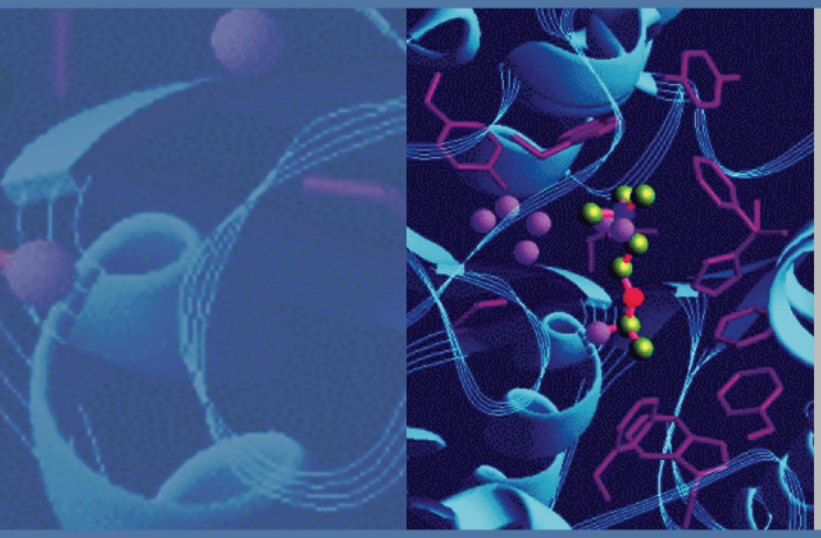

TSQ Series

Getting Started Guide

70111-97152 Revision C March 2009

DOCUMENTATION SURVEY

Thermo SCIENTIFIC

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Software requirements: Xcalibur 2.1; TSQ 2.1

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EN 55011: 2007, A2: 2007
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EN 61000-3-3: 1995, A1: 2001, A2: 2005
EN 61000-4-2: 1995, A1: 1999, A2: 2001
EN 61000-4-3: 2006
EN 61000-4-4: 2004
EN 61000-4-5: 2005
EN 61000-4-6: 2007
EN 61000-4-11: 2004
EN 61326-1: 2006

FCC Class A: CFR 47 Part 15: 2007

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EN 61000-3-3: 1998	EN 61000-4-6: 2001
EN 61000-4-2: 2000	EN 61000-4-11: 1994, A1: 2001
EN 61000-4-3: 2002	EN 61326-1: 1998
FCC Class A, CFR 47 Part 15: 2005	CISPR 11: 1999, A1: 1999, A2: 2002

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EN 61000-3-2: 2006	EN 61000-4-5: 2006
EN 61000-3-3: 1995, A1: 2001, A2: 2005	EN 61000-4-6: 2001
EN 61000-4-2: 2001	EN 61000-4-11: 2004
EN 61000-4-3: 2006	EN 61326-1: 2006
FCC Class A, CFR 47 Part 15: 2007	CISPR 11: 1999, A1: 1999, A2: 2002

Low Voltage Safety Compliance

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EN 61000-4-3: 2002
EN 61000-4-4: 1995, A1: 2000, A2: 2001
EN 61000-4-5: 2001
EN 61000-4-6: 2003
EN 61000-4-11: 2001
EN 61326: 1997, A1: 1998, A2: 2001, A3: 2003

FCC Class A, CFR 47 Part 15: 2005
CISPR 11: 1999, A1: 1999, A2: 2002

Low Voltage Safety Compliance

This device complies with the Low Voltage Directive EN 61010-1:2001 and harmonized standard EN 61010-1: 2001.

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Contents

Preface

About This Guide

The *TSQ Series Getting Started Guide* provides you with information on how to set up, calibrate, and tune the TSQ Series mass spectrometer, and acquire MS data. You can perform these procedures from EZ Tune or Tune Master.

Related Documentation

In addition to this guide, Thermo Fisher Scientific provides the following documents for the TSQ™ Series mass spectrometer:

- *TSQ Series Preinstallation Requirements Guide*
- *TSQ Series Getting Connected Guide*
- *TSQ Series Hardware Manual*
- *H-ESI Probe User Guide*
- *HESI-II Probe User Guide*
- *Ion Max and Ion Max-S API Source Hardware Manual*

The software also provides Help.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

CAUTION Highlights hazards to humans, property, or the environment. Each Caution notice is accompanied by an appropriate Caution symbol.

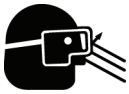
IMPORTANT Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

Table 1 lists the Caution notices that appear in the *TSQ Series Getting Started Guide*.

Table 1. Caution symbols and their meanings

Symbol	Meaning
	**General Hazard:** A hazard is present that is not included in the following categories. This symbol also appears on the instrument. Refer to the instructions in the instrument manual for details on the hazard.
	**Chemical:** Hazardous chemicals might be present in the instrument. Wear gloves when handling toxic, carcinogenic, mutagenic, corrosive, or irritant chemicals. Use only approved containers and procedures for disposing of waste oil.
	**Heat:** Allow heated components to cool before servicing the instrument.
	**Eye Hazard:** Eye damage could occur from splattered chemicals or airborne particles. Wear safety glasses when handling chemicals or servicing the instrument.
	**Sharp Object:** A sharp object is present in the instrument. Proceed with caution.
	**Electric Shock:** An electric shock hazard is present in the instrument. Proceed with caution.

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Introduction

TSQ Series mass spectrometers are members of the Thermo Scientific family of mass spectrometers. TSQ Series mass spectrometers are high-performance triple-stage quadrupole mass spectrometers. Each includes a syringe pump, a divert/inject valve, an atmospheric pressure ionization (API) source, and the Xcalibur™ data system. Typically, samples are introduced using any of these methods:

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

When performing analysis by LC/MS, separated components from an LC column are analyzed in the mass spectrometer. When performing analysis by direct infusion or flow injection, no chromatographic separation of sample components occurs before analysis. In either case, the Xcalibur data system stores and processes the data from the mass spectrometer.

This introduction answers the following questions:

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

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

- Electrospray ionization (ESI)
- Heated electrospray ionization (H-ESI)

- Atmospheric pressure chemical ionization (APCI)

Typically, ESI or H-ESI provides the best analysis of polar compounds such as amines, peptides, and proteins; and APCI provides the best analysis of non-polar compounds such as steroids.

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 ESI or H-ESI

The ESI and H-ESI modes generally produce mass spectra consisting of singly-charged ions, but the charge 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 ESI and H-ESI modes transfer ions in solution into the gas phase. ESI and H-ESI can analyze any samples that previously were not suitable for mass analysis (for example, heat-labile compounds or high molecular weight compounds). You can use ESI and H-ESI to analyze any polar compound that makes a preformed ion in solution. The term *preformed ion* can include adduct ions. For example, you can analyze polyethylene glycols 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 ESI and H-ESI, the TSQ can analyze compounds with molecular weights that are greater than 100 000 u because of multiple charging. ESI and H-ESI are 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 ESI and H-ESI modes in either positive or negative ion polarity mode. The polarity of the preformed ions in solution determines the ion polarity mode: Acidic molecules form negative ions in high pH solution, and basic molecules form positive ions in low pH solution. You use a positively-charged electrospray needle to generate positive ions and a negatively-charged needle to generate negative ions.

You can vary the flow rate from the LC into the mass spectrometer from less than 1 µL/min (using a Nanospray ion source) to 1000 µL/min (using the standard ESI and H-ESI sources). See Table 4. 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 ESI and H-ESI processes are 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. (In ESI and H-ESI, because the buffer and the buffer strength both have a noticeable effect on sensitivity, take care to choose these variables correctly.)

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

To generate stable electrospray, follow these recommendations:

- Refrain from using non-volatile salts and buffers in the solvent system. For example, avoid the use of phosphates and salts that contain sodium or potassium. 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 ESI and H-ESI, APCI is a soft ionization technique. APCI provides molecular weight information for compounds of low polarity that are somewhat volatile. Typically, you use APCI to analyze small molecules with molecular weights up to approximately 1000 u.

APCI is a gas phase ionization technique. Accordingly, 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 200 and 2000 µL/min. See Table 5.

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, APCI produces fewer negative ions than positive ions, the negative ion polarity mode can be more specific—that is, the negative ion polarity mode typically generates less chemical noise than does the positive mode. As a result, 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 (see Table 2).

Table 2. Techniques for introducing samples into the mass spectrometer

	Sample introduction technique	Analytical technique	Figure reference
Direct infusion	Direct infusion (with syringe pump)*	ESI or H-ESI automatic tuning and calibrating ESI, H-ESI, or APCI analysis of a pure analyte in solution	Figure 15 (ESI), Figure 16 (H-ESI), Figure 17, Figure 18
LC flow without chromatographic separation	Auto loop injection into LC flow (with syringe pump)	ESI, H-ESI, or APCI automatic optimization with compound of interest ESI, H-ESI, or APCI analysis of a pure analyte in solution	Figure 30 (ESI), Figure 31 (H-ESI), Figure 32, Figure 33, Figure 55 (APCI)
	Manual loop injection into LC flow	ESI, H-ESI, or APCI automatic optimization of tune with analyte of interest ESI, H-ESI, or APCI analysis of a pure analyte in solution	Figure 40 (ESI), Figure 42, Figure 43
	Infusion into LC flow (with syringe pump)*	ESI, H-ESI, or APCI analysis of a pure analyte in solution	The chapter "Making Plumbing Connections to Run Samples on the TSQ" in *TSQ Series Getting Connected Guide*
	Autosampler injections into LC flow	ESI, H-ESI, or APCI analysis of one or more solutions	
LC flow with chromatographic separation	Autosampler injections onto a column via LC flow	ESI, H-ESI, or APCI analysis of one or more mixtures	

*Provides a steady state introduction of sample into the mass spectrometer.

Note To find additional plumbing diagrams for alternative ion source / sample introduction combinations, refer to the chapter "Making Plumbing Connections to Run Samples on the TSQ Series mass spectrometer" in the *TSQ Series Getting Connected Guide*.

Compound optimization solutions, such as the reserpine sample solution, can contaminate your system at high concentrations. For best results, use the LC flow technique of auto loop injection to introduce optimization solutions into the mass spectrometer.

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

You can 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 introduces sample at a steady rate and at higher solvent flow rates. Use it especially for optimizing tune parameters in ESI, H-ESI, and APCI modes on an analyte of interest. This technique of introducing a solution of pure analyte at a steady rate also works well in ESI, H-ESI, and APCI modes.

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

An LC autosampler can also introduce samples into an LC flow. This technique works in ESI, H-ESI, and APCI modes 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 works in ESI, H-ESI, and APCI modes to separate the analytes before they are introduced sequentially into the mass spectrometer.

For plumbing diagrams for the various methods of sample introduction, see subsequent chapters in this manual and the *TSQ Series Getting Connected Guide*.

What Types of Buffers Do I Use?

To obtain the highest performance for your assays, use volatile buffers whenever possible, instead of nonvolatile ones. Many volatile buffer solutions are available and 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 salt buildup in the ion source and can cause a loss of sensitivity.

For LC applications that require nonvolatile buffers, use these guidelines for best performance:

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

Note You might 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 ESI, H-ESI, and HESI-II probes can generate ions from liquid flows of 1 µL/min to 1.0 mL/min. With this flow rate range you can use a variety of separation techniques: capillary LC, microbore LC, and analytical LC. An optional nanospray ion source is available for 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 separation techniques such as microbore LC, analytical LC, and semi-preparative LC.

As you change the rate of flow of solvents entering the mass spectrometer, you must adjust several of the mass spectrometer parameters:

- For ESI, adjust the temperatures of the ion transfer tube and adjust the gas flow rates for the sheath gas and auxiliary gas.
- For H-ESI, adjust the temperatures of the ion transfer tube and the vaporizer, and adjust the gas flow rates for the sheath gas and auxiliary gas.
- For APCI, adjust the temperatures 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 3 provides guidelines for setting H-ESI operating parameters for various LC solvent flow rates, Table 4 provides guidelines for setting ESI operating parameters, and Table 5 provides guidelines for setting APCI operating parameters.

Table 3. Guidelines for setting operating parameters for LC/H-ESI/MS

Liquid flow rate (µL/min)	Capillary (ion transfer tube) temperature (°C)*	Vaporizer temperature (°C)†	Sheath gas pressure (arbitrary units)	Auxiliary gas flow (arbitrary units)	Spray voltage (V)
5	240	Off to 50	5	0 to 10‡	+3000 (-2500)**
200	350	250 to 300	35	30 (H-ESI probe) 10 (HESI-II probe)	+3000 (-2500)
500	380	300 to 400 (H-ESI probe) 300 to 500 (HESI-II probe)	60	50 (H-ESI probe) 20 (HESI-II probe)	+3000 (-2500)
1000	400	350 to 450 (H-ESI probe) 500 (HESI-II probe)	75	60 (H-ESI probe) 20 (HESI-II probe)	+3000 (-2500)

*Always optimize the tube lens voltage or S-lens rf voltage (TSQ Vantage) whenever you change the temperature of the ion transfer tube.

†Compound dependent

‡Aux gas flow must be greater than 0 if the vaporizer is on

**Negative ion mode

Table 4. Guidelines for setting operating parameters for LC/ESI/MS

Liquid flow rate (µL/min)	Suggested column ID size (mm)	Spray voltage (V)	Capillary temperature (°C)	Sheath gas (arbitrary units)	Auxiliary gas (arbitrary units)
≤ 10	Capillary	3000 (-2500)*	200 to 250	5 to 30	Off
50 to 100	1.0	3000 (-2500)	250 to 300	10 to 30	5 to 10
200 to 400	2.1 to 4.6	3500 (-2500)	300 to 350	20 to 40	10 to 20
≥ 400	4.6	4000 (-3500)	350	30 to 75	10 to 40

*Negative ion mode

Table 5. Guidelines for setting operating parameters for LC/APCI/MS

Liquid flow rate (µL/min)	Capillary temperature (°C)	APCI vaporizer temperature (°C)	Sheath gas (arbitrary units)	Auxiliary gas (arbitrary units)	Corona discharge current (µA)
200	250	350	25	5	+4 (-10)*
1000	250	450	45	5	+4 (-10)

*Negative ion mode

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 Series mass spectrometer. You tune and calibrate in either ESI or H-ESI mode before you acquire data in the ESI, H-ESI, or APCI mode.

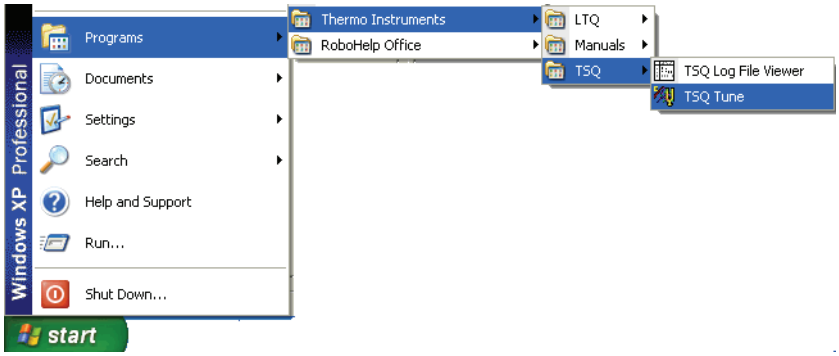
Contents

Placing the LC/MS System in Standby

The LC/MS system must be placed in standby before you can remove the ion source.

❖ To place the LC/MS system in standby

1. If necessary, stop the flow of solvent to the API source as follows:
   a. If the EZ Tune window is not already open, choose **Start > Programs > Thermo Instruments > TSQ > TSQ Tune**, from the Windows™ taskbar, to open the EZ Tune window. See Figure 1.



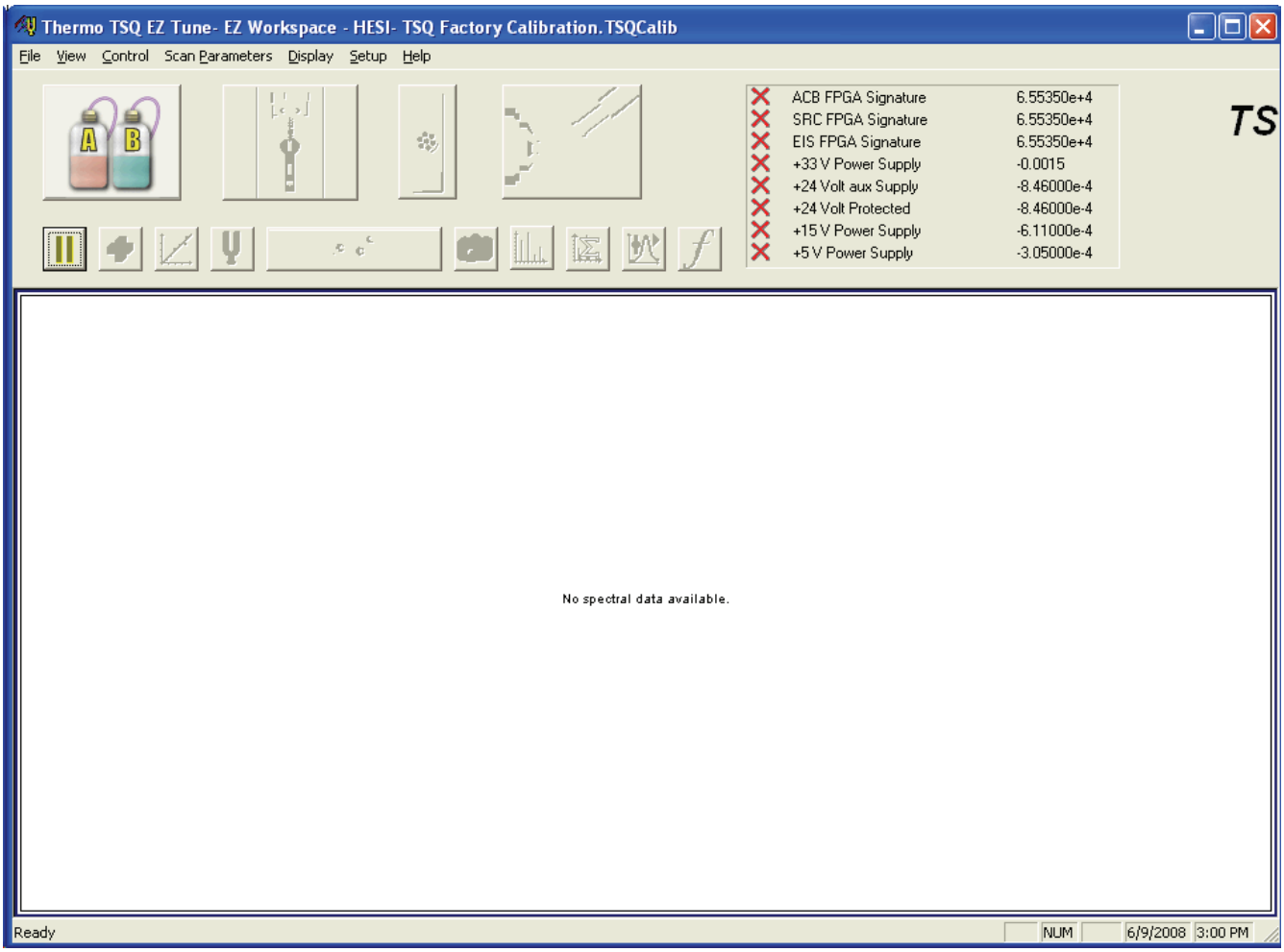
   b. Choose **Setup > Inlet Direct Control** to open the Inlet Direct Control dialog box.
   c. Display the LC page and click (Stop) to stop the LC pump.

On 
Off 
Standby 

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.)

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

Figure 1. EZ Tune window, with mass spectrometer in standby



The LC/MS system is now in Standby mode, and you can safely remove the ion source.

Go to the next topic, "Removing the APCI Probe."

Removing the APCI Probe

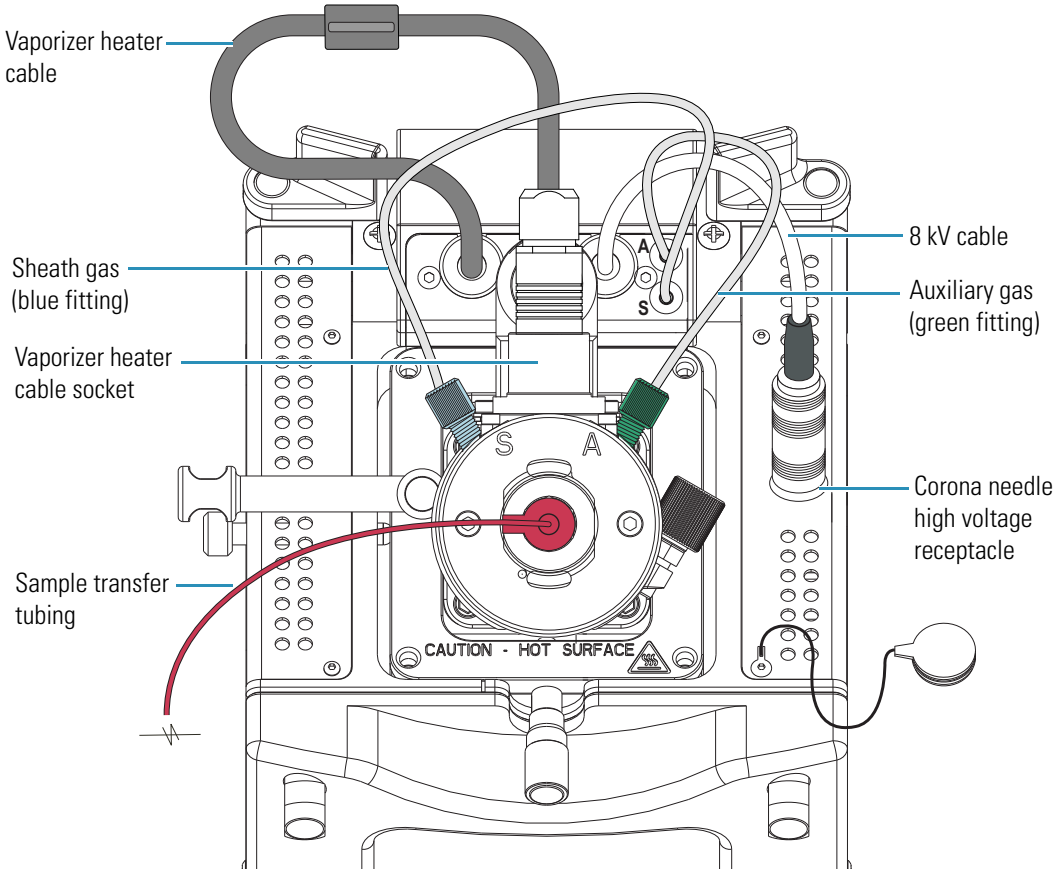
This topic describes how to remove the APCI probe.

Note The following procedures assume that you are familiar with your instrument and the Xcalibur data system. If you need additional guidance, refer to the TSQ Series Help, *TSQ Series Getting Connected*, *Ion Max and Ion Max-S API Source Hardware Manual*, or the *TSQ Series Hardware Manual*.

❖ To remove the APCI probe

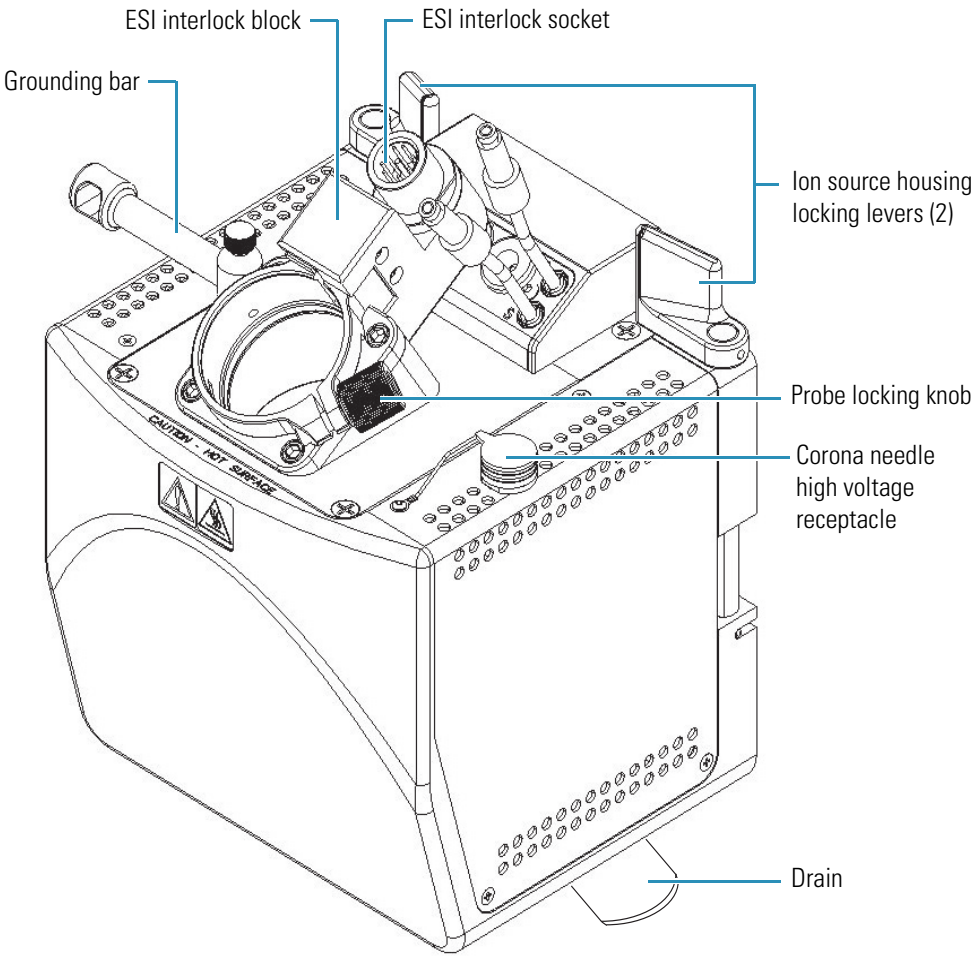
1. Unplug the vaporizer heater cable from the vaporizer heater cable socket on the APCI probe. See Figure 2.

Figure 2. Ion Max™ with APCI probe installed



2. If you plan to operate in ESI mode, connect the vaporizer heater cable to the ESI interlock socket on the ion source housing. See Figure 3.

Figure 3. Ion Max-S™ housing, detail of components (similar to the Ion Max)



3. Disconnect the sample transfer tubing from the APCI probe. See Figure 2.
4. Disconnect the auxiliary gas line (green colored fitting) from the APCI probe.
5. Disconnect 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 °C. Always allow the heated vaporizer to cool to room temperature (for approximately 20 minutes) 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 prevents 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 counterclockwise 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 counterclockwise.

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

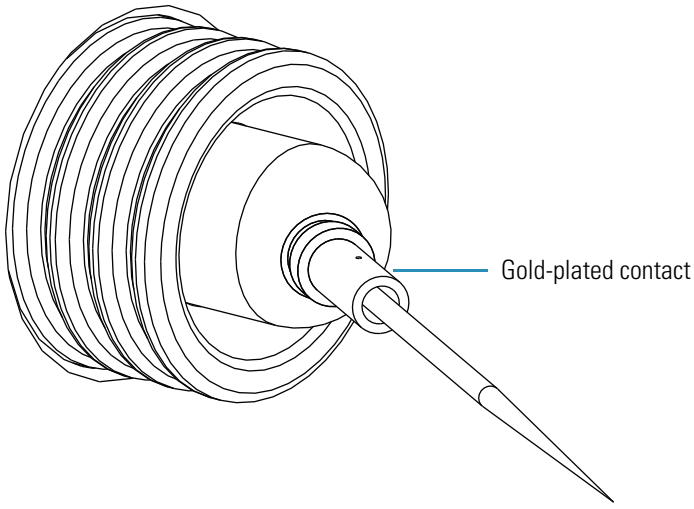
8. Remove the corona discharge needle as follows:

   a. Remove the Ion Max or Ion Max-S source housing as described in "Removing the Ion Max or Ion Max-S Ion Source Housing" on page 15.

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

   b. Remove the corona needle: Using pliers, grasp the needle by the gold-plated contact and pull the needle straight out of the socket. See Figure 4.

Figure 4. Corona needle, rear view



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

If you want to install the optional ion sweep cone, go to "Installing the Ion Sweep Cone" on page 15.

If you do not want to install the ion sweep cone, go to "Installing the Ion Sweep Cone" on page 15.

Removing the Ion Max or Ion Max-S Ion Source Housing

You must remove the Ion Max or Ion Max-S ion source housing to access the ion sweep cone and to remove the APCI corona discharge needle.

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

❖ To remove the ion source housing

1. Remove the drain tube from the ion source housing drain. See Figure 3.
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 it straight off of the ion source mount assembly. Place the housing in a safe location for temporary storage.

Go to the next topic, "Installing the Ion Sweep Cone."

Installing the Ion Sweep Cone

The ion sweep cone is a metallic cone over the ion transfer tube. The ion sweep cone channels the sweep gas towards the entrance of the ion transfer tube. 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 you can analyze without loss of signal intensity. In addition, keeping the ion transfer tube entrance cleaner reduces the need for ion source maintenance.

❖ To install the ion sweep cone

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. You place the gas inlet on the ion sweep cone in this port. See Figure 5 and Figure 6.

