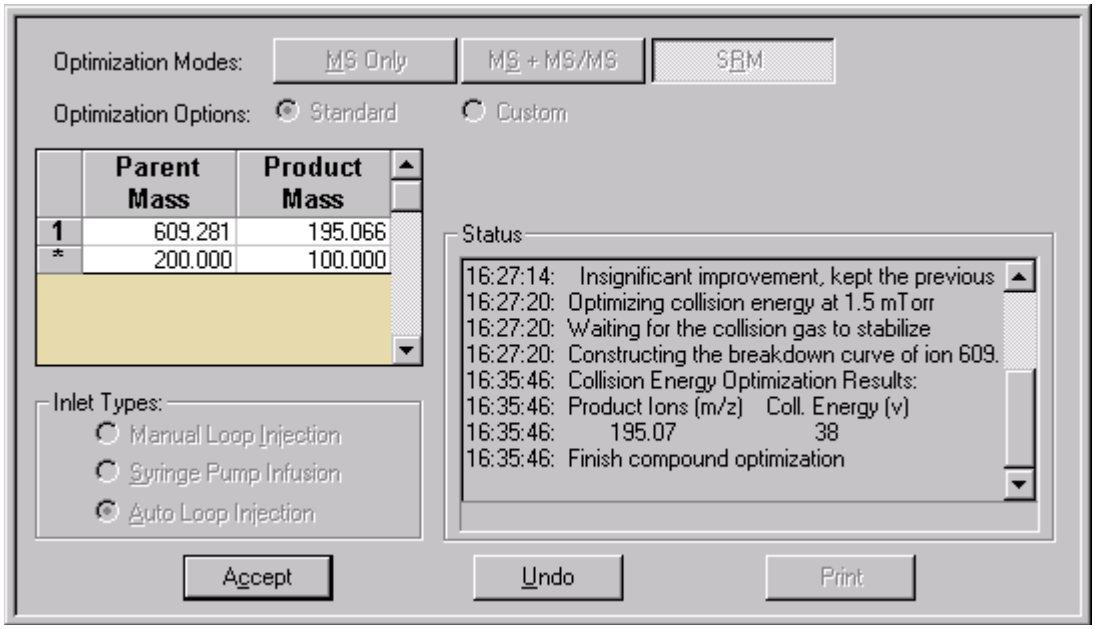


Figure 4-11. Compound Optimization view, showing the successful completion of compound optimization

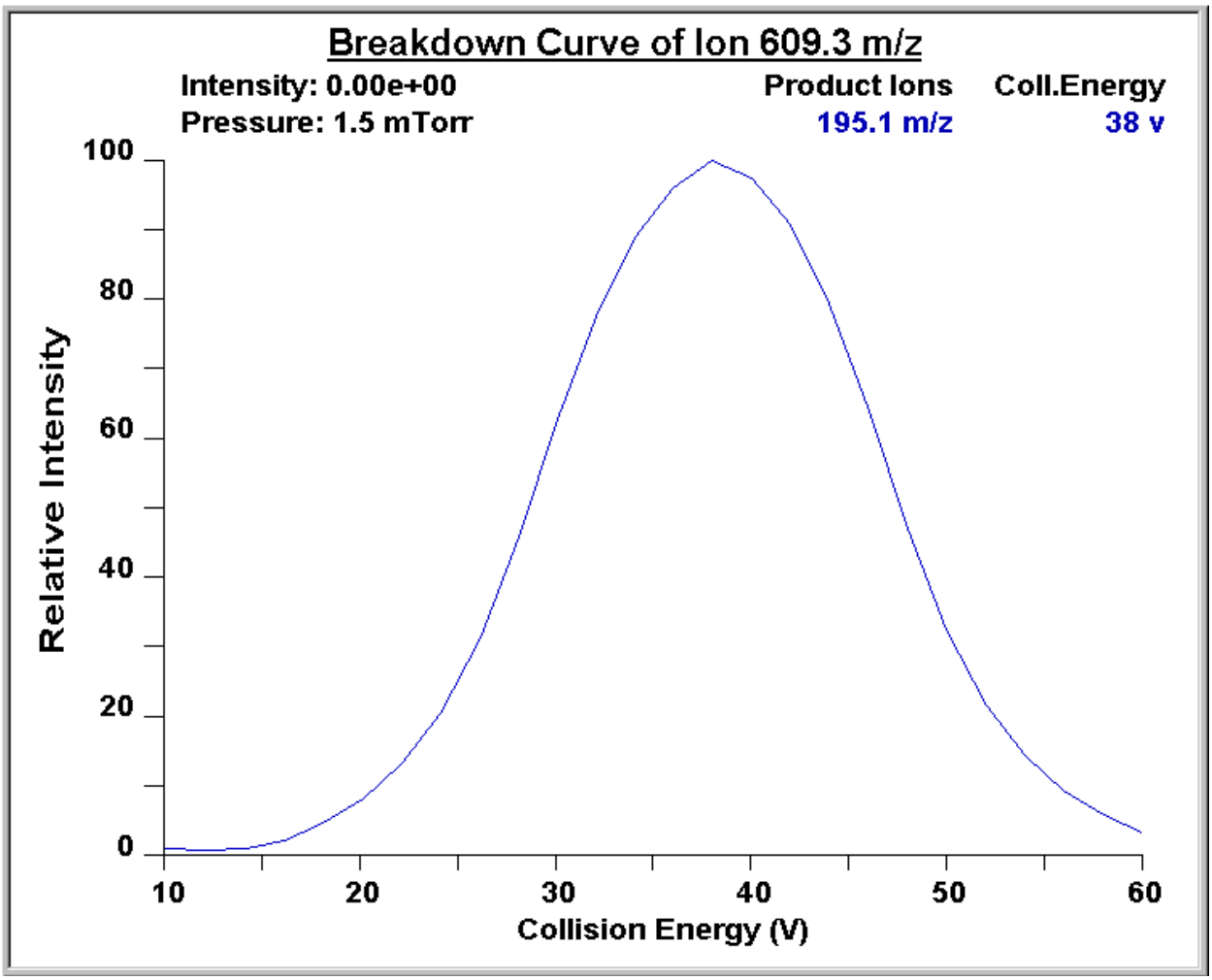


Figure 4-12. Breakdown curve of reserpine showing the relative intensity of the product ion at *m/z* 195.066 as a function of collision energy

4. If errors occurred during the compound optimization procedure, restore the previous mass spectrometer compound sensitive device settings by completing the following steps:

   a. Click on **Undo** to restore the prior device settings.

   b. Click on **Accept** to reload the prior device settings to the mass spectrometer.

   c. Troubleshoot and correct the situation that caused the optimization to fail.

   d. Go to step 3 of this procedure and restart the compound optimization procedure.

5. Click on **Accept** to accept the results of the compound optimization.

Note Save the Tune Method while the mass spectrometer is On if any of the ion source parameters have been changed from their initial settings. ▲

6. Save the Tune Method file by doing the following:

   a. Click on **Save Tune As** to open the Save As dialog box. See Figure 4-13.

   b. Enter a file name (such as **H-ESI_reserpine**, or the name of your compound) for your Tune Method file in the File Name text box.

   c. Click on **Save** to save the Tune Method file.

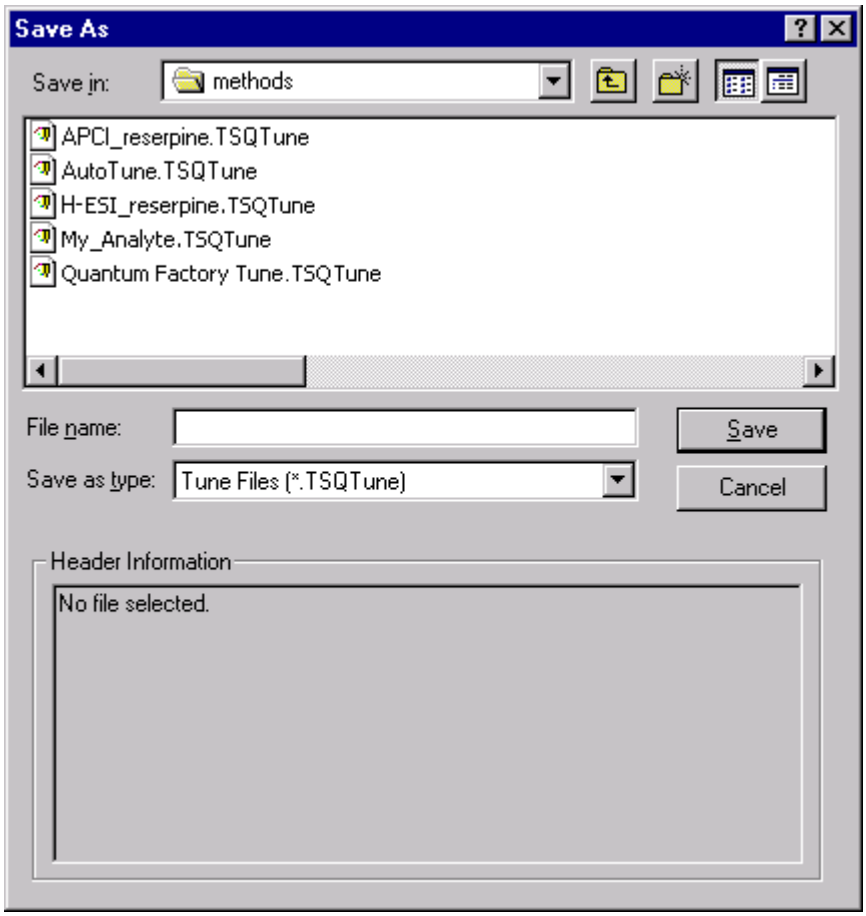


Figure 4-13. Tune Method Save As dialog box

The mass spectrometer is now optimized in H-ESI/MS/MS mode for the compound reserpine (or for your compound).

Go to the next chapter: "Acquiring H-ESI/MS/MS Data with Quantum Ultra Tune Master".

Chapter 5 Acquiring H-ESI/MS/MS Data with Tune Master

To acquire data using Tune Master, you must first have the following:

- A H-ESI source installed
- A sample introduction system set up
- A calibrated instrument
- A Tune Method created for your analyte of interest

This chapter provides information on acquiring sample data using Tune Master in the H-ESI/SRM mode. This experiment uses reserpine but you can follow the same procedure with your analyte of interest.

This chapter contains the following topics:

- Setting Up to Introduce Sample by Manual Loop Injection in H-ESI Mode
- Acquiring H-ESI/MS/MS Data in the SRM Scan Mode

Setting Up to Introduce Sample by Manual Loop Injection in H-ESI Mode

This topic describes how to introduce sample by manual loop injection into the solvent flow from an LC. The plumbing connections for H-ESI sample introduction by manual loop injection are shown in Figure 5-1.

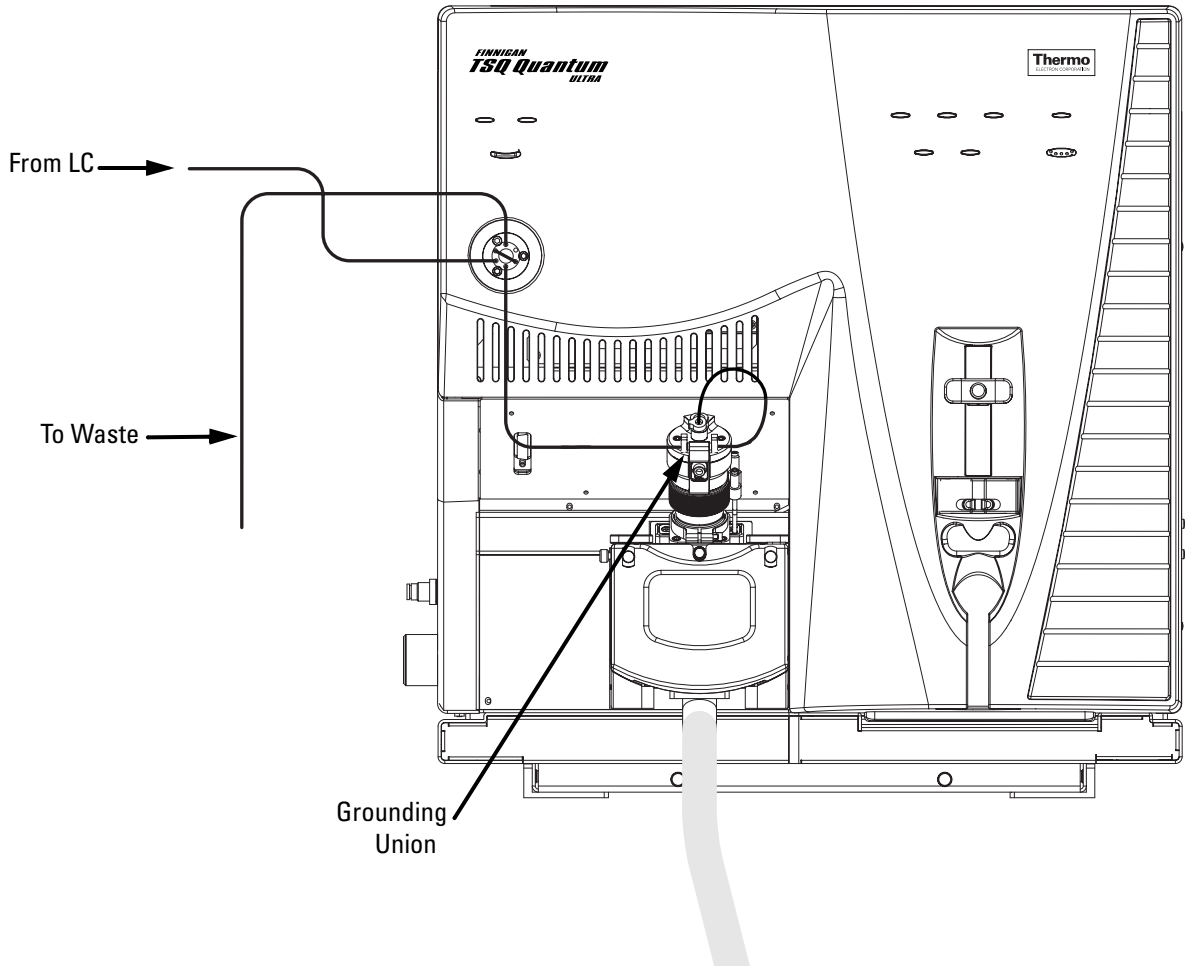


Figure 5-1. H-ESI/MS plumbing connections for sample introduction by manual loop injection into the solvent flow from an LC

Make the plumbing connections for manual loop injection as follows:

1. Stop the flow of solvent to the H-ESI source as follows:

   a. On the Control / Scan Mode toolbar, click on the AS/LC Direct Control button to display the Inlet Direct Control view in the top right corner of the workspace. See Figure 5-2.

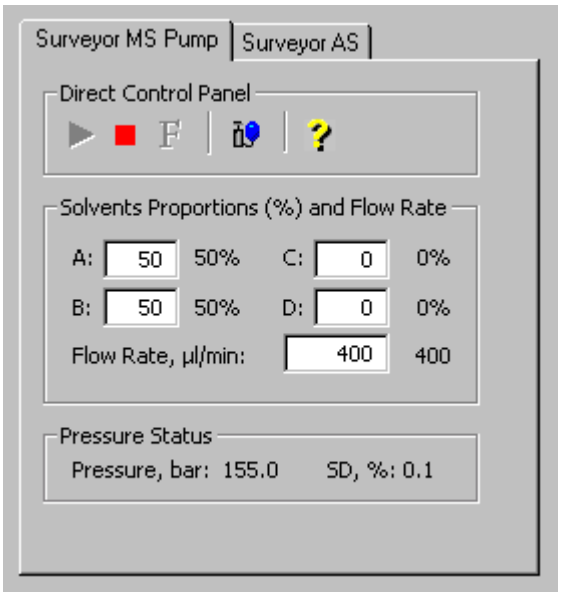


Figure 5-2. Inlet Direct Control view, showing pump On

b. In the Direct Control Panel group box, click on the Stop button to stop the flow of solvent.

2. Click on the On/Standby button on the Control / Scan Mode toolbar to place the mass spectrometer in Standby.

3. Remove the syringe from the syringe pump holder as follows:

   a. Lift the handle off the syringe while depressing the black release button on the syringe pump handle.

   b. Remove the syringe.

   c. Remove the tip of the syringe needle from the end of the Teflon tube on the syringe adapter assembly. See Figure 5-3.

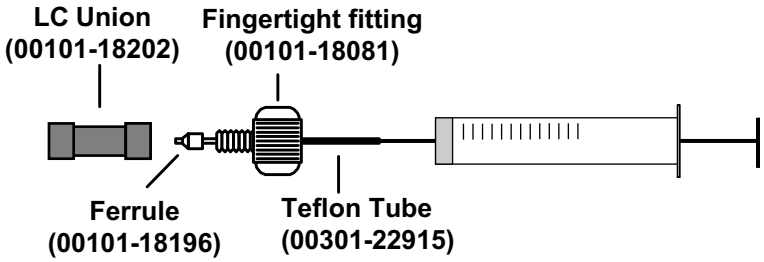


Figure 5-3. Syringe and syringe adapter assembly

4. Remove the sample transfer line that is installed between the syringe adapter assembly and port 5 of the divert/inject valve. Port 5 is now used as the injection port. See Figure 5-4.

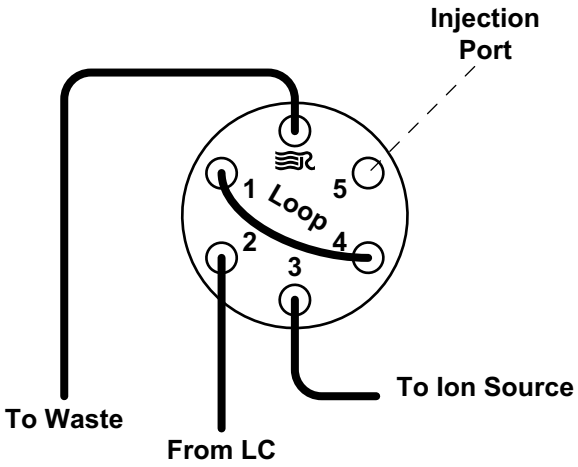


Figure 5-4. Divert/inject valve, showing plumbing for manual loop injection

5. Install the needle port fitting into the divert/inject valve as follows:

   a. Inserting the liner tube, RheFlex® ferrule, and the threaded portion of the RheFlex nut into port 5 of the divert/inject valve. See Figure 5-5.

   b. Carefully tighten the nut with your fingers.

The mass spectrometer is now set up for manual loop injection.

Go to the next topic: "Acquiring H-ESI/MS/MS Data in the SRM Scan Mode".

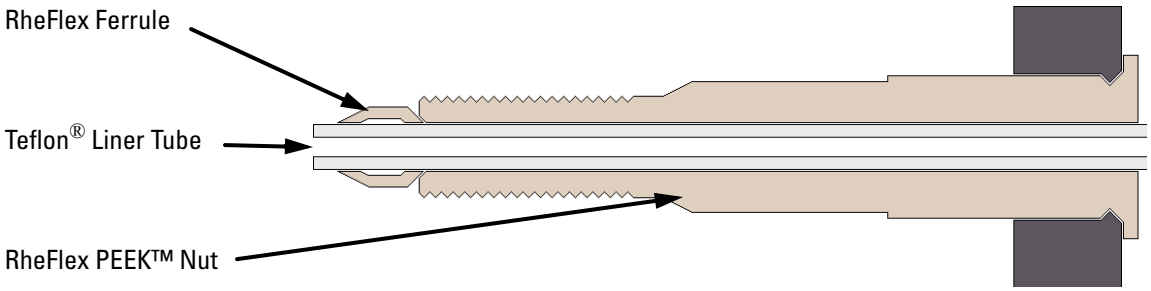


Figure 5-5. Needle port fitting, P/N 00110-22030

Acquiring H-ESI/MS/MS Data in the SRM Scan Mode


On


Standby

Use the following procedure to acquire a file of reserpine data in the SRM scan mode. Tune Master automatically saves the data you acquire on your hard disk.

1. Click on the On/Standby button on the Control / Scan Mode toolbar to turn On the mass spectrometer.

- If you want to acquire data with the currently displayed Tune Method, go to step 3.
- If you want to acquire data with a different Tune Method than the one currently displayed, you first need to open the desired Tune Method as described in step 2.