Figure 5. Sweep gas supply port in the API cone seal of the capillary heater cage

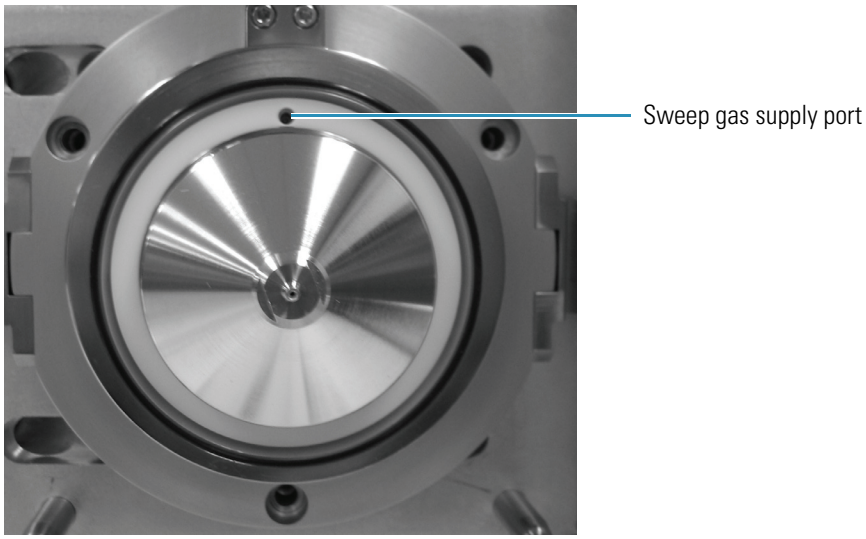
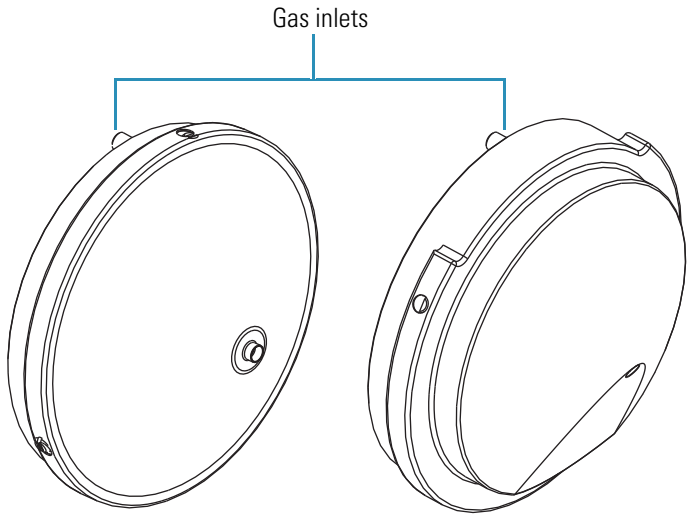


Figure 6. Ion sweep cones for FAIMS and APPI (left) and H-ESI, ESI, and APCI (right), showing the gas inlets



CAUTION AVOID BURNS. At operating temperatures, 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 minutes) 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 or Ion Max-S Ion Source Housing."

Installing the Ion Max or Ion Max-S Ion Source Housing

❖ **To reinstall the Ion Max or Ion Max-S ion source housing**

CAUTION Ensure that the guide pins are exactly aligned with the guide pin holes when pressing the ion source housing onto the ion source. Otherwise, the connectors on the ion source housing can become damaged.

1. Carefully align the two guide pin holes on the back of the ion source housing with the ion source housing guide pins on the mass spectrometer. Carefully press the ion source housing onto the ion source mount. See Figure 7 and Figure 8.

Figure 7. Rear view of the Ion Max ion source housing (similar to the Ion Max-S source housing)

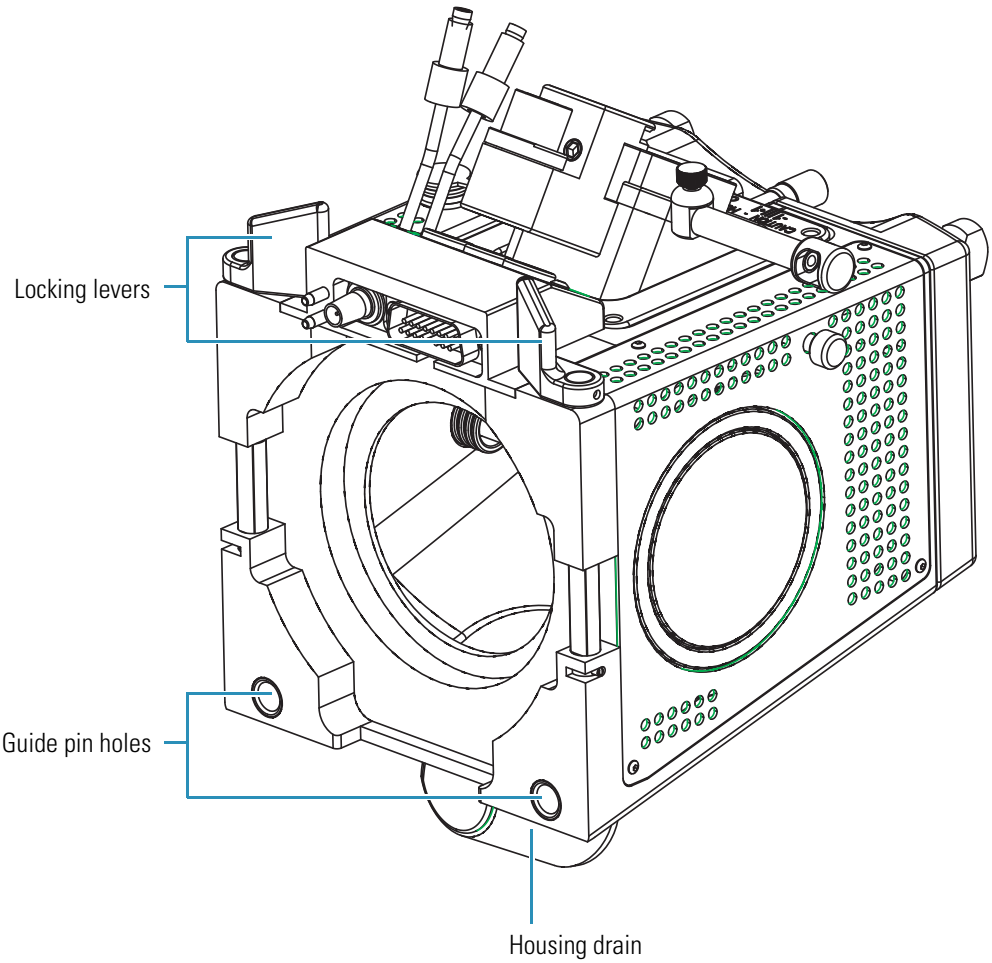
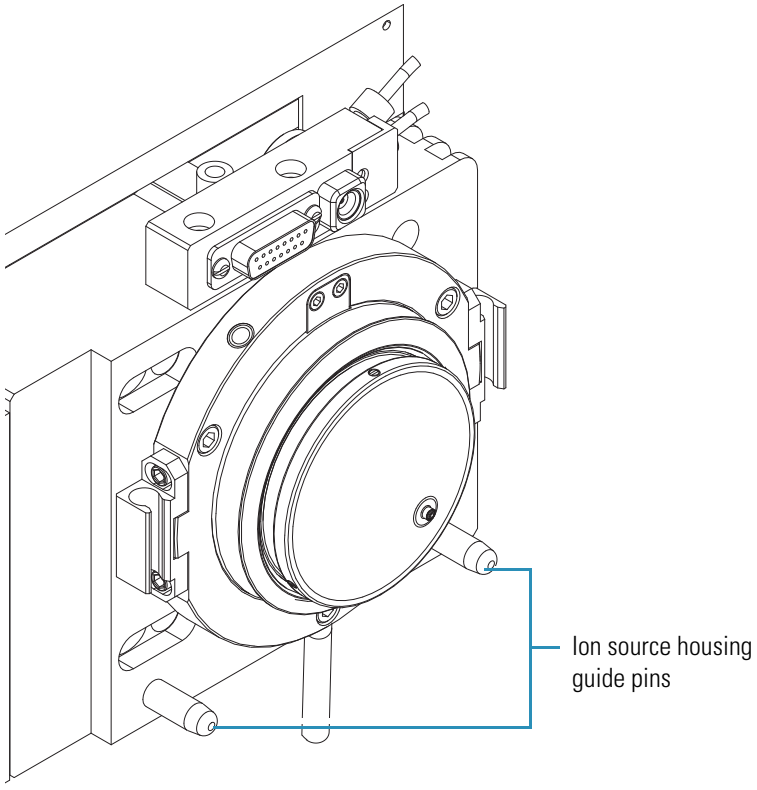


Figure 8. Ion source mount showing ion source housing guide pins



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 forepump. 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 is vented to a fume exhaust system.

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

Go to the next topic, "Installing the ESI, H-ESI, or HESI-II Probe."

Installing the ESI, H-ESI, or HESI-II Probe

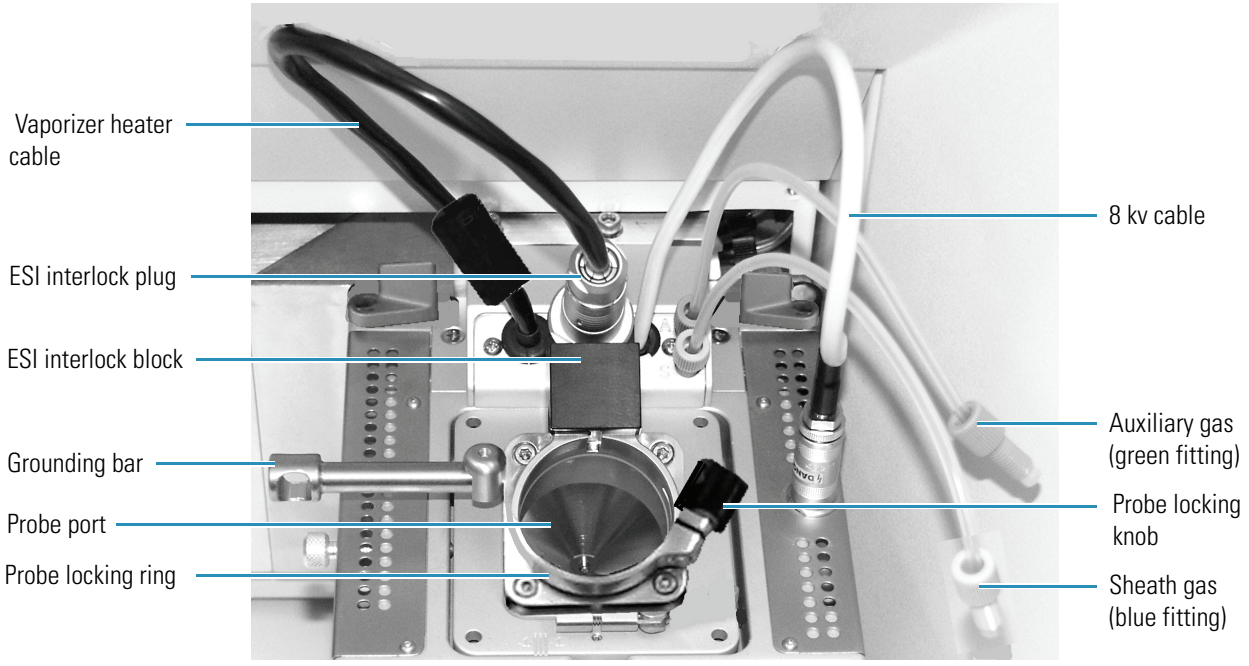
❖ **To install the ESI, H-ESI, or HESI-II probe**

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

Note If your ESI or H-ESI probe does not already have a sample tube (fused-silica capillary) and safety sleeve attached, you must follow the procedure for installing a sample tube and PEEK safety sleeve in "Installing a New Fused-Silica Sample Tube and PEEK Safety Sleeve" section of the *Ion Max and Ion Max-S API Source Hardware Manual*.

2. Ensure that the probe locking ring is opened to its widest position. See Figure 9.

Figure 9. Ion Max-S ion source housing with no API probe installed



3. Insert the ESI, H-ESI, or HESI-II probe into the port in the ion source housing, aligning the guide pin (see Figure 10 and Figure 12) on the probe body at minus 45 degrees from the ESI interlock block.

Figure 10. ESI probe

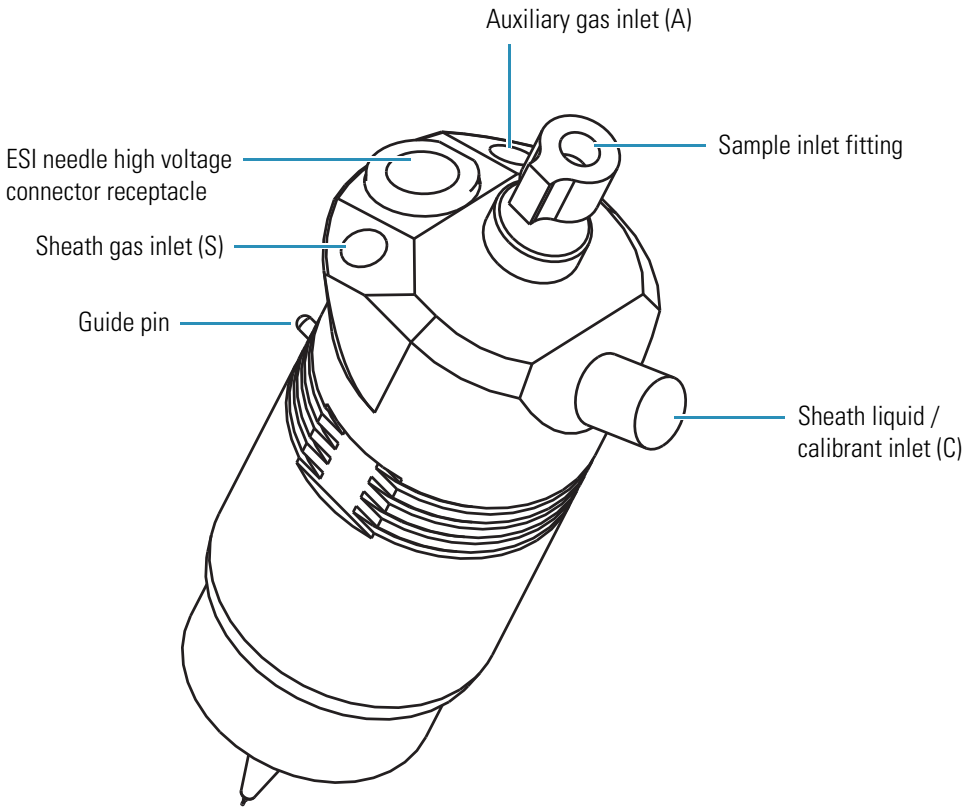


Figure 11. H-ESI probe

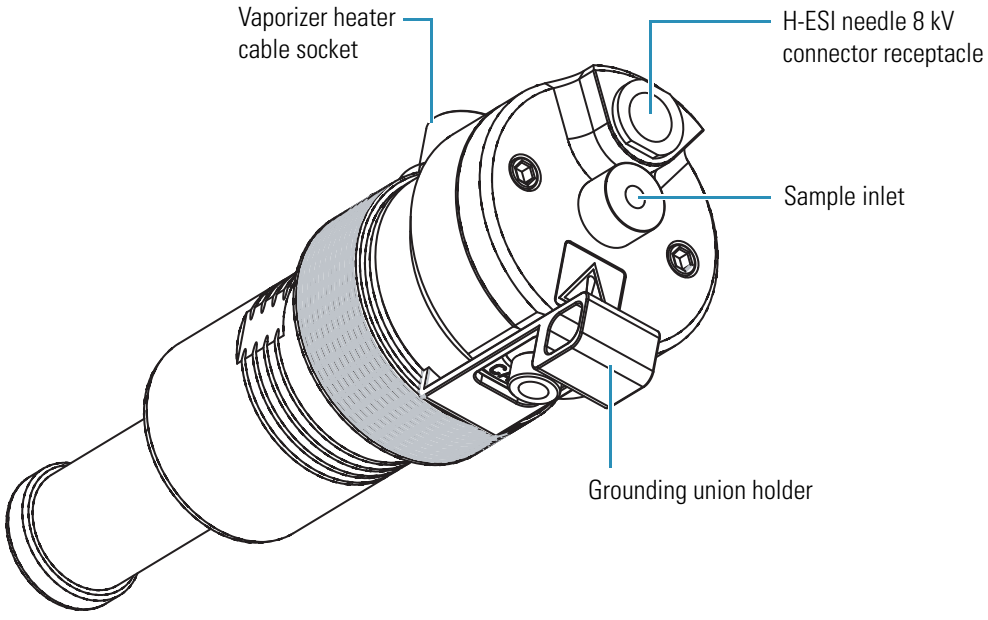
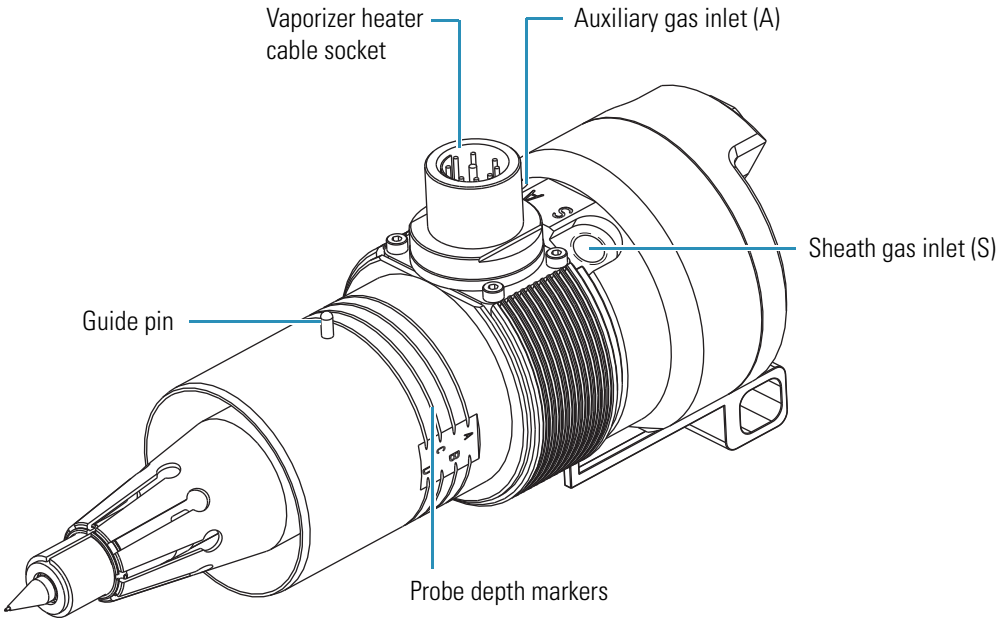


Figure 12. HESI-II probe



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 might have to pull the probe slightly toward you 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 bar on the Ion Max or Ion Max-S source (ESI) or the grounding union holder on the H-ESI or HESI-II probe. See Figure 13 (ESI) and Figure 14 (HESI-II).
8. Connect the sheath gas fitting (blue) to the sheath gas inlet (S) on the probe manifold.
9. Connect the auxiliary gas fitting (green) to the auxiliary gas inlet (A) on the probe manifold.
10. For H-ESI or HESI-II, connect the vaporizer heater cable to the vaporizer heater cable socket on the H-ESI or HESI-II probe.

Figure 13. ESI probe installed

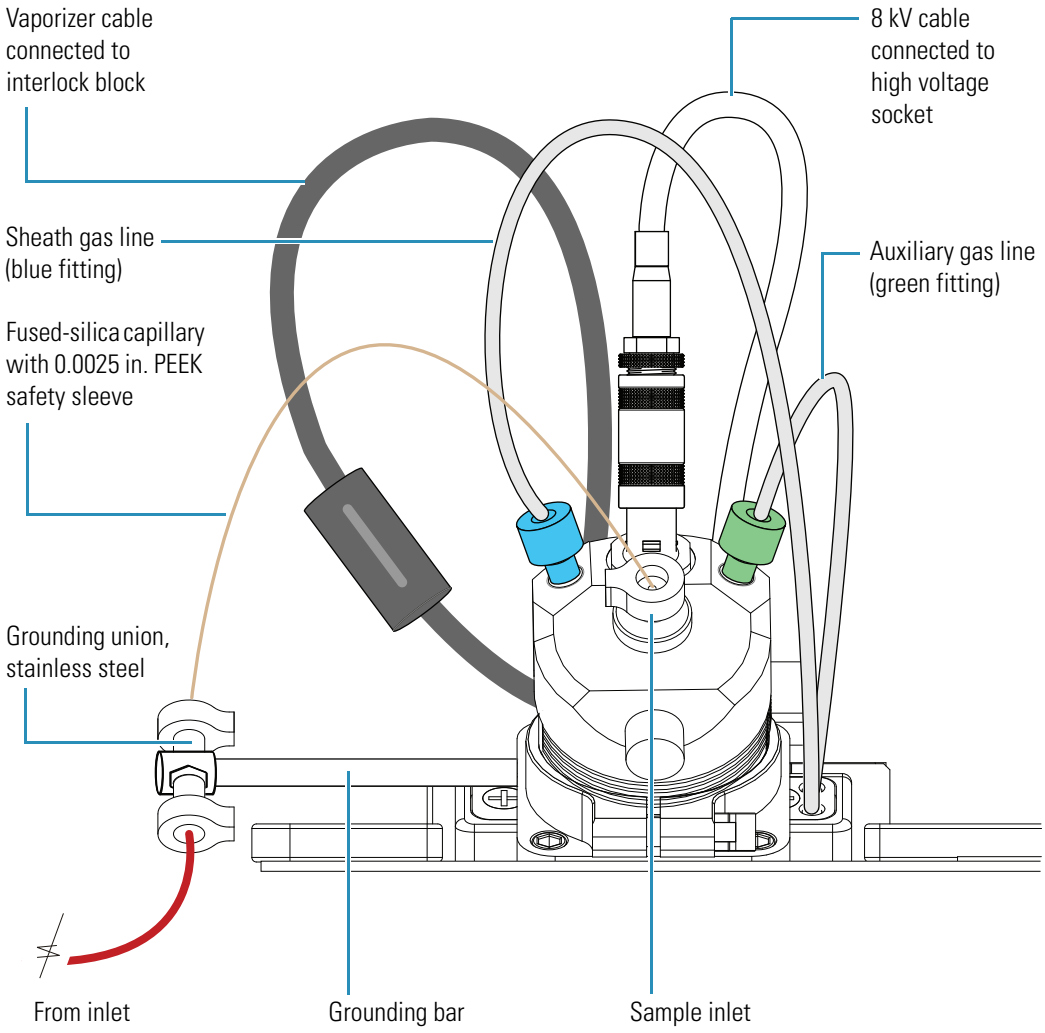
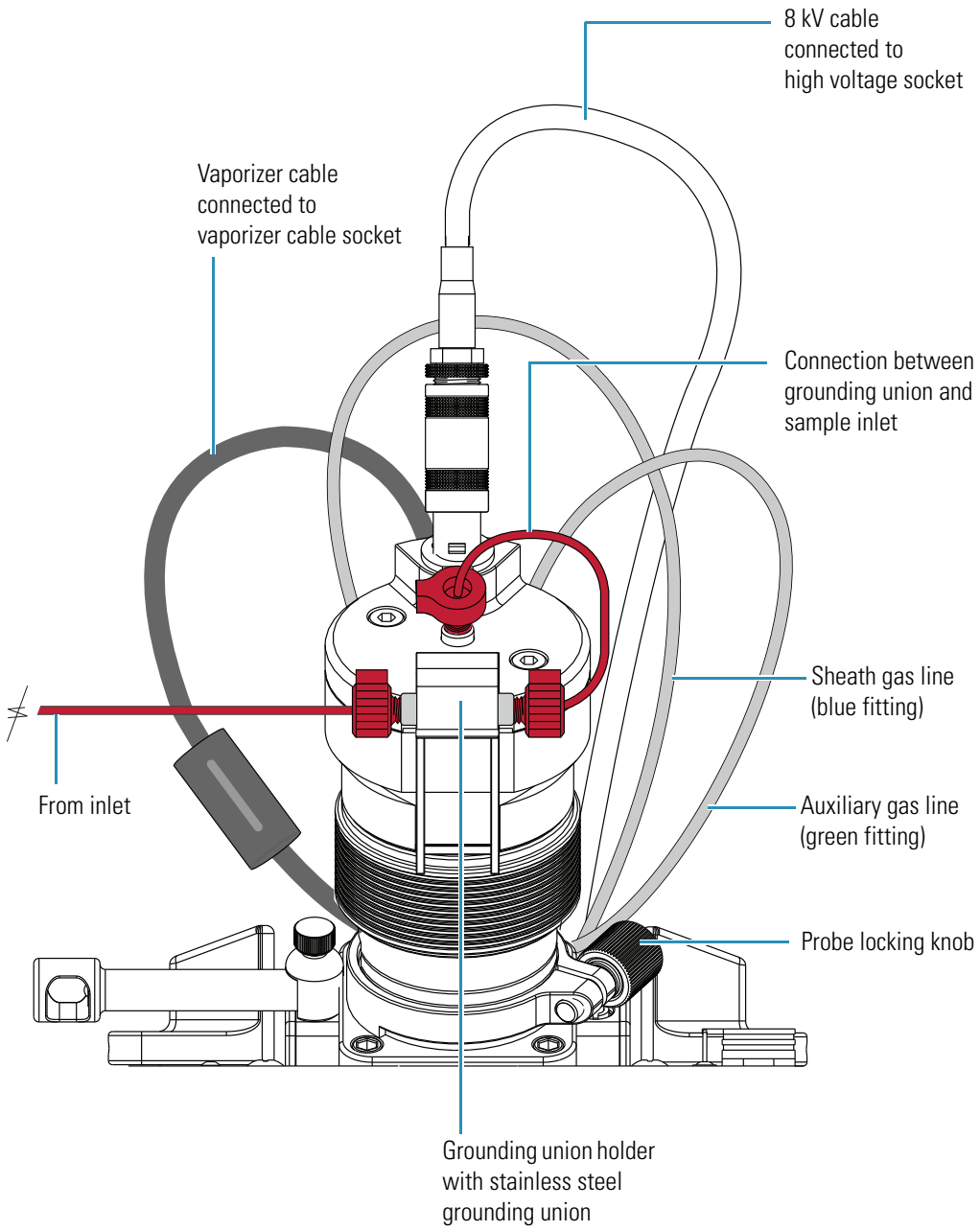


Figure 14. HESI-II probe installed



11. Connect the 8 kV cable to the ESI needle high voltage receptacle on the ESI, H-ESI, or HESI-II probe. See Figure 10 and Figure 11. Tighten the locking ring on the 8 kV connector.
12. Connect the sample transfer tubing (LC line) to the grounding union.

The ESI, H-ESI, or HESI-II source is now properly installed on the mass spectrometer.

Note Before you analyze samples with the ESI, H-ESI, or HESI-II ion source, you must change to ESI or H-ESI source mode in Quantum EZ Tune or Tune Master. Choose **Setup > Change Ion Source > ESI** or **Setup > Change Ion Source > HESI**.

Keep the LC/MS system in Standby mode and go to the next chapter, "Tuning and Calibrating the Mass Spectrometer in ESI/MS or H-ESI/MS Mode."

Tuning and Calibrating the Mass Spectrometer

This chapter contains procedures for tuning and calibrating the mass spectrometer. The procedures use a tuning and calibration solution, which is introduced directly into the mass spectrometer in low flow mode. For optimum performance over the mass range of the detector, you must tune and calibrate every month or up to a maximum of three months.

Note In addition to the tuning and calibrating described in this chapter, you must perform high mass calibration for TSQ Quantum™ Access™, TSQ Quantum Access MAX, TSQ Quantum Ultra EMR™, and TSQ Vantage™ EMR, and accurate mass calibration for TSQ Quantum Ultra™ AM and TSQ Vantage AM.

Tuning and calibrating your mass spectrometer requires the following actions described in this chapter:

- Infusing a low concentration tuning and calibration solution that contains polytyrosine - 1,3,6 directly into the ESI, H-ESI, or HESI-II source by using a syringe pump.
- Testing 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.
- Running the automatic tuning and calibration procedure.
- Saving the tune and calibration files.

Contents

Setting Up to Introduce Sample by Direct Infusion

The sample introduction device that you use for the ESI or H-ESI tuning and calibration procedure is a syringe pump. With a syringe pump you can infuse the tuning and calibration solution directly into the ESI, H-ESI, or HESI-II source for extended periods.

The syringe and the syringe pump are located on the front panel of your TSQ Series mass spectrometer. The plumbing connections for ESI/MS and H-ESI/MS sample introduction from the syringe pump are shown in Figure 15 and Figure 16.

Figure 15. ESI/MS plumbing connections for sample introduction by syringe pump direct infusion

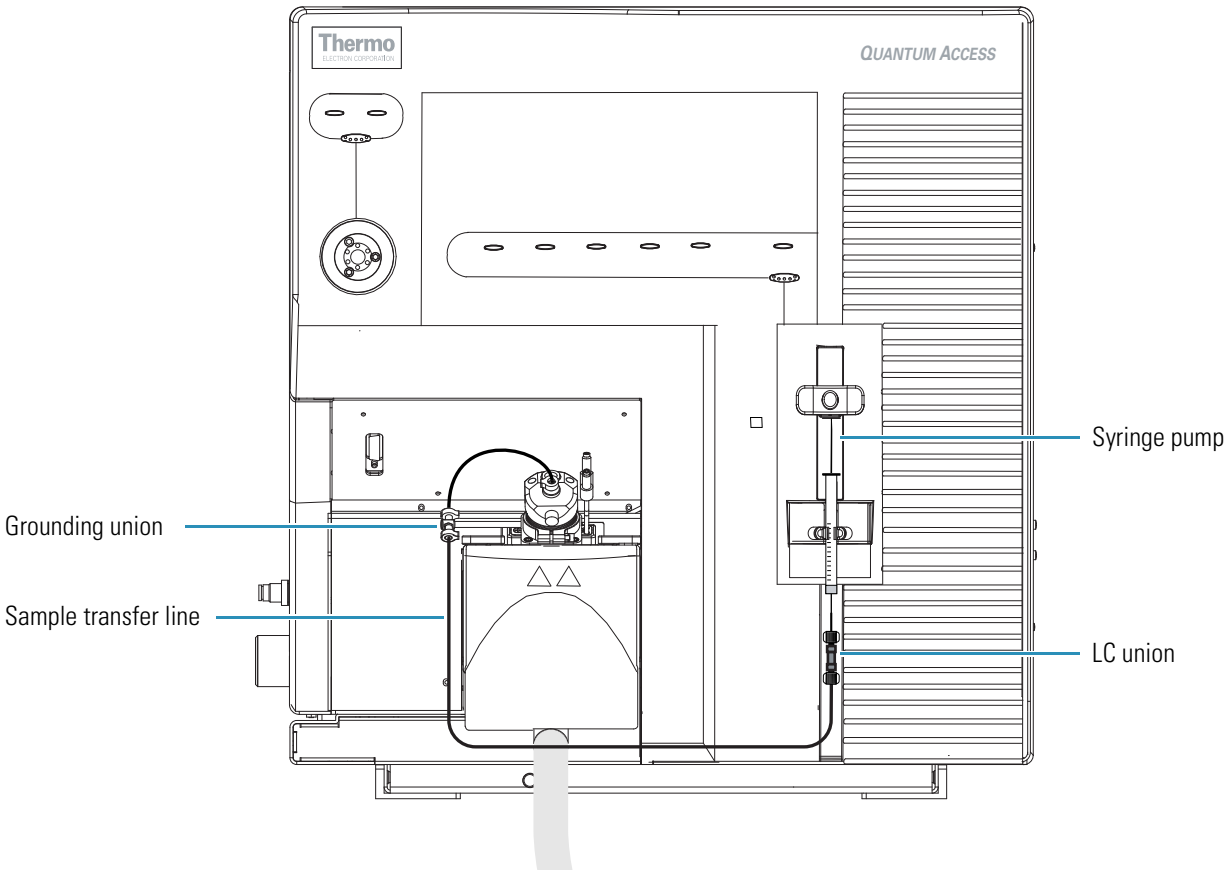
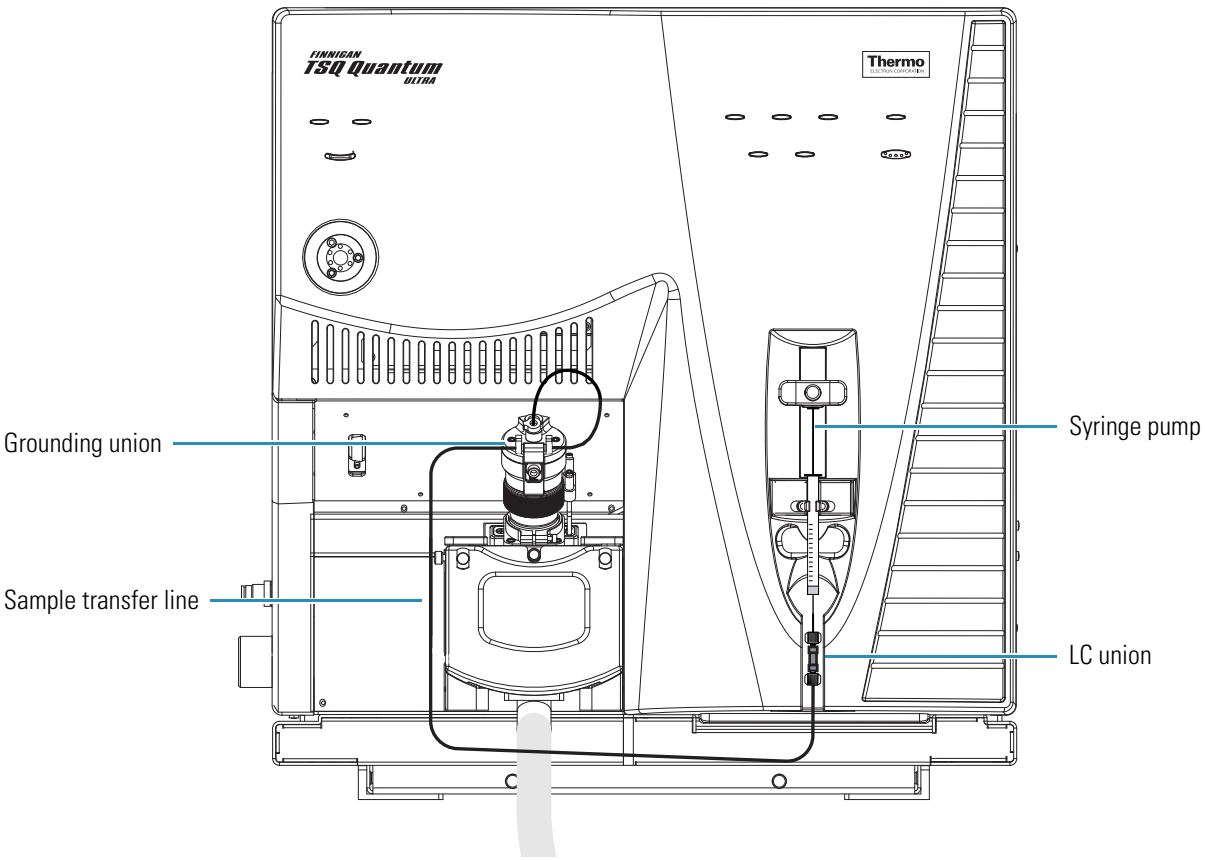


Figure 16. H-ESI/MS plumbing connections for sample introduction by syringe pump direct injection



To introduce solutions for tuning and calibrating, you must install a syringe that contains the tuning and calibration solution onto the syringe pump.

Before starting this procedure, you must also place your LC/MS system in Standby mode, as described in "Placing the LC/MS System in Standby" on page 10.

❖ **To set up the syringe pump for introducing tuning and calibration solution into the ESI, H-ESI, or HESI-II source**

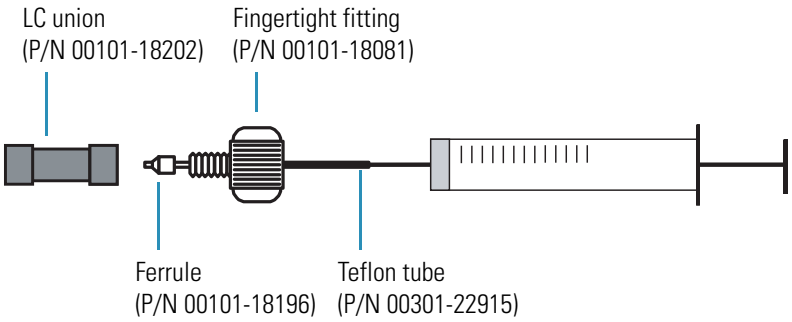
Note To minimize the possibility of cross-contamination, for your tuning and calibration solution, use a different syringe and a different sample transfer line from the ones used for your samples and compound optimization solution.

1. Load a clean, 500 µL syringe with about 420 µL of the polytyrosine - 1,3,6 tuning and calibration solution. (For the procedure for preparing the tuning and calibration solution, see Appendix C, "Solution Formulations.")

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

2. 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 17.

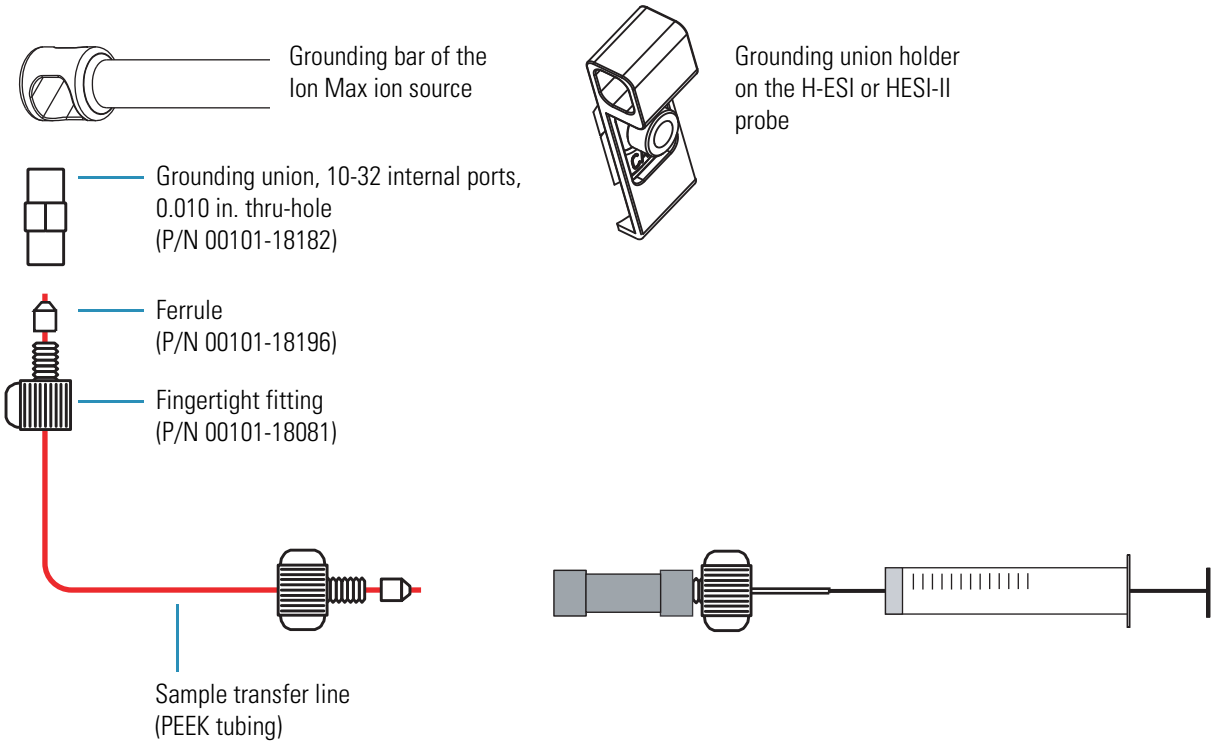
Figure 17. Syringe and syringe adapter assembly



3. Connect the syringe adapter assembly to the LC union.
4. Connect the PEEK transfer line tubing to the LC union and grounding union (see Figure 18). Both the LC union and the grounding union have 10-32, coned-bottom receiving ports.
5. Insert the grounding union in the grounding bar or grounding union holder.

CAUTION Ensure that the grounding union is completely inserted in the grounding bar or grounding union holder. Otherwise, electric shock can occur.

Figure 18. Plumbing connections for the infusion line between the LC union and the grounding union



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.

Go to the next section. "Setting Up for Automatic Tuning and Calibrating."

Setting Up for Automatic Tuning and Calibrating

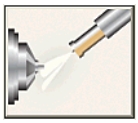
To ensure optimum performance of the automatic tuning and calibration procedure, ensure proper setup of the instrument.

CAUTION If the TSQ mass spectrometer runs out of nitrogen, the instrument automatically turns off to prevent the possibility of atmospheric oxygen from entering the ion source. The presence of oxygen in the ion source when the MS detector is on can be unsafe. In addition, if the TSQ mass spectrometer automatically turns off during an analytical run, you might lose data.

❖ To set up the mass spectrometer for tuning and calibrating

1. From the Windows taskbar, choose **Start > Programs > Thermo Instruments > TSQ > TSQ Tune** to open EZ Tune.

2. In EZ Tune, click 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.) Turning on the mass spectrometer initiates the following events:
   - The mass spectrometer begins scanning.
   - Nitrogen begins to flow through the ESI, H-ESI, or HESI-II probe.
   - The mass spectrometer applies a high voltage to the ESI, H-ESI, or HESI-II probe.
   - EZ Tune shows a real-time mass spectrum in the Spectrum view.



3. Click the **Ion Source Devices** button to display the Ion Source Devices dialog box. See Figure 19 or Figure 20.
4. Set the values for several of the compound dependent devices to the values shown in Figure 19 or Figure 20:
   a. In the Ion Source Devices dialog box, select a device to highlight it. (Make sure the check box is clear.)
   b. In the Device box, type the setting for the device, or use the up or down arrow.
   c. Perform steps step 4a and b for all devices listed in Figure 19 or Figure 20.
   d. Wait until the device readbacks are similar to the set values.

Figure 19. Ion Source Devices dialog box of the TSQ Vantage

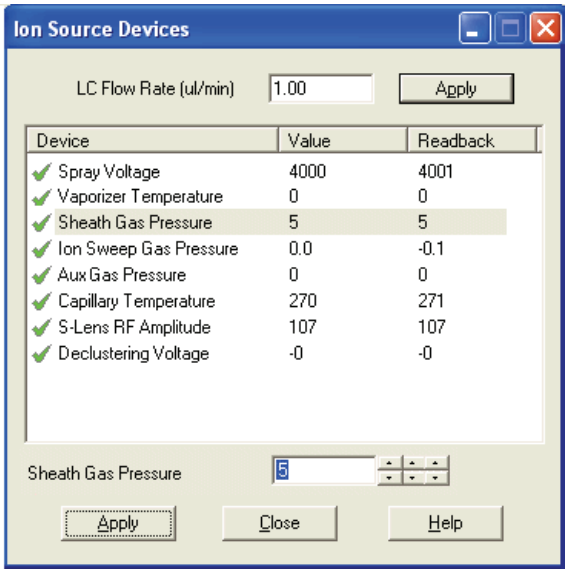
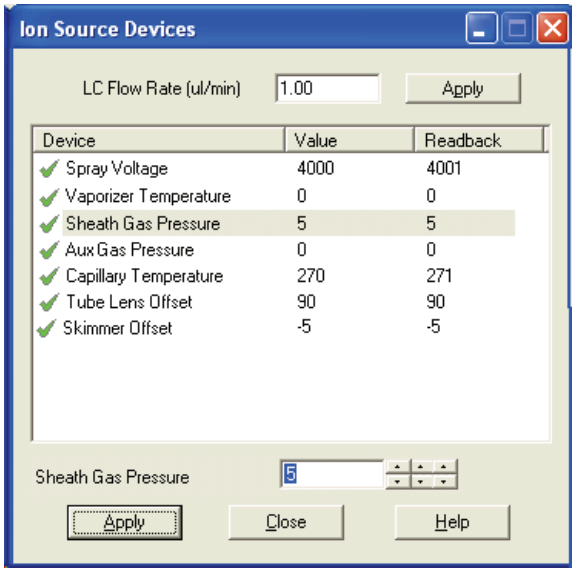


Figure 20. Ion Source Devices dialog box of the TSQ Quantum Ultra, TSQ Quantum Access, and TSQ Quantum Access MAX



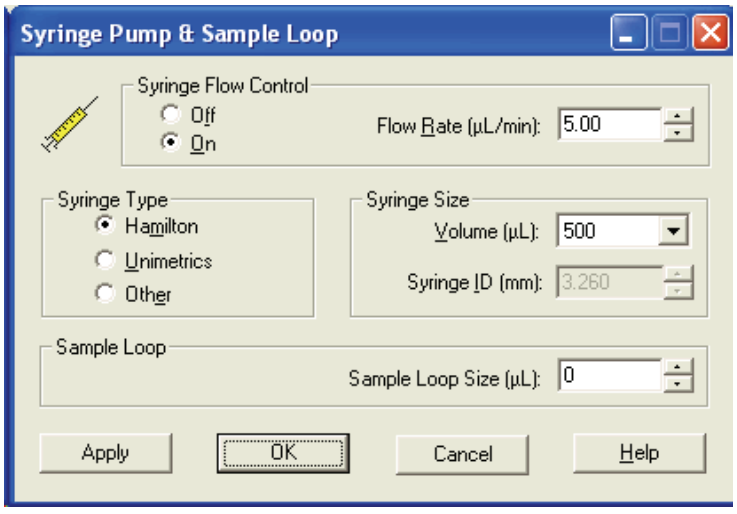
5. Configure the syringe pump to inject the polytyrosine - 1,3,6 tuning and calibration solution and start the syringe pump:

   a. Choose **Setup > Syringe Pump & Sample Loop** to display the Syringe Pump and Sample Loop dialog box. See Figure 21.

   b. In the Syringe Flow Control area, select the **On** option to activate the Flow Rate box.

   c. In the Flow Rate box, enter **2.00** to set a flow rate of 2.00 µL/min. (To achieve the best ion beam intensity and stability, you can adjust the flow rate between 1.00 to 10.00 µL/min.)

Figure 21. Syringe Pump and Sample Loop dialog box



- If you are using either a Unimetrics™ or Hamilton™ syringe, go to step 5d.
- For all other syringe types, go to step 5f.

d. In the Syringe Type area, select the **Unimetrics** or **Hamilton** option, as appropriate.

e. In the Syringe Size area, select **500** (or the size of your syringe) from the Volume list 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 5g.

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

i. In the Syringe Type area, select the **Other** option to specify that you are using a syringe other than a Unimetrics or Hamilton syringe and to activate the Syringe ID box under Syringe Size.

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

iii. In the Syringe ID box, enter the inner diameter of your syringe.

g. To apply these settings and start the syringe pump, click **Apply**. 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.

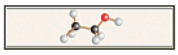
To establish a stable ion beam, go to the next section, "Establishing a Stable Ion Beam."

Establishing a Stable Ion Beam

Before you start the tuning and calibration procedure, you must establish a stable ion beam. The intensity and stability of the ion beam largely depend on the performance of the ion source. To optimize the ion beam intensity and stability, adjust the sheath gas pressure.

❖ To establish a stable ion beam

1. Set the scan parameters to prepare for observing the intensity and stability of the ion beam (see Figure 22):



a. In EZ Tune, click the **Define Scan** button to display the Define Scan dialog box.

b. In the Define Scan dialog box, select **Full Scan** for the Scan Type. The Scan Parameters area is enabled. See Figure 22.

c. For Scan Mode, select **Q1MS** to select the Q1MS scan mode.

d. In the Scan Parameters area under Scan Range, for Entry Mode select the **Center Mass** option. The Center Mass list and Scan Width box are enabled.

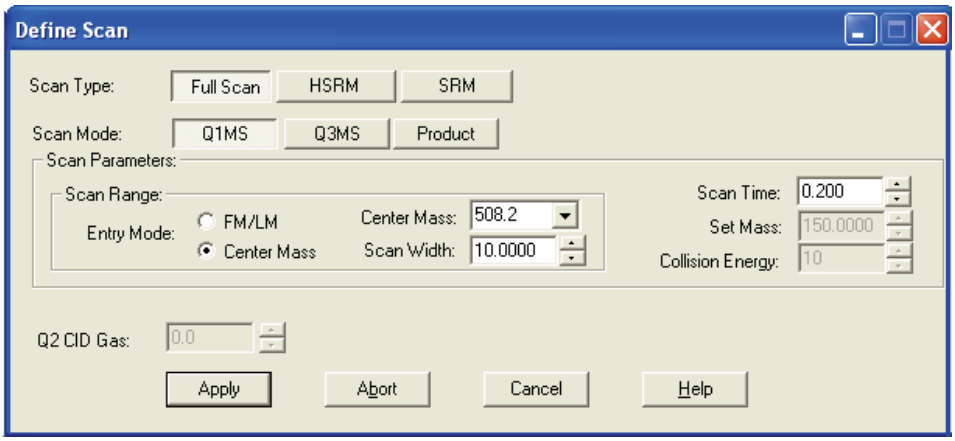
e. In the Center Mass list, select **508.208** to set the center of the scan range to 508.208 u.

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

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

h. To apply these scan parameters, click **Apply**. See Figure 22.

Figure 22. Typical settings for establishing a stable ion current in the Define Scan dialog box



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

Profile 

Centroid 

3. Place the mass spectrometer 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 the **Profile/Centroid** button to change the data type to profile.

Positive Polarity 

Negative Polarity 

4. Place the mass spectrometer in the positive ion polarity mode. 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.

5. Ensure that the other compound dependent devices are set to the values shown in Figure 19 or Figure 20.

6. Determine if you have a stable ion beam:

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

   b. Choose **Display > Zoom > Normalize** so that you can observe the relative intensity of the ion at *m/z* 508.208. See Figure 23.

   Note You can adjust the sheath gas pressure between 0 and 15 psi to establish a stable ion beam. Sheath gas pressure that is too low can cause a loss of signal stability, whereas pressure that is too high can cause a loss of peak intensity.

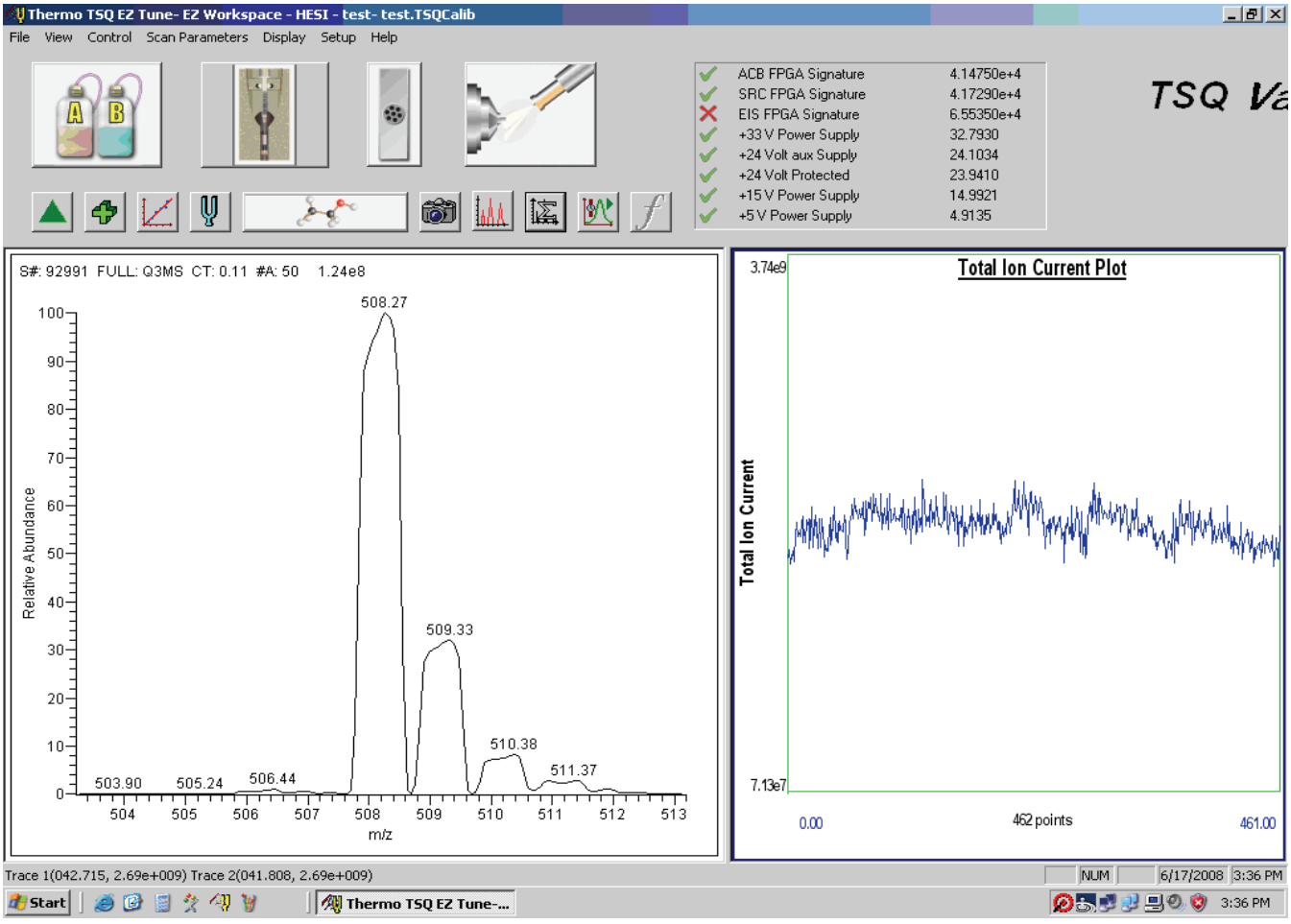
   c. Observe the height of the peak at *m/z* 508.208 in the Spectrum view.

- 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 percent from scan to scan.
- If the top of the peak is unsteady, you must adjust the pressure of the sheath gas to establish a stable ion beam. You can also observe the ion current fluctuation in the Graph view in the lower right corner of the workspace.

Note There is coupling between the spray voltage, the sheath gas pressure, and the sample solution flow rate. Because of this, you might need to vary these three parameters to obtain a stable ion beam.

After you have established a stable ion beam, adjust the scan parameters to observe the other polytyrosine tuning peaks in the Spectrum view. See the description in the next section, "Verifying Operation in the ESI/MS or H-ESI/MS Mode."

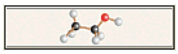
Figure 23. Real-time spectrum and TIC plot for polytyrosine 3, demonstrating a stable ion beam



Verifying Operation in the ESI/MS or H-ESI/MS Mode

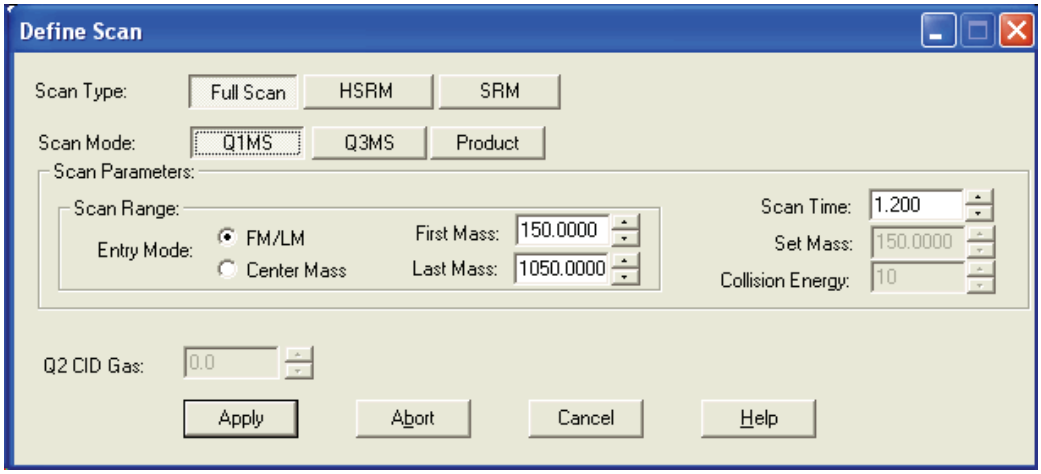
You are now ready to verify the proper operation of the mass spectrometer. To do this, inject the polytyrosine tuning and calibration solution directly into the ESI, H-ESI, or HESI-II source, and monitor the mass spectrum of the solution.

❖ To monitor the mass spectrum of the tuning and calibration solution



1. In EZ Tune, click the **Define Scan** button to display the Define Scan dialog box.
2. In the Define Scan dialog box, set the scan parameters for verifying the operation of the mass spectrometer in Q1:
   a. In the Scan Parameters area under Scan Range, select the **FM/LM** option for Entry Mode. This displays the First Mass and Last Mass boxes. See Figure 24.
   b. To set the low endpoint of the scan range to 150.000 u, in the First Mass box enter **150.000.**
   c. To set the high endpoint of the scan range to 1050.000 u, in the Last Mass box enter **1050.000**.
   d. In the Scan Time box, enter **1.20** to set the scan time to 1.20 s.
   e. To use these scan parameters, click **Apply**.

Figure 24. Typical settings for testing the mass spectrometer operation in the Q1MS scan mode from the Define Scan dialog box



3. Apply spectrum averaging:
   a. Choose **Scan Parameters > Configure Spectrum Averaging** to open the Configure Spectrum Averaging dialog box.
   b. Select **Average** and enter **10** in the box.
   c. Click **Apply** and **OK**.

4. Monitor the tuning and calibration solution in Q1:

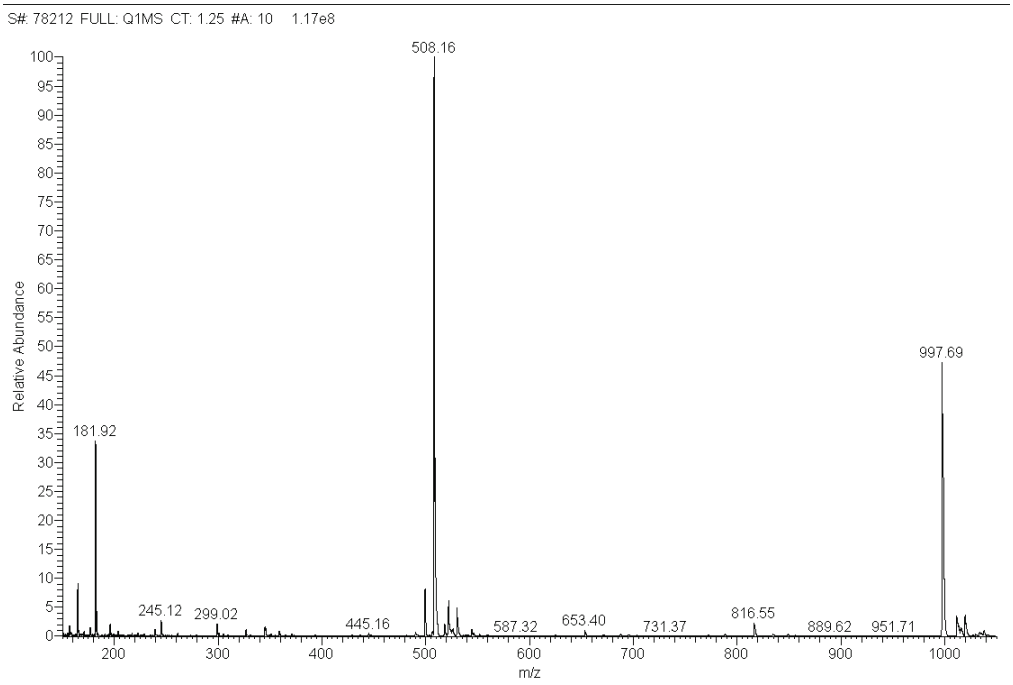
   a. Choose **Display > Zoom > Normalize** to normalize the spectrum.

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

      - Polytyrosine 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 25.

Figure 25. A real-time spectrum of polytyrosine tuning and calibration solution in the Spectrum view



5. As tuning and calibration solution is detected and the readback values fluctuate, determine the answers to 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 10^6 counts for TSQ Quantum Access, TSQ Quantum Access MAX, and TSQ Quantum Ultra, or 10^7 counts (in profile mode) for TSQ Vantage?
   - Is the signal stable, varying by less than about 15 percent from scan to scan?

- Are the peaks for the tuning and calibration solution symmetrical, well resolved, and unsplit?

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

Note You can adjust tuning parameters to establish a good polytyrosine tuning and calibration signal. You can adjust sheath gas pressure between 0 and 15 psi, auxiliary gas flow between 0 and 5 (arbitrary units), and the tuning and calibration solution flow rate between 1 and 15 µL/min.

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

- Adjust the sheath gas pressure or the auxiliary gas flow rate 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 ESI needle.
- Ensure that the entrance of the ion transfer capillary is clean.
- Ensure that the solution entering the probe is free of air bubbles and that the tubing and connectors are free of leaks.

6. Set the scan parameters for verifying the operation of the mass spectrometer in Q3:
   a. In the Define Scan dialog box (Figure 24), for Scan Mode select **Q3MS** to enable Q3 scanning.
   b. Verify that the scan parameters have not changed from those set for Q1.
   c. To apply these scan settings, click **Apply**.
7. Again, observe the values of the normalized ion current signal in the Spectrum view. If the spectrum meets the requirements of step 5 above, then your mass spectrometer is operating properly in the Q3MS mode.

You are now ready to tune and calibrate the mass spectrometer. Keep your TSQ system as it is, and go to the next section, "Tuning and Calibrating Automatically in the ESI/MS or H-ESI/MS Positive Ion Mode."