2. Open the Tune Method file that stores reserpine tune settings, or the settings for your analyte, as follows:

   a. On the File / Display toolbar, click on the Open File button to display the Open dialog box. See Figure 5-6.

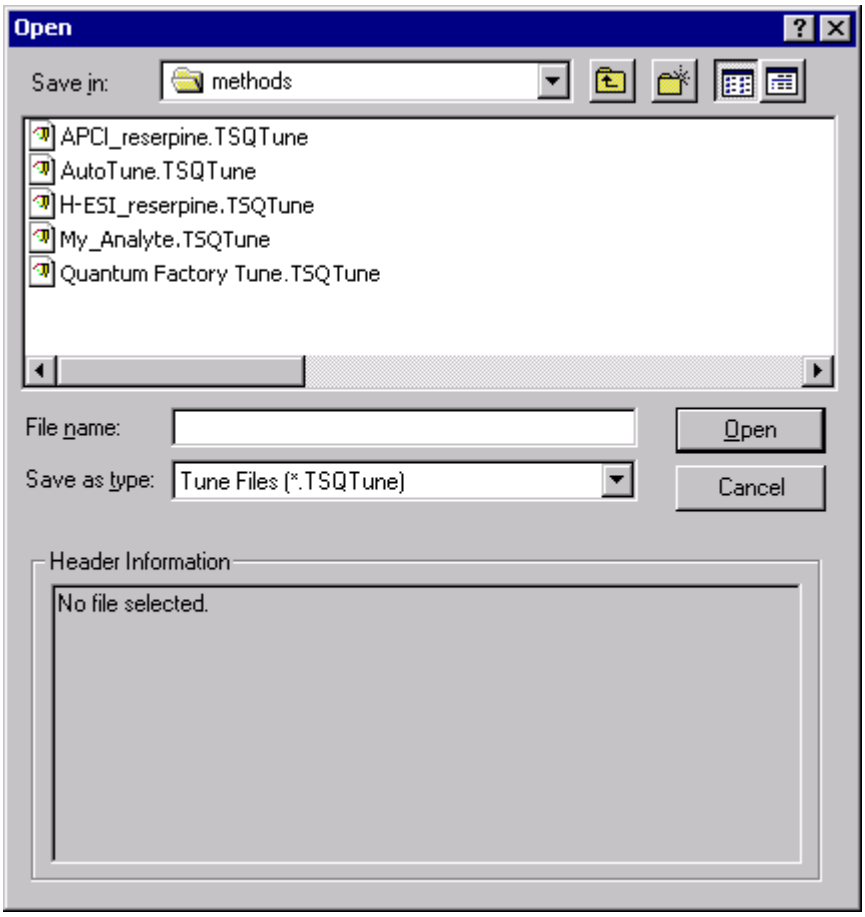


Figure 5-6. Open Tune Method dialog box

b. Confirm that the folder *C:\Xcalibur\methods* is displayed. Select the file *H-ESI_reserpine.TSQTune* (or your Tune Method).

c. Click on **Open** to open the file. Tune Master downloads the Tune Method settings to the mass spectrometer.

3. Start the flow of solvent as follows:

a. On the Control / Scan Mode toolbar, click on the AS/LC Direct Control button to display the Inlet Direct Control view in the top right corner of the workspace. See Figure 5-7.

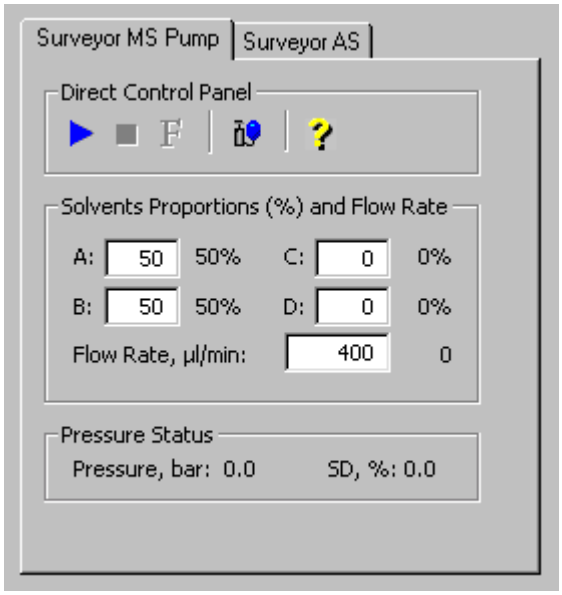


Figure 5-7. Inlet Direct Control view, showing pump Off

Note The following procedure assumes that isopropyl alcohol and HPLC grade water are in the solvent bottles labeled A and B, respectively. ▲

b. Set up the Surveyor MS Pump to deliver a solution of 50:50 isopropyl alcohol–water, at 400 µL/min, as follows:

i. In the Inlet Direct Control view, in the Solvents Proportions (%) and Flow Rate group box, enter **50** in the text box labeled *A* to specify a delivery proportion of 50% solvent A.

ii. Enter **50** in the text box labeled *B* to specify a delivery proportion of 50% solvent B.

iii. In the Flow Rate text box, enter **400** to set a flow rate of 400 µL/min.

c. In the Direct Control Panel group box, click on the Start button to start the Surveyor MS pump.

4. On the Control / Scan Mode toolbar, click on the Instrument Method Development Workspace button to open the Instrument Method Development workspace. See Figure 5-8.

Note If you just completed compound optimization using reserpine as described in Chapter 4, then the following settings will be selected by default when you switch to the Method Development workspace. ▲

5. Define the scan parameters, as required, for acquiring SRM data, by doing the following:

   a. In the Define Scan view in the top left corner of the workspace, select **Scan Type: SRM** to select the Selected Reaction Monitoring (SRM) scan type.

   b. In the SRM table, verify that a single reaction is listed, and verify that the parent mass is 609.281 and the product mass is 195.066.

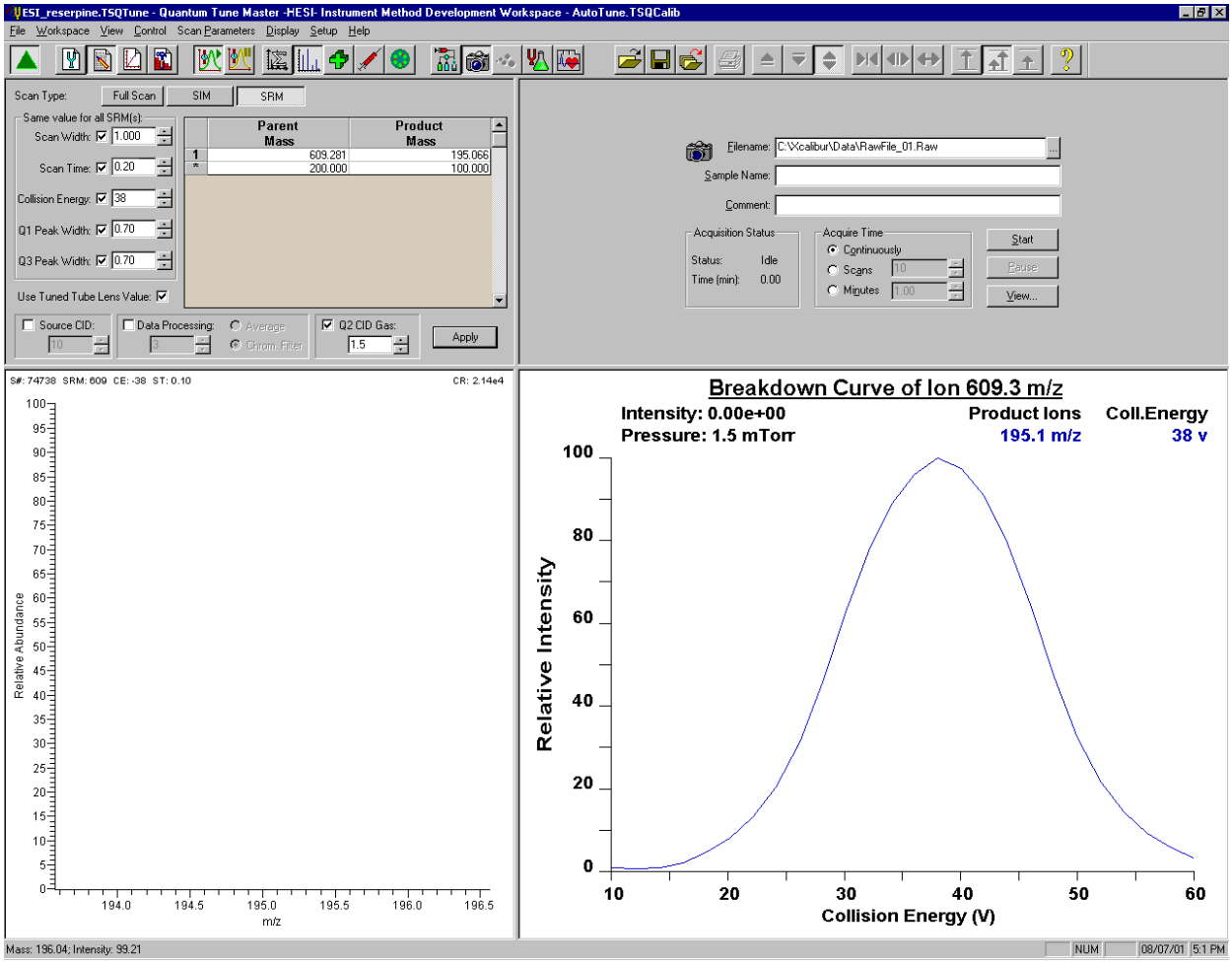


Figure 5-8. Instrument Method Development workspace

Note In the Define Scan view, the Same Value For All SRM(s) group box allows you to select global parameters for your SRM scan. Any parameter that you define as global has the same value for each reaction that you are monitoring. To define a global parameter, select the check box for the parameter and set its value by using the spin box. ▲

c. In the Same Value For All SRM(s) group box, ensure that all the global parameter check boxes are selected, and verify or enter the following values into the appropriate spin boxes:

   i. In the Scan Width spin box, enter **1.000** to set the scan width to 1.000 u.

   ii. In the Scan Time spin box, enter **0.20** to set the scan time to 0.20 s.

   iii. Verify that the collision energy in the Collision Energy spin box is approximately equal to the value of 38 that you entered prior

to compound optimization. (The automatic optimization might have changed the value of the collision energy.)

iv. In the Q1 Peak Width spin box, enter **0.70** to set the peak width to 0.70 u.

v. In the Q3 Peak Width spin box, enter **0.70** to set the peak width to 0.70 u.

d. Select the Use Tuned Tube Lens Value check box.

e. Ensure that the Source CID check box is left unchecked.

f. Specify that a 3 s chromatography filter be used for the data acquisition as follows:

i. Select the Data Processing check box to activate the data processing spin box and option buttons.

ii. Select the Chrom. Filter option button to specify that a chromatography filter be used.

iii. Enter **3** in the Data Processing spin box to designate a 3 s chromatography filter.

g. Set the collision cell gas settings as follows:

i. In the Q2 CID Gas group box, select the Q2 CID Gas check box to specify that collision gas be used.

ii. Enter **1.5** in the Q2 CID Gas spin box to set the collision cell gas pressure to 1.5 mTorr.

6. Click on **Apply** to apply the scan parameters to the mass spectrometer.

7. On the Control / Scan Mode toolbar, click on the Display TIC button to begin recording the total ion current in the Graph view in the bottom right corner of the workspace. See Figure 5-9.

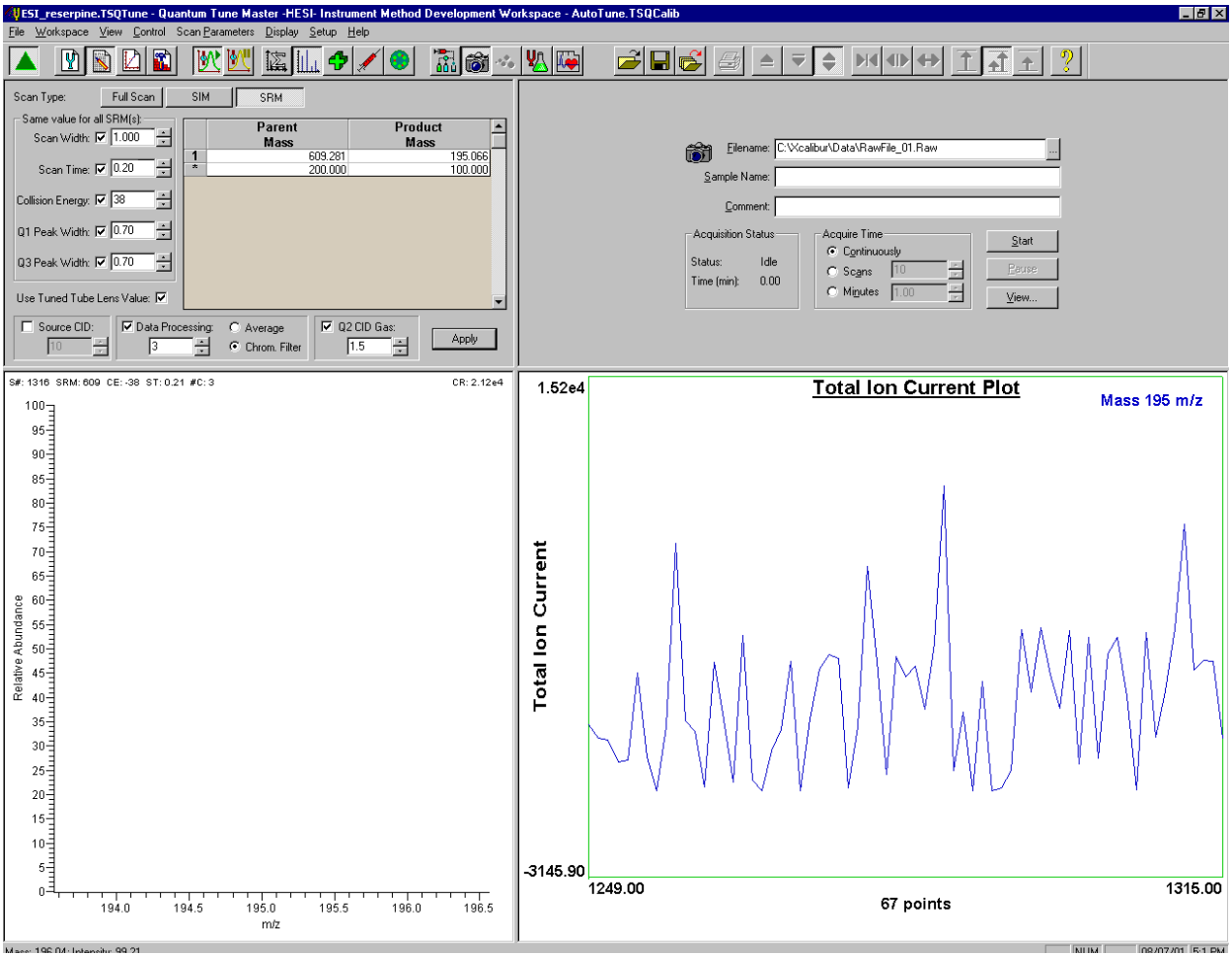


Figure 5-9. Instrument Method Development workspace, showing SRM scan type

8. Specify the acquisition parameters as follows:

Browse

a. In the Acquire Data view in the top right corner of the work space, in the Filename text box, enter **C:\Xcalibur\Data\reserpine_01.raw** to specify a path and filename. (If desired, use the browse button to select a different file folder.)

b. In the Sample Name text box, enter **reserpine** to specify the sample identity. If you are not using reserpine, type the name of your analyte.

c. In the Comment text box, enter a comment about your experiment. For example, enter **SRM, H-ESI, 10 pg, loop** to specify the scan mode, ionization mode, sample amount, and method of sample introduction. Xcalibur includes the comment on hard copies of your data.

d. In the Acquire Time group box, select the Continuously option button to specify that data be continuously acquired until you stop the acquisition.


Load/Detector


Inject/Waste

9. On the Control / Scan Mode toolbar, ensure that the Divert/Inject Valve button is in the Load state. If the Divert/Inject Valve button is in the Inject state (as shown at the left), click on the Divert/Inject Valve button to switch the Divert/Inject Valve to the Load position.

10. In the Acquire Data view, click on **Start** to begin acquiring data to the file *reserpine_01.raw*. Tune Master serially appends a numeric date and time to your file name if that name already exists in the specified folder, such as *C:\Xcalibur\Data\reserpine_010502092159.raw*.

Note To minimize the possibility of cross-contamination, use a different syringe and a different sample transfer line for your tuning and calibration solution than you do for your samples and compound optimization solution. ▲

11. Fill the sample loop with reserpine solution as follows:

    a. Ensure that the syringe is loaded with 420 µL of the 2 pg/µL reserpine solution. (Refer to **Appendix A: Solution Formulations** for the procedure for preparing the reserpine solution.)

Note Be sure to wipe off the tip of the needle with a clean, lint-free tissue before reinserting it into the syringe adapter assembly to minimize the possibility of cross-contamination of the assembly. ▲

    b. Carefully insert the tip of the syringe needle into the end of the Teflon liner tube on the needle port.

    c. Overfill the sample loop with reserpine solution from the syringe.

12. Press the blue Divert/Inject Valve button on the front panel of the TSQ Quantum Ultra to inject the reserpine solution into the LC solvent flow.

13. Observe the reserpine product peak at *m/z* 195.066, or that of your analyte of interest, in the Spectrum view.

14. Repeat the following sequence several times to obtain consecutive loop injections of reserpine in the SRM scan mode. Wait approximately 1 min between injections.

a. Press the blue Divert/Inject Valve button on the TSQ Quantum Ultra to return the Divert/Inject valve to the Load position. Overfill the loop with the 2 pg/µL solution of reserpine.

b. Press the Divert/Inject Valve button again to inject the reserpine solution into the LC solvent flow. Then, observe the Spectrum view.

c. Wait approximately 1 min before the next injection.

d. Repeat steps 14.a through 14.c several times.

15. Click on **Stop** in the Acquire Data dialog box to end the data acquisition. See Figure 5-10.

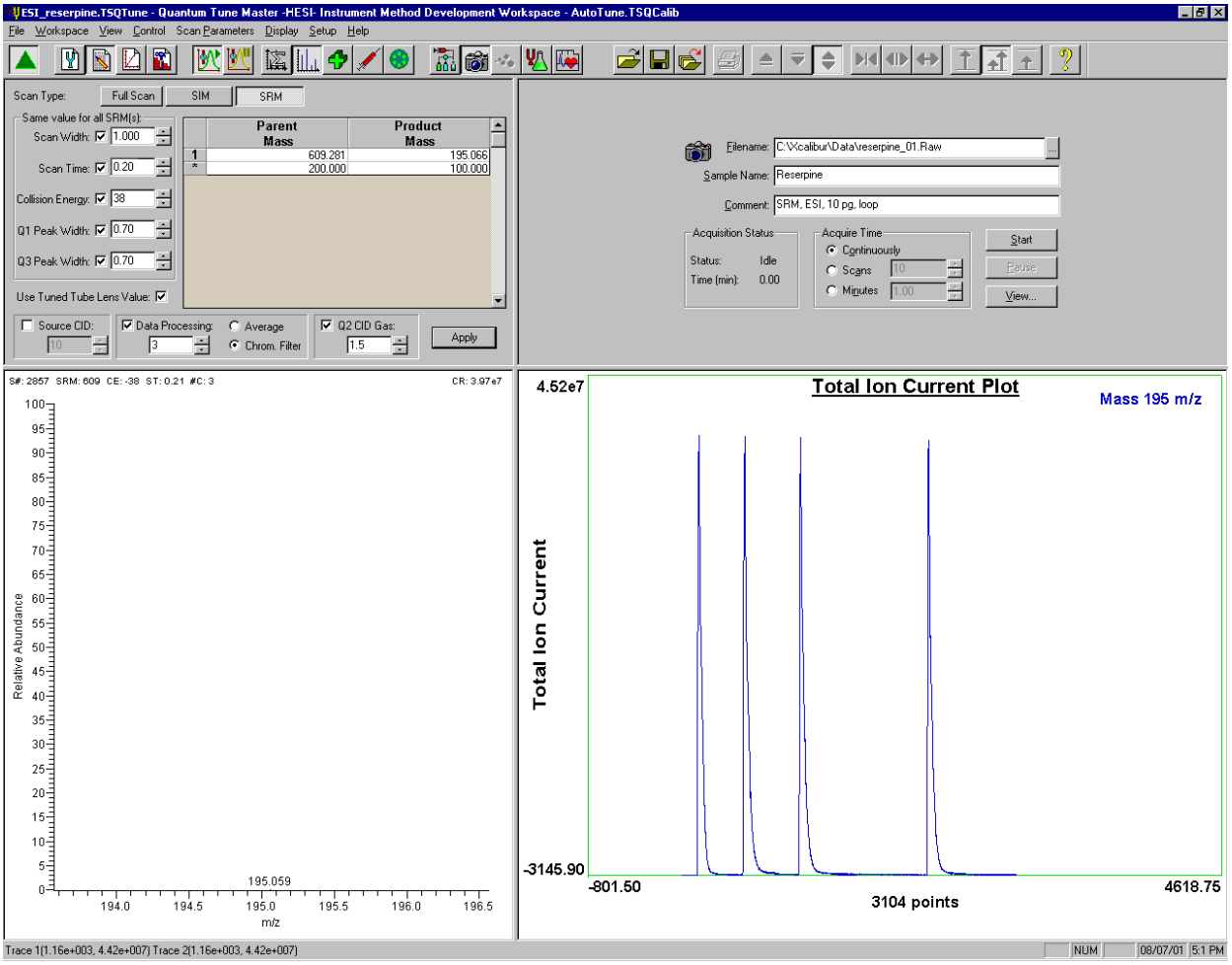


Figure 5-10. Instrument Method Development workspace, showing an SRM scan acquisition

A file of reserpine data in the SRM scan mode is now stored on the hard drive (with a name such as *reserpine_010502092159.raw*).

Note For more information about reviewing the data you acquire using TSQ Quantum Ultra with Xcalibur, refer to *Finnigan Xcalibur Getting Productive: Qualitative Analysis*. ▲

16. To integrate the chromatogram in the raw file you just acquired using the Xcalibur Qual Browser window, click on **View**. See Figure 5-11.

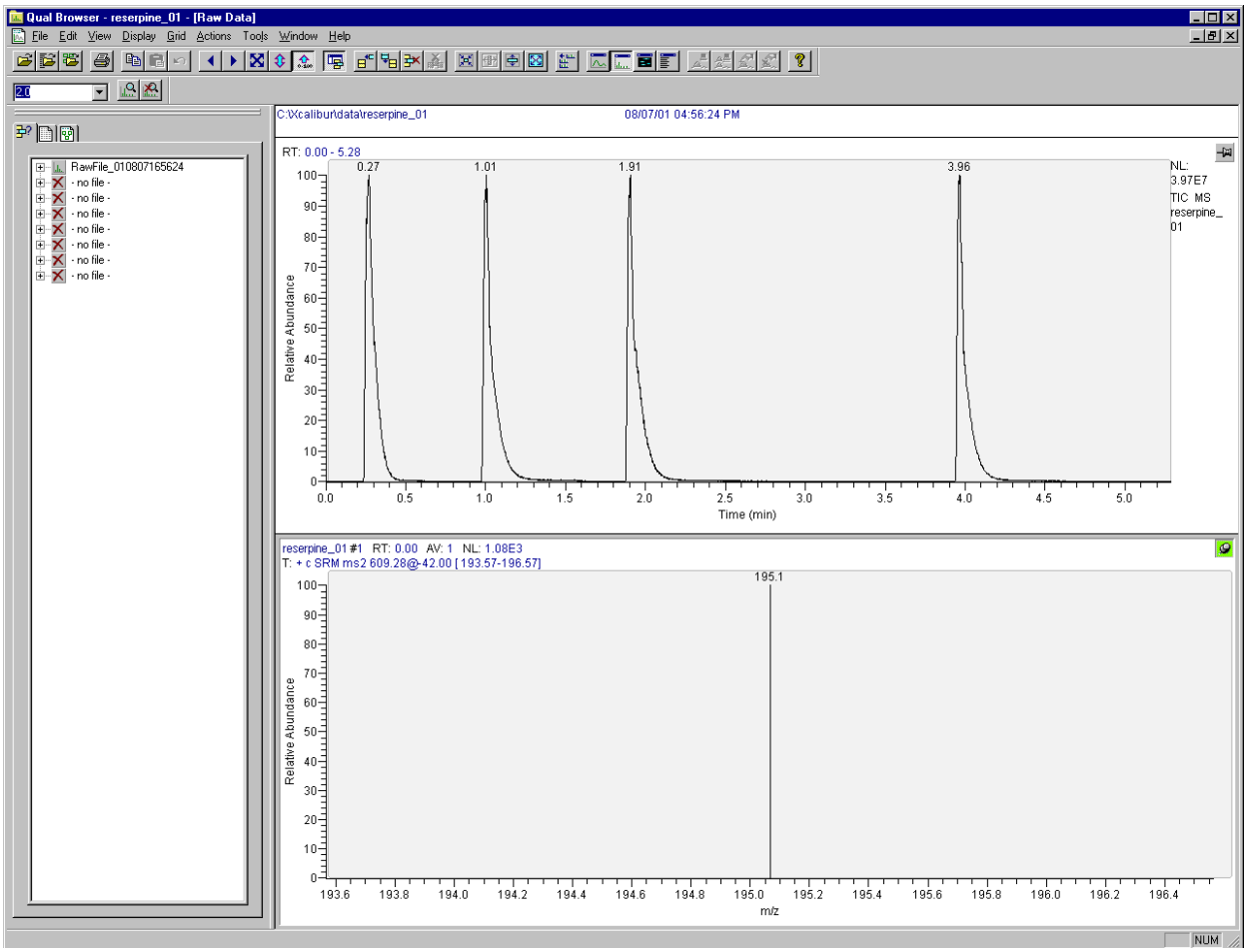


Figure 5-11. Qual Browser window, showing loop injections of reserpine in the Chromatogram view (top) and the centroid at *m/z* 195.066 in the Spectrum view (below)

If you want to acquire data using the APCI source, you must first change the API source as described in "Chapter 6: Setting Up the Ion Source for Acquiring Data in APCI/MS/MS Mode".

Chapter 6 Setting Up the Ion Source for Acquiring Data in APCI/MS/MS Mode

This chapter provides information on setting up the ion source for acquiring data in the APCI/MS/MS mode.

This chapter contains the following topics:

- Removing the H-ESI Probe
- Removing the Ion Max Ion Source Housing
- Removing the Ion Sweep Cone
- Installing the Ion Max Ion Source Housing
- Installing the APCI Probe

Removing the H-ESI Probe

Remove the H-ESI source as follows:

1. Place the LC/MS system in Standby:

   a. Stop the flow of solvent to the H-ESI source:

   i. On the Control / Scan Mode toolbar, click on the AS/LC Direct Control button to display the Inlet Direct Control view in the top right corner of the workspace. See Figure 6-1.

   ii. In the Direct Control Panel group box, click on the Stop button to stop the flow of solvent.

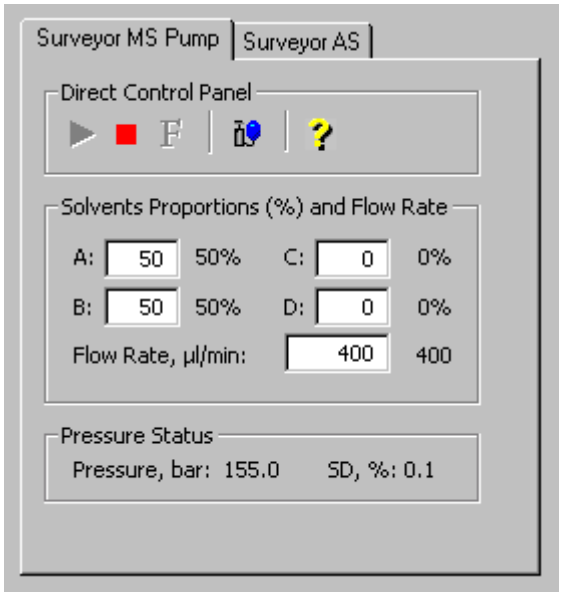


Figure 6-1. Inlet Direct Control view, showing the pump On

   b. Turn Off the spray voltage as follows:

   i. Click on the Optimize Compound Dependent Devices button or choose **Display > Compound Dependent Devices** to display the Optimize Compound Dependent Devices view in the top right corner of the workspace. See Figure 6-2.

   ii. In the Device spin box, enter **0** to set the spray voltage to 0 V.

   c. Turn Off the H-ESI vaporizer as follows:

   i. Click on Vaporizer Temperature in the Device list.

   ii. In the Device spin box, enter **0** to turn off the vaporizer.

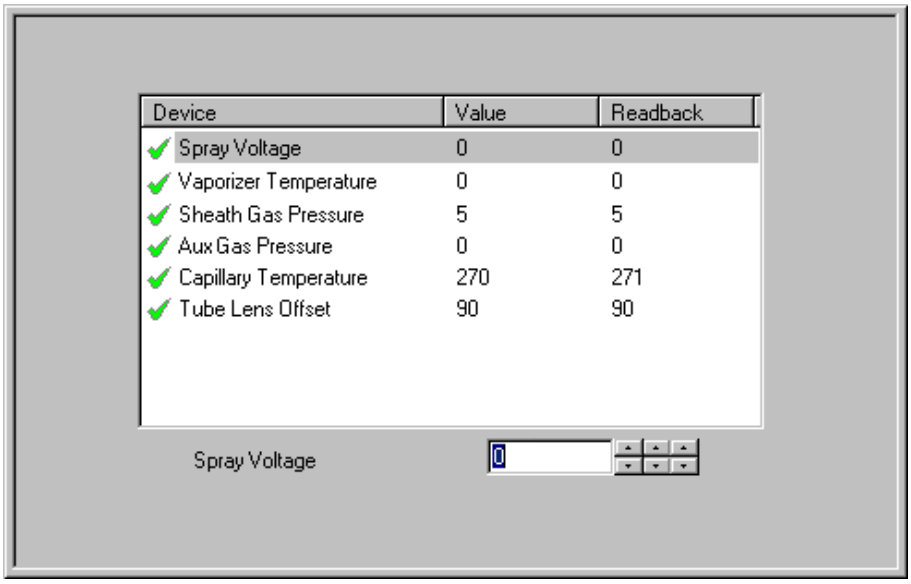


Figure 6-2. Optimize Compound Dependent Devices view


On


Standby

d. Click on the On/Standby button on the Control / Scan Mode toolbar to place the mass spectrometer in Standby.

CAUTION AVOID BURNS. At operating temperatures, the H-ESI vaporizer can severely burn you! The H-ESI vaporizer typically operates between 350 and 450 °C. **Always allow the heated vaporizer to cool to room temperature (for approximately 20 min) before you remove or touch the H-ESI probe.** ▲

2. Disconnect the sample transfer tubing (LC line) from the grounding union (stainless steel ZDV fitting). See Figure 6-3.

3. Disconnect the 8 kV cable from the H-ESI needle high voltage receptacle as follows:

   a. Unlock the cable by twisting the locking ring counter-clockwise.

   b. Unplug the 8 kV cable from the H-ESI needle high voltage receptacle.

4. Unplug the H-ESI vaporizer cable from the cable socket on the H-ESI probe.

5. Connect the H-ESI vaporizer cable to the ESI interlock socket on the ion source housing. See Figure 2-10 on page 2-14.

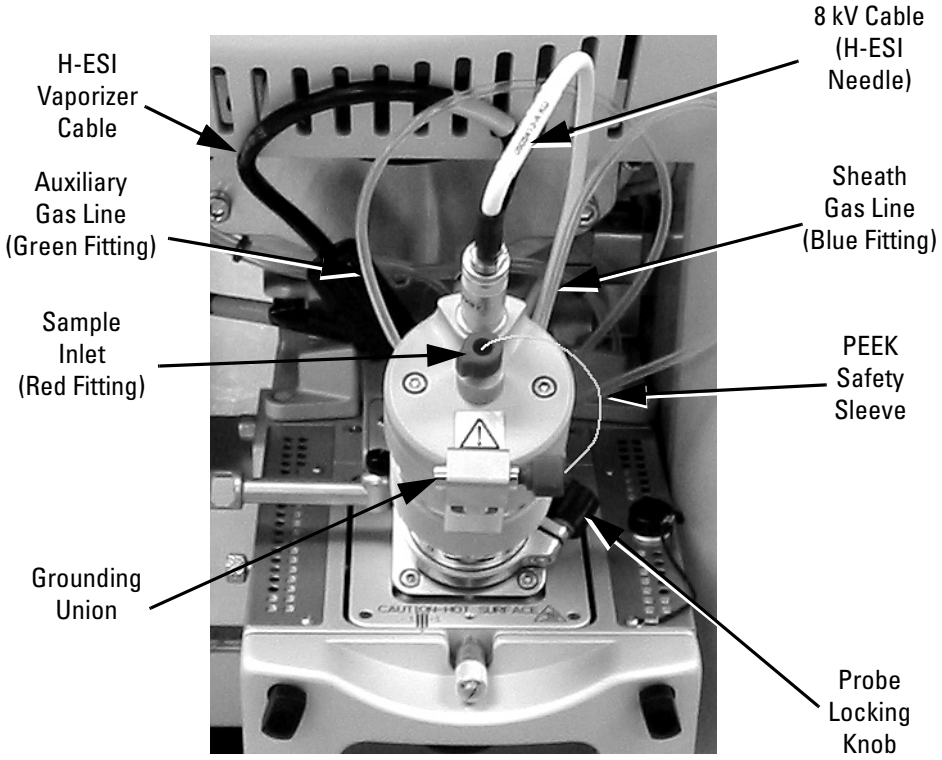


Figure 6-3. H-ESI probe installed on the Ion Max housing

6. Disconnect the auxiliary gas fitting (green) from the auxiliary gas inlet (A) on the probe manifold.

7. Disconnect the sheath gas fitting (blue) from the sheath gas inlet (S) on the probe manifold.

8. Unlock the probe locking ring by turning the probe locking knob counterclockwise.

9. Carefully pull the probe straight back in the port in the housing until it meets with the slot in the ESI interlock block. The guide pin on the probe manifold will prevent you from twisting the probe until the pin is aligned with the slot in the ESI interlock block. Once the probe is all the way back and aligned with the slot, turn the probe 45 degrees counter-clockwise to free the probe from the alignment notch. Be careful not to break the fused-silica sample tube or PEEK safety sleeve.

10. Pull the probe straight out to remove it from the ion source housing.

11. Store the H-ESI probe in its original shipping container.

Removing the Ion Max Ion Source Housing

You need to remove the Ion Max ion source housing to access the ion sweep cone.

Note Disconnect any external liquid lines connected to the ion source housing before removing the ion source housing. ▲

Remove the ion source housing as follows:

1. Remove the drain tube from the ion source housing drain. See Figure 6-4.

2. Rotate the ion source housing locking levers 90 degrees to release the ion source housing from the ion source mount assembly.

3. Remove the ion source housing by pulling straight off of the ion source mount assembly, and place the housing in a safe location for temporary storage.

Go to the next topic: “Removing the Ion Sweep Cone”.

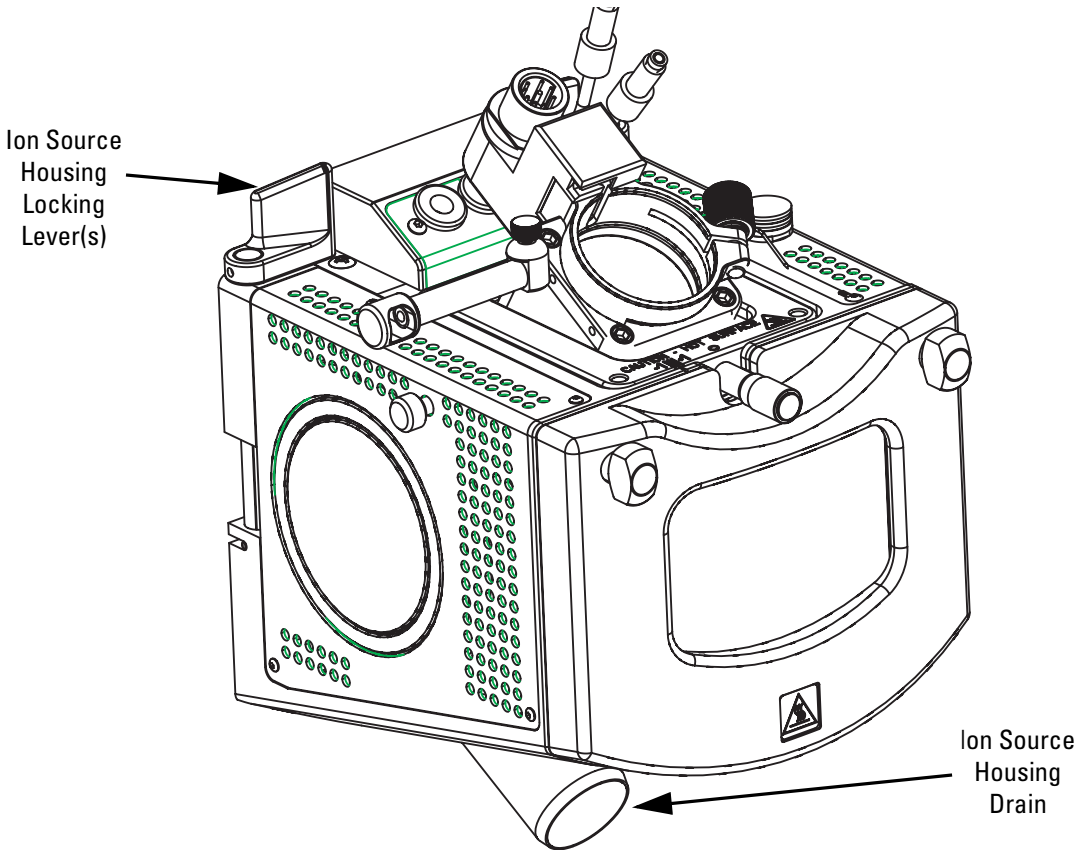


Figure 6-4. Ion Max, detail of components

Removing the Ion Sweep Cone

The ion sweep cone is not used for APCI operation.

Remove the ion sweep cone as follows:

1. Put on a pair of talc-free gloves.

CAUTION AVOID BURNS. At operating temperatures, the ion transfer tube can severely burn you! The ion transfer tube typically operates between 200 and 400 °C. **Always allow the ion sweep cone to cool to room temperature (for approximately 20 min) before you touch or remove this component.** Always be careful not to touch the entrance end of the ion transfer tube when it is exposed. ▲

2. Grasp the outer ridges of the ion sweep cone and pull the cone straight off of the API cone seal.

3. Store the ion sweep cone in its original shipping container.

Go to the next topic: “Installing the Ion Max Ion Source Housing”.

Installing the Ion Max Ion Source Housing

Reinstall the Ion Max ion source housing as follows:

1. Carefully align the two guide pin holes on the rear of the ion source housing with the ion source housing guide pins on the mass spectrometer, and carefully press the ion source housing onto the ion source mount. See Figure 6-5 and Figure 6-6.