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

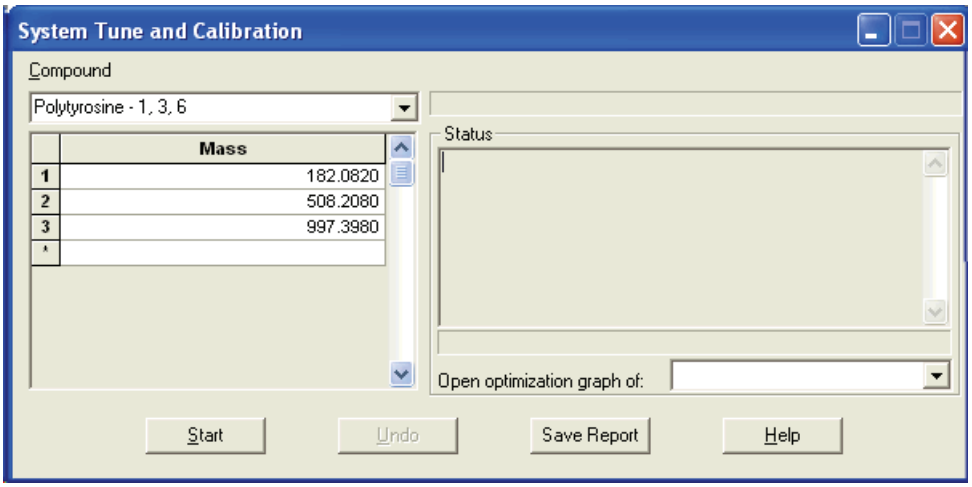
You are now ready to tune and calibrate the mass spectrometer for ESI or H-ESI operation. The automatic tuning and calibration procedure first tunes the instrument with tuning and calibration solution; this establishes a stable spray of solution and ensures that enough ions are detected to perform a 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 ESI/MS or H-ESI positive ion mode

1. In EZ Tune, choose **Setup > System Tune and Calibration** to display the System Tune and Calibration dialog box. See Figure 28.

   Figure 26. System Tune and Calibration dialog box

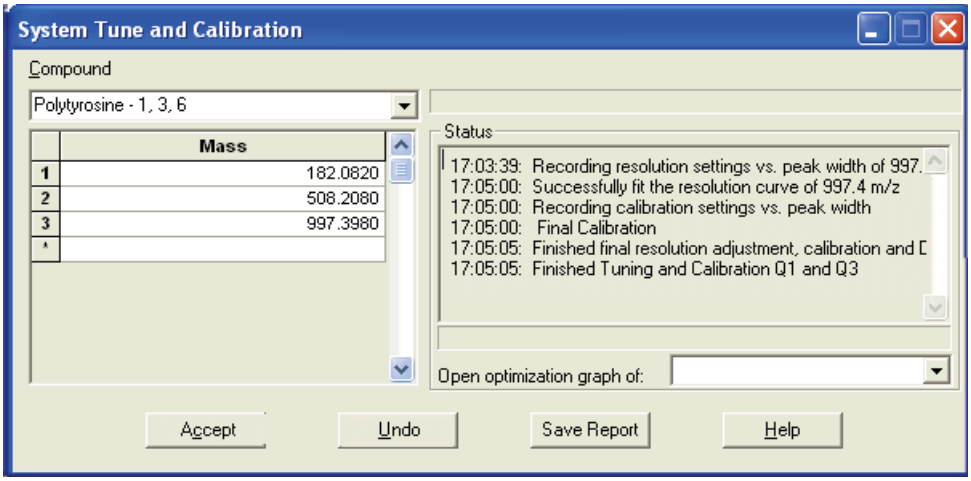


2. In the System Tune and Calibration dialog box, select **Polytyrosine – 1,3,6** from the Compound list. This automatically selects the three positively charged polytyrosine ions that the TSQ system uses for automatic tuning and calibrating (Figure 28).

3. To start the automatic tuning and calibration procedure, click **Start**.

   The Status box displays real-time messages about the system tune and calibration so that you can monitor the progress of each subprocedure. After EZ Tune completes a subprocedure, it reports the result (for example, whether it passed or failed). At the end of the entire procedure, the Status box displays a summary. See Figure 27.

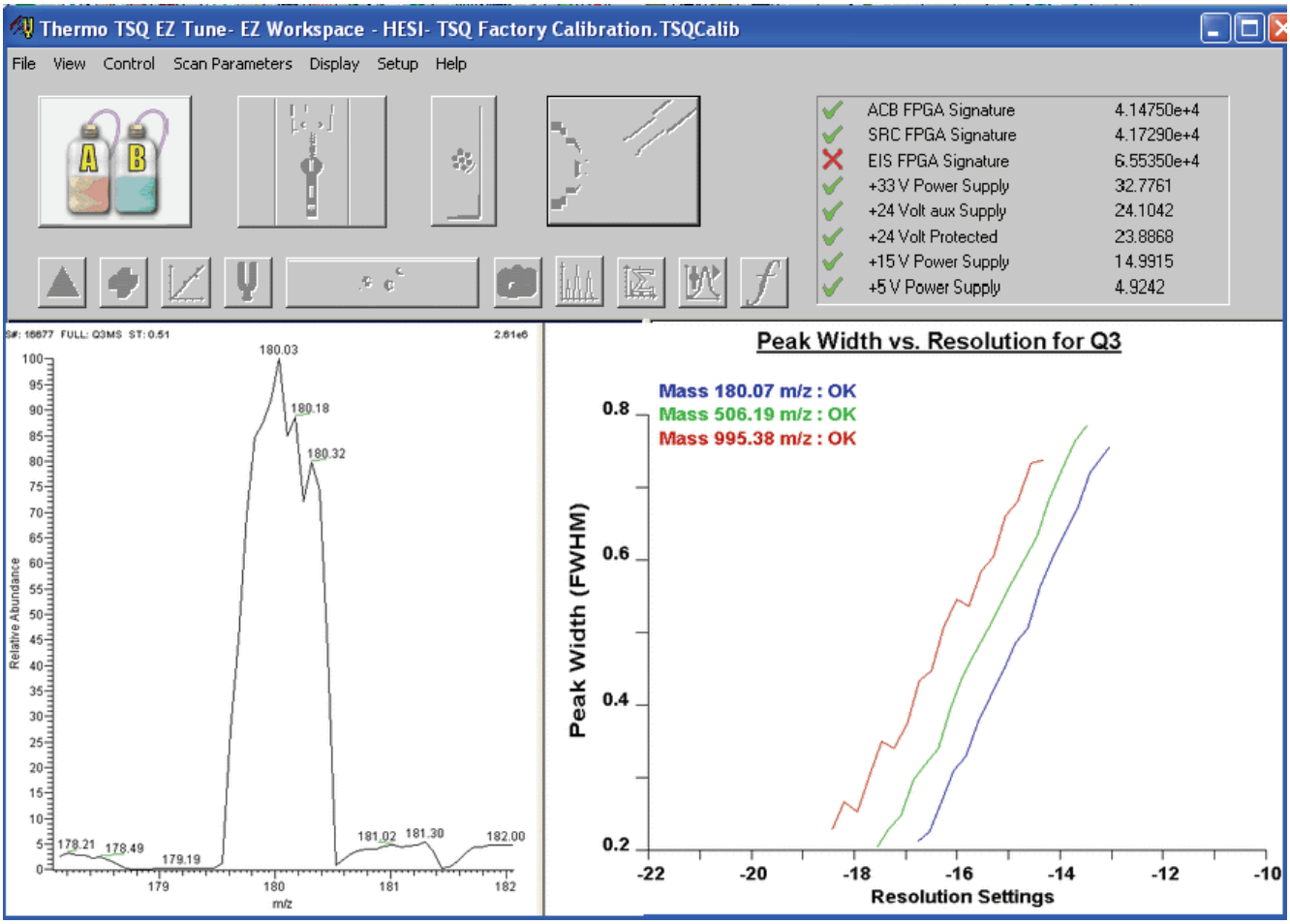
Figure 27. System tune and calibration status



- If errors occur during the automatic tuning and calibration procedure, go to step 4.
- If the automatic tuning and calibration procedure finishes without errors, go to step 5.

4. 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 these steps:
   a. Click **Undo** to restore the prior tuning and calibration settings.
   b. Click **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 3 and restart the tuning and calibration procedure.

Figure 28. EZ Tune during the tuning and calibration procedure



5. To accept the results of the tuning and calibration procedure, click **Accept**.

   After you accept the results of the tuning and calibration procedure, a message box prompts you to copy the positive ion tuning and calibration settings to the negative ion mode, or not.

   - If you have already tuned and calibrated the instrument successfully in the negative ion mode, click **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 **Yes**.

   Note If you intend to perform high sensitivity negative ion mass spectrum analysis, for optimum results, perform a full tune and calibration of the instrument in the negative ion mode. This procedure is found in the next section, "Tuning and Calibrating Automatically in the ESI/MS or H-ESI/MS Negative Ion Mode" on page 41.

- To save the new calibration parameters as the "current" calibration, click **Save Calib**. When you click Save Calibration, the old "current" calibration becomes the new "previous" calibration.

  The TSQ displays the Save As dialog box.

6. Save the Tune Method file:
   a. In the File Name box, enter a name for your Tune Method file, such as **ESI Polytyrosine Tune.TSQTune**.
   b. Click **Save** to save the Tune Method file.

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

Tuning and calibrating in negative polarity mode gives you a couple of options:

- Performing automatic tuning and calibration in the negative ion mode. If you intend to perform high sensitivity analysis in the negative ion mode, go to the next section, "Tuning and Calibrating Automatically in the ESI/MS or H-ESI/MS Negative Ion Mode."
- Copying the positive ion tune file to negative polarity mode. Choose **Scan Parameters > Copy Tune Values** to copy the positive ion tune file to negative polarity mode. This changes the signs of all polarity-dependent settings of the current tune file and maintains the polarity-independent settings. These settings are not preserved unless you save the file.

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

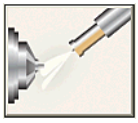
After you have tuned and calibrated the instrument in the positive ion mode, you have the option of tuning and calibrating the instrument in the negative ion mode.

- 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 the rest of this section. Instead, go to "Flushing the System after Tuning and Calibrating" on page 43.
- If you have *not* copied the positive ion tuning and calibration parameters to the negative ion mode, or you intend to perform mass spectrum analysis of high sensitivity negative ions, tune and calibrate the mass spectrometer in the negative ion mode as described in this section.

❖ To tune and calibrate the mass spectrometer automatically in the ESI/MS or H-ESI/MS negative ion mode

Positive polarity 
Negative polarity 

1. On the Control/Scan Mode toolbar, click the **Polarity** button to change the detector to the negative ion polarity mode.



2. Click the **Ion Source Devices** button to display the Ion Source Devices dialog box.
3. Set the spray voltage to -3000 V:
   a. In the Device column, select **Spray Voltage** to highlight it. The label of the Device box changes to Spray Voltage.
   b. In the Spray Voltage box, enter **3000** to set the spray voltage to -3000 V.
4. Change the sheath gas pressure to 15 psi:
   a. In the Device column, select **Sheath Gas Pressure** to highlight it. The label of the Device box changes to Sheath Gas.
   b. In the Sheath Gas box, enter **15** to change the sheath gas pressure to 15 psi.
5. Choose **Setup > System Tune and Calibration** to display the System Tune and Calibration dialog box.
6. From the Compound list, select **Polytyrosine – Neg**. This selects the three negatively charged polytyrosine ions that the TSQ system uses for automatic tuning and calibration.
7. To start the automatic tuning and calibration procedure, click **Start**.

   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.

   - If errors occur during the automatic tuning and calibration procedure, go to step 8.
   - If the automatic tuning and calibration procedure finishes without errors, go to step 9.

8. 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 **Undo** to restore the prior tuning and calibration settings.
   b. Click **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 7 and restart the tuning and calibration procedure.
9. To accept the results of the tuning and calibration procedure, click **Accept**.

   After you accept the results of the tuning and calibration procedure, a message box prompts you to copy the negative ion tuning and calibration settings to the positive ion mode, or not:

   - If you have already tuned and calibrated the instrument successfully in the positive ion mode, click **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 **Yes**.

  Note If you intend to perform mass spectrum analysis of high sensitivity positive ions, for optimum results perform a full tune and calibration of the instrument in the positive ion mode.

- To save the new calibration parameters as the "current" calibration, click **Save Calibration**. When you click Save Calibration, the old "current" calibration becomes the new "previous" calibration.

  The TSQ displays the Save As dialog box.

10. Save the Tune Method file:
    a. In the File Name box, enter a name for your Tune Method file, such as **ESI Polytyrosine Neg Ion Tune.TSQTune**.
    b. Click **Save** to save the Tune Method file.

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

You must clean the system before optimizing the mass spectrometer with your compound. To clean the system, go to the next section, Flushing the System after Tuning and Calibrating.

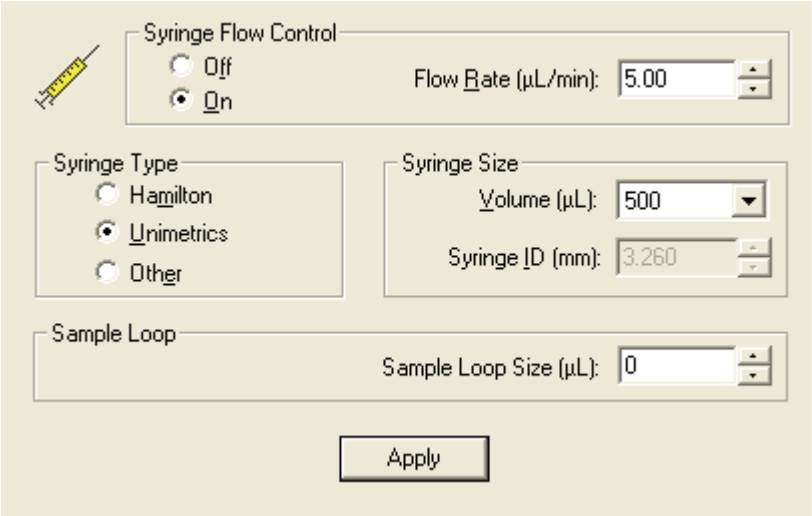
Flushing the System after Tuning and Calibrating

This section describes how to clean your mass spectrometer after performing the tuning and calibration procedure. For best results, clean the mass spectrometer before acquiring data on your analyte of interest.

❖ To clean the mass spectrometer

1. Turn off the flow of liquid from the syringe pump:
   a. In EZ Tune, 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 29.
   b. In the Syringe Flow Control area, select the **Off** option and then click **Apply** to stop the syringe pump.

Figure 29. Pump is on in the Syringe Pump and Sample Loop view



On 

Standby 

2. If necessary, click 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:
   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.
5. Flush the sample transfer line, sample tube, and ESI, H-ESI, or HESI-II probe:

   Note The solvent that you use to flush the syringe, sample transfer line, sample tube, and ESI, H-ESI, or HESI-II 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. 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 ESI, H-ESI, or HESI-II probe with the solution by slowly depressing the syringe plunger.
   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 Chapter 4, "Optimizing the Mass Spectrometer with Your Compound in ESI/MS/MS or H-ESI/MS/MS Mode."

Optimizing the Mass Spectrometer with Your Compound in ESI/MS/MS or H-ESI/MS/MS Mode

This chapter provides information on fine tuning the mass spectrometer in the ESI/MS/MS or 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 result from automatic tuning are useful for a wide range of applications. You can use them often without further tuning of your mass spectrometer. For certain applications, however, you might need to optimize several mass spectrometer parameters. For instance, the following parameters affect ESI or H-ESI performance and signal quality:

- Spray voltage
- Sheath gas pressure
- Auxiliary gas flow rate
- Capillary (ion transfer tube) temperature
- Tube lens offset voltage (TSQ Quantum Access, TSQ Quantum Access MAX, and TSQ Quantum Ultra)
- S-lens rf amplitude (TSQ Vantage)

For H-ESI, in addition to the above parameters, the vaporizer temperature is important.

Note The TSQ can perform either a standard optimization or a custom optimization. During a standard optimization, the TSQ optimizes the collision energy, the tube lens offset voltage (TSQ Quantum Access, TSQ Quantum Access MAX, and TSQ Quantum Ultra) or the S-lens rf amplitude (TSQ Vantage), and the voltages applied to the ion optics until the ion transmission of your analyte is maximized. For a custom optimization, you can select which of the above settings the TSQ optimizes.

The optimum settings for these parameters depend on the solvent flow rate and on the structure of your analyte. In general, you must fine tune the mass spectrometer parameters whenever you change the solvent flow rate conditions of your particular application.

The capillary (ion transfer tube) is heated to maximize the ion transmission to the mass spectrometer. You set the capillary temperature so that it is proportional to the flow rate of your solution. See the guidelines in Table 2 (H-ESI) or Table 4 (ESI) on page 7.

Note Ensure that you have performed the TSQ 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, follow the procedure in Chapter 3, "Tuning and Calibrating the Mass Spectrometer."

Optimizing the mass spectrometer for your compound in the ESI/MS/MS or H-ESI/MS/MS mode requires the following actions described in this chapter:

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

Contents

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

To introduce your compound by auto loop injection, follow these procedures. The plumbing connections for ESI/MS and H-ESI/MS sample introduction from the syringe pump into the solvent flow from an LC are shown in Figure 30 and Figure 31.

Figure 30. ESI/MS plumbing connections for sample introduction by auto loop injection into the solvent flow from an LC

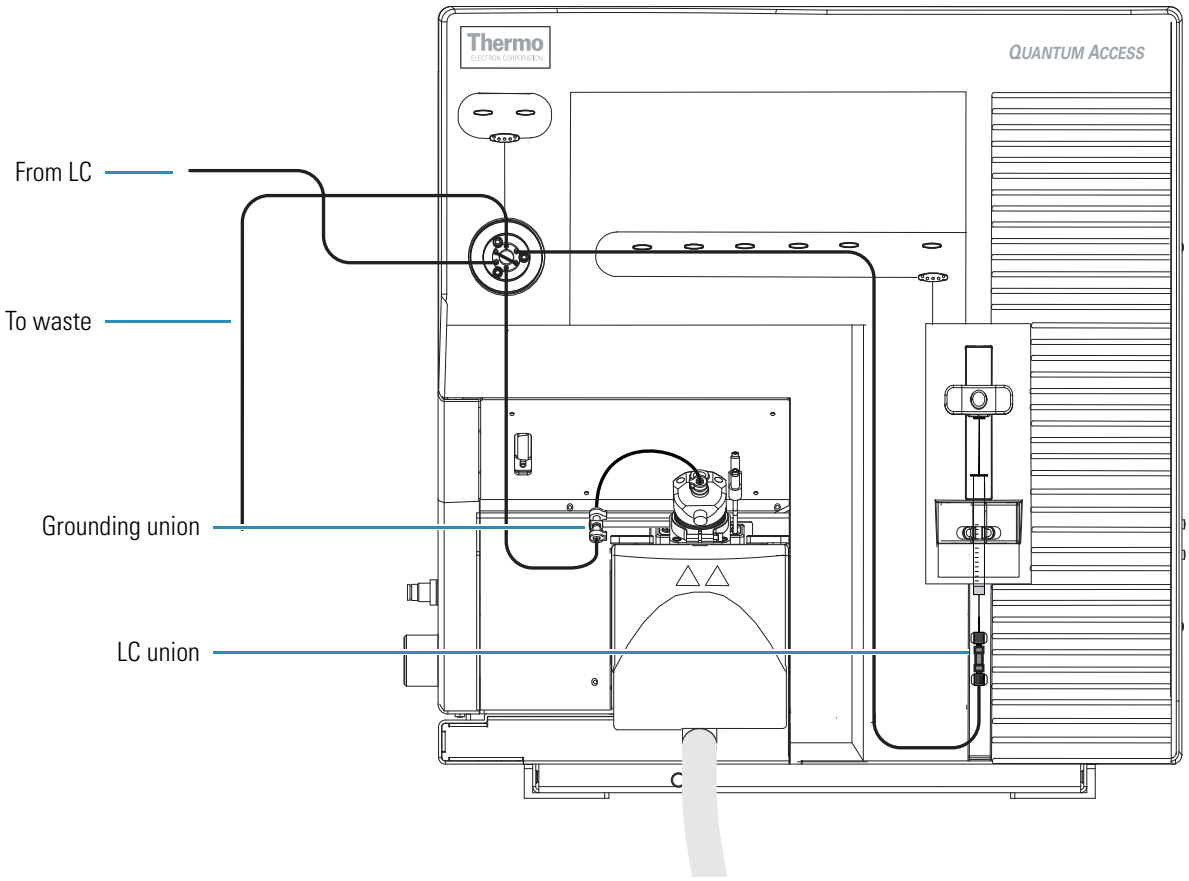
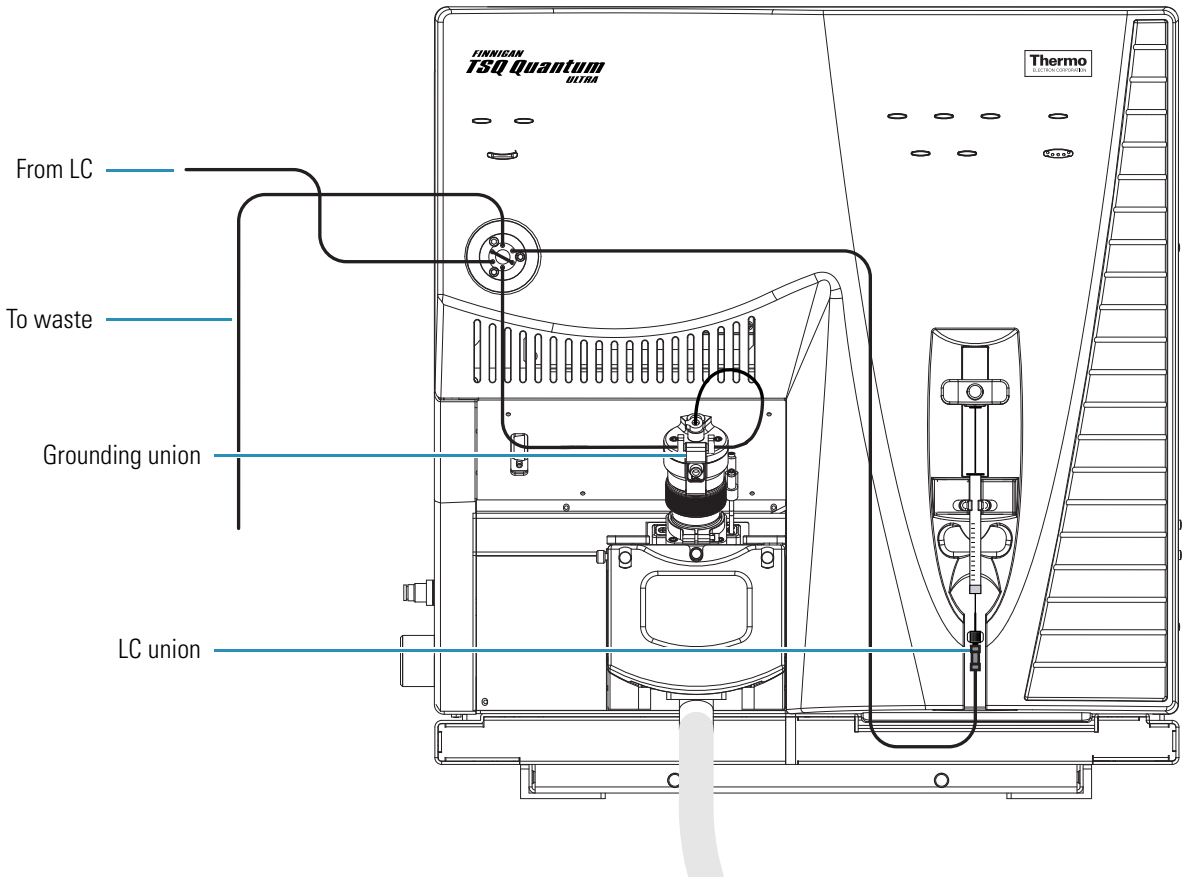


Figure 31. H-ESI/MS plumbing connections for sample introduction by auto loop injection into the solvent flow from an LC



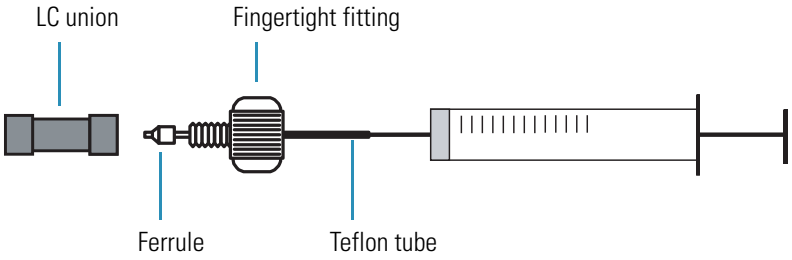
Note You can use the reserpine sample solution described in "Solution Formulations" on page 117, or you can use your compound of interest.

The following procedures assume that you are familiar with your TSQ instrument and with Tune Master. If you need additional guidance, refer to the TSQ Help, *TSQ Series Getting Connected Guide*, or the *TSQ Series Hardware Manual.*

❖ **To make the plumbing connections for sample introduction from the syringe pump into the solvent flow from an LC**

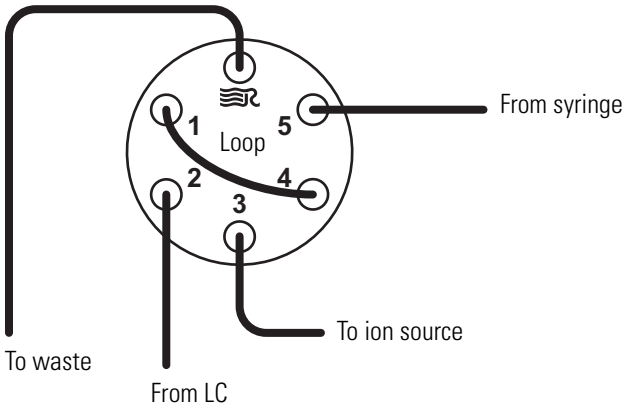
1. Remove the syringe from the syringe pump holder:
   a. Lift the handle off the syringe while depressing the black release button on the syringe pump handle.
   b. Remove the syringe.
   c. Remove the tip of the syringe needle from the end of the Teflon tube on the syringe adapter assembly. See Figure 32.

Figure 32. Syringe and syringe adapter assembly



2. Remove the sample transfer line installed between the syringe adapter assembly and the grounding union on the ion source.
3. Install a sample transfer line between the syringe adapter assembly and the divert/inject valve:
   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 33.

Figure 33. Plumbing for auto loop injection of the divert/inject valve



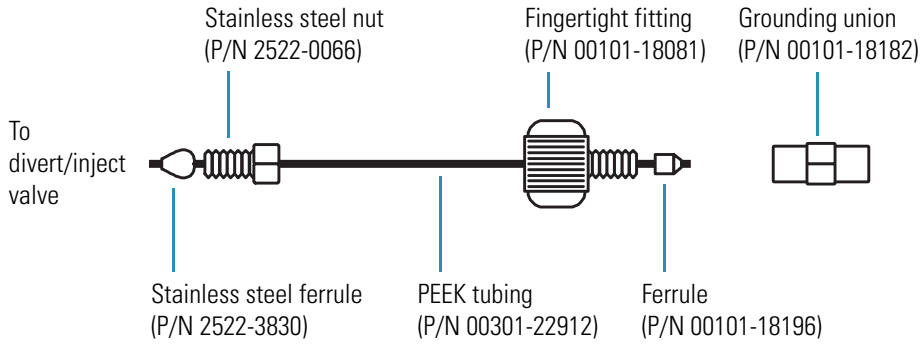
Note To minimize the possibility of cross-contamination, for your tuning and calibration solution, use a different syringe and a different sample transfer line from the ones used for your samples and compound optimization solution.

4. Load a clean, 500 µL Unimetrics syringe with 420 µL of the 2 pg/µL (TSQ Quantum Access and TSQ Quantum Access MAX), 200 fg/µL (TSQ Quantum Ultra, TSQ Quantum Ultra AM, TSQ Quantum Ultra EMR), or 100 fg/µL (TSQ Vantage, TSQ Vantage AM, and TSQ Vantage EMR) reserpine sample solution, or your analyte. (For the procedure to prepare the reserpine solution, see "Reserpine Solutions" on page 122.)

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

5. 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 32).
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:
   a. Gather the necessary fittings for installing a sample transfer line (see Figure 34).
   b. Connect an appropriate length of tubing fitted with a nut and a ferrule to port 3 of the divert/inject valve (Figure 33).
   c. Connect the other end of the tubing with a fingertight fitting and a ferrule to the grounding union on the ion source (Figure 30, Figure 31, and Figure 34).

Figure 34. Sample transfer line, installed between the divert/inject valve and the grounding union



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:
    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:
    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 section, "Setting Up to Optimize in ESI/MS/MS or H-ESI/MS/MS Mode with Your Compound."

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

❖ **To set up the mass spectrometer to optimize the compound dependent devices for your compound in ESI/MS/MS or H-ESI/MS/MS mode**

1. From the Windows taskbar, choose **Start > Programs > Thermo Instruments > TSQ > TSQ Tune** to open EZ Tune.

On 

Standby 

Positive Polarity 

Negative Polarity 

2. In EZ Tune, click the **On/Standby** button on the Control/Scan Mode toolbar to turn on the mass spectrometer.
3. If necessary, change the ion polarity mode to positive ion polarity. On the Control/Scan Mode toolbar, click the **Polarity** button to change the ion polarity mode of the mass spectrometer.
   - If you want to optimize the currently displayed Tune Method, go to step 5.
   - If you want to optimize a different Tune Method from the one currently displayed, first open the desired Tune Method as described in step 4.
4. Open the Tune Method file that stores reserpine tune settings, or the settings for your analyte:
   a. On the File/Display toolbar, click the **Open File** button,  , to display the Open dialog box.
   b. Confirm that the folder C:\Xcalibur\methods is displayed. Select a Tune Method file
   c. Click **Open** to open the file. Tune Master downloads the Tune Method parameters to the mass spectrometer.
5. Choose **Display > Compound Dependent Devices** to display the Compound Dependent Devices dialog box.
6. Set the values for the compound dependent devices:
   a. Ensure that **Spray Voltage** is selected (highlighted) in the Device Display table.
   b. In the Optimize Compound Dependent Devices view, enter **4000** in the Spray Voltage box to change the spray voltage to 4000 V.
   c. Set the pressure of the sheath gas:
      i. In the Device Display table, select **Sheath Gas Pressure** to highlight it.
      ii. In the Sheath Gas Pressure box, enter **30** to set the sheath gas pressure to 30 units.
   d. Set the flow rate of the auxiliary gas:
      i. Select **Aux Valve Flow** in the Device Display table to highlight it.
      ii. In the Aux Valve Flow box, enter **10** to set the auxiliary gas flow rate to 10 units.

e. Set the temperature of the capillary (ion transfer tube):

   i. Select **Capillary Temperature** in the Device Display table to highlight it.

   ii. In the Capillary Temperature box, enter **350** to set the capillary temperature to 350 °C.

f. Set the ion source fragmentation (CID) collision energy:

   i. For the TSQ Quantum Access, TSQ Quantum Access MAX, or the TSQ Quantum Ultra, select **Skimmer Offset** in the Device Display table to highlight it.

   ii. In the Skimmer Offset box, enter **0** to set the collision energy to 0 V.

   iii. For the TSQ Vantage, select **Declustering Voltage** in the Device Display table to highlight it.

   iv. In the Declustering Voltage box, enter **0** to set the collision energy to 0 V.

g. Set the collision pressure:

   i. Select **Collision Pressure** in the Device Display table to highlight it.

   ii. In the Collision Pressure box, enter **1.5** to set the collision pressure to 1.5 mTorr.

h. Set the collision energy:

   i. Select **Collision Energy** in the Device Display table to highlight it.

   ii. In the Collision Energy box, enter **-38** to set the collision energy to -38 eV.

i. Click **Apply** to apply the settings.

Ensure that the readbacks in the Device Display table are approximately equal to the set values. (You might need to wait a few minutes for the capillary temperature to stabilize at the set value.)

7. Configure the Syringe Pump to automatically inject the reserpine sample solution into the sample loop:

   a. Choose **Setup > Syringe Pump & Sample Loop** to display the Syringe Pump and Sample Loop dialog box. See Figure 35.

   b. Select the **Off** option in the Syringe Flow Control area to turn off the syringe pump.

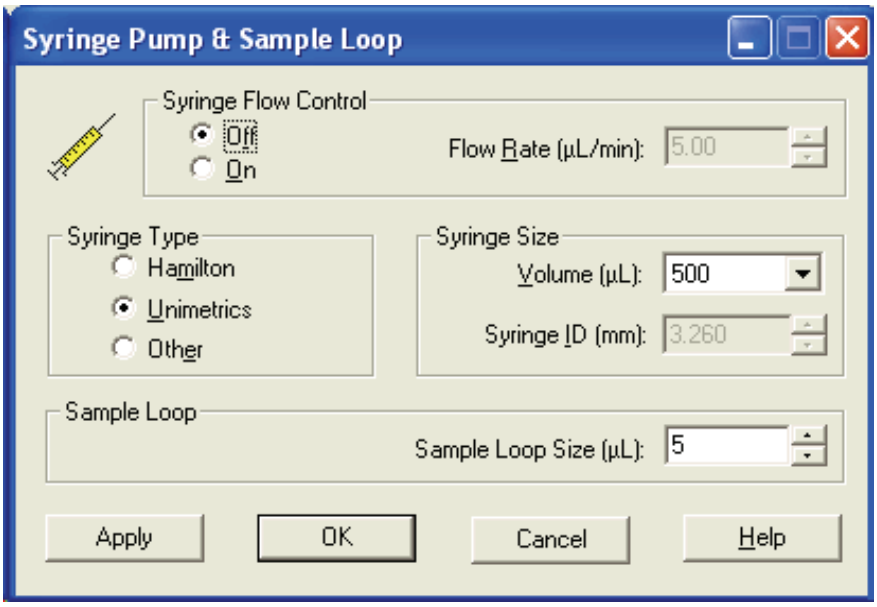
      - If you are using a Unimetrics or Hamilton syringe, go to step 7c.
      - If you are *not* using a Unimetrics or Hamilton syringe, go to step 7e.

   c. In the Syringe Type area, select the **Unimetrics** (or **Hamilton**) option, as appropriate.

   d. In the Syringe Size area, select **500** (or the size of your syringe) from the Volume list 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 7f.

Figure 35. Auto loop injection setup in the Syringe Pump and Sample Loop dialog box



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

   i. In the Syringe Type area, select the **Other** option. This specifies that you are using a syringe other than Unimetrics or Hamilton syringe and enables the Syringe ID box.

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

   iii. In the Syringe ID box, enter the inner diameter of your syringe.

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

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

8. Start the flow of solvent:

   a. Choose **Setup > Inlet Direct Control** button to display the Inlet Direct Control dialog box. See Figure 36.

   Note The following procedure assumes that isopropyl alcohol and LCMS-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:

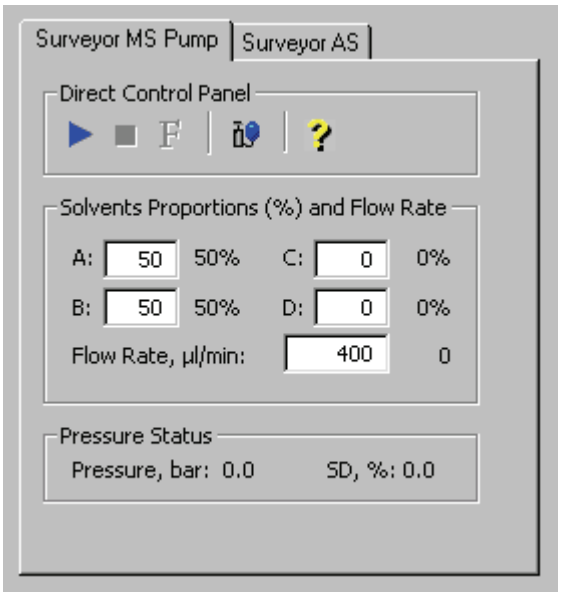
      i. In the Inlet Direct Control view, in the Solvents Proportions (%) and Flow Rate area, type **50** in the box labeled *A* to specify a delivery proportion of 50% solvent A.

      ii. In the box labeled *B,* type **50** to specify a delivery proportion of 50% solvent B.

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

c. To start the Surveyor MS pump, in the Direct Control Panel area, click ▶ (Start).

Figure 36. Pump is off in the Inlet Direct Control dialog box



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 ESI/MS/MS or H-ESI/MS/MS mode. Go to the next section, "Optimizing in ESI/MS/MS or H-ESI/MS/MS Mode Automatically with Your Compound."

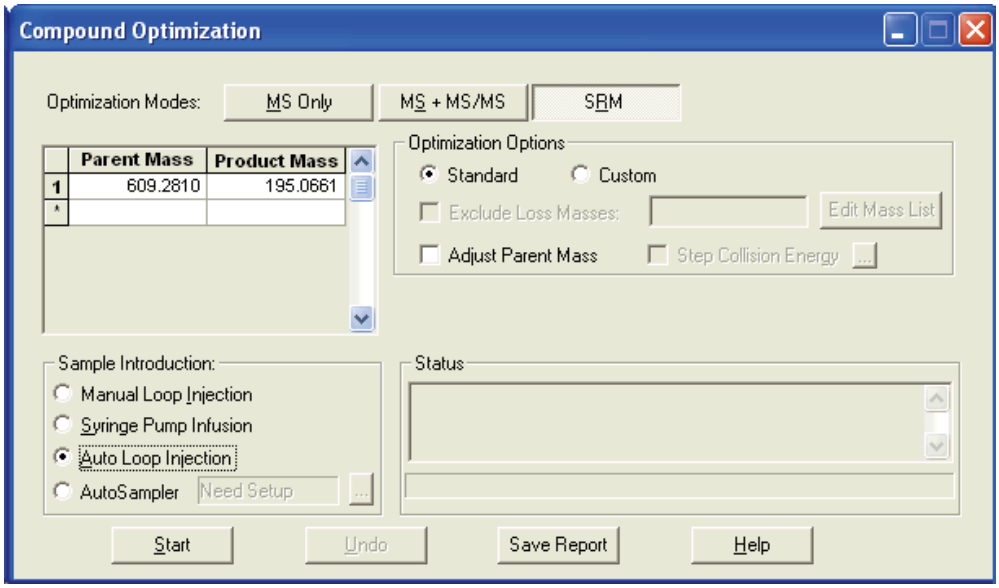
Optimizing in ESI/MS/MS or H-ESI/MS/MS Mode Automatically with Your Compound

To maximize the ion transmission of your compound, optimize your mass spectrometer. Performing optimization fine tunes compound dependent parameters such as spray voltage, capillary temperature, and tube lens offset. Thermo Fisher Scientific recommends that you optimize the mass spectrometer only after you have successfully tuned and calibrated the instrument.

❖ **To automatically optimize the mass spectrometer in the ESI/MS/MS or H-ESI/MS/MS mode for the reserpine transition from *m/z* 609.281 to *m/z* 195.066**

1. From the Windows taskbar, choose **Start > Programs > Thermo Instruments > TSQ > TSQ Tune** to open EZ Tune.
2. Choose **Setup > Compound Optimization** button to display the Compound Optimization dialog box. See Figure 37.

Figure 37. Compound Optimization dialog box showing the settings for reserpine optimization



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

   a. For Optimization Modes, select **SRM**. This enables you to optimize a selected reaction.

   b. In the Optimization Options area, select the **Standard** option to tune the default selection of devices. (In this configuration, the tube lens offset voltage (TSQ Quantum Access, TSQ Quantum Access MAX, and TSQ Quantum Ultra) or the S-lens rf amplitude (TSQ Vantage), and the collision energy are the default compound sensitive settings that are optimized.)

   c. In the Optimization table, in the Parent Mass column, type **609.281** to set the parent mass of the SRM reaction to the ion at *m/z* 609.281.

   d. In the Product Mass column, type **195.066** to set the product mass of the SRM reaction to the ion at *m/z* 195.066.

   Note You must select the inlet type option 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 Sample Introduction area, select the **Auto Loop Injection** option to have the TSQ system automatically inject the optimization solution.

4. Click **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 you receive this message, reload the syringe and click **OK** to continue the optimization.

When the compound optimization completes successfully, the message to finish compound optimization appears in the Status box. See Figure 38.

- If the compound optimization procedure finishes without errors, and the breakdown curve of the *m/z* 609.281 ion is Gaussian-shaped (as in Figure 39) or is a smooth, positive-sloped curve, go to step 6.
- If errors occur during the compound optimization procedure; or if the breakdown curve of the *m/z* 609.281 ion oscillates, contains multiple peaks, or is excessively noisy, go to step 5.

Figure 38. Successful completion of compound optimization as shown in the Status box of the Compound Optimization view

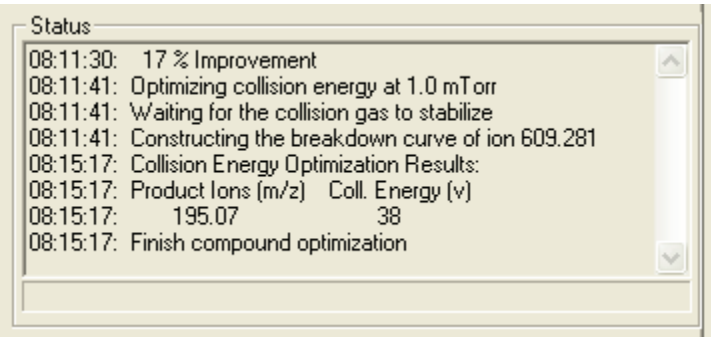
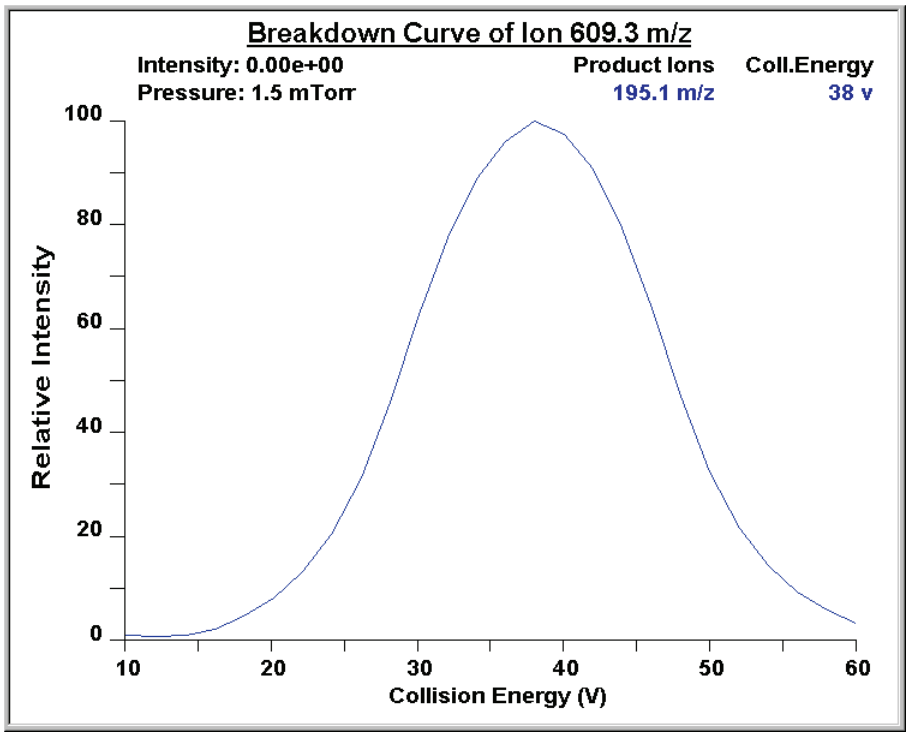


Figure 39. Breakdown curve of reserpine showing the relative intensity of the product ion at *m/z* 195.066 as a function of collision energy



5. If errors occurred during the compound optimization procedure, restore the previous mass spectrometer compound sensitive device settings as follows:
   a. Click **Undo** to restore the prior device settings.
   b. Click **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 4 of this procedure and restart the compound optimization procedure.
6. Click **Accept** to accept the results of the compound optimization.

> **Note** If any of the ion source parameters have been changed from their initial settings, save the Tune Method while the mass spectrometer is on or else the settings will be lost.

7. Save the Tune Method file as follows:
   a. Click **Save Tune As** in the Compound Optimization view to open the Save As dialog box.
   b. In the File Name box, enter a file name (such as **ESI_reserpine.TSQTune**, or the name of your compound) for your Tune Method file.
   c. Click **Save** to save the Tune Method file.

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

Go to Chapter 5, "Acquiring ESI/SRM or H-ESI/SRM Data."

Acquiring ESI/SRM or H-ESI/SRM Data

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

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

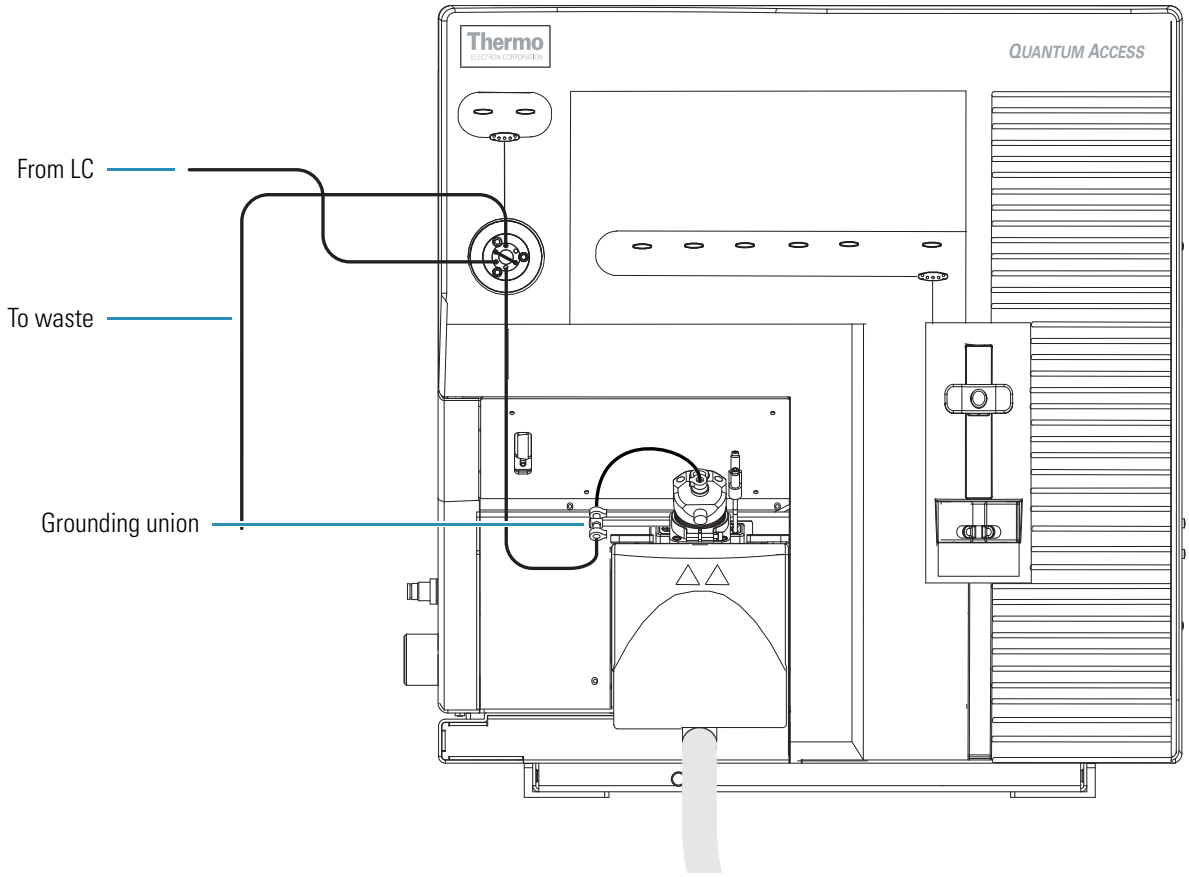
This chapter provides information on acquiring sample data using Tune Master in the ESI/SRM or H-ESI/SRM mode. This experiment uses reserpine, but you can follow the same procedure with your analyte of interest.

Contents

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

Follow the next procedure to introduce sample by manual loop injection into the solvent flow from an LC. The plumbing connections for ESI sample introduction by manual loop injection are shown in Figure 40. (For H-ESI, the grounding union resides in the grounding union holder on the H-ESI or HESI-II probe.)

Figure 40. ESI/MS plumbing connections for sample introduction by manual loop injection into the solvent flow from an LC



❖ **To make the plumbing connections for manual loop injection**

1. Open Tune Master if it is not already open:
   a. Right-click on **Start** and choose **Explore**.
   b. Browse to C:\Thermo\Instruments\TSQ\System\Programs.
   c. Double-click on **TSQTune**.
2. Stop the flow of solvent to the ESI, H-ESI, or HESI-II source:

   a. In Tune Master, on the Control/Scan Mode toolbar, click the **AS/LC Direct Control** button to display the Inlet Direct Control view in the top right corner of the workspace.
   b. In the Direct Control Panel area, click  (Stop) to stop the flow of solvent.

On 

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

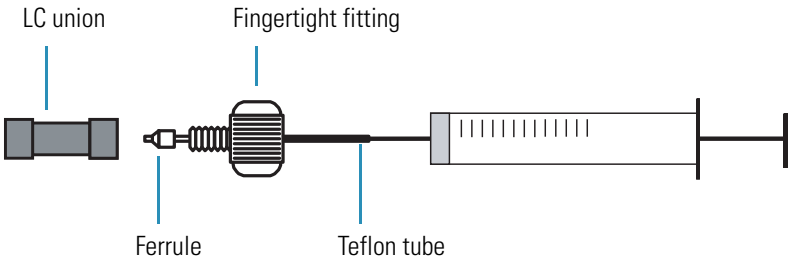
Standby 

4. Remove the syringe from the syringe pump holder:
   a. Lift the handle off the syringe while depressing the black release button on the syringe pump handle.

b. Remove the syringe.

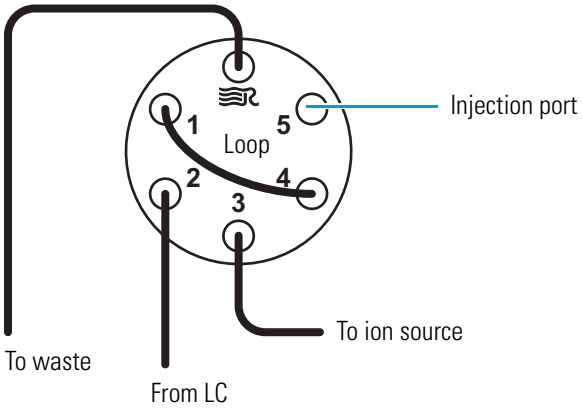
c. Remove the tip of the syringe needle from the end of the Teflon tube on the syringe adapter assembly. See Figure 41.

Figure 41. Syringe and syringe adapter assembly



5. 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 42.

Figure 42. Plumbing for manual loop injection in the divert/inject valve



6. Install the needle port fitting (P/N 00110-22030) into the divert/inject valve:

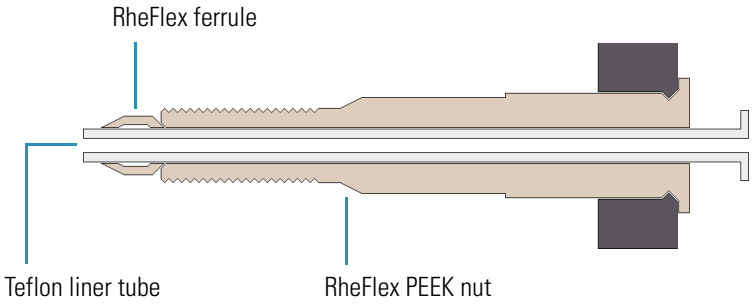
   a. Insert the liner tube, RheFlex™ ferrule, and the threaded portion of the RheFlex nut (see Figure 43) into port 5 of the divert/inject valve.

   b. Carefully tighten the nut with your fingers.

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

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

Figure 43. Needle port fitting



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

❖ To acquire a file of reserpine data in the SRM scan mode

Note Tune Master automatically saves the data you acquire to your hard drive.

1. Open Tune Master if it is not already open:
   a. Right-click on **Start** and choose **Explore**.
   b. Browse to C:\Thermo Instruments\TSQ\System\Programs.
   c. Double-click on **TSQTune**.

On 

Standby 

2. In Tune Master, click 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 4.
   - If you want to acquire data with a Tune Method that is different from the one currently displayed, first 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:
   a. On the File/Display toolbar, click the **Open File** button,  , to display the Open dialog box.
   b. Confirm that the folder *C:\Xcalibur\methods* is displayed. Select the file **ESI_reserpine.TSQTune** (or your Tune Method).
   c. Click **Open** to open the file. Tune Master downloads the Tune Method settings to the mass spectrometer.
4. Start the flow of solvent:

   a. On the Control/Scan Mode toolbar, click the **AS/LC Direct Control** button to display the Inlet Direct Control view in the top right corner of the workspace. See Figure 44.

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

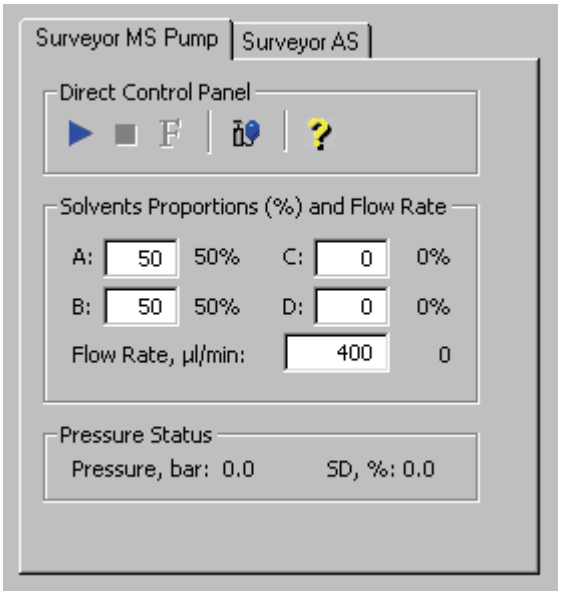
b. Set up the Surveyor MS Pump to deliver a solution of 50:50 isopropyl alcohol/water, at 400 µL/min:

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

ii. In the box labeled *B,* type **50** to specify a delivery proportion of 50% solvent B.

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

Figure 44. Inlet Direct Control view, showing pump is off



c. In the Direct Control Panel area, click ▶ (Start) to start the Surveyor MS pump.

5. On the Control/Scan Mode toolbar, click the **Instrument Method Development Workspace** button to open the Instrument Method Development Workspace. See Figure 45.

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

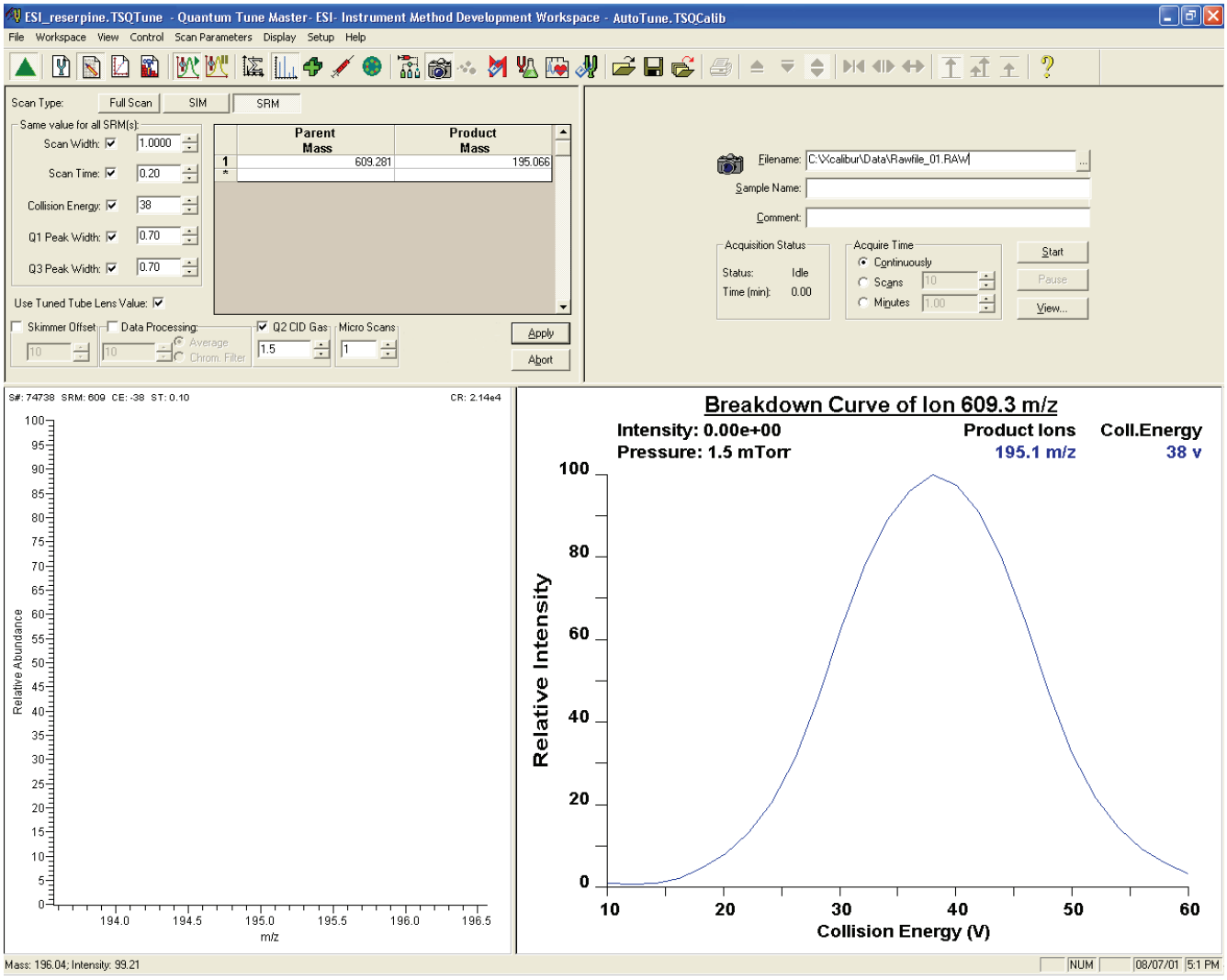
6. Set any required scan parameters for acquiring SRM data:

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

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

-Or-
Enter the parent and product masses of your analyte.

Figure 45. Instrument Method Development Workspace



Note In the Define Scan view, use the Same value for all SRM(s) area 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 in the adjacent box.

c. In the Same value for all SRM(s) area, ensure that all the global parameter check boxes are selected, and verify or enter the following values into the appropriate boxes:

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

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

iii. Verify that the collision energy in the Collision Energy box is approximately equal to 38, the value 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 box, enter **0.70** to set the peak width to 0.70 u.

v. In the Q3 Peak Width 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 **Skimmer Offset** check box is cleared.

f. For the data acquisition, specify the use of a 3 s chromatography filter:

i. Select the **Data Processing** check box to activate the data processing box and options.

ii. Select the **Chrom. Filter** option to specify the use of a chromatography filter.

iii. In the **Data Processing** box, enter **3** to designate a 3 s chromatography filter.

g. Set the collision cell gas settings:

i. In the Q2 CID Gas box, select the **Q2 CID Gas** check box to specify the use of collision gas.

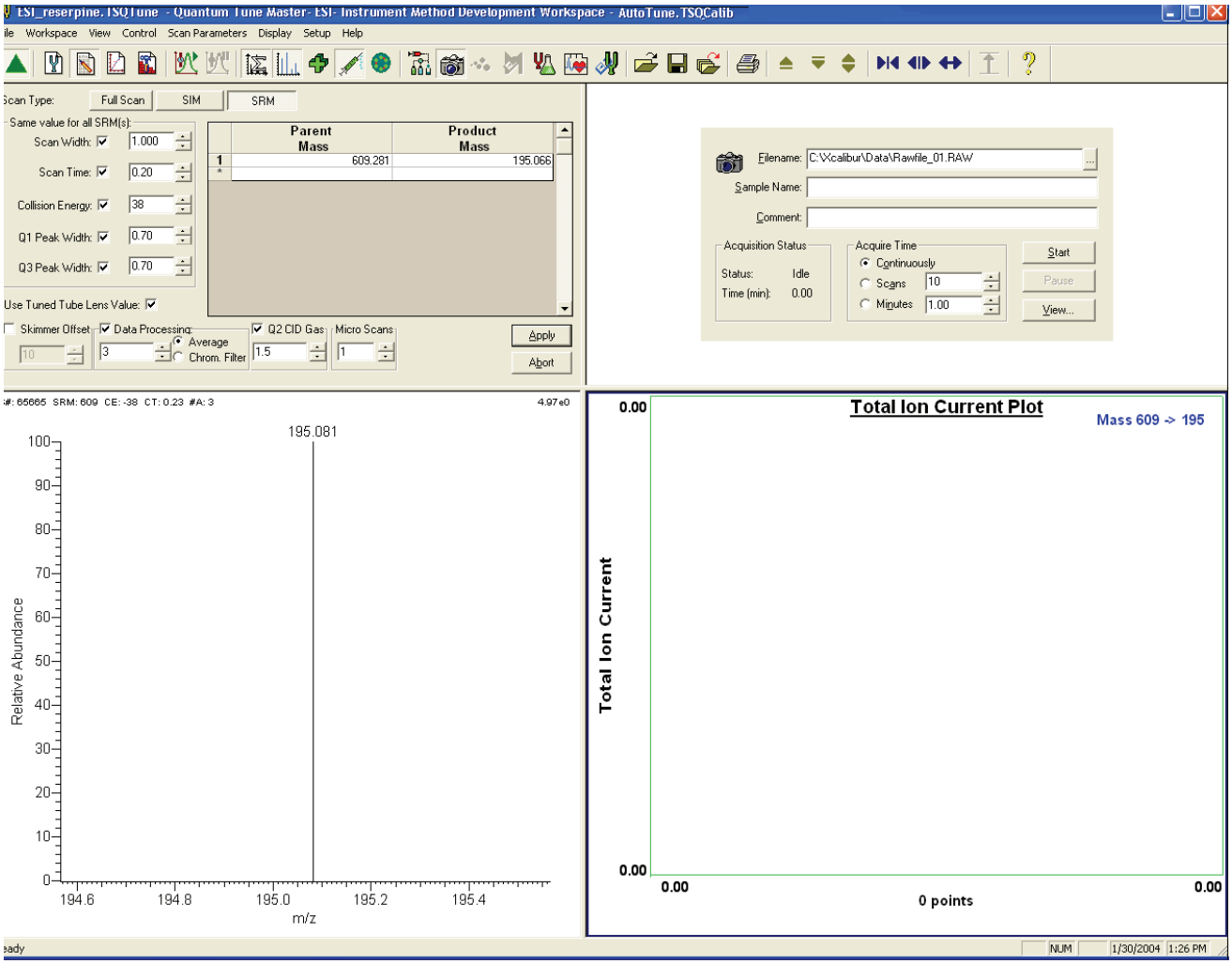
ii. In the Q2 CID Gas box, enter **1.5** to set the collision cell gas pressure to 1.5 mTorr.

h. Confirm that Micro Scans is set to **1**.