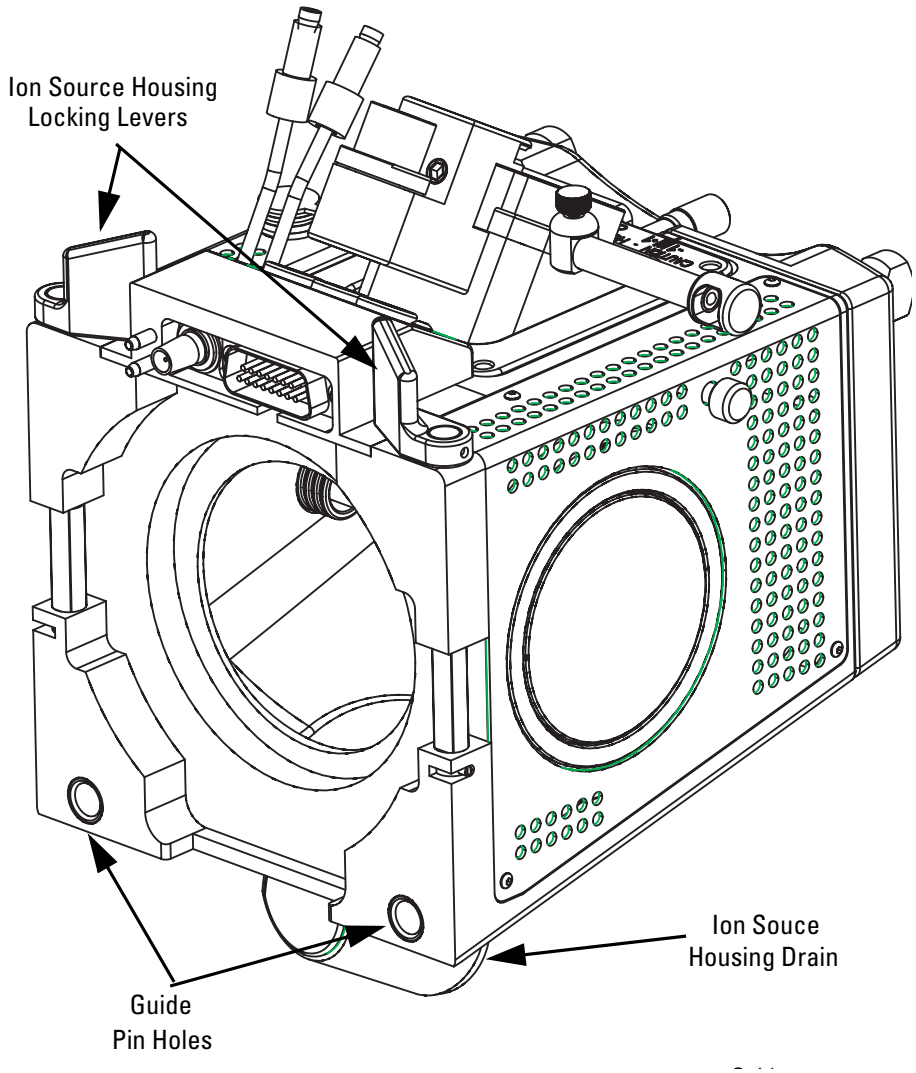


Figure 6-5. Rear view of the Ion Max ion source housing

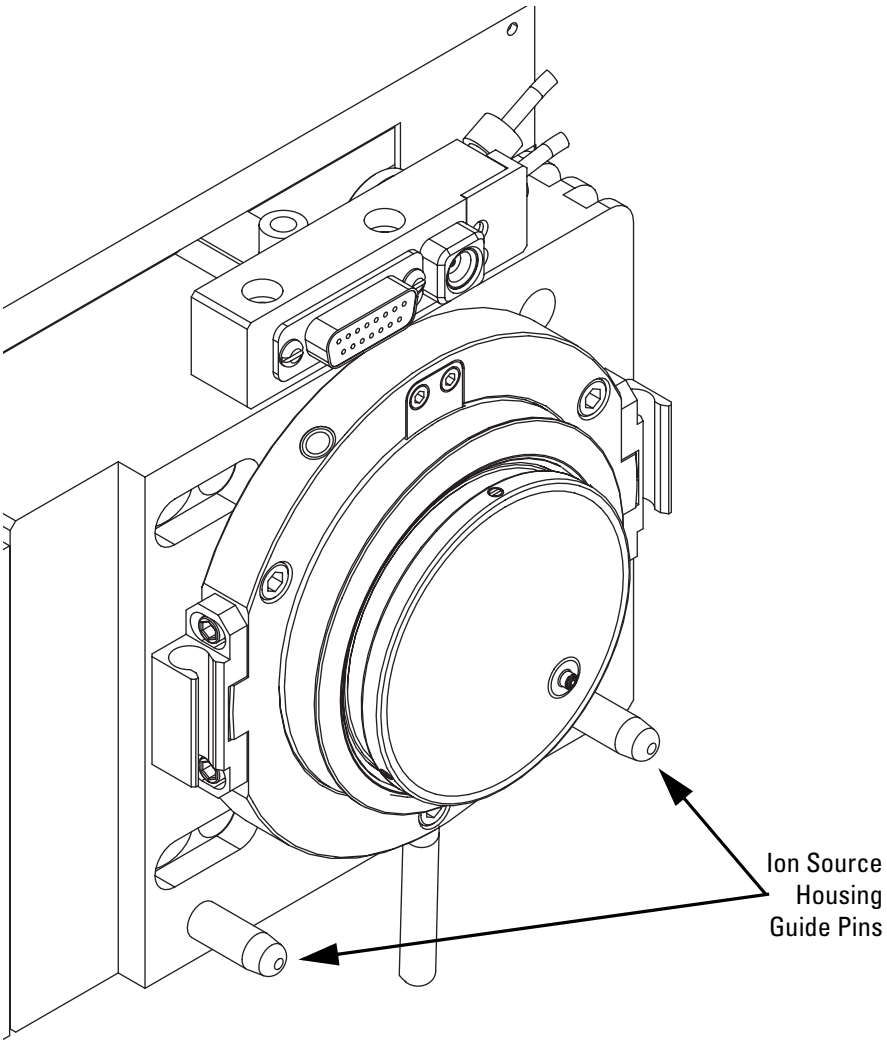


Figure 6-6. Ion source mount showing ion source housing guide pins

2. Rotate the ion source housing locking levers 90 degrees to lock the ion source housing onto the ion source mount assembly.

Caution Prevent solvent waste from backing up into the ion source and mass spectrometer. Always ensure that liquid in the drain tube is able to drain to a waste container and that the outlet of the drain tube is above the level of liquid in the waste container. ▲

Caution Do **not** vent the API source drain tube (or any vent tubing connected to the waste container) to the same fume exhaust system to which you have connected the forepumps. The analyzer optics can become contaminated if the API source drain tube and the (blue) forepump exhaust tubing are connected to the same fume exhaust system.

Your laboratory must be equipped with at least two fume exhaust systems. Route the (blue) forepump exhaust tubing to a dedicated fume exhaust system. Route the drain tube from the API source to a waste container. Vent the waste container to a dedicated fume exhaust system. ▲

3. Reinstall the ion source drain tube as follows:

    a. Connect the 1-in. ID Tygon tubing (P/N 00301-22922) to the ion source housing drain fitting.

    b. Attach the free end of the hose to a waste container. Ideally, the waste container should be vented to a fume exhaust system.

The Ion Max is now properly installed on the mass spectrometer.

Go to the next topic: “Installing the APCI Probe”.

Installing the APCI Probe

Install the APCI probe as follows:

1. Install the corona needle as follows:

CAUTION AVOID INJURY. The corona discharge needle is very sharp and can puncture your skin. Handle it with care. ▲

   a. Unlock the ion source housing door by turning the locks 90 degrees so that the knobs are horizontal.

   b. Open the ion source housing door.

   c. Using pliers, grasp the needle by the gold plated contact and push the needle straight into the socket. See Figure 6-7.

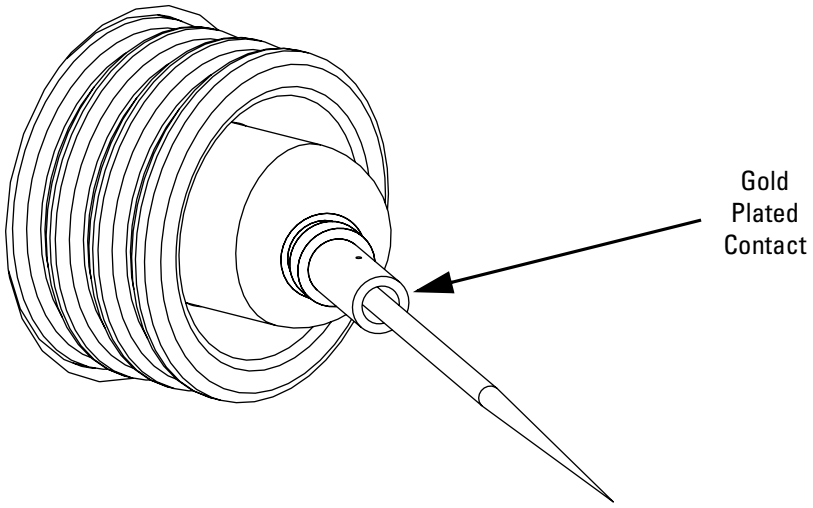


Figure 6-7. Corona needle, view from rear

   d. Make sure the tip of the needle is aligned with the path of travel between the APCI probe and the ion source interface on the instrument.

   e. Close and lock the ion source housing door.

2. Connect the 8 kV cable to the corona needle high voltage receptacle as follows:

   a. Plug the 8 kV cable into the corona needle high voltage receptacle on the right side of the top of the ion source housing. See Figure 2-10 on page 2-14.

   b. Lock the cable by twisting the locking ring clockwise.

3. Be sure to loosen the probe locking ring (widest open position) before attempting to install the probe.

4. Insert the APCI probe into the port in the ion source housing, align the guide pin on the probe body at a 45 degree angle from the ESI interlock block. See Figure 6-8.

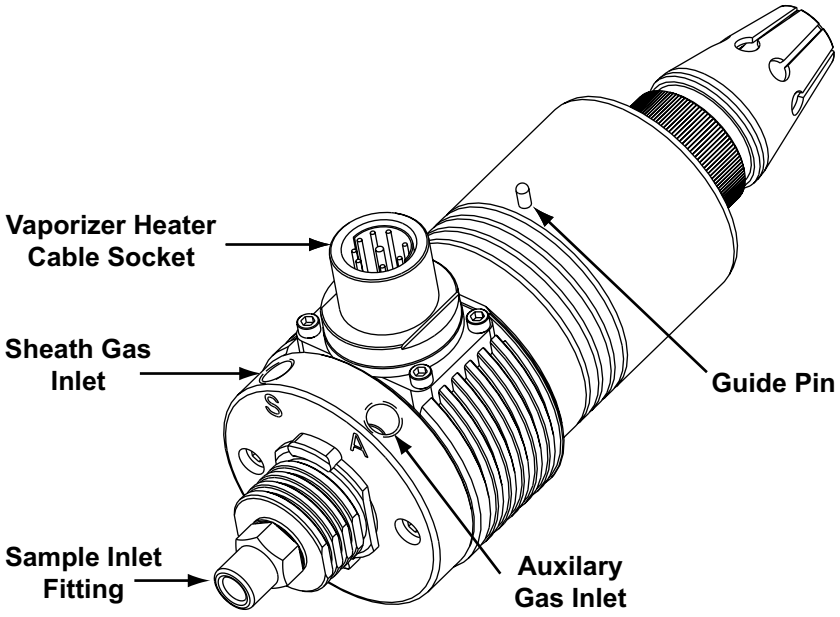


Figure 6-8. APCI probe

5. Push the probe into the port until the guide pin meets with the locking ring on the housing.

6. Turn the probe 45 degrees clockwise and align the guide pin with the slot in the ESI interlock block (you may need to pull the probe towards you slightly to properly align the pin with the notch). Once you have turned the probe far enough to align the pin with the alignment notch at the rear of the port, push the probe straight in until the guide pin stops at the bottom of the alignment notch.

7. Seat the probe all the way down into the alignment notch.

8. Lock the probe in place by turning the probe locking knob clockwise.

9. Unplug the vaporizer heater cable from the ESI interlock plug on the ion source housing.

10. Connect the vaporizer heater cable to the vaporizer heater cable socket on the APCI probe.

11. Connect the auxiliary gas line (green colored fitting) to the inlet on the APCI probe marked *A*.

12. Connect the sheath gas line (blue colored fitting) to the inlet on the APCI probe marked *S*.

13. Connect the sample transfer line to the APCI probe inlet.

The APCI source is now properly installed on the mass spectrometer.

Caution Prevent solvent waste from backing up into the ion source and mass spectrometer. Always ensure that liquid in the drain tube is able to drain to a waste container and that the outlet of the drain tube is above the level of liquid in the waste container. ▲

Note Before you analyze samples with the APCI source, you need to change to APCI source mode in Tune Master. The next time you turn On the TSQ Quantum Ultra, place Tune Master in the APCI source mode by choosing **Setup > Change Ion Source > APCI**. ▲

Leave the LC/MS system in Standby and go to the next chapter: "Optimizing the Mass Spectrometer with Your Compound in APCI/MS/MS Mode".

Chapter 7 Optimizing the Mass Spectrometer with Your Compound in APCI/MS/MS Mode

This chapter provides information on fine tuning the mass spectrometer in the APCI/MS/MS mode using your analyte as the tuning compound. You optimize the sensitivity of the mass spectrometer for your analyte with an automatic tuning procedure.

The Tune Methods that are provided with your TSQ Quantum Ultra are useful for a wide range of applications. They can often be used without further tuning of your mass spectrometer. However, for certain applications you might need to optimize several mass spectrometer parameters. For instance, the parameters that affect APCI performance and signal quality are as follows:

- Discharge current
- APCI vaporizer temperature
- Sheath gas pressure
- Auxiliary gas pressure
- Capillary temperature
- Tube lens offset voltage

The optimum settings for these parameters depend on the solvent flow rate and on the structure of your analyte. In general, you need to fine tune the mass spectrometer parameters whenever you change the solvent flow rate conditions of your particular application. When you optimize the mass spectrometer parameters using the automatic tuning procedure, the procedure adjusts all the parameters listed above and the voltages applied to the ion optics until the ion transmission of your analyte is maximized.

Note Ensure that you have performed the TSQ Quantum Ultra tuning and calibration procedure, within the previous three months, before you optimize the tune for your compound. If you need to tune and calibrate the system, refer to the procedure in the "Chapter 3: Tuning and Calibrating the Mass Spectrometer in H-ESI/MS Mode". ▲

To optimize the mass spectrometer for your compound in the APCI/MS/MS mode, you do the following:

- Set up the syringe pump and divert/injection valve for auto loop injection.
- Set up the mass spectrometer for your specific compound from the Tune Master.
- Run the automatic compound optimization procedure to fine tune the mass spectrometer parameters that are compound dependent.
- Save the new Tune Method.

This chapter contains the following topics:

- Setting Up to Introduce Sample by Auto Loop Injection in APCI Mode
- Setting Up to Optimize in APCI/MS/MS Mode with Your Compound
- Optimizing in APCI/MS/MS Mode Automatically with Your Compound

Setting Up to Introduce Sample by Auto Loop Injection in APCI Mode

The following procedure describes how to introduce your compound by auto loop injection. The plumbing connections for APCI/MS sample introduction from the syringe pump into the solvent flow from an LC are shown in Figure 7-1.

Note You can use the reserpine sample solution described in "Appendix A: Solution Formulations", or you can use your compound of interest. ▲

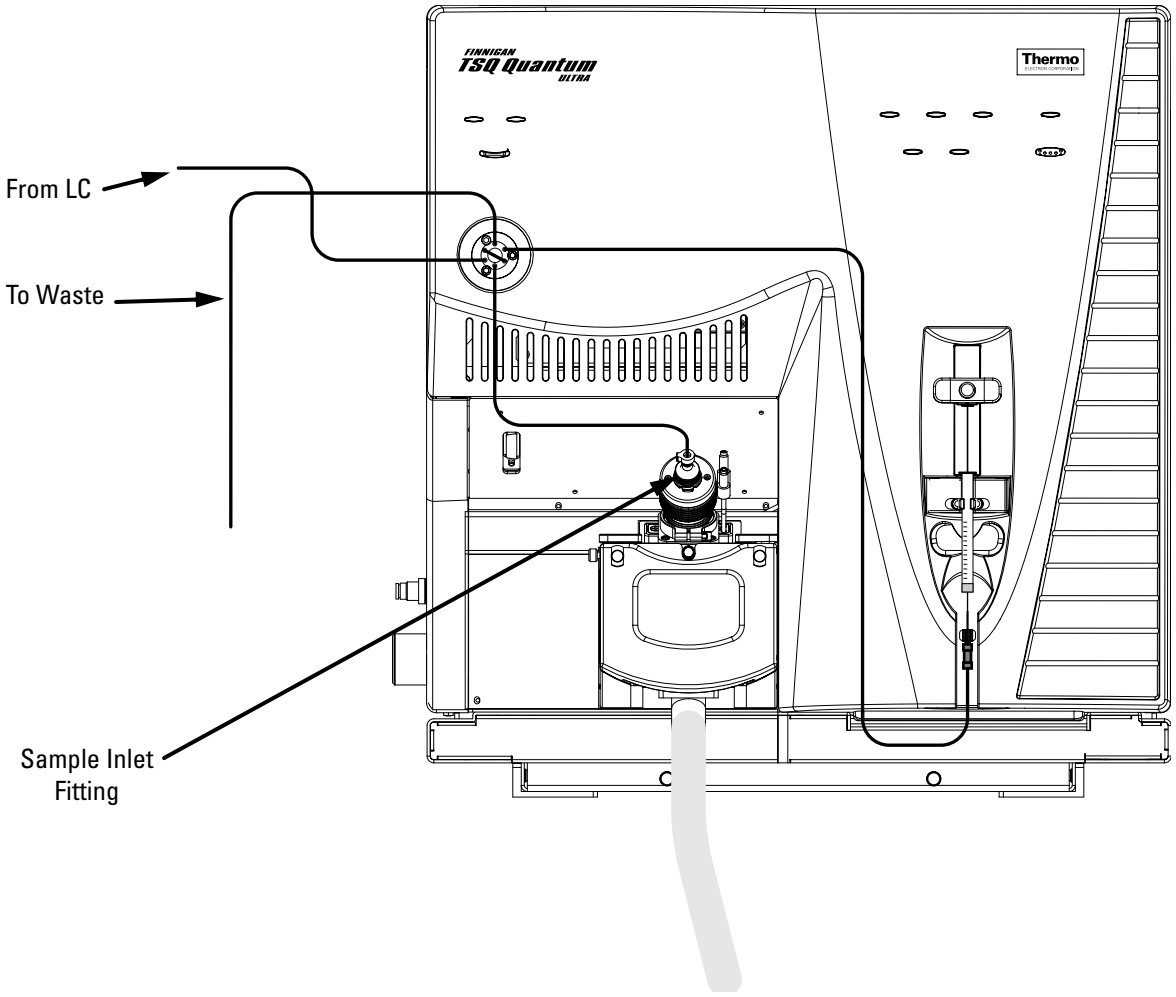


Figure 7-1. APCI/MS plumbing connections for sample introduction by auto loop injection into the solvent flow from an LC

Make the plumbing connections for APCI/MS sample introduction from the syringe pump into the solvent flow from an LC, as follows:

1. Remove the syringe from the syringe pump holder as follows:

a. Lift the handle off the syringe while depressing the black release button on the syringe pump handle.

b. Remove the syringe.

c. Remove the tip of the syringe needle from the end of the Teflon tube on the syringe adapter assembly. See Figure 7-2.

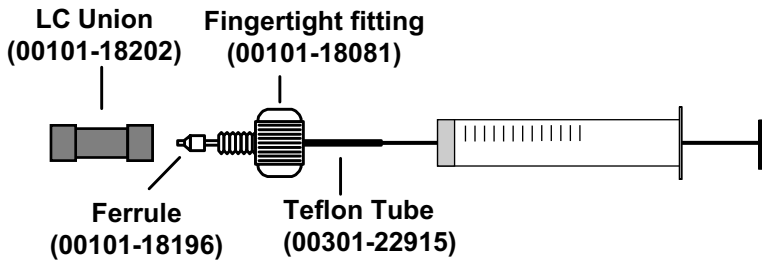


Figure 7-2. Syringe and syringe adapter assembly

2. Remove the sample transfer line installed between the syringe adapter assembly and the APCI probe.

3. Install a sample transfer line between the syringe adapter assembly and the divert/inject valve as follows:

   a. Connect an appropriate length of tubing to the LC union on the syringe adapter assembly.

   b. Connect the other end of the tubing fitted with a nut and a ferrule to port 5 of the divert/inject valve. See Figure 7-3.