7. Click **Apply** to apply the scan parameters to the mass spectrometer.

8. On the Control/Scan Mode toolbar, click the **Display TIC** button to begin recording the total ion current in the Graph view in the lower right corner of the workspace. See Figure 46.

Figure 46. SRM scan type in the Instrument Method Development Workspace



9. Specify the acquisition parameters:

   a. In the Acquire Data view in the top right corner of the workspace, in the Filename box, enter **C:\Xcalibur\Data\reserpine_01.raw** to specify a path and file name. (You can use the browse button, ..., to select a different file folder.)

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

   c. In the Comment box, type a comment about your experiment. For example, type **SRM, 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 area, select the **Continuously** option to specify that data be continuously acquired until you stop the acquisition.

Load/ Detector 
Inject/ Waste 

10. 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 the **Divert/Inject Valve** button to change it to the Load position.
11. In the Acquire Data view, click **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, for example:

    C:\Xcalibur\Data\reserpine_010502092159.raw

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

12. Fill the sample loop with reserpine solution or your analyte:
    a. Ensure that the syringe is loaded with 420 µL of the 2 pg/µL (TSQ Quantum Access or TSQ Quantum Access MAX), 200 fg/µL (TSQ Quantum Ultra, TSQ Quantum Ultra AM, or TSQ Quantum Ultra EMR), or 100 fg/µL (TSQ Vantage, TSQ Vantage AM, or TSQ Vantage EMR) reserpine solution. For the procedure for preparing the reserpine solution, see Appendix C, "Solution Formulations."

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

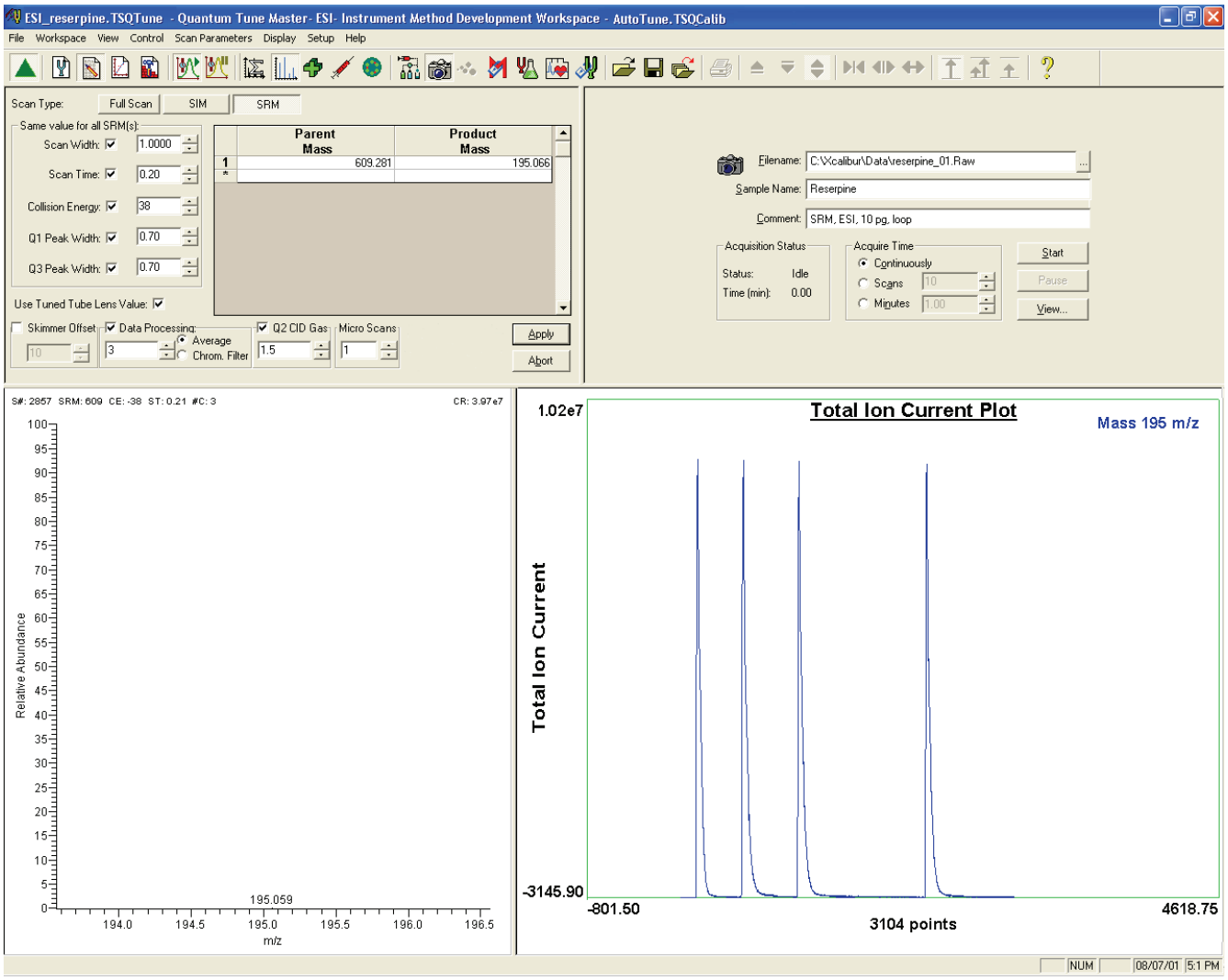
    b. Carefully insert the tip of the syringe needle into the end of the Teflon liner tube on the needle port.
    c. Overfill the sample loop with reserpine solution from the syringe.
13. To inject the reserpine solution into the LC solvent flow, press the blue Divert/Inject Valve button on the front panel of the TSQ Series mass spectrometer.
14. Observe the reserpine product peak at *m/z* 195.066, or that of your analyte of interest, in the Spectrum view.
15. Repeat the following sequence several times to obtain consecutive loop injections of reserpine in the SRM scan mode. Wait approximately 1 minute between injections.
    a. Press the blue Divert/Inject Valve button on the TSQ mass spectrometer to return the Divert/Inject valve to the Load position. Overfill the loop with the 2 pg/µL (TSQ Quantum Access or TSQ Quantum Access MAX), 200 fg/µL (TSQ Quantum Ultra, TSQ Quantum Ultra AM, or TSQ Quantum Ultra EMR), or 100 fg/µL (TSQ Vantage, TSQ Vantage AM, or TSQ Vantage EMR) 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 minute before the next injection.
    d. Repeat steps 15a through 15c several times.

Note You can optimize a setting by varying the setting and repeating step 14.

16. To end the data acquisition, click **Stop** in the Acquire Data dialog box. See Figure 47.

A .raw file of reserpine data in the SRM scan mode is now stored on the hard drive.

Figure 47. An SRM scan acquisition in the Instrument Method Development Workspace

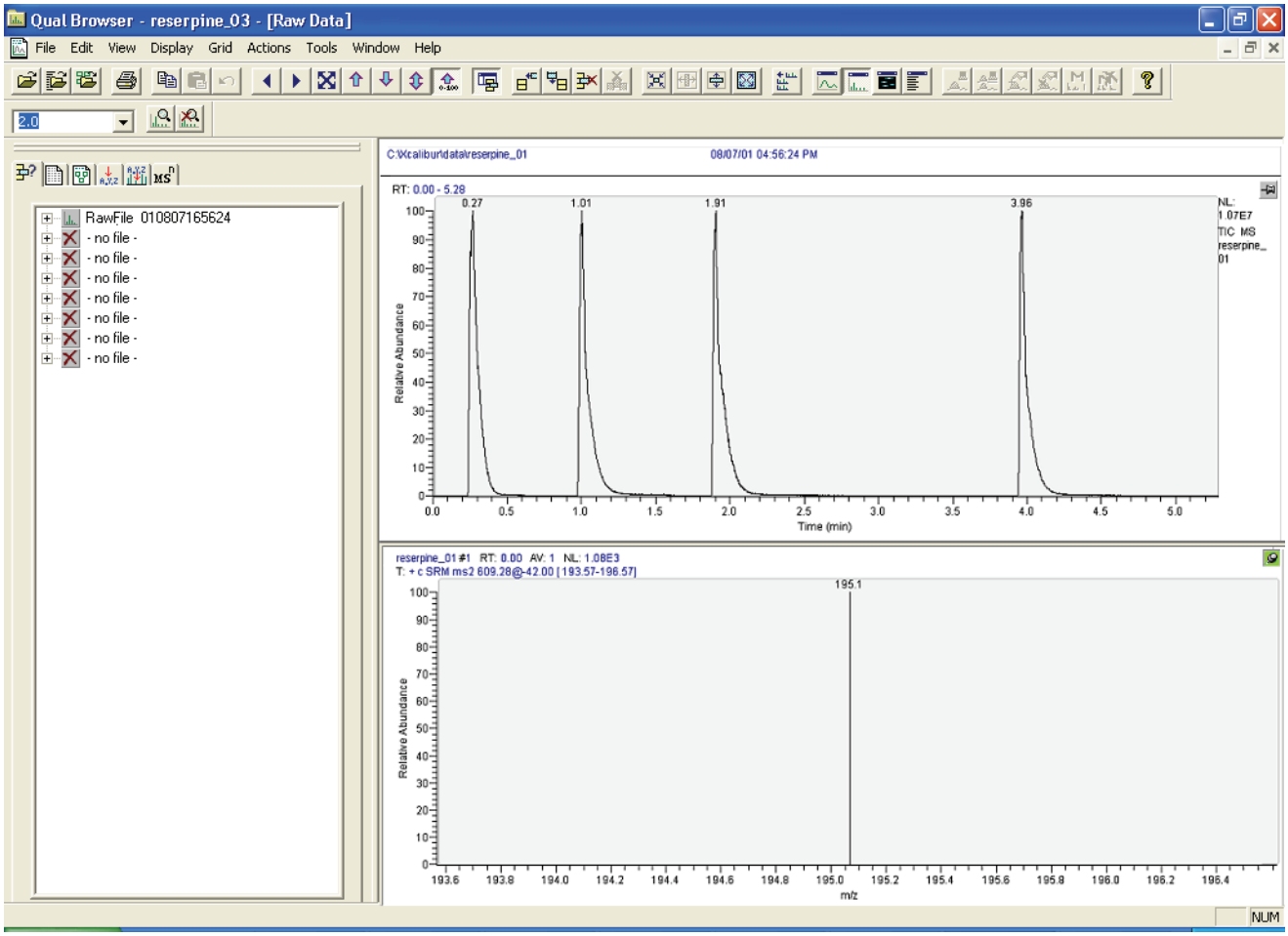


Note For more information about reviewing the data you acquire using the TSQ system with Xcalibur, refer to *Thermo Xcalibur Qualitative Analysis User Guide.*

17. To integrate the chromatogram in the raw file you just acquired using the Xcalibur Qual Browser window, click **View**. See Figure 48.

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."

Figure 48. Loop injections of reserpine in the Chromatogram view (top) and the centroid at *m/z* 195.066 in the Spectrum view (below) of the Qual Browser window



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

To set up the ion source for acquiring data in the APCI/MS/MS mode, follow these procedures.

Contents

Removing the ESI, H-ESI, or HESI-II Probe

❖ **To remove the ESI, H-ESI, or HESI-II probe**

1. If necessary, stop the flow of solvent to the API source as follows:
   a. If the EZ Tune window is not already open, choose **Start > Programs > Thermo Instruments > TSQ > TSQ Tune**, from the Windows™ taskbar, to open the EZ Tune window.
   b. Choose **Setup > Inlet Direct Control** to open the Inlet Direct Control dialog box.
   c. Display the LC page and click  (Stop) to stop the LC pump.

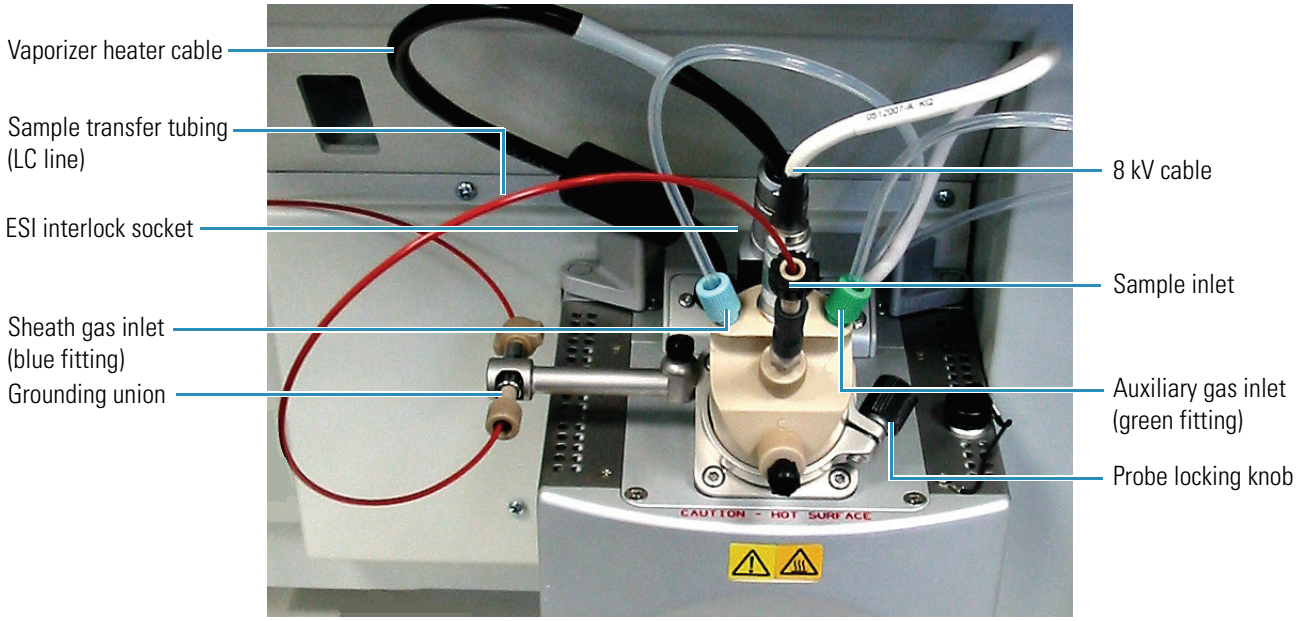
   On 
   Off 
   Standby 

   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.)
2. If the mass spectrometer is on, click the **On/Standby** button to place the mass spectrometer in Standby mode. When the mass spectrometer is in Standby mode, the TSQ turns off the ion source sheath gas, auxiliary gas, and high voltage.

3. Disconnect the sample transfer tubing from the stainless steel grounding union (ZDV fitting), as shown in Figure 49.
4. Unplug the 8 kV cable from the ESI needle high voltage receptacle (Figure 49):
   a. Unlock the cable by twisting the locking ring counterclockwise.
   b. Unplug the 8 kV cable from the ESI needle high voltage receptacle.
5. For H-ESI, unplug the vaporizer heater cable from the vaporizer heater cable socket on the H-ESI or HESI-II probe.
6. Disconnect the auxiliary gas fitting (green) from the auxiliary gas inlet (A) on the probe manifold (Figure 49).
7. Disconnect the sheath gas fitting (blue) from the sheath gas inlet (S) on the probe manifold.
8. If you have an ESI probe installed, remove the stainless steel grounding union (ZDV fitting) from the grounding bar on the ion source housing.

CAUTION AVOID BURNS. At operating temperatures, the HESI-II (or H-ESI) vaporizer can severely burn you. The HESI-II (or H-ESI) vaporizer typically operates in excess of 300 °C. **Always allow the heated vaporizer to cool to room temperature (for approximately 20 min) before you remove or touch the H-ESI or HESI-II probe**.

Figure 49. Ion Max ion source housing with ESI probe installed



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

10. 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 prevents you from twisting the probe until the pin is aligned with the slot in the ESI interlock block.

    After the probe is all the way back and aligned with the slot, turn the probe 45 degrees counterclockwise to free the probe from the alignment notch. Be careful not to break the fused-silica sample tube or PEEK safety sleeve.

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

12. Store the ESI probe in its original shipping container.

Removing the Ion Max or Ion Max-S Ion Source Housing

To access the ion sweep cone and to install the corona discharge needle, first remove the Ion Max or Ion Max-S ion source housing.

Note Disconnect any external liquid lines connected to the ion source housing before you remove the ion source housing.

❖ To remove the ion source housing

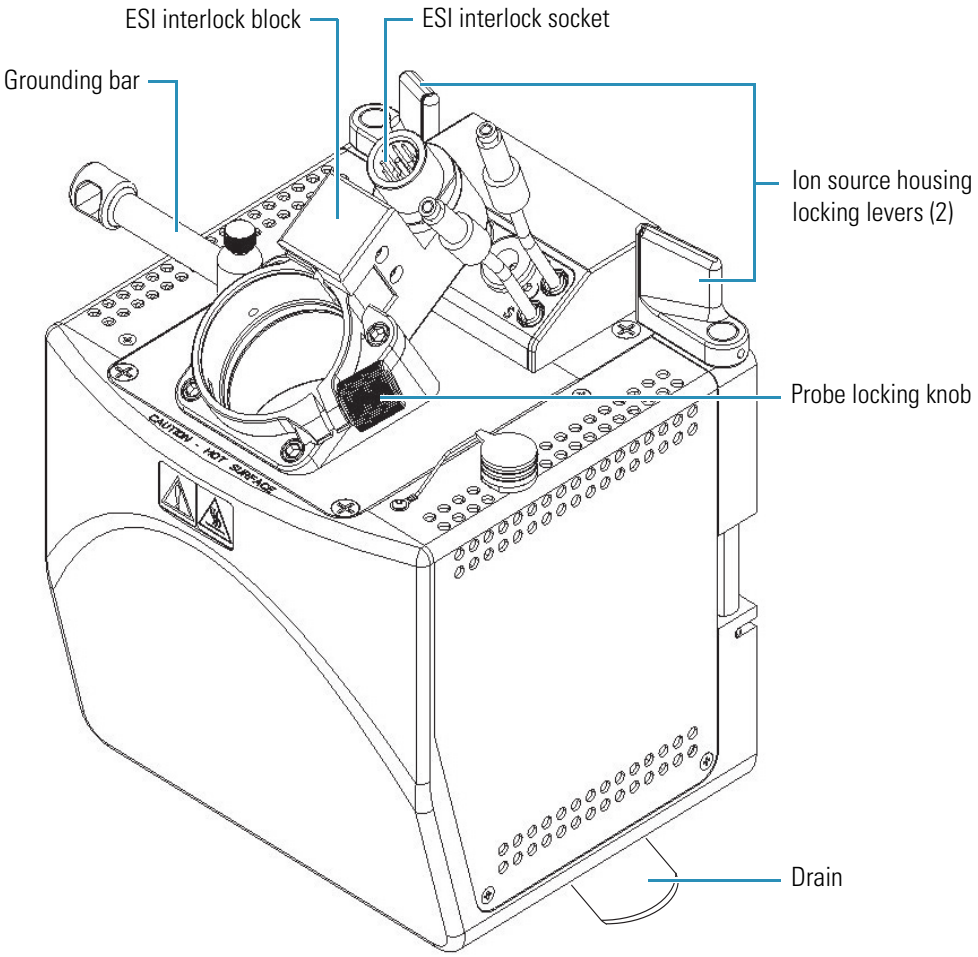
1. Remove the drain tube from the ion source housing drain (see Figure 50).

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 it straight off of the ion source mount assembly.

4. Go to “Removing the Ion Sweep Cone.”

Figure 50. Ion Max-S housing, detail of components (similar to the Ion Max)



Removing the Ion Sweep Cone

Remove the ion sweep cone, as it is not used for APCI operation.

❖ **To remove the ion sweep cone**

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 minutes) 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 "Installing the Corona Discharge Needle."

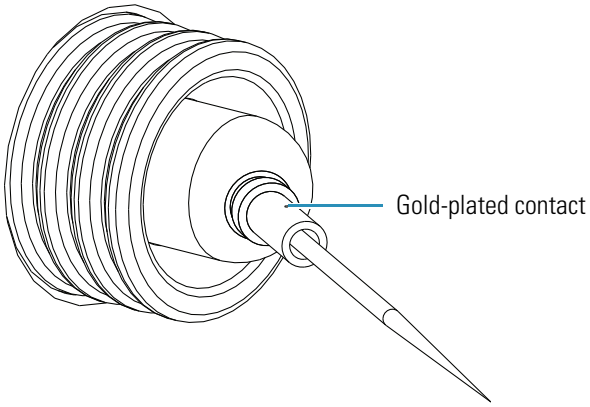
Installing the Corona Discharge Needle

❖ **To install the corona discharge needle**

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

1. Position the Ion Max source housing to access it from behind.
2. Using pliers, grasp the needle by the gold-plated contact and push the needle straight into the socket. See Figure 51.

Figure 51. Rear view of the corona discharge needle



3. Ensure that the tip of the needle is aligned with the path of travel between the APCI probe and the ion source interface on the instrument.

Go to "Installing the Ion Max or Ion Max-S Ion Source Housing."

Installing the Ion Max or Ion Max-S Ion Source Housing

❖ **To reinstall the Ion Max or Ion Max-S ion source housing**

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 52 and Figure 53.

Figure 52. Rear view of the Ion Max ion source housing

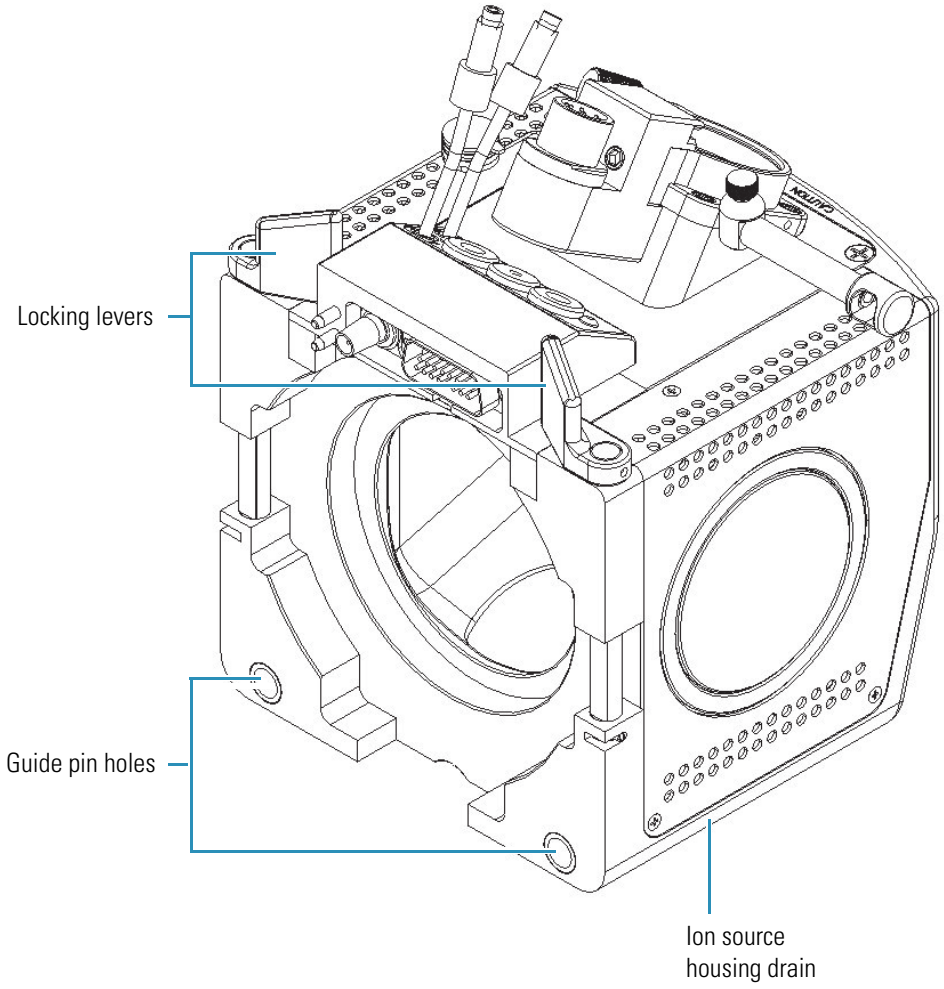
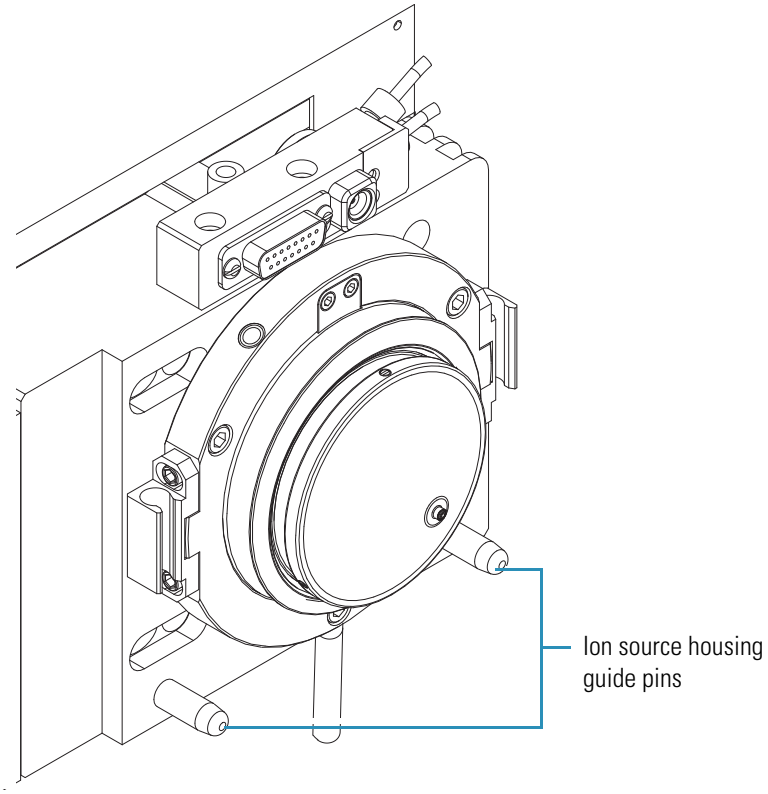


Figure 53. Ion source housing guide pins on the ion source mount



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.

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 forepump. 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:
   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 is vented to a fume exhaust system.

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

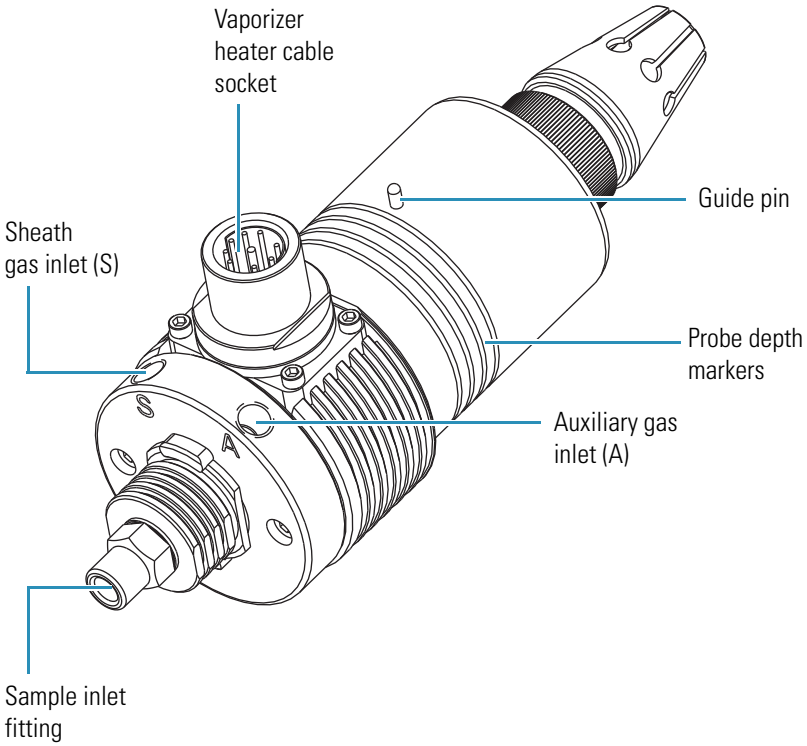
Go to "Installing the APCI Probe."

Installing the APCI Probe

❖ **To install the APCI probe**

1. Connect the 8 kV cable to the corona needle high voltage receptacle:
   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 on page 12.
   b. Lock the cable by twisting the locking ring clockwise.
2. Turn the probe locking knob counterclockwise to open the probe locking ring to its widest open position.
3. Insert the APCI probe into the port in the ion source housing, aligning the guide pin on the probe body at a 45-degree angle from the ESI interlock block. See Figure 54.

Figure 54. APCI probe



4. Push the probe into the port until the guide pin meets with the locking ring on the housing.
5. Turn the probe 45 degrees clockwise and align the guide pin with the slot in the ESI interlock block (you might need to pull the probe toward you slightly to properly align the pin with the notch). After 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. Seat the probe all the way down into the alignment notch.
7. Lock the probe in place by turning the probe locking knob clockwise.
8. Unplug the vaporizer heater cable from the ESI interlock plug on the ion source housing.
9. Connect the vaporizer heater cable to the vaporizer heater cable socket on the APCI probe.
10. Connect the Auxiliary gas line (green colored fitting) to the inlet on the APCI probe marked ***A***.
11. Connect the Sheath gas line (blue colored fitting) to the inlet on the APCI probe marked ***S***.
12. Connect the sample transfer tubing 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 must change to APCI source mode in EZ Tune by choosing **Setup > Change Ion Source > APCI**.