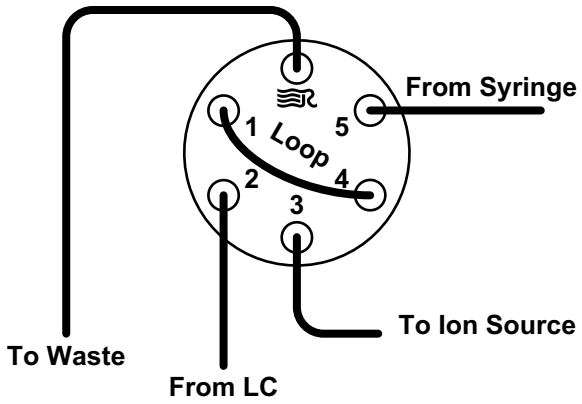


Figure 7-3. Divert/inject valve, showing plumbing for auto loop injection

Note To minimize the possibility of cross-contamination, use a different syringe and a different sample transfer line for your tuning and calibration solution than you do for your samples and compound optimization solution. ▲

4. Load a clean, 500-µL Unimetrics syringe with 420 µL of the 2 pg/µL reserpine sample solution. (Refer to "Appendix A: Solution Formulations" for the procedure for preparing the reserpine solution.)

Note Be sure to wipe off the tip of the needle with a clean, lint-free tissue before reinserting it into the syringe adapter assembly to minimize the possibility of cross-contamination of the assembly. ▲

5. While holding the plunger of the syringe in place, carefully reinsert the tip of the syringe needle into the end of the Teflon tube on the syringe adapter assembly. See Figure 7-2.

6. Place the syringe into the syringe holders of the syringe pump.

7. While squeezing the black release button on the syringe pump handle, push the handle down until it just contacts the syringe plunger.

8. Install a sample transfer line between the divert/inject valve and the APCI probe as follows:

   a. Gather the necessary fittings for installing a sample transfer line. See Figure 7-4.

   b. Connect an appropriate length of tubing fitted with a nut and a ferrule to port 3 of the divert/inject valve. See Figure 7-3.

   c. Connect the other end of the tubing with a fingertight fitting and a ferrule to the sample inlet fitting (LC inlet). See Figure 7-1.

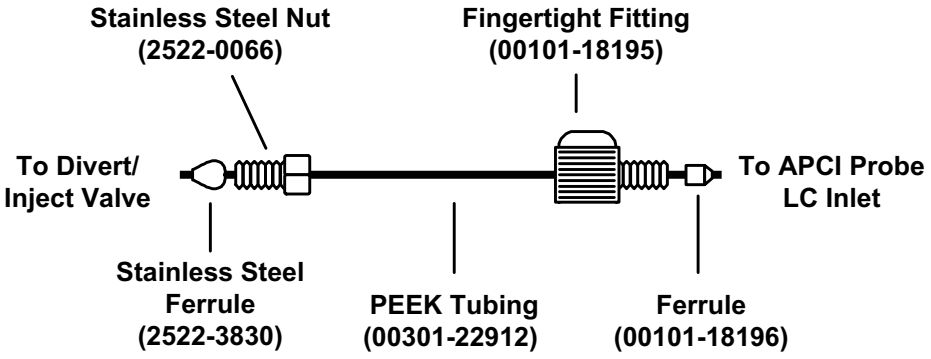


Figure 7-4. Sample transfer line, installed between the divert/inject valve and the APCI probe

9. Install a 5 µL sample loop with nuts and ferrules between ports 1 and 4 of the divert/inject valve.

10. Install a solvent line between the LC system and the divert/inject valve as follows:

    a. Connect an appropriate length of tubing with a proper fitting and a ferrule to the outlet of the LC system.

    b. Connect the other end of the tubing with a nut and ferrule to port 2 of the divert/inject valve.

11. Install a waste line on the divert/inject valve and direct the outlet to a waste container as follows:

    a. Connect an appropriate length of tubing with a nut and ferrule to port 6 of the divert/inject valve (port 6 is labeled with the Rheodyne logo).

    b. Insert the other end of the tubing into the waste container.

You have completed setting up to introduce your compound by auto loop injection. Go to the next topic: “Setting Up to Optimize in APCI/MS/MS Mode with Your Compound”.

Setting Up to Optimize in APCI/MS/MS Mode with Your Compound

Use the following procedure to set up the mass spectrometer to optimize automatically on your compound in APCI/MS/MS mode.


On


Standby

1. Click on the On/Standby button on the Control / Scan Mode toolbar to turn On the mass spectrometer.

2. Tune Master must be placed in the APCI source mode before analyzing samples with the APCI source. Choose **Setup > Change Ion Source > APCI** to place Tune Master in the APCI source mode.

3. If desired, open an existing Tune Method as follows:

   a. On the File / Display toolbar, click on the Open File button to display the Open dialog box. See Figure 7-5.

   b. Confirm that the path is *C:\Xcalibur\methods* and then select the desired file.

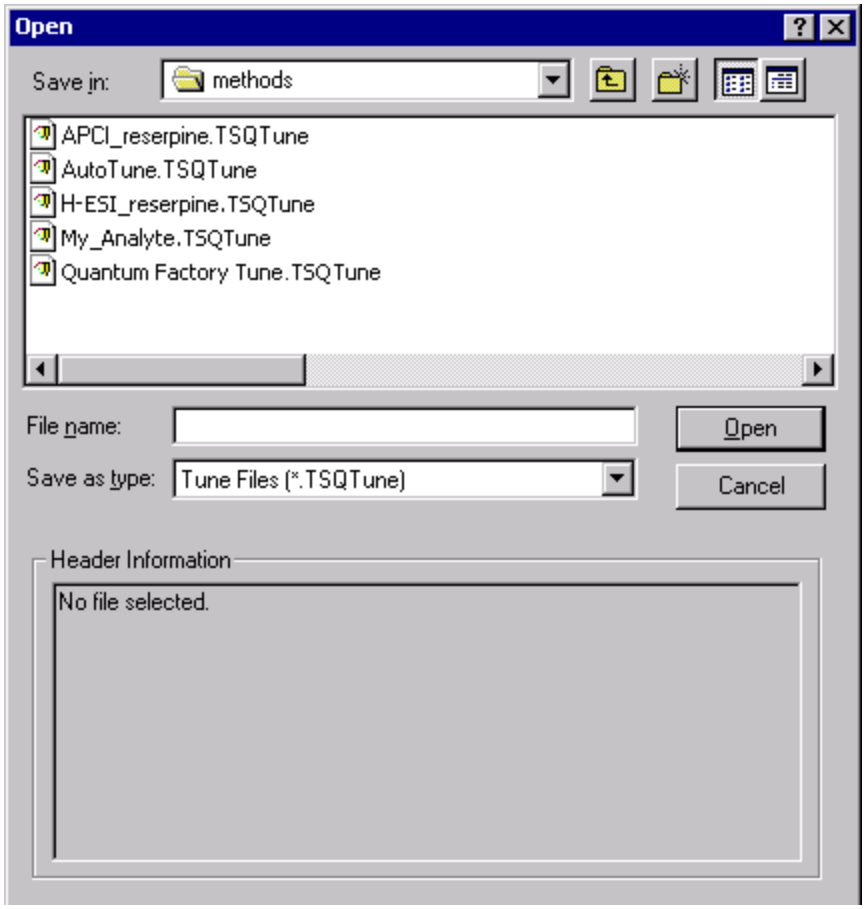


Figure 7-5. Open Tune Method dialog box

c. Click on **Open** to open the file. Tune Master downloads the Tune Method parameters to the mass spectrometer.

4. Click on the Optimize Compound Dependent Devices button on the Control / Scan Mode toolbar or choose **Display > Compound Dependent Devices** to display the Optimize Compound Dependent Devices view in the top right corner of the workspace. See Figure 7-6.

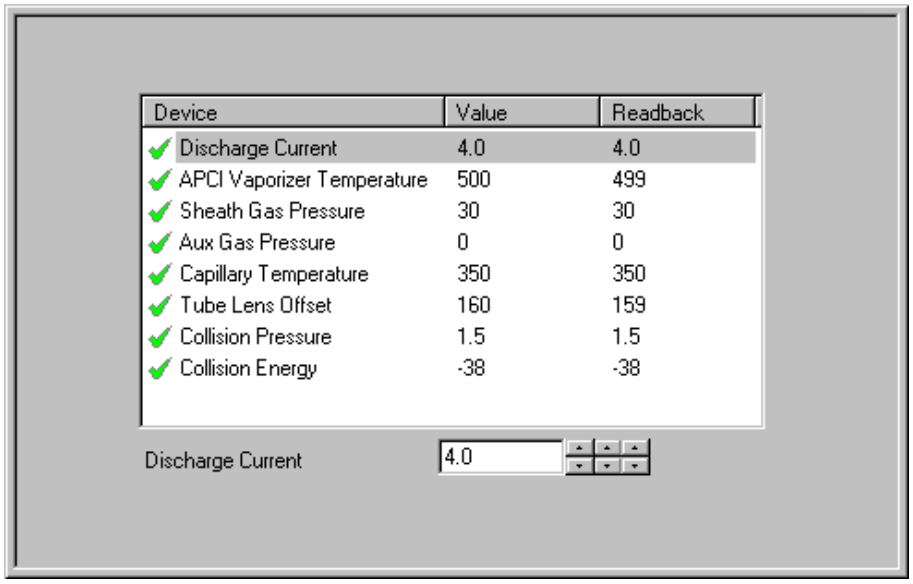


Figure 7-6. Optimize Compound Dependent Devices view, APCI settings

Note You might find that the presence of chemical contamination in the APCI vaporizer creates chemical noise in the mass spectrum. If this occurs, recondition the APCI vaporizer. To recondition the APCI vaporizer, you start LC solvent flow, elevate the temperature of the APCI vaporizer, and increase the sheath gas and auxiliary gas pressures for approximately 30 min to drive off the chemical contamination.

Typical values used for reconditioning the APCI vaporizer are as follows:

LC flow rate = 400 µL/min
Vaporizer temperature = 600 °C
Sheath gas pressure = 80 psi
Auxiliary gas pressure = 15 units ▲

5. Set the values for the compound dependent devices as follows:

    a. Make sure that Discharge Current is selected in the Device Display box.

    b. In the Optimize Compound Dependent Devices view, enter **4.0** in the Device spin box to set the discharge current to 4.0 µA.

    c. Set the temperature of the APCI vaporizer to 500 °C as follows:

        i. In the Device Display box, click on APCI Vaporizer Temperature. This changes the Device spin box label to *APCI Vaporizer Temperature* and enables you to set the APCI vaporizer temperature.

        ii. In the Device spin box, enter **500** to set the vaporizer temperature to 500 °C.

    d. Set the pressure of the sheath gas to 30 psi as follows:

        i. Click on Sheath Gas Pressure in the Device Display box.

        ii. Enter **30** in the Device spin box to set the sheath gas pressure to 30 psi.

    e. Set the pressure of the auxiliary gas to 0 units as follows:

        i. Click on Aux Gas Pressure in the Device Display box.

        ii. Enter **0** in the Device spin box to set the auxiliary gas pressure to 0 units.

    f. Set the temperature of the ion transfer capillary to 350 °C as follows:

        i. Click on Capillary Temperature in the Device Display box.

        ii. Enter **350** in the Device spin box to set the capillary temperature to 350 °C.

    g. Set the collision pressure to 1.5 mTorr as follows:

        i. Click on Collision Pressure in the Device Display box.

        ii. Enter **1.5** in the Device spin box to set the collision pressure to 1.5 mTorr.

    h. Set the collision energy to -38 eV as follows:

        i. Click on Collision Energy in the Device Display box.

        ii. Enter **-38** in the Device spin box to set the collision energy to -38 eV.

Ensure that the readbacks in the Device Display box are approximately equal to the set values. (You might need to wait for a few minutes for the capillary and vaporizer temperatures to stabilize at their set values.)

6. Configure the Syringe Pump to automatically inject the reserpine sample solution into the sample loop as follows:

   a. Choose **Setup > Syringe Pump & Sample Loop** to display the Syringe Pump and Sample Loop view in the top right corner of the workspace. See Figure 7-7.

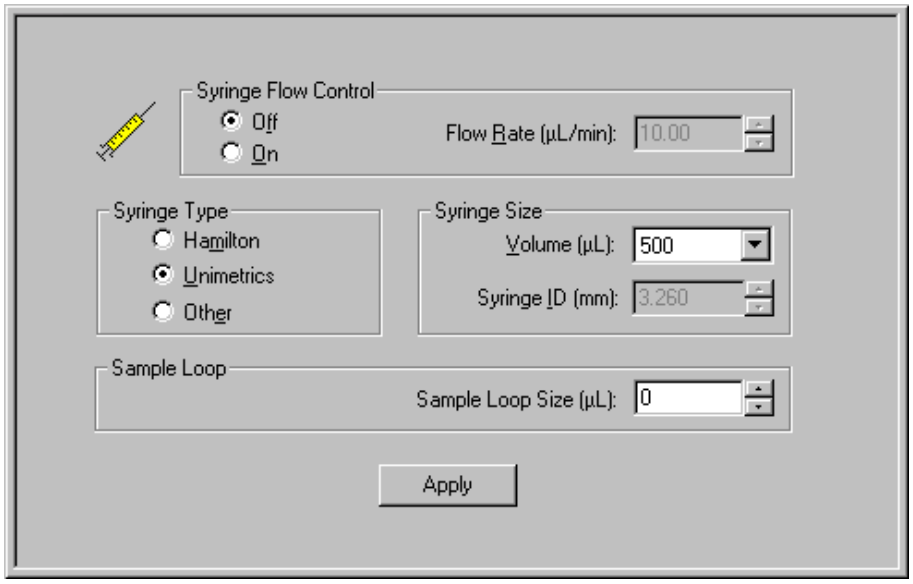


Figure 7-7. Syringe Pump and Sample Loop view, showing auto loop injection setup

   b. Select the Off option button in the Syringe Flow Control group box to turn Off the syringe pump.

- If you are using either a Unimetrics or Hamilton syringe, go to step 6.c.
- If you are *not* using either a Unimetrics or Hamilton syringe, go to step 6.e.

   c. In the Syringe Type group box, select the Unimetrics (or Hamilton) option button to specify a Unimetrics (or Hamilton) syringe.

   d. In the Syringe Size group box, select *500* (or the size of your syringe) from the Volume list box to specify that the volume of your syringe is 500 µL.

When you specify the syringe type and syringe volume, Tune Master automatically sets the proper syringe ID value. Go to step 6.f.

e. If you are using a make of syringe other than Unimetrics or Hamilton, you need to manually specify the syringe ID by doing the following:

i. Select the Other option button in the Syringe Type group box. This specifies that you are using a syringe other than Unimetrics or Hamilton syringe and enables the Syringe ID spin box.

ii. In the Syringe Size group box, select the volume of your syringe from the Volume list box.

iii. Enter the inner diameter of your syringe in the Syringe ID spin box.

f. In the Sample Loop group box, enter 5 in the Sample Loop Size spin box to specify a loop size of 5 µL.

g. Click on **Apply** to apply these settings. The syringe pump is now configured to fill the sample loop with the appropriate amount of sample.

7. Start the flow of solvent as follows:

a. On the Control / Scan Mode toolbar, click on the AS/LC Direct Control button to display the Inlet Direct Control view in the top right corner of the workspace. See Figure 7-8.

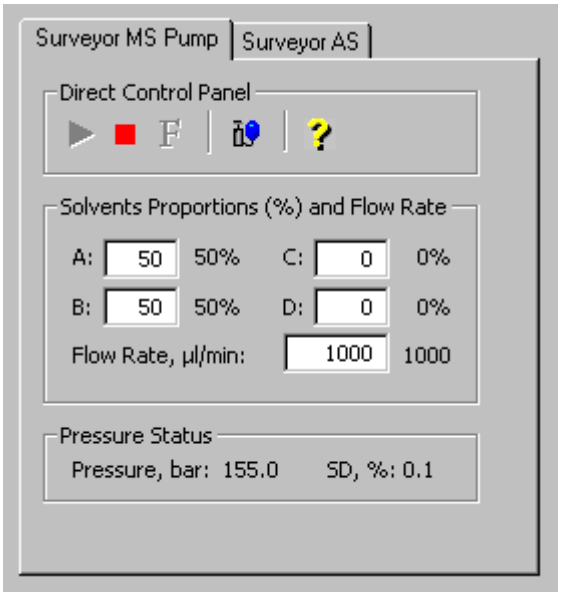


Figure 7-8. Inlet Direct Control view, showing high flow setup

Note The following procedure assumes that isopropyl alcohol and HPLC grade water are in the solvent bottles labeled A and B, respectively. ▲

b. Set up the Surveyor MS Pump to deliver a solution of 50:50 isopropyl alcohol–water at 1000 µL/min, as follows:

   i. In the Inlet Direct Control view, in the Solvents Proportions (%) and Flow Rate group box, enter **50** in the text box labeled *A* to specify a delivery proportion of 50% solvent A.

   ii. Enter **50** in the text box labeled *B* to specify a delivery proportion of 50% solvent B.

   iii. In the Flow Rate text box, enter **1000** to set a flow rate of 1000 µL/min.

c. In the Direct Control Panel group box, click on the Start button to start the Surveyor MS pump.

The system is now set up to automatically deliver reserpine to the ion source for optimizing the mass spectrometer with your compound.

Next you will optimize the compound dependent devices for your compound in APCI/MS/MS mode. Go to the next topic: “Optimizing in APCI/MS/MS Mode Automatically with Your Compound”.

Optimizing in APCI/MS/MS Mode Automatically with Your Compound

You optimize the mass spectrometer to maximize the ion transmission of your compound. Optimization is performed to fine tune compound dependent parameters such as discharge current, capillary temperature, and tube lens offset. It is recommended that you optimize the mass spectrometer only after you have successfully tuned and calibrated the instrument.

Use the following procedure to automatically optimize the mass spectrometer in the APCI/MS/MS mode for the reserpine transition from *m/z* 609.281 to *m/z* 195.066.

1. On the Control / Scan Mode toolbar, click on the Compound Optimization Workspace button to display the Compound Optimization workspace. See Figure 7-9.

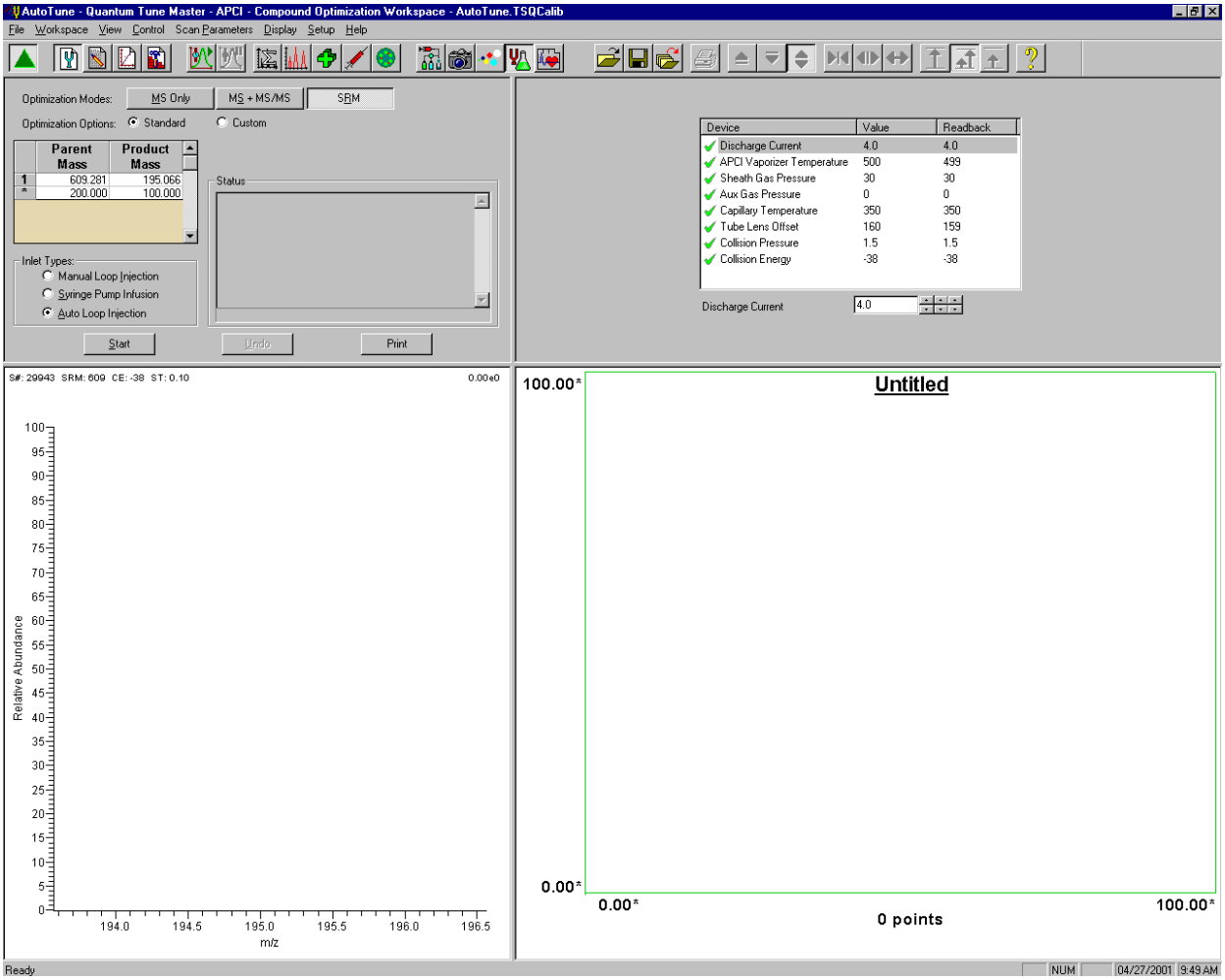


Figure 7-9. Compound Optimization workspace, APCI mode

2. Set the optimization parameters for monitoring the reserpine transition from *m/z* 609.281 to *m/z* 195.066 as follows:

   a. In the Compound Optimization view in the top left corner of the workspace, select **Optimization Modes: SRM** to enable you to optimize a selected reaction. See Figure 7-10.

   b. Select the Optimization Options: Standard option button to tune the default selection of devices. (Tube lens offset and collision energy are the default compound sensitive devices that are optimized in this configuration.)

   c. In the Optimization table, enter the parent mass **609.281** to set the parent mass of the SRM reaction to the ion at *m/z* 609.281.

   d. Enter the product mass **195.066** to set the product mass of the SRM reaction to the ion at *m/z* 195.066.