Leave the LC/MS system in standby and go to Chapter 7, "Optimizing the Mass Spectrometer in APCI/MS/MS Mode."

Optimizing the Mass Spectrometer in APCI/MS/MS Mode

This chapter provides information on fine tuning the mass spectrometer in the APCI/MS/MS mode. This experiment uses reserpine, but you can follow the same procedure with your analyte. You optimize the sensitivity of the mass spectrometer for an analyte by using an automatic tuning procedure.

The following parameters affect APCI performance and signal quality:

- Discharge current
- APCI vaporizer temperature
- Sheath gas pressure
- Auxiliary gas flow rate
- Capillary temperature
- S-lens rf amplitude (TSQ Vantage)
- Tube lens offset voltage (TSQ Quantum Access, TSQ Quantum Access MAX, and TSQ Quantum Ultra)

Note The TSQ can perform either a standard optimization or a custom optimization. During a standard optimization, the TSQ optimizes the collision energy, the tube lens offset voltage (TSQ Quantum Access, TSQ Quantum Access MAX, and TSQ Quantum Ultra) or the S-lens rf amplitude (TSQ Vantage), and the voltages applied to the ion optics until the ion transmission of your analyte is maximized. For a custom optimization, you can select which of the above settings the TSQ optimizes.

The optimum settings for these parameters depend on the solvent flow rate and on the structure of your analyte. In general, you must fine tune the mass spectrometer parameters whenever you change the solvent flow rate conditions of your particular application.

Note Ensure that you have performed the TSQ 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, follow the procedure in Chapter 3, "Tuning and Calibrating the Mass Spectrometer."

To optimize the mass spectrometer for your compound in the APCI/MS/MS mode requires the following actions described in this chapter:

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

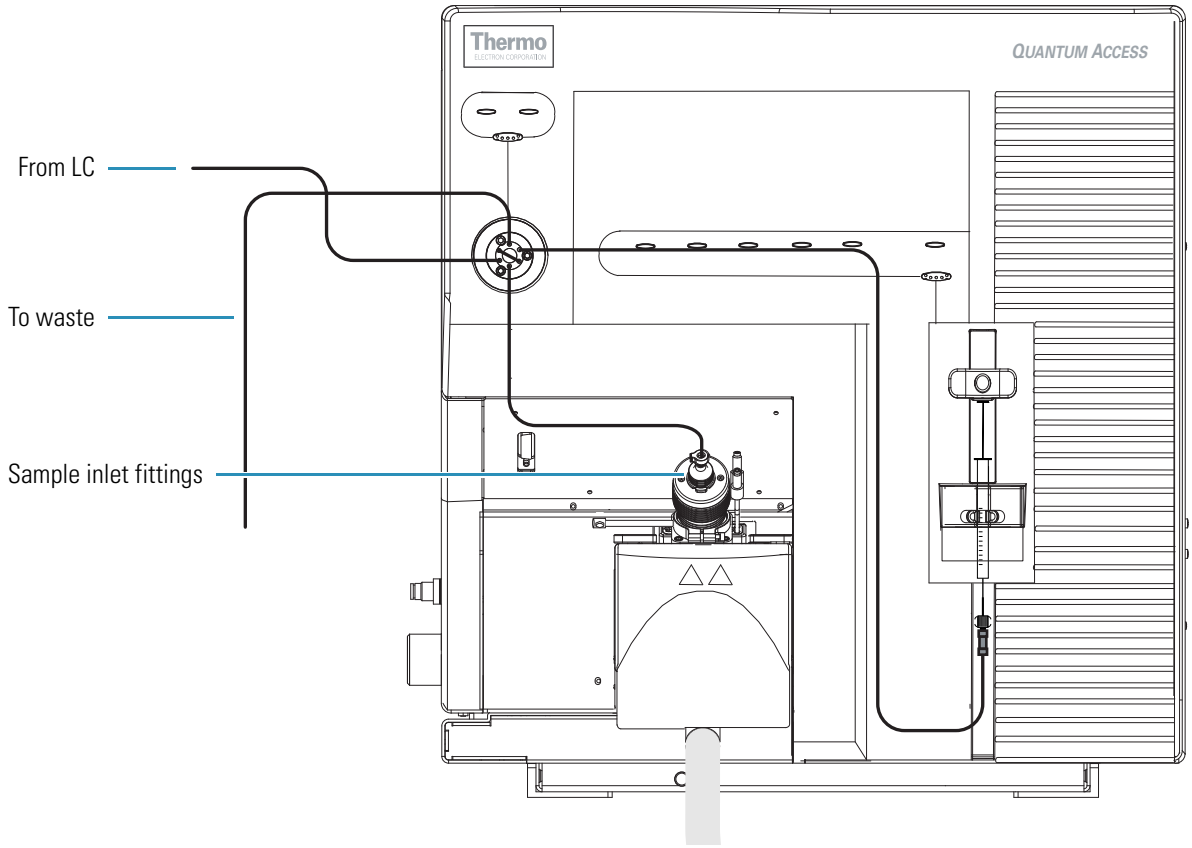
Contents

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

To introduce your compound by auto loop injection, follow these steps. The plumbing connections for APCI/MS sample introduction from the syringe pump into the solvent flow from an LC are shown in Figure 55.

Note You can use the reserpine sample solution described in Appendix C, "Solution Formulations." You can also use your compound of interest.

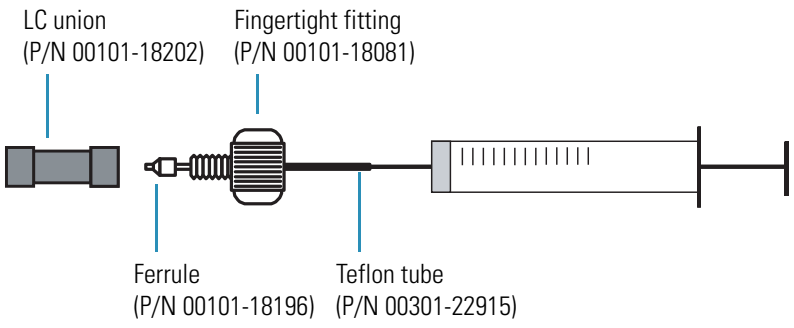
Figure 55. APCI/MS plumbing connections for sample introduction by auto loop injection into the solvent flow from an LC



❖ **To make the plumbing connections for APCI/MS sample introduction from the syringe pump into the solvent flow from an LC**

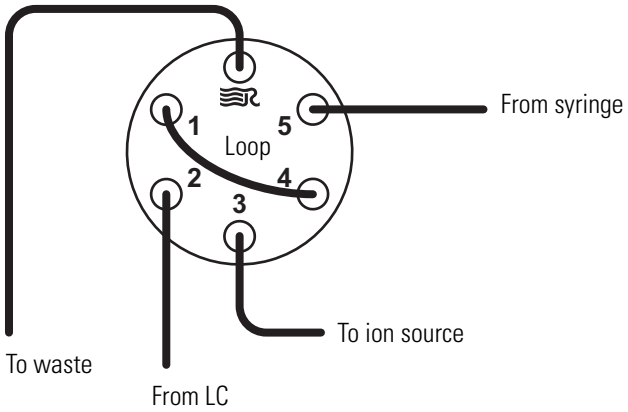
1. Remove the syringe from the syringe pump holder:
   a. Lift the handle off the syringe while depressing the black release button on the syringe pump handle.
   b. Remove the syringe.
   c. Remove the tip of the syringe needle from the end of the Teflon tube on the syringe adapter assembly. See Figure 56.

Figure 56. 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:
   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 57.

Figure 57. Divert/inject valve, showing plumbing for auto loop injection



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

4. Load a clean, 500 µL Unimetrics syringe with 420 µL of the 2 pg/µL (TSQ Quantum Access or TSQ Quantum Access MAX), 200 fg/µL (TSQ Quantum Ultra, TSQ Quantum Ultra AM, or TSQ Quantum Ultra EMR), or 100 fg/µL (TSQ Vantage, TSQ Vantage AM, or TSQ Vantage EMR) reserpine sample solution, or your analyte. For the procedure to prepare the reserpine solution, see Appendix C, "Solution Formulations."

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

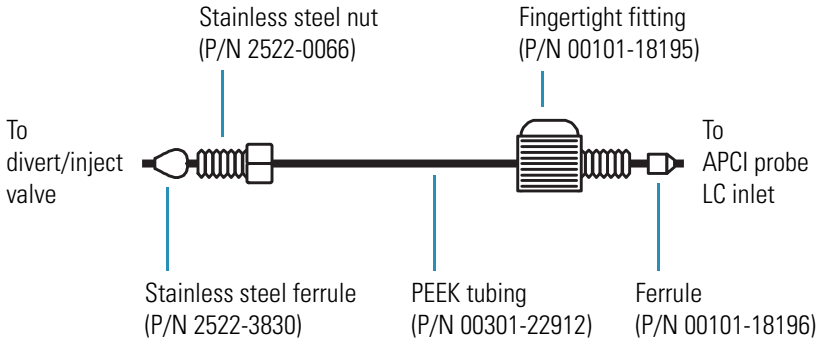
5. While holding the plunger of the syringe in place, carefully reinsert the tip of the syringe needle into the end of the Teflon tube on the syringe adapter assembly (see Figure 56).
6. Place the syringe into the syringe holder of the syringe pump.
7. While squeezing the black release button on the 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:

a. Gather the necessary fittings for installing a sample transfer line (see Figure 58).

b. Connect an appropriate length of tubing fitted with a nut and a ferrule to port 3 of the divert/inject valve (Figure 57).

c. Connect the other end of the tubing with a fingertight fitting and a ferrule to the sample inlet fitting (LC inlet) (Figure 55).

Figure 58. 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:

    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:

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

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

❖ **To set up the mass spectrometer to optimize automatically on your compound in APCI/MS/MS mode**

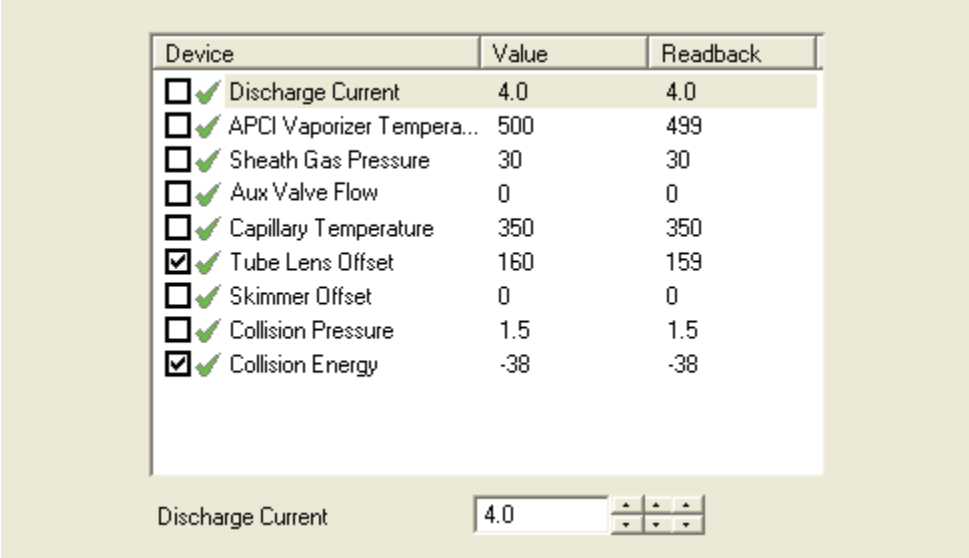
1. From the Windows taskbar, choose **Start > Programs > Thermo Instruments > TSQ > TSQ Tune** to open EZ Tune.

On 

Standby 

2. In EZ Tune, click the **On/Standby** button on the Control/Scan Mode toolbar to turn on the mass spectrometer.
3. Before you can analyze samples with the APCI source, place the TSQ in the APCI source mode by choosing **Setup > Change Ion Source > APCI**.
4. If desired, open an existing Tune Method:
   a. On the File/Display toolbar, click the **Open File** button,  , to display the Open dialog box.
   b. Confirm the path, *C:\Xcalibur\methods,* and then select the desired file.
   c. Click **Open** to open the file. EZ Tune downloads the Tune Method parameters to the mass spectrometer.
5. Choose **Display > Compound Dependent Devices** to display the Compound Dependent Devices dialog box. See Figure 59.

Figure 59. APCI settings in the Compound Dependent Devices dialog box (TSQ Quantum Access, TSQ Quantum Access MAX, or TSQ Quantum Ultra)



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, start LC solvent flow, elevate the temperature of the APCI vaporizer, and increase the sheath gas and auxiliary gas pressures for approximately 30 minutes to drive off the chemical contamination.

Here are typical values for reconditioning the APCI vaporizer:

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

6. Set the values for the compound dependent devices:
   a. Ensure that **Discharge Current** is selected (highlighted) in the Device Display table.
   b. In the Optimize Compound Dependent Devices dialog box, enter **4.0** in the Discharge Current box to set the discharge current to 4.0 µA.
   c. Set the temperature of the APCI vaporizer 350 °C:
      i. In the Device Display table, select **APCI Vaporizer Temperature** to highlight it.
      ii. In the APCI Vaporizer Temperature box, enter **350**.
   d. Set the pressure of the sheath gas to 30 units:
      i. In the Device Display table, select **Sheath Gas Pressure** to highlight it.
      ii. In the Device box now labeled Sheath Gas Pressure, enter **30**.
   e. Set the flow rate of the auxiliary gas to 0 units:
      i. In the Device Display table, select **Aux Valve Flow** to highlight it.
      ii. In the Aux Valve Flow box, enter **0** to set the auxiliary gas flow rate to 0 units.
   f. Set the temperature of the ion transfer capillary 200 °C:
      i. Select **Capillary Temperature** in the Device Display table.
      ii. In the Capillary Temperature box, enter **200** to set the capillary temperature to 200 °C.
   g. Set the ion source fragmentation (skimmer offset) to 0:
      i. Select **Skimmer Offset** in the Device Display table.
      ii. In the Skimmer Offset box, enter **0** to set the collision energy to 0 V.
   h. Set the collision gas pressure to 1.5 mTorr:
      i. Select **Collision Pressure** in the Device Display table.
      ii. In the Collision Pressure box, enter **1.5** to set the collision gas pressure to 1.5 mTorr.

i. Set the collision energy to -38 eV:

i. Select **Collision Energy** in the Device Display table.

ii. In the Collision Energy box, enter **-38** to set the collision energy to -38 eV.

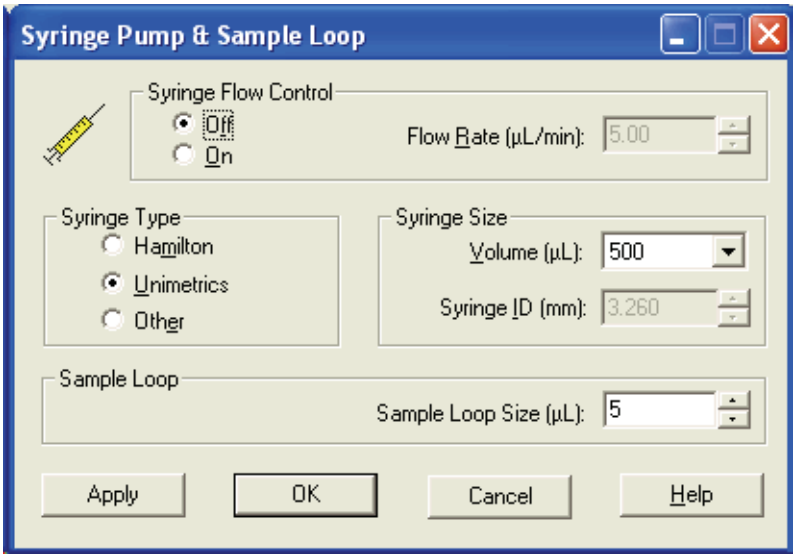
j. Click **Apply** to apply the settings.

Ensure that the readbacks in the Device Display table 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.)

7. Configure the Syringe Pump to automatically inject the reserpine sample solution into the sample loop:

a. Choose **Setup > Syringe Pump & Sample Loop** to display the Syringe Pump and Sample Loop dialog box in the top right corner of the workspace. See Figure 60.

Figure 60. Auto loop injection setup in the Syringe Pump and Sample Loop dialog box



b. Select the **Off** option in the Syringe Flow Control area to turn off the syringe pump.

- If you are using a Unimetrics or Hamilton syringe, go to step 7c.
- If you are *not* using a Unimetrics or Hamilton syringe, go to step 7e.

c. In the Syringe Type area, select the **Unimetrics** (or **Hamilton**) option, as appropriate.

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

When you specify the syringe type and syringe volume, EZ Tune automatically sets the proper syringe ID value. Go to step 7f.

e. If you are using a make of syringe other than Unimetrics or Hamilton, manually specify the syringe ID as follows:

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

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

iii. In the Syringe ID box, enter the inner diameter of your syringe.

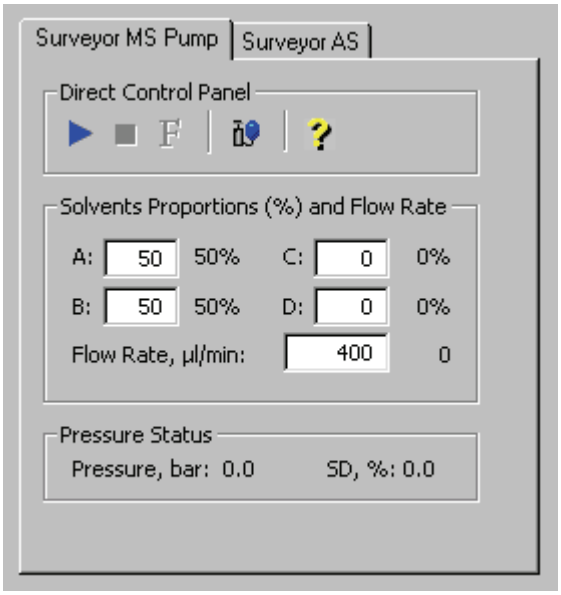
f. In the Sample Loop area, enter **5** in the Sample Loop Size box to specify a loop size of 5 µL.

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

8. Start the flow of solvent:

a. Choose **Setup > Inlet Direct Control** button to display the Inlet Direct Control dialog box.. See Figure 61.

Figure 61. Pump is off in the Inlet Direct Control dialog box



Note The following procedure assumes that isopropyl alcohol and LCMS-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:

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

ii. In the box labeled *B,* enter **50** to specify a delivery proportion of 50% solvent B.

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

c. In the Direct Control Panel area, click (Start) 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 "Optimizing in APCI/MS/MS Mode Automatically with Your Compound."

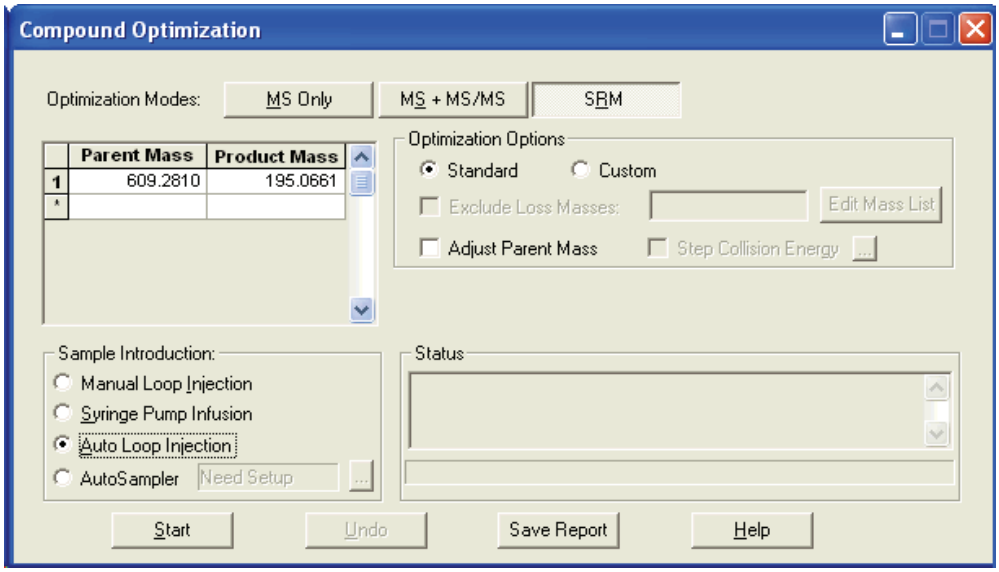
Optimizing in APCI/MS/MS Mode Automatically with Your Compound

You optimize the mass spectrometer to maximize the ion transmission of your compound. Performing optimization fine tunes compound dependent parameters such as discharge current, capillary temperature, and tube lens offset. Thermo Fisher Scientific recommends that you optimize the mass spectrometer only after you have successfully tuned and calibrated the instrument.

❖ **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. In EZ Tune, choose **Setup > Compound Optimization** button to display the Compound Optimization dialog box. See Figure 62.

Figure 62. Compound Optimization dialog box



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

   a. For Optimization Modes, select **SRM**. In this mode you can optimize a selected reaction.

   b. In the Optimization Options area, select the **Standard** option to tune the default selection of devices. (The collision energy and the tube lens offset (TSQ Quantum Access, TSQ Quantum Access MAX, and TSQ Quantum Ultra) or the S-lens rf

amplitude (TSQ Vantage) are the default compound sensitive devices that the TSQ optimizes for this option.)

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

d. In the Product Mass column, enter **195.066** to set the product mass of the SRM reaction to the ion at *m/z* 195.066.

> **Note** You must select the inlet type option that is appropriate for the inlet mode you are using to introduce your sample into the mass spectrometer. This procedure uses the Auto Loop Injection option.

e. In the Inlet Types area, select the **Auto Loop Injection** option to have the TSQ system automatically inject the optimization solution.

3. Click **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 you receive this message, reload the syringe and click **OK** to continue optimization.

The Status box in the Compound Optimization dialog box displays the message, *Finish compound optimization,* when the compound optimization has completed successfully. See Figure 63.

- If the compound optimization procedure finishes without errors, and the breakdown curve of the *m/z* 609.3 ion is Gaussian-shaped (as in Figure 64) 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 *m/z* 609.3 oscillates, contains multiple peaks, or is excessively noisy; go to step 4.

Figure 63. The successful completion of compound optimization viewed in the Status box of the Compound Optimization Workspace

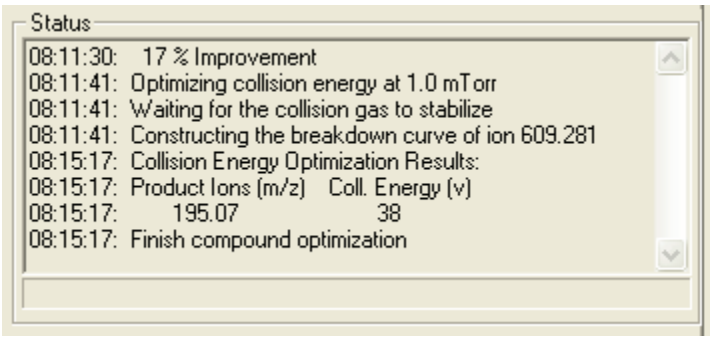
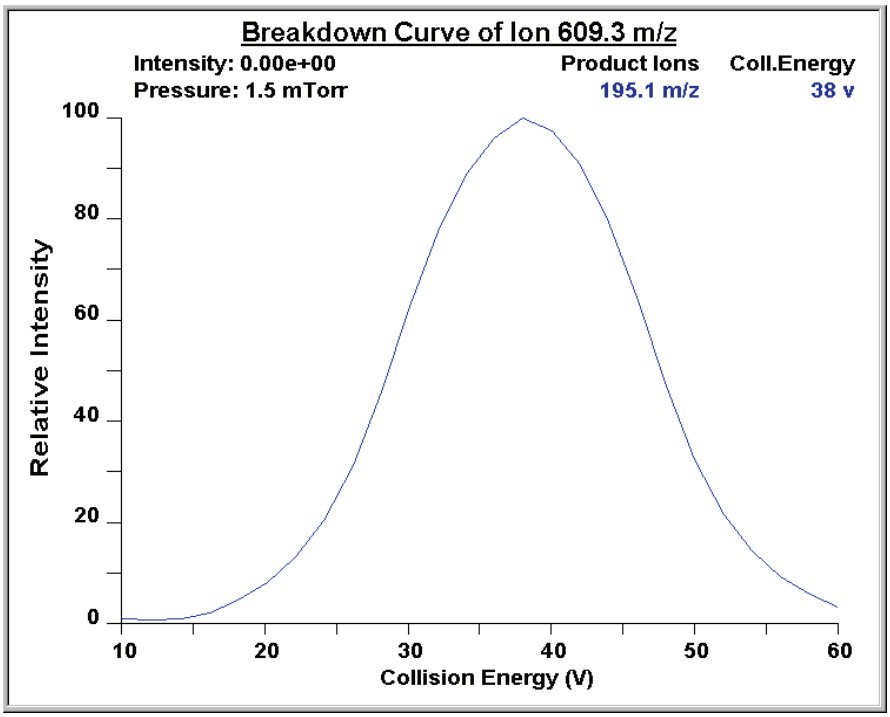


Figure 64. Breakdown curve of reserpine showing the relative intensity of the product ion at *m/z* 195.066 as a function of collision energy



4. If errors occurred during the compound optimization procedure, restore the previous mass spectrometer compound sensitive device settings by completing these steps:
   a. Click **Undo** to restore the prior device settings.
   b. Click **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 **Accept** to accept the results of the compound optimization.

   Note If any of the ion source parameters have changed from their initial settings, save the Tune Method while the mass spectrometer is on or else the settings will be lost.

6. Save the Tune Method file as follows:
   a. Click **Save Tune As** to open the Save As dialog box.
   b. In the File Name box, enter a file name (such as **APCI_reserpine.TSQTune**, or the name of your compound) for your Tune Method file.
   c. Click **Save** to save the Tune Method file.

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

Go to Chapter 8, "Acquiring APCI/MS/MS Data."

Acquiring APCI/MS/MS Data

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.

Contents

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

Follow these procedures 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 65.

❖ To make the plumbing connections for manual loop injection

1. Open Tune Master if it is not already open:
   a. Right-click on **Start** and choose **Explore**.
   b. Browse to C:\Thermo Instruments\TSQ\System\Programs.
   c. Double-click on **TSQTune**.
2. Stop the flow of solvent to the APCI source:

   a. In Tune Master, on the Control/Scan Mode toolbar, click the **AS/LC Direct Control** button to display the Inlet Direct Control view in the top right corner of the workspace. See Figure 66.
   b. In the Direct Control Panel area, click  (Stop) to stop the flow of solvent.

Figure 65. APCI/MS plumbing connections for sample introduction by manual loop injection into the solvent flow from an LC

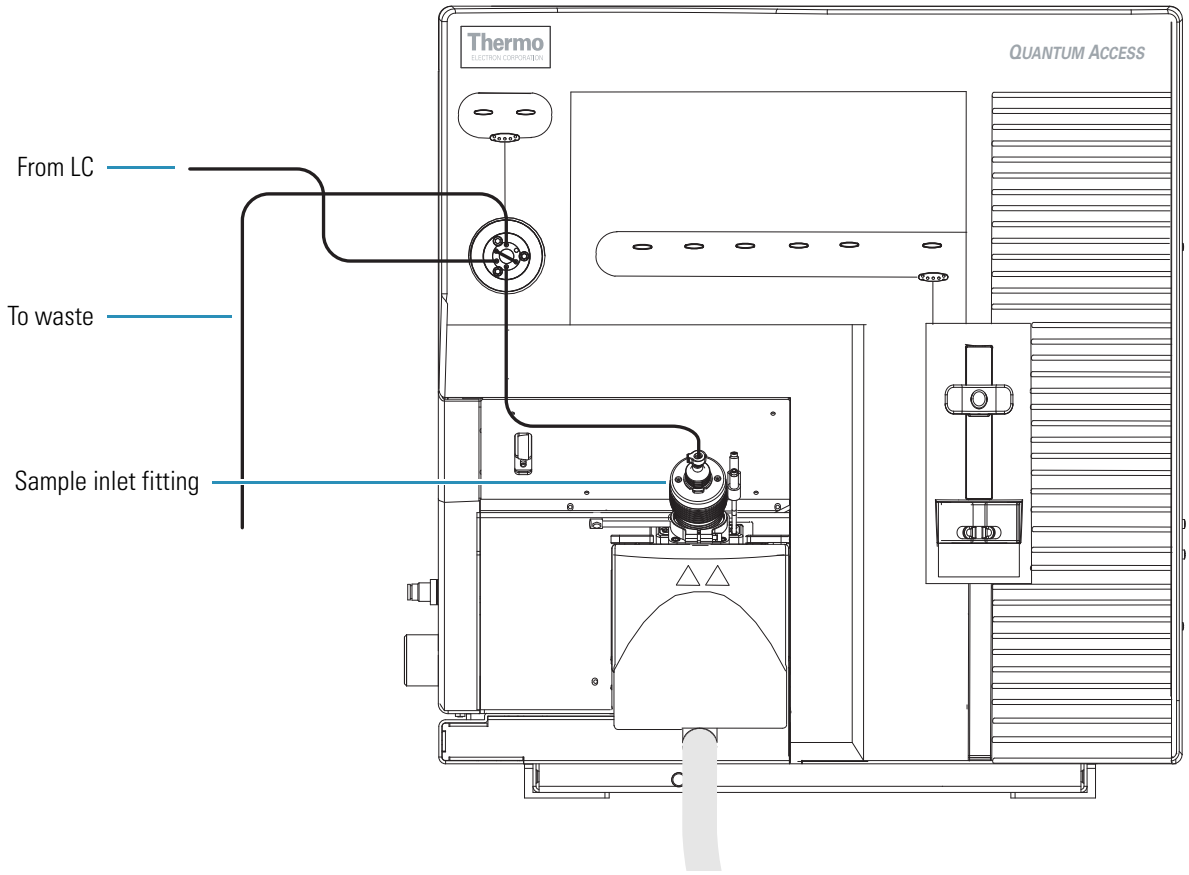
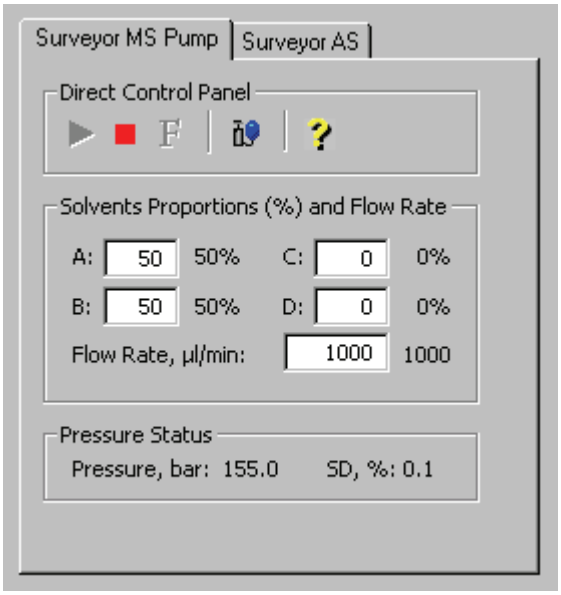


Figure 66. The pump is on in the Inlet Direct Control view

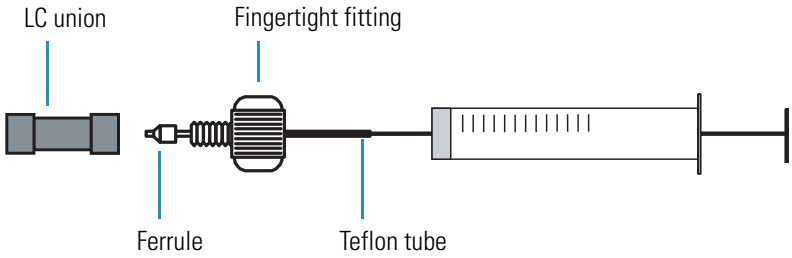


On 

Standby 

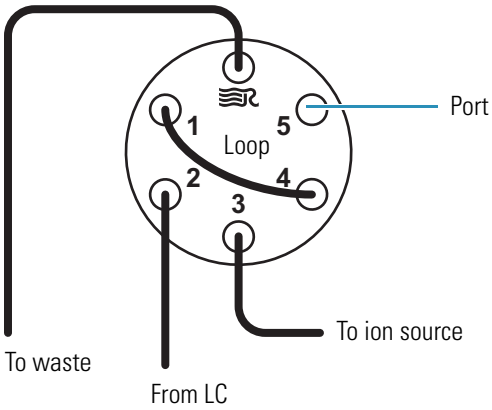
3. Click the **On/Standby** button on the Control/Scan Mode toolbar to place the mass spectrometer in standby.
4. Remove the syringe from the syringe pump holder:
   a. Lift the handle off the syringe while depressing the black release button on the syringe pump handle.
   b. Remove the syringe.
   c. Remove the tip of the syringe needle from the end of the Teflon tube on the syringe adapter assembly. See Figure 67.