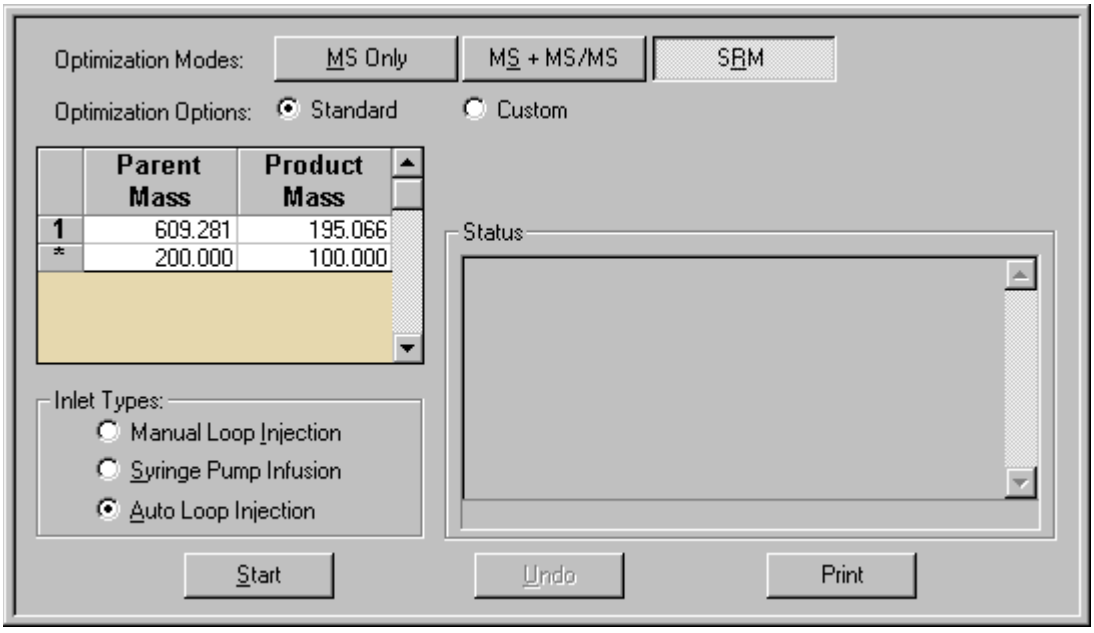


Figure 7-10. Compound Optimization view

Note You need to select inlet type option button appropriate to the inlet mode you use to introduce your sample into the mass spectrometer. This procedure uses the Auto Loop Injection option. ▲

   e. In the Inlet Types group box, select the Auto Loop Injection option button to have the TSQ Quantum Ultra automatically inject the optimization solution.

3. Click on **Start** to start the automatic tuning procedure.

Note If the syringe runs out of sample during the compound optimization procedure, the instrument pauses the automatic tuning and displays the message: *Syringe out of sample, Reload and click OK*. If this occurs, reload the syringe and click on **OK** to continue the optimization. ▲

The message *Finish compound optimization* is displayed in the Status box in the Compound Optimization view when the compound optimization has completed successfully. See Figure 7-11.

- If the compound optimization procedure finishes without errors and the breakdown curve of the 195.066 fragment is Gaussian-shaped (as in Figure 7-12) or is a smooth, positive-sloped curve, go to step 5.
- If errors occur during the compound optimization procedure or the breakdown curve of the 195.066 fragment oscillates, contains multiple peaks, or is excessively noisy, go to step 4.

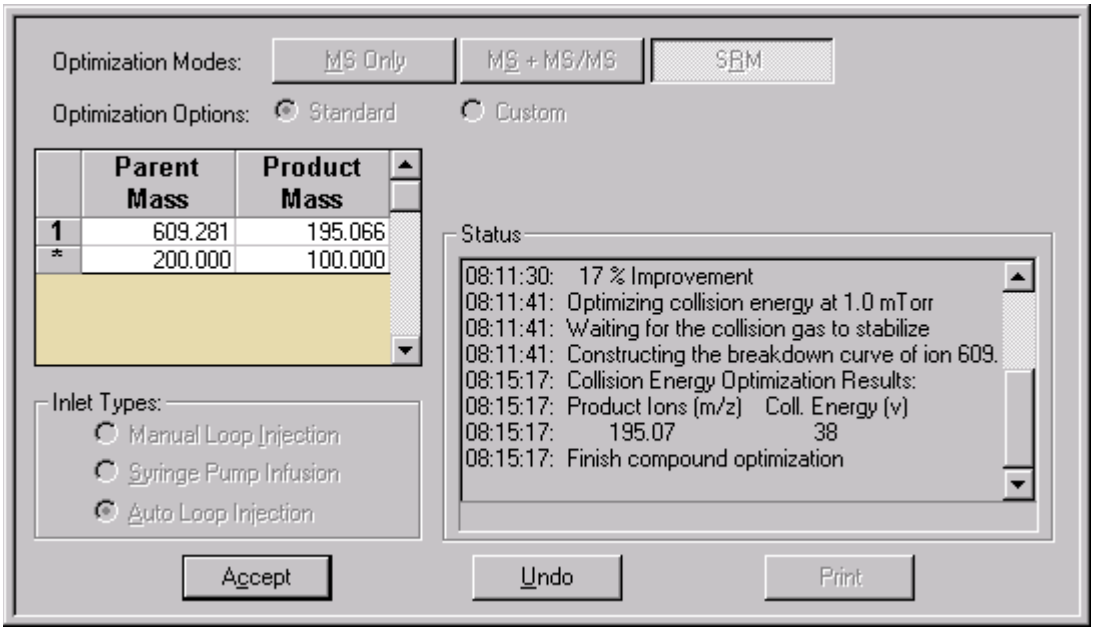


Figure 7-11. Compound Optimization workspace, showing the successful completion of compound optimization

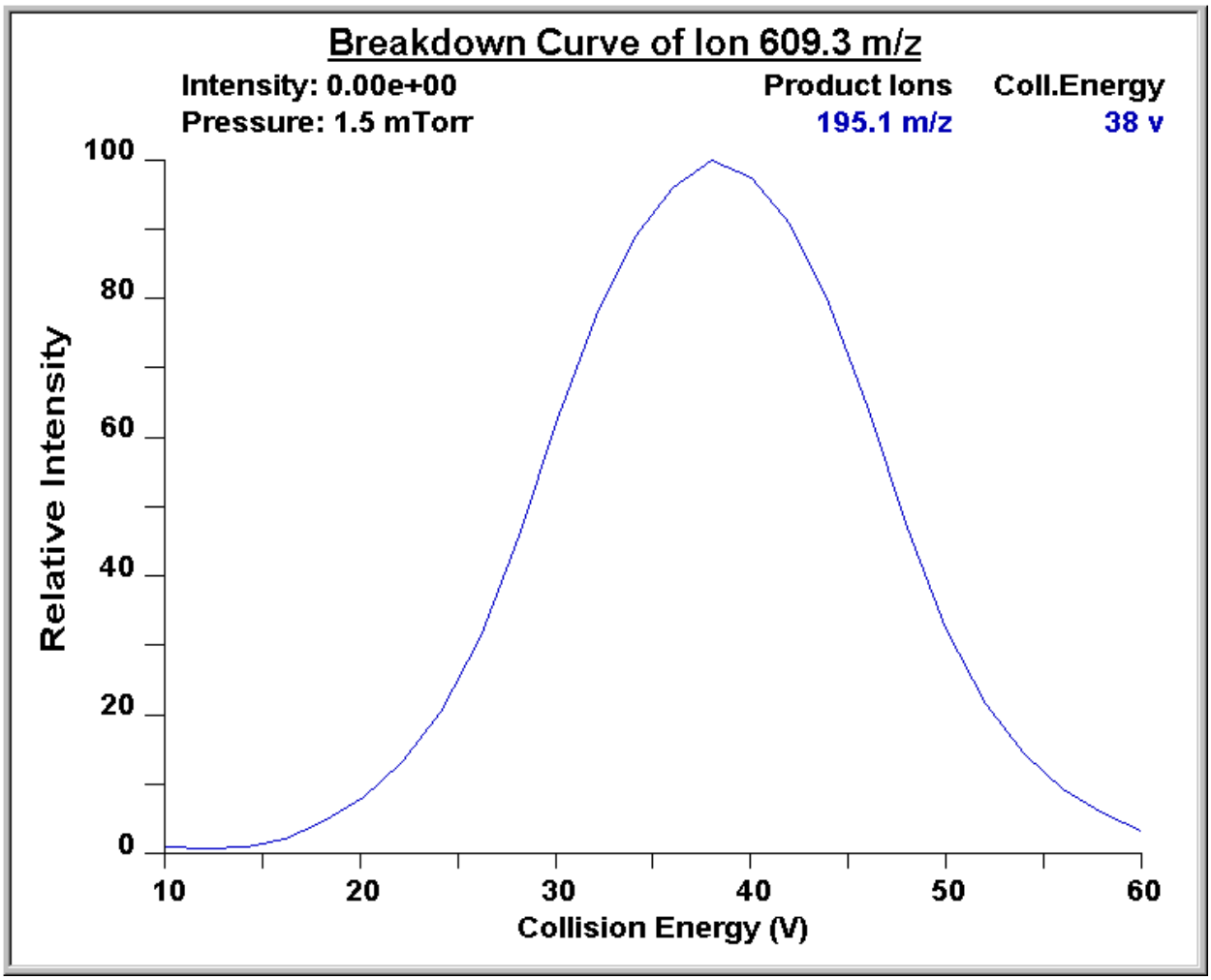


Figure 7-12. Breakdown curve of reserpine showing the relative intensity of the product ion at *m/z* 195.066 as a function of collision energy

4. If errors occurred during the compound optimization procedure, restore the previous mass spectrometer compound sensitive device settings by completing the following steps:

   a. Click on **Undo** to restore the prior device settings.

   b. Click on **Accept** to reload the prior device settings to the mass spectrometer.

   c. Troubleshoot and correct the situation that caused the optimization to fail.

   d. Go to step 3 of this procedure and restart the compound optimization procedure.

5. Click on **Accept** to accept the results of the compound optimization.

Note Save the Tune Method while the mass spectrometer is On if any of the ion source parameters have been changed from their initial settings. ▲

6. Save the Tune Method file by doing the following:

   a. Click on **Save Tune As** to open the Save As dialog box. See Figure 7-13.

   b. Enter a file name (such as **APCI_reserpine**, or the name of your compound) for your Tune Method file in the File Name text box.

   c. Click on **Save** to save the Tune Method file.

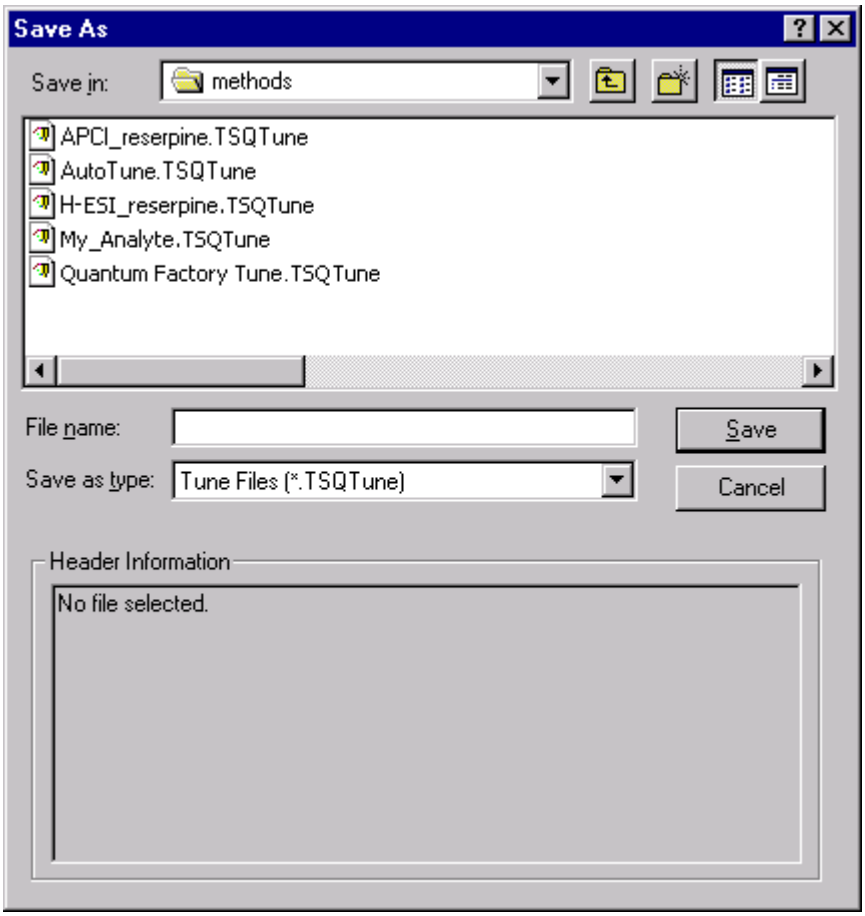


Figure 7-13. Tune Method Save As dialog box

The mass spectrometer is now optimized in APCI/MS/MS mode for the compound reserpine (or for your compound).

Go to the next chapter: "Acquiring APCI/MS/MS Data with Tune Master".

Chapter 8 Acquiring APCI/MS/MS Data with Tune Master

This chapter provides information on acquiring sample data using Tune Master in the APCI/SRM mode. This experiment uses reserpine, but you can follow the same procedure with your analyte of interest.

This chapter contains the following topics:

- Setting Up to Introduce Sample by Manual Loop Injection in APCI Mode
- Acquiring APCI/MS/MS Data in the SRM Scan Mode

Setting Up to Introduce Sample by Manual Loop Injection in APCI Mode

This topic describes how to introduce sample by manual loop injection into the solvent flow from an LC. The plumbing connections for APCI sample introduction by manual loop injection are shown in Figure 8-1.

Make the plumbing connections for manual loop injection as follows:

1. Stop the flow of solvent to the HESI source as follows:

   a. On the Control / Scan Mode toolbar, click on the AS/LC Direct Control button to display the Inlet Direct Control view in the top right corner of the workspace. See Figure 8-2.

   b. In the Direct Control Panel group box, click on the Stop button to stop the flow of solvent.

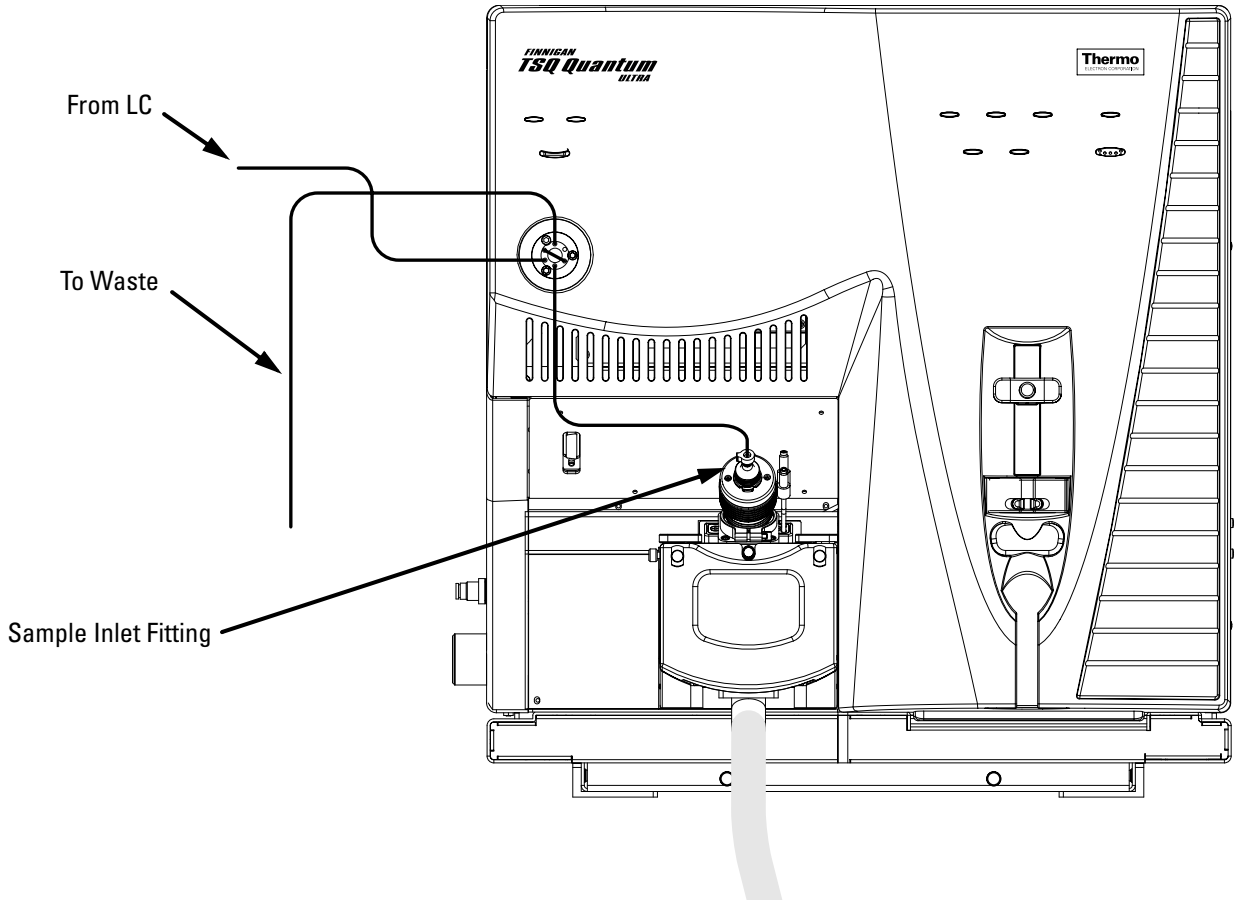


Figure 8-1. APCI/MS plumbing connections for sample introduction by manual loop injection into the solvent flow from an LC

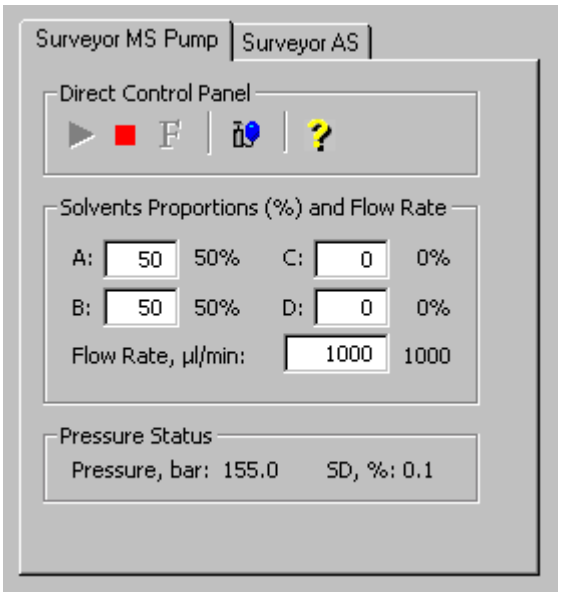


Figure 8-2. Inlet Direct Control view, showing pump On

2. Click on the On/Standby button on the Control / Scan Mode toolbar to place the mass spectrometer in Standby.

3. Remove the syringe from the syringe pump holder as follows:

   a. Lift the handle off the syringe while depressing the black release button on the syringe pump handle.

   b. Remove the syringe.

   c. Remove the tip of the syringe needle from the end of the Teflon tube on the syringe adapter assembly. See Figure 8-3.

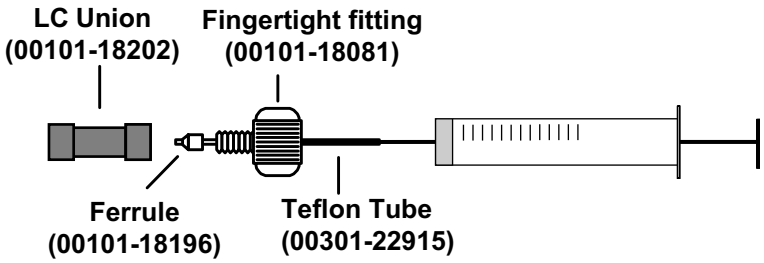


Figure 8-3. Syringe and syringe adapter assembly

4. Remove the sample transfer line that is installed between the syringe adapter assembly and port 5 of the divert/inject valve. Port 5 is now used as the injection port. See Figure 8-4.

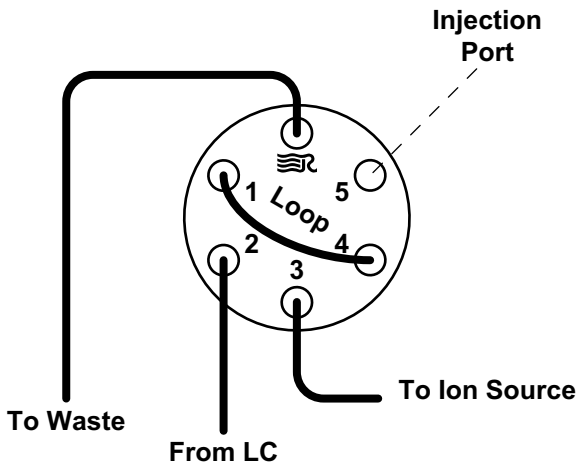


Figure 8-4. Divert/inject valve, showing plumbing for manual loop injection

5. Install the needle port fitting into the divert/inject valve as follows:

    a. Inserting the liner tube, RheFlex ferrule, and the threaded portion of the RheFlex nut (see Figure 8-5) into port 5 of the divert/inject valve.

    b. Carefully tighten the nut with your fingers.

The mass spectrometer is now set up for manual loop injection.

Go to the next topic: “Acquiring APCI/MS/MS Data in the SRM Scan Mode”.

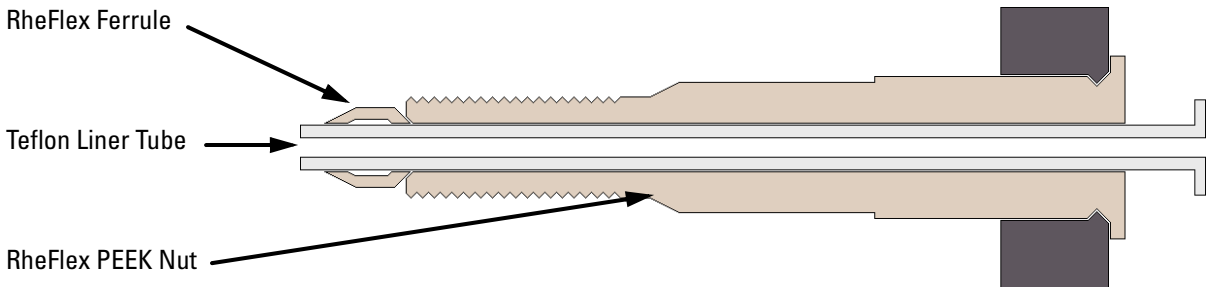


Figure 8-5. Needle port fitting, P/N 00110-22030

Acquiring APCI/MS/MS Data in the SRM Scan Mode


On


Standby

Use the following procedure to acquire a file of reserpine data in the SRM scan mode. Tune Master automatically saves the data you acquire on your hard disk.

1. Click on the On/Standby button on the Control / Scan Mode toolbar to turn On the mass spectrometer.

- If you want to acquire data with the currently displayed Tune Method, go to step 3.
- If you want to acquire data with a different Tune Method than the one currently displayed, you first need to open the desired Tune Method as described in step 2.

2. Open the Tune Method file that stores reserpine tune settings, or the settings for your analyte, as follows:

   a. On the File / Display toolbar, click on the Open File button to display the Open dialog box. See Figure 8-6.

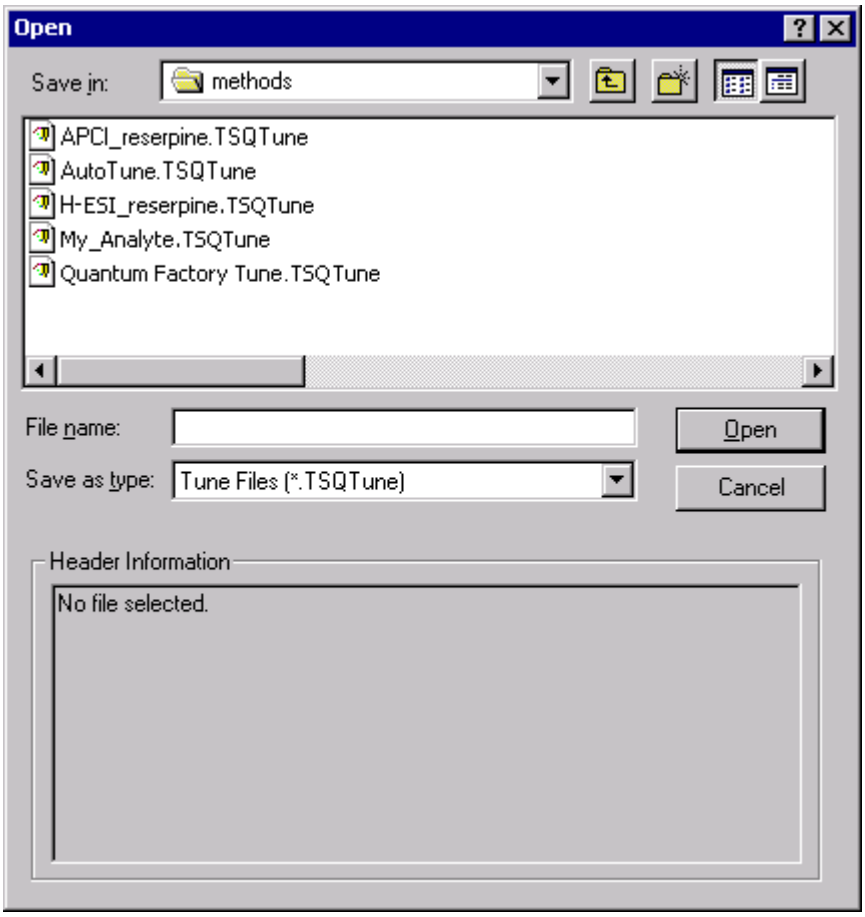


Figure 8-6. Open Tune Method dialog box

b. Confirm that the folder *C:\Xcalibur\methods* is displayed. Select the file *H-ESI_reserpine.TSQTune* (or your Tune Method).

c. Click on **Open** to open the file. Tune Master downloads the Tune Method settings to the mass spectrometer.

3. Start the flow of solvent as follows:

a. On the Control / Scan Mode toolbar, click on the AS/LC Direct Control button to display the Inlet Direct Control view in the top right corner of the workspace. See Figure 8-7.

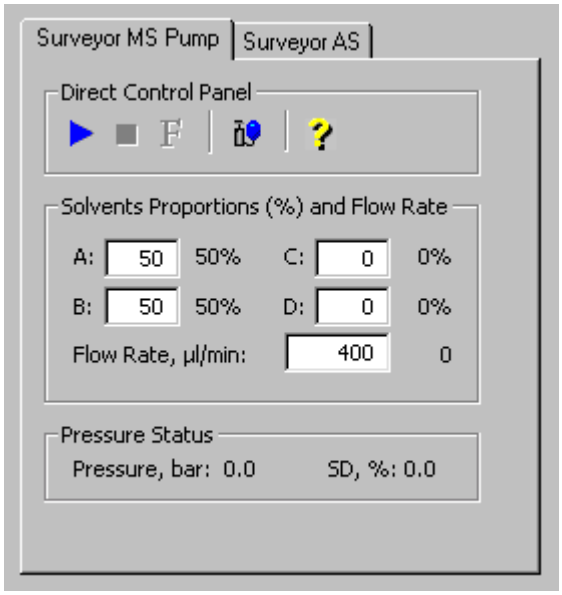


Figure 8-7. Inlet Direct Control view, showing pump Off

Note The following procedure assumes that isopropyl alcohol and HPLC grade water are in the solvent bottles labeled A and B. ▲

b. Set up the Surveyor MS Pump to deliver a solution of 50:50 isopropyl alcohol–water at 400 µL/min, as follows:

i. In the Inlet Direct Control view, in the Solvents Proportions (%) and Flow Rate group box, enter **50** in the text box labeled *A* to specify a delivery proportion of 50% solvent A.

ii. Enter **50** in the text box labeled *B* to specify a delivery proportion of 50% solvent B.

iii. In the Flow Rate text box, enter **400** to set a flow rate of 400 µL/min.

c. In the Direct Control Panel group box, click on the Start button to start the Surveyor MS pump.

4. On the Control / Scan Mode toolbar, click on the Instrument Method Development Workspace button to open the Instrument Method Development workspace. See Figure 8-8.

Note If you just completed compound optimization using reserpine as described in Chapter 4, then the following settings will be selected by default when you switch to the Method Development workspace. ▲

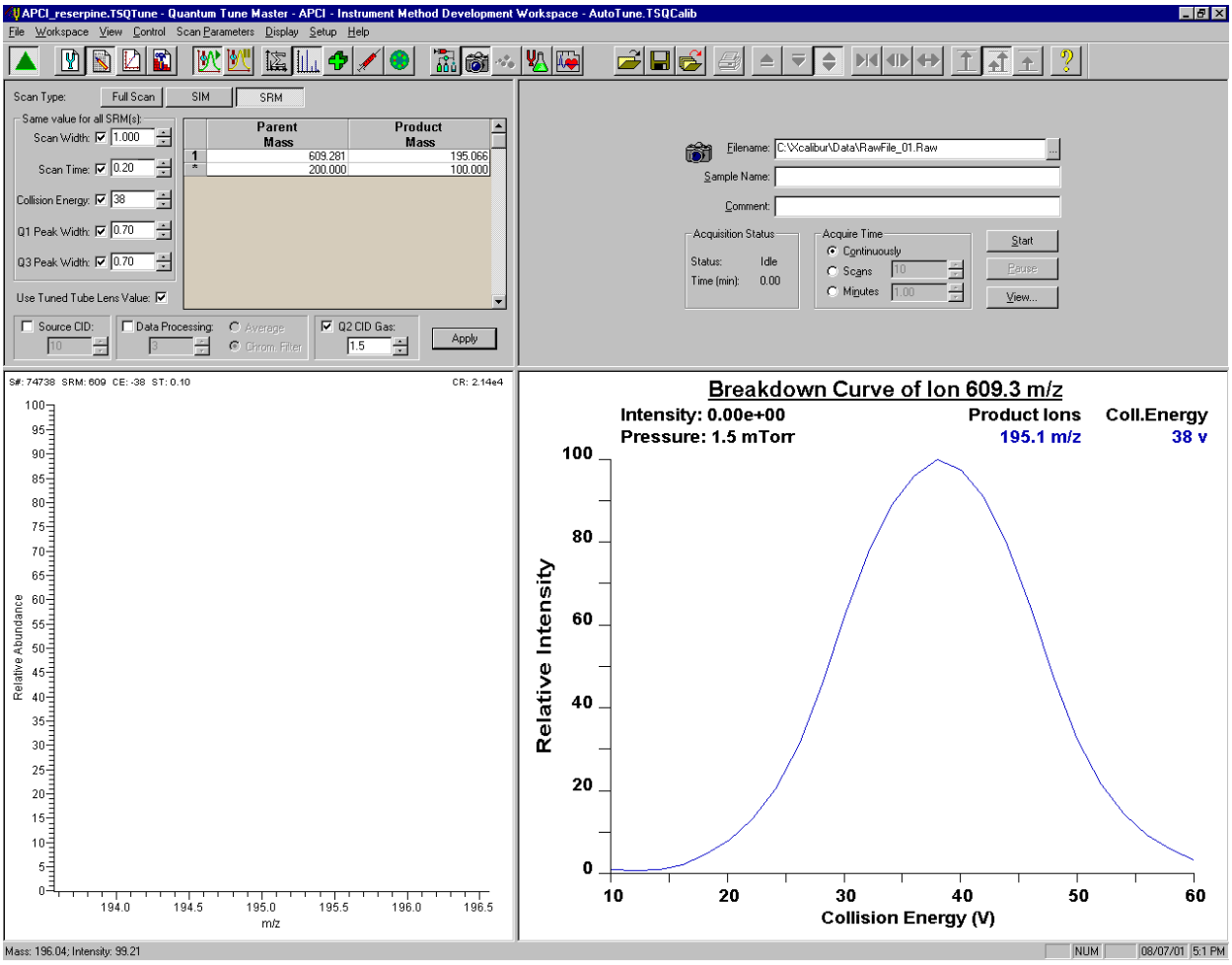


Figure 8-8. Instrument Method Development workspace

5. Define the scan parameters, as required, for acquiring SRM data, by doing the following:

   a. In the Define Scan view in the top left corner of the workspace, select **Scan Type: SRM** to select the Selected Reaction Monitoring (SRM) scan type.

   b. In the SRM table, verify that a single reaction is listed, and verify that the parent mass is 609.281 and the product mass is 195.066.

Note In the Define Scan view, the Same Value For All SRM(s) group box allows you to select global parameters for your SRM scan. Any parameter that you define as global has the same value for each reaction that you are monitoring. To define a global parameter, select the check box for the parameter and set its value by using the spin box. ▲

c. In the Same Value For All SRM(s) group box, ensure that all the global parameter check boxes are selected, and verify or enter the following values into the appropriate spin boxes.

   i. In the Scan Width spin box, enter **1.000** to set the scan width to 1.000 u.

   ii. In the Scan Time spin box, enter **0.20** to set the scan time to 0.20 s.

   iii. Verify that the collision energy in the Collision Energy spin box is approximately equal to the value of 38 that you entered prior to compound optimization. (The automatic optimization might have changed the value of the collision energy.)

   iv. In the Q1 Peak Width spin box, enter **0.70** to set the peak width to 0.70 u.

   v. In the Q3 Peak Width spin box, enter **0.70** to set the peak width to 0.70 u.

d. Select the Use Tuned Tube Lens Value check box.

e. Ensure that the Source CID check box is left unchecked.

f. Specify that a 3 s chromatography filter be used for the data acquisition as follows:

   i. Select the Data Processing check box to activate the Data Processing spin box and option buttons.

   ii. Select the Chrom. Filter option button to specify that a chromatography filter be used.

   iii. Enter **3** in the Data Processing spin box to designate a 3 s chromatography filter.

g. Set the collision cell gas settings as follows:

   i. Select the Q2 CID Gas check box to specify that collision gas be used.

   ii. Enter **1.5** in the Q2 CID Gas spin box to set the collision cell gas pressure to 1.5 mTorr.

6. Click on **Apply** to apply the scan parameters to the mass spectrometer.

7. On the Control / Scan Mode toolbar, click on the Display TIC button to begin recording the total ion current in the Graph view in the bottom right corner of the workspace. See Figure 8-9.

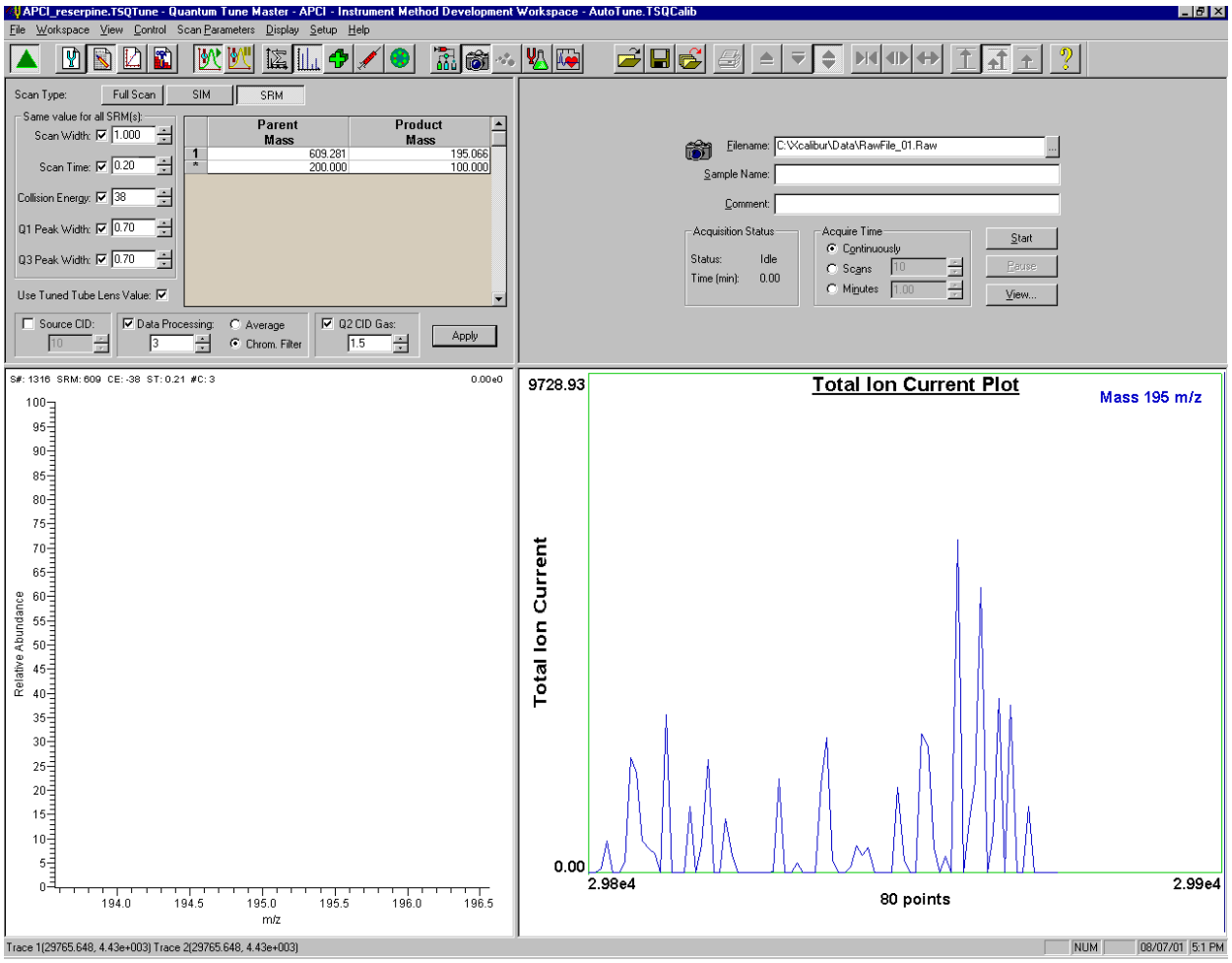


Figure 8-9. Instrument Method Development workspace, showing SRM scan type

8. Specify the acquisition parameters as follows:

Browse

a. In the Acquire Data view in the top right corner of the work space, enter **C:\Xcalibur\Data\reserpine_01.raw** in the Filename text box to specify a path and filename. (If desired, use the browse button to select a different file folder.)

b. In the Sample Name text box, enter **reserpine** (or the name of your analyte) to specify the sample identity.

c. In the Comment text box, enter a comment about your experiment. For example, enter **SRM, APCI, 10 pg, loop** to specify the scan mode, ionization mode, sample amount, and method of sample

introduction. Xcalibur includes the comment on hard copies of your data.

d. In the Acquire Time group box, select the Continuously option button to specify that data be continuously acquired until you stop the acquisition.


Load/Detector


Inject/Waste

9. On the Control / Scan Mode toolbar, ensure that the Divert/Inject Valve button is in the Load state. If the Divert/Inject Valve button is in the Inject state (as shown at the left), click on the Divert/Inject Valve button to switch the Divert/Inject Valve to the Load position.

10. In the Acquire Data view, click on **Start** to begin acquiring data to the file *reserpine_01.raw*. Tune Master serially appends a numeric date and time to your file name if that name already exists in the specified folder, such as *C:\Xcalibur\Data\reserpine_010502092159.raw*.

Note To minimize the possibility of cross-contamination, use a different syringe and a different sample transfer line for your tuning and calibration solution than you do for your samples and compound optimization solution. ▲

11. Fill the sample loop with reserpine solution as follows:

    a. Ensure that the syringe is loaded with 420 µL of the 2 pg/µL reserpine solution. (Refer to "Appendix A: Solution Formulations" for the procedure for preparing the reserpine solution.)

Note Be sure to wipe off the tip of the needle with a clean, lint-free tissue before reinserting it into the syringe adapter assembly to minimize the possibility of cross-contamination of the assembly. ▲

    b. Carefully insert the tip of the syringe needle into the end of the Teflon liner tube on the needle port.

    c. Overfill the sample loop with reserpine solution from the syringe.

12. Press the blue Divert/Inject Valve button on the front panel of the TSQ Quantum Ultra to inject the reserpine solution into the LC solvent flow.

13. Observe the reserpine product peak at *m/z* 195.066, or that of your analyte of interest, in the Spectrum view.

14. Repeat the following sequence several times to obtain consecutive loop injections of reserpine in the SRM scan mode. Wait approximately 1 min between injections.

    a. Press the blue Divert/Inject Valve button on the TSQ Quantum Ultra to return the Divert/Inject valve to the Load position. Overfill the loop with the 2 pg/µL solution of reserpine.

    b. Press the Divert/Inject Valve button again to inject the reserpine solution into the LC solvent flow. Then, observe the Spectrum view.

    c. Wait 1 min before the next injection.

    d. Repeat steps 14.a through 14.c several times.

15. Click on **Stop** in the Acquire Data dialog box to end the data acquisition.

    A file of reserpine data in the SRM scan mode is now stored on the hard drive (with a name such as *reserpine_010502092159.raw*).

Note For more information about reviewing the data you acquire using TSQ Quantum Ultra with Xcalibur, refer to *Finnigan Xcalibur Getting Productive: Qualitative Analysis*. ▲

16. To integrate the chromatogram in the raw file you just acquired using the Xcalibur Qual Browser window, click on **View**. See Figure 8-10.

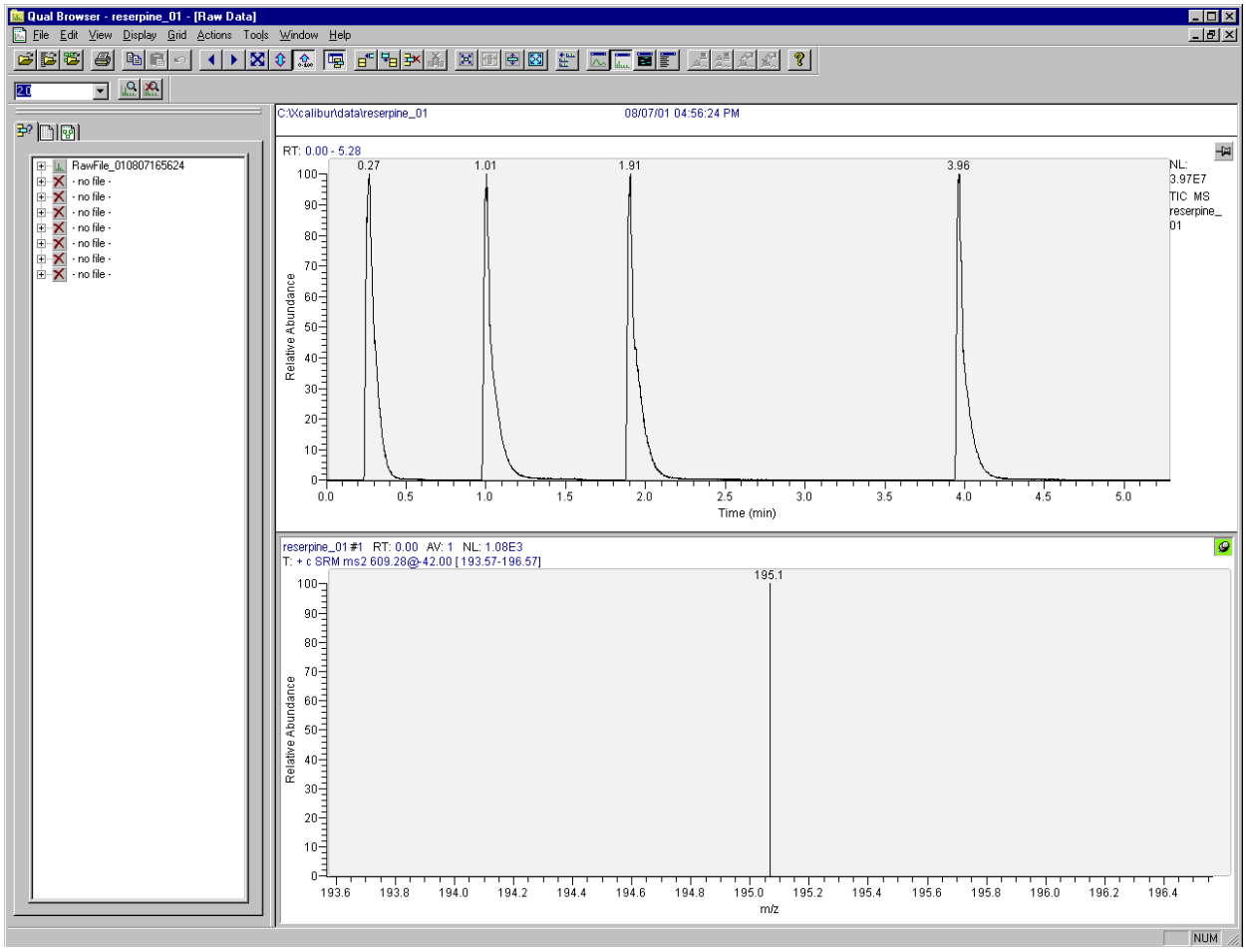


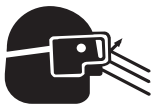
Figure 8-10. Qual Browser window, showing loop injections of reserpine in the Chromatogram view (top) and the centroid at *m/z* 195.066 in the Spectrum view (bottom)

Appendix A Solution Formulations

This appendix provides instructions for preparing the tuning and calibration solution and the reserpine solution that is used to optimize the tune of the mass spectrometer.

The topics in this appendix are as follows:

- Tuning and Calibration Solution
- Reserpine Solutions



CAUTION AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS. Always wear protective gloves and safety glasses when you use solvents or corrosives. Also, contain waste streams and use proper ventilation. Refer to your supplier's Material Safety Data Sheet (MSDS) for the proper handling of a particular solvent. ▲

Always take safety precautions when you handle chemicals and unknown samples. **READ AND UNDERSTAND THE HAZARDS OF THE CHEMICALS USED IN THE FOLLOWING PREPARATIONS.** Dispose of all laboratory reagents by the appropriate method for a specific reagent or solvent.

Material Safety Data Sheets (MSDS) provide summarized information on the hazards and toxicity of specific chemical compounds. MSDSs also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures for the remedy of spills or leaks. Producers and suppliers of chemical compounds are required by law to provide their customers with the most current health and safety information in the form of an MSDS. Read the MSDSs for each chemical you use. Examples of potentially hazardous chemicals used in procedures throughout this manual are as follows:

- Acetic acid
- Methanol
- Reserpine

Tuning and Calibration Solution

This topic provides two procedures for preparing solutions of polytyrosine – 1, 3, 6 that are suitable for tuning and calibrating the mass spectrometer.

The first procedure describes how to reconstitute the tuning and calibration solution using the 20-mL vial (P/N 00301-22924) containing pre-weighed amounts of the polytyrosine components in dry powder form (the appearance is that of a residue). This vial is supplied in the accessory kit.

The second procedure provides instructions for preparing the tuning and calibration solution from your stock of dry chemicals.

Your accessory kit also contains a 20-mL vial of polytyrosine – 1, 3, 6 (P/N 00301-22924) in solution—no dilution is required. The concentrations of the components in this solution are suitable for immediate injection into your mass spectrometer.

Table A-1 provides a summary of the polytyrosine standards supplied in the accessory kit.

Table A-1. Polytyrosine standards supplied in the accessory kit

Standard Description (on label)	Thermo Electron Part Number	C S Bio Company Product Number
Polytyrosine Standard Liquid Form	00301-22924	CS0272L
Polytyrosine Standard Solid Form	00301-22925	CS0272S

Preparing the Polytyrosine – 1, 3, 6 Tuning and Calibration Solution Using the Premixed Vial

Reconstitute the polytyrosine – 1, 3, 6 tuning and calibration solution from the vial of polytyrosine solid (P/N 00301-22925) as follows:

1. Obtain the vial of premixed polytyrosine chemicals from the accessory kit.

2. Dissolve the polytyrosine residue in the vial to a total volume of 20 mL with 0.1% formic acid in 50:50 methanol–water. This yields a solution of 4 ng/µL of Tyr, 12 ng/µL of $(Tyr)_3$, and 24 ng/µL of $(Tyr)_6$.

3. Label the vial *Polytyrosine – 1, 3, 6 Tuning and Calibration Solution* and store it in a refrigerator until it is needed.

Preparing the Polytyrosine – 1, 3, 6 Tuning and Calibration Solution from Your Stock of Dry Chemicals

Prepare 250 mL of the polytyrosine – 1, 3, 6 tuning and calibration solution from your stock of dry chemicals, as follows:

1. Weigh out and deliver into a clean, dry, 250-mL flask 1 mg of L-tyrosine, 3 mg of $(Tyr)_3$, and 6 mg of $(Tyr)_6$.

2. Dissolve the polytyrosine mix with 0.1% formic acid in 50:50 methanol–water to a total volume of 250 mL. This yields a solution of 4 ng/µL of Tyr, 12 ng/µL of $(Tyr)_3$, and 24 ng/µL of $(Tyr)_6$.

3. Transfer the solution to a clean vial labeled *Polytyrosine – 1, 3, 6 Tuning and Calibration Solution*, and store it in a refrigerator until it is needed.

Table A-2 provides a summary of the compounds used in the preparation of the tuning and calibration solution.

Table A-2. Polytyrosine tuning and calibration stock standard summary

Compound	Formula	MW	Vendor	Vendor P/N
L-Tyrosine	$C_9H_{11}NO_3$	181.19	Sigma	T8566
Tyr-Tyr-Tyr	$C_{27}H_{29}N_3O_7$	507.54	Sigma	T2007
$(Tyr)_6$	$C_{54}H_{56}N_6O_{13}$	997.07	Sigma	T1780

Note You can order standard chemicals directly from Thermo Electron, or you can contact these chemical suppliers:

Sigma Chemical Company
P.O. Box 14508
St. Louis, MO, USA 63178-9916
(800) 325-3010 (in the USA or Canada)
[1] (314) 771-3750 (outside the USA or Canada)

C S Bio Company
1300 Industrial Road
San Carlos, CA, USA 94070
(800) 627-2461 (in the USA or Canada)
[1] (650) 802-0880 (outside the USA or Canada) ▲

Reserpine Solutions

Follow the directions given below to prepare a stock solution of reserpine. Then, use serial dilutions of the stock solution to make the compound optimization solution.

Reserpine Stock Solution

Prepare a stock solution of 1 µg/µL reserpine in 1% acetic acid in methanol:

1. Obtain the 1 gram vial of reserpine in your accessory kit. Weigh out 1 mg of reserpine and transfer the sample to a polypropylene microcentrifuge tube.
2. Dissolve the reserpine sample in a total volume of 1 mL of 1% acetic acid in methanol.
3. Ensure that the sample is thoroughly dissolved in solution.
4. Label the vial *Reserpine Stock Solution (1 µg/µL).*

Reserpine Sample Solution

Prepare 1 mL of the 2 pg/µL (3.29 fmol/µL) reserpine compound optimization solution in 1% acetic acid in methanol, as follows:

1. Pipet 20 µL of the stock solution (1 µg/µL) of reserpine into a clean polypropylene microcentrifuge tube.
2. Add 980 µL of 1% acetic acid in methanol to the tube.
3. Mix this solution (20 ng/µL) thoroughly.
4. Transfer 10 µL of the 20 ng/µL solution into a clean polypropylene tube.
5. Add 990 µL of 1% acetic acid in methanol to the tube.
6. Mix this solution (200 pg/µL) thoroughly.
7. Transfer 10 µL of the 200 pg/µL solution into a clean polypropylene tube.
8. Add 990 µL of 1% acetic acid in methanol to the tube.
9. Mix this solution (2 pg/µL) thoroughly.
10. Label the vial *Reserpine Sample Solution (2 pg/µL)* and store it in a refrigerator until it is needed.

Appendix B Instrument Method Development Guidelines

This appendix provides guidelines for developing instrument methods. The settings provided here are chosen to demonstrate various analytical techniques using a polytyrosine sample and therefore might not be directly applicable to your application. To develop an instrument method for your application, you first need to fine tune the instrument by optimizing on your compound.

The instrument settings found in the following tables can be applied to the mass spectrometer using the Instrument Method Development workspace of Tune Master. This allows you to immediately assess instrument performance with your analyte before you build a method. Once you achieve the desired result using Tune Master, the scan event can be copied and pasted into the Instrument Setup window.

To copy the scan event from Tune Master, right-click in the Define Scan view of the Instrument Method Development workspace and select **Copy Scan Event** from the shortcut menu. To paste the scan event into Instrument Setup, right-click on the Scan Editor page of Instrument Setup, and select **Paste Scan Event** from the shortcut menu.

Table B-1. Instrument parameters for Full Scan Q1MS

Entry Mode	First Mass (*m/z*)	Last Mass (*m/z*)	Scan Time (s)	Q1 Peak Width (u)	Source CID	Data Processing	Q2 CID Gas
FM/LM	150.000	1050.000	0.65	0.70	Off	10 spectrum avg.	Off

Table B-2. Instrument parameters for Full Scan Q3MS

Entry Mode	Center Mass (*m/z*)	Scan Width (u)	Scan Time (s)	Q3 Peak Width (u)	Source CID	Data Processing	Q2 CID Gas
Center Mass	182.082	6.000	0.20	0.20	Off	10 spectrum avg.	Off

Table B-3. Instrument parameters for Full Scan Parent MS/MS

Entry Mode	Center Mass (*m/z*)	Scan Width (u)	Scan Time (s)	Product Mass (*m/z*)	Collision Energy (eV)	Q1 Peak Width (u)	Q3 Peak Width (u)	Source CID	Data Processing	Q2 CID Gas (mTorr)
Center Mass	182.082	10.000	0.20	136.076	20	0.70	0.70	Off	10 spectrum average	0.8[a]

[a]Q2 CID gas pressure is reduced in the parent MS/MS scan mode to preserve peak shape and peak resolution.

Table B-4. Instrument parameters for Full Scan Product MS/MS

Entry Mode	Center Mass (*m/z*)	Scan Width (u)	Scan Time (s)	Parent Mass (*m/z*)	Collision Energy (eV)	Q1 Peak Width (u)	Q3 Peak Width (u)	Source CID	Data Processing	Q2 CID Gas (mTorr)
Center Mass	182.082	10.000	0.20	182.082	18	0.70	0.70	Off	10 spectrum average	1.5

Table B-5. Instrument parameters for Full Scan Neutral Loss MS/MS

Entry Mode	Center Mass (*m/z*)	Scan Width (u)	Scan Time (s)	Neutral Loss Mass (*m/z*)	Collision Energy (eV)	Q1 Peak Width (u)	Q3 Peak Width (u)	Charge State	Source CID	Data Processing	Q2 CID Gas (mTorr)
Center Mass	182.082	6.000	0.20	17.027	10	0.70	0.70	1 (for both Q1 & Q3)	Off	10 spectrum average	1.5

Table B-6. Instrument parameters for SIM Q1MS or Q3MS scan mode

Mass (*m/z*)	Scan Width (u)	Scan Time (s)	Q1 Peak Width (u)	Q3 Peak Width (u)	Use Tuned Lens Value	Source CID	Data Processing	Q2 CID Gas
182.082	6.000	0.20	0.70	0.70	On	Off	10 spectrum avg.	Off
508.208	6.000	0.20	0.70	0.70	On	Off	10 spectrum avg.	Off
997.398	6.000	0.20	0.70	0.70	On	Off	10 spectrum avg.	Off

Table B-7. Instrument parameters for SIM Parent MS/MS scan mode

Mass (*m/z*)	Scan Width (u)	Scan Time (s)	Product Mass (*m/z*)	Collision Energy (eV)	Q1 Peak Width (u)	Q3 Peak Width (u)	Use Tuned Lens Value	Source CID	Data Processing	Q2 CID Gas
182.082	6.000	0.20	136.076	20	0.70	0.70	On	Off	10 spectrum avg.	0.8[a]
508.208	6.000	0.20			0.70	0.70	On	Off	10 spectrum avg.	0.8*

[a]Q2 CID gas pressure is reduced in the parent MS/MS scan mode to preserve peak shape and peak resolution.

Table B-8. Instrument parameters for SIM Product MS/MS scan mode

Mass (*m/z*)	Scan Width (u)	Scan Time (s)	Parent Mass (*m/z*)	Collision Energy (eV)	Q1 Peak Width (u)	Q3 Peak Width (u)	Use Tuned Lens Value	Source CID	Data Processing	Q2 CID Gas
136.076	6.000	0.20	182.082	18	0.70	0.70	On	Off	10 spectrum avg.	1.5
165.055	6.000	0.20			0.70	0.70	On	Off	10 spectrum avg.	1.5

Table B-9. Instrument parameters for SIM Neutral Loss MS/MS scan mode

Mass (*m/z*)	Scan Width (u)	Scan Time (s)	Neutral Loss Mass (*m/z*)	Peak Width (u)	Collision Energy (eV)	Use Tuned Lens Value	Source CID	Data Processing	Q2 CID Gas
182.082	6.000	0.20	17.027	0.70	18	On	Off	10 spectrum avg.	1.5

Table B-10. Instrument parameters for SRM MS/MS scan mode

Scan Width (u)	Scan Time (s)	Q1 Peak Width (u)	Q3 Peak Width (u)	Use Tuned Lens Value	Parent Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (eV)	Source CID	Data Processing	Q2 CID Gas
6.000	0.20	0.70	0.70	On	182.082	136.076	18	Off	10 spectrum avg.	1.5
6.000	0.20	0.70	0.70	On	182.082	165.055	10	Off	10 spectrum avg.	1.5
6.000	0.20	0.70	0.70	On	508.208	136.076	38	Off	10 spectrum avg.	1.5
6.000	0.20	0.70	0.70	On	508.208	299.140	24	Off	10 spectrum avg.	1.5
6.000	0.20	0.70	0.70	On	997.398	136.076	60	Off	10 spectrum avg.	1.5
6.000	0.20	0.70	0.70	On	997.398	299.140	54	Off	10 spectrum avg.	1.5

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