Figure 67. Syringe and syringe adapter assembly



5. 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 68.

Figure 68. Plumbing for manual loop injection in the divert/inject valve

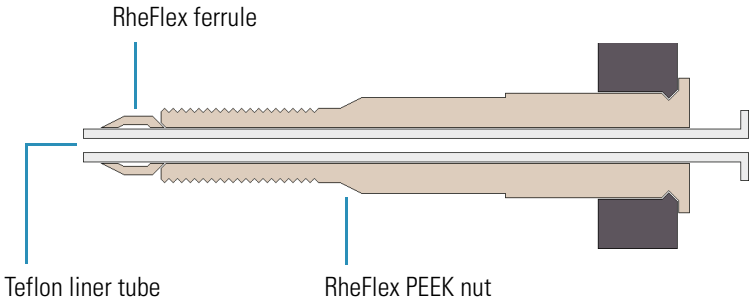


6. Install the needle port fitting (P/N 00110-22030) into the divert/inject valve:
   a. Insert the liner tube, RheFlex ferrule, and the threaded portion of the RheFlex nut (see Figure 69) into port 5 of the divert/inject valve.
   b. Carefully tighten the nut with your fingers.

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

Go to "Acquiring APCI/MS/MS Data in the SRM Scan Mode."

Figure 69. Needle port fitting



Acquiring APCI/MS/MS Data in the SRM Scan Mode

❖ **To acquire a file of reserpine data in the SRM scan mode**

On 

Standby 

1. In Tune Master, click 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 Tune Method that is different from the one currently displayed, first 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:
   a. On the File/Display toolbar, click the **Open File** button,  , to display the Open dialog box.
   b. Confirm that the folder, *C:\Xcalibur\methods,* is displayed. Select the file **APCI_reserpine.TSQTune** (or your Tune Method).
   c. Click **Open** to open the file. Tune Master downloads the Tune Method settings to the mass spectrometer.
3. Start the flow of solvent:

   a. On the Control/Scan Mode toolbar, click the **AS/LC Direct Control** button to display the Inlet Direct Control view in the top right corner of the workspace. See Figure 70.

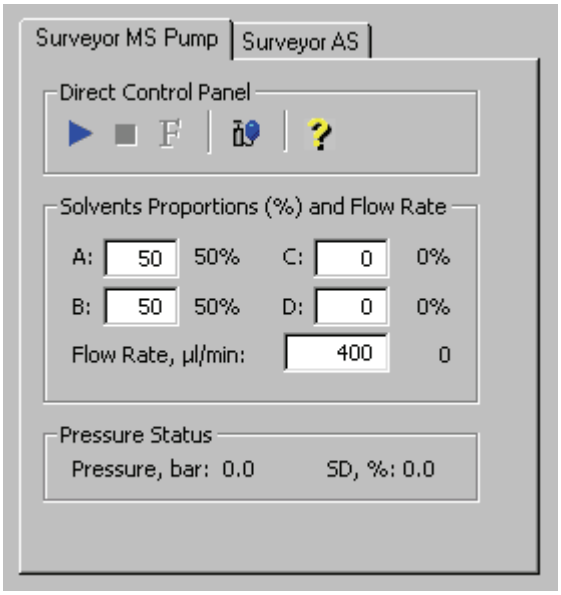
> **Note** The following procedure assumes that isopropyl alcohol and LCMS-grade water are in the solvent bottles labeled A and B.

   b. Set up the Surveyor MS Pump to deliver a solution of 50:50 isopropyl alcohol/water at 400 µL/min:
      i. In the Inlet Direct Control view, in the Solvents Proportions (%) and Flow Rate area, type **50** in the box labeled *A* to specify a delivery proportion of 50% solvent A.

ii. In the box labeled *B,* type **50** to specify a delivery proportion of 50% solvent B.

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

Figure 70. LC pump is off in the Inlet Direct Control view



c. In the Direct Control Panel area, click ▶ (Start) to start the Surveyor MS pump.

4. On the Control/Scan Mode toolbar, click the **Instrument Method Development Workspace** button to open the Instrument Method Development Workspace. See Figure 71.

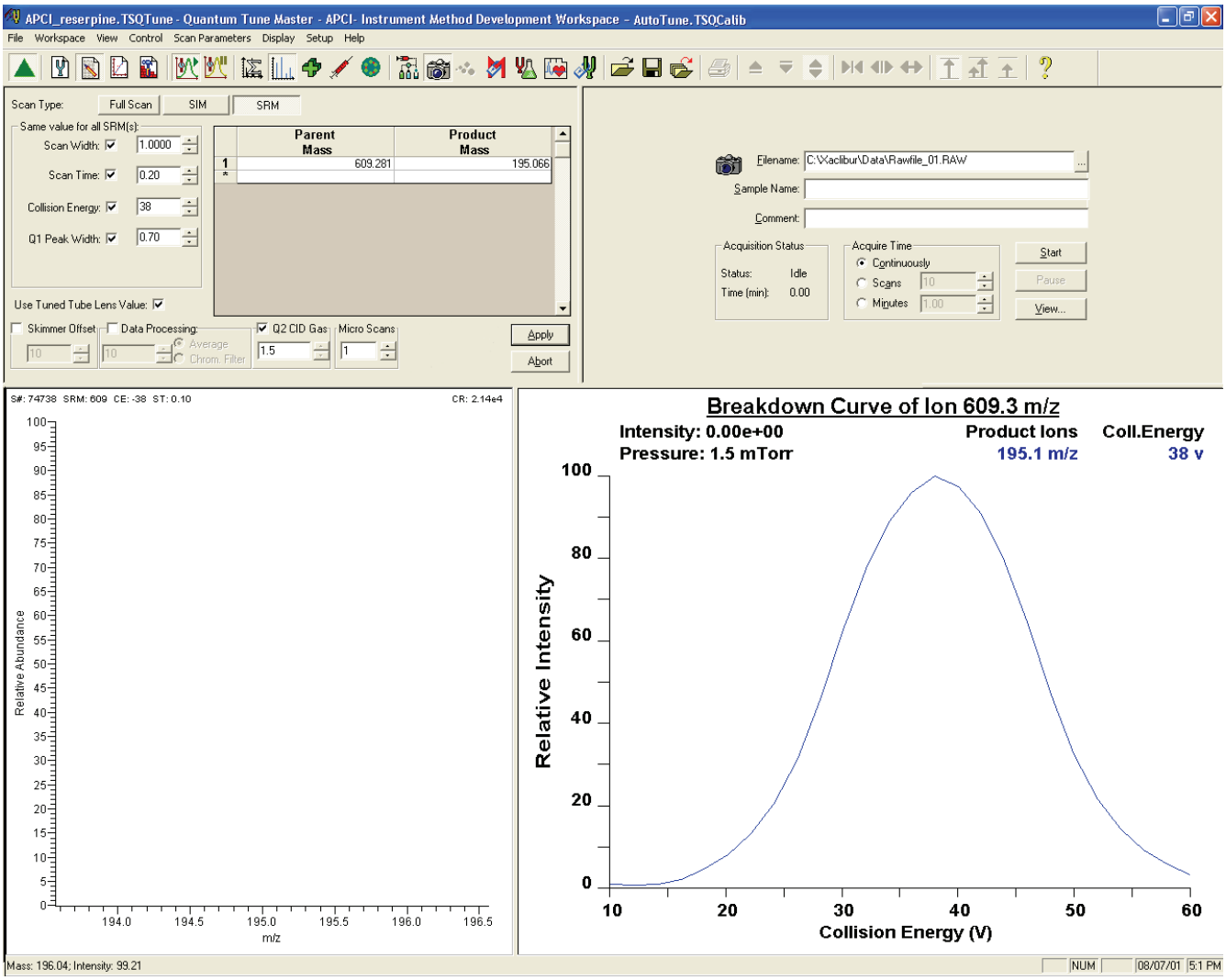
Note If you just completed compound optimization using reserpine as described in Chapter 4, then the following settings are selected by default when you switch to the Method Development Workspace.

5. Set any required scan parameters for acquiring SRM data:

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

b. In the corresponding SRM table, verify that a single reaction is listed. Enter the reserpine parent mass **609.281** and product mass **195.066**.
-Or-
Enter the parent and product masses of your analyte.

Figure 71. Instrument Method Development Workspace



Note In the Define Scan view, use the Same value for all SRM(s) area 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 in the adjacent box.

c. In the Same value for all SRM(s) area, ensure that all the global parameter check boxes are selected. Then verify the following values (or enter them) in the appropriate boxes.

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

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

   iii. Verify that the collision energy in the Collision Energy box is approximately equal to 38, the value 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 box, enter **0.70** to set the peak width to 0.70 u.

v. In the Q3 Peak Width 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 **Skimmer Offset** check box is cleared.

f. Specify the use of a 3 s chromatography filter for the data acquisition:

i. Select the **Data Processing** check box to activate the Data Processing box and options.

ii. Select the **Chrom. Filter** option to specify the use of a chromatography filter.

iii. In the Data Processing box, enter **3** to designate a 3 s chromatography filter.

g. Set the collision cell gas settings:

i. Select the **Q2 CID Gas** check box to specify the use of collision gas.

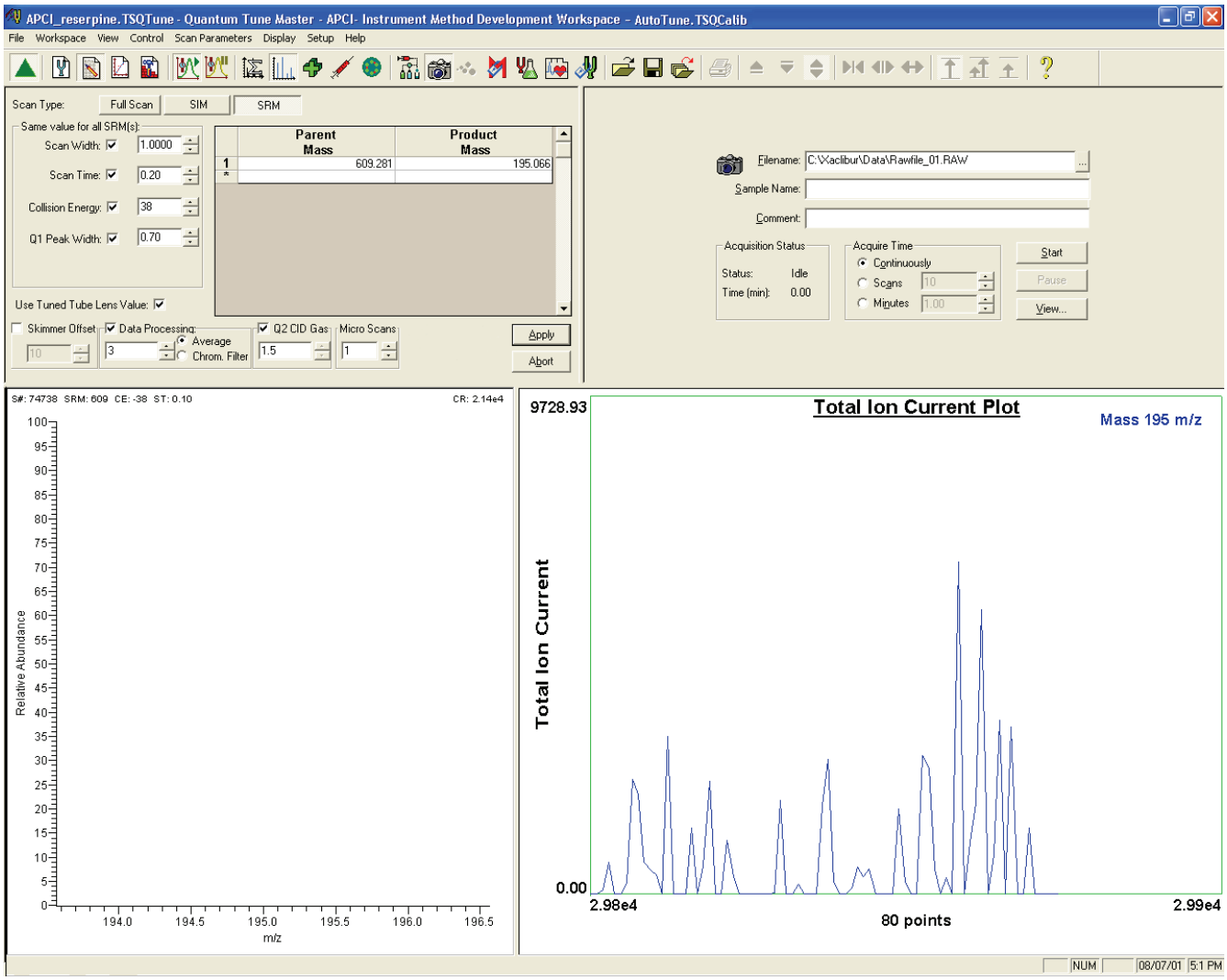
ii. In the Q2 CID Gas box, enter **1.5** to set the collision cell gas pressure to 1.5 mTorr.

h. Confirm that Micro Scans is set to **1**.

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

7. On the Control/Scan Mode toolbar, click the **Display TIC** button to begin recording the total ion current in the Graph view in the lower right corner of the workspace. See Figure 72.

Figure 72. SRM scan type in the Instrument Method Development Workspace



8. Specify the acquisition parameters:

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

   b. In the Sample Name box, type **reserpine** (or the name of your analyte) to specify the sample identity.

   c. In the Comment box, type a comment about your experiment. For example, type **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 area, select the **Continuously** option 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 the **Divert/Inject Valve** button to change it to the Load position.
10. In the Acquire Data view, click **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, for example:

    C:\Xcalibur\Data\reserpine_010502092159.raw

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

11. Fill the sample loop with reserpine solution:
    a. Ensure that the syringe is loaded with 420 µL of the 2 pg/µL (TSQ Quantum Access or TSQ Quantum Access MAX), 200 fg/µL (TSQ Quantum Ultra, TSQ Quantum Ultra AM or TSQ Quantum Ultra EMR), or 100 fg/µL (TSQ Vantage, TSQ Vantage AM, or TSQ Vantage EMR) reserpine solution or your analyte. (For the procedure to prepare the reserpine solution, see Appendix C, "Solution Formulations.")

       Note To minimize the possibility of cross-contamination of the assembly, be sure to wipe off the tip of the needle with a clean, lint-free tissue before reinserting it into the syringe adapter 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. To inject the reserpine solution into the LC solvent flow, press the blue Divert/Inject Valve button on the front panel of the TSQ mass spectrometer.
13. In the Spectrum view, observe the reserpine product peak at *m/z* 195.066 (or that of your analyte of interest).
14. Repeat the following sequence several times to obtain consecutive loop injections of reserpine in the SRM scan mode. Wait approximately 1 minute between injections.
    a. Press the blue Divert/Inject Valve button on the TSQ mass spectrometer to return the Divert/Inject valve to the Load position. Overfill the loop with the reserpine solution.
    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 minute before the next injection.
    d. Repeat steps 14a through 14c several times.

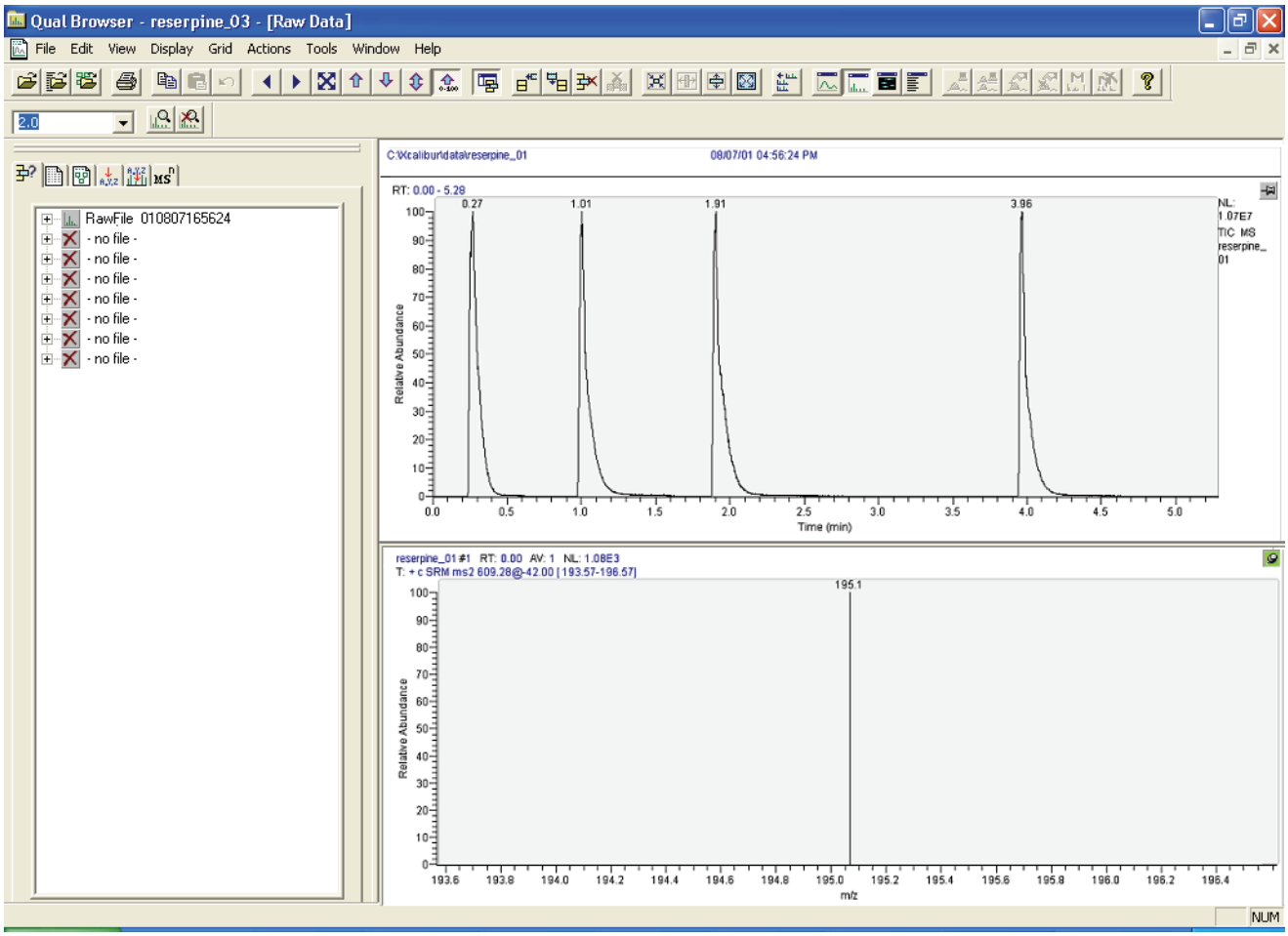
Note You can optimize a setting by varying the setting and repeating step 14.

15. To end the data acquisition, click **Stop** in the Acquire Data dialog box.

    A file of reserpine data in the SRM scan mode is now stored on the hard drive.

16. To integrate the chromatogram in the raw file you just acquired using the Xcalibur Qual Browser window, click **View**. See Figure 73.

Figure 73. Loop injections of reserpine in the Chromatogram view (top) and the centroid at *m/z* 195.066 in the Spectrum view (bottom) in the Qual Browser window



High Mass Calibration

The TSQ Quantum Access, TSQ Quantum Access MAX, TSQ Quantum Ultra EMR, and TSQ Vantage EMR can operate in the high mass range (*m/z* 1500 to 3000). To acheive the optimum mass accuracy and signal strength for these instruments you must perform high mass calibration in addition to the automatic calibration described in Chapter 3, "Tuning and Calibrating the Mass Spectrometer." The high mass calibration procedure involves optimizing the lens parameters for the high mass peaks, performing a mass calibration, and then appending the calibration file for polytyrosine. The procedure uses Ultramark 1621 as the calibrant.

❖ To calibrate the TSQ Quantum Access, TSQ Quantum Access MAX, TSQ Quantum Ultra EMR, or TSQ Vantage EMR for higher masses

1. Using Tune Master, complete the standard automatic tuning and calibration procedure detailed in Chapter 3, "Tuning and Calibrating the Mass Spectrometer."
2. Save the tune file as *ESI Polytyrosine Tune.TSQTune* and the calibration file as *ESI Polytyrosine Calibration.TSQCalib*.

3. Click the **Compound Optimization Workspace** button on the Control/Scan Mode toolbar to display the Compound Optimization Workspace.
4. Use the Device list and Device box (upper-right corner) to set the ion source parameters to the following values:
   - Spray voltage: 4000 V
   - Sheath gas pressure: 0 to 3 units
   - Aux gas pressure: 0 units
   - Capillary temperature: 270 °C
   - HESI-II vaporizer temperature: 0 °C
   - Tube lens voltage: 70 V
   - Skimmer offset: 0 V

❖ To perform high mass calibration in positive polarity mode

Positive Polarity 

Negative Polarity 

1. Place the mass spectrometer in the positive ion polarity mode. (Ensure that the Polarity button is in the positive polarity state.)
2. Infuse Ultramark 1621 at a flow rate of 1 to 3 µL/min.
   a. Load a clean syringe with the Ultramark 1621 calibration solution and install the syringe in the syringe pump. To prepare the Ultramark 1621 calibration solution, see "Ultramark 1621 High Mass Calibration Solution" on page 120.
   b. Choose **Setup > Syringe Pump & Sample Loop** to display the Syringe Pump and Sample Loop view in the top right corner of the workspace.
   c. Enter or select the relevant settings and click **Apply** to start infusion.

In Full Scan mode your spectrum should look similar to the spectrum shown in Figure 74.

Figure 74. Ultramark 1621 positive ion spectrum

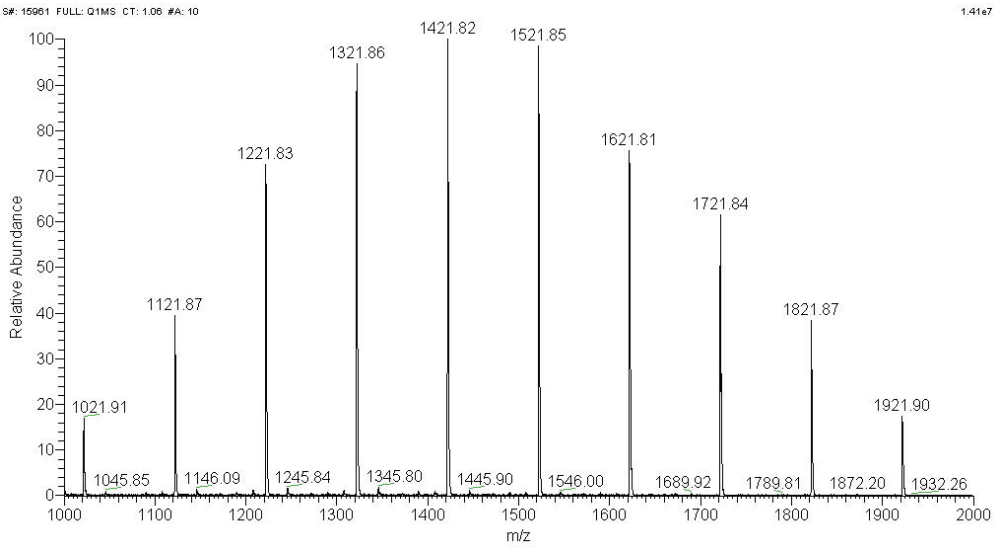


Table 6 lists the Ultramark 1621 positive ions.

Table 6. Mass-to-charge ratios of Ultramark 1621 positive ions (Sheet 1 of 2)

Ultramark positive ions *(m/z)*
822.0162
922.0098
1022.0034
1121.9970
1221.9906
1321.9843

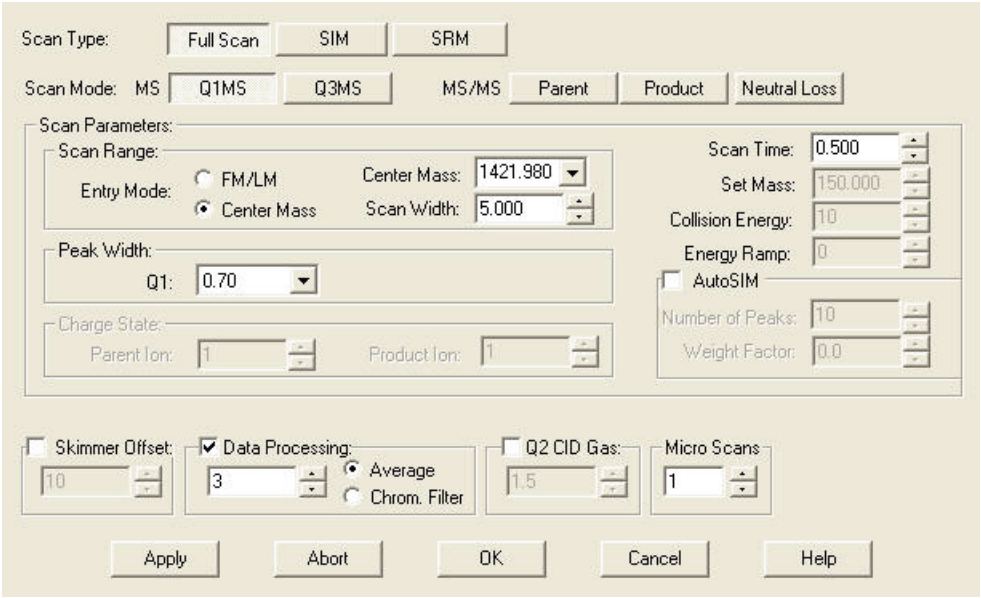
Table 6. Mass-to-charge ratios of Ultramark 1621 positive ions (Sheet 2 of 2)

Ultramark positive ions *(m/z)*
1421.9779
1521.9715
1621.9651
1721.9587
1821.9523
1921.9459
2021.9395
2121.9332

3. In Tune Master, on the Control/Scan Mode toolbar, click the **Instrument Method Development Workspace** button to display the Instrument Method Development Workspace.

4. In the Define Scan view, set the parameter values to those shown in Figure 75, and click **Apply**.

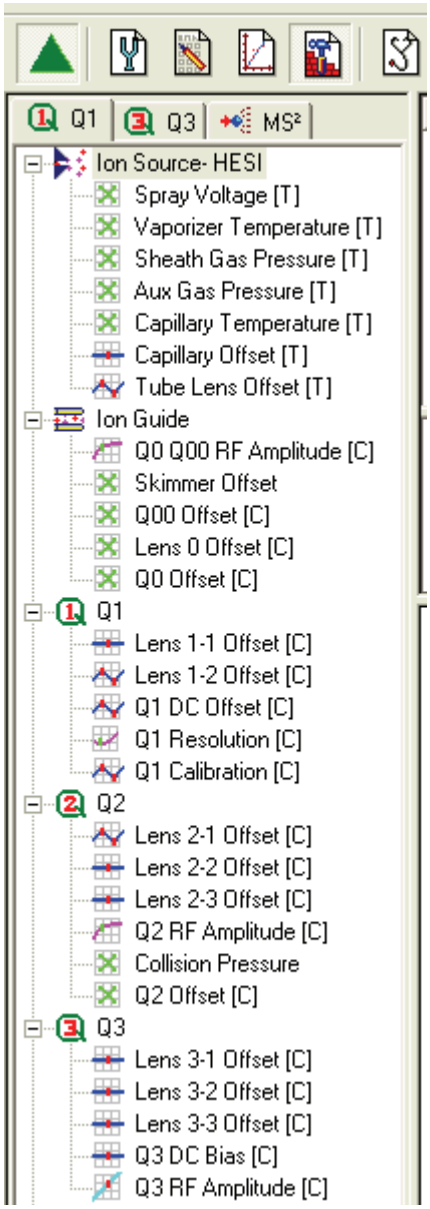
Figure 75. The settings for high mass calibration in positive polarity mode in the Define Scan view



5. Click the **Full Instrument Control** button to display the Full Instrument Control Workspace.

6. Click the **Q1** tab of the Device view. See Figure 76.

Figure 76. Device view of the Full Instrument Control Workspace



7. Select **Tube Lens Offset** and click **Optimize**. After the optimization procedure is complete, click **Accept**.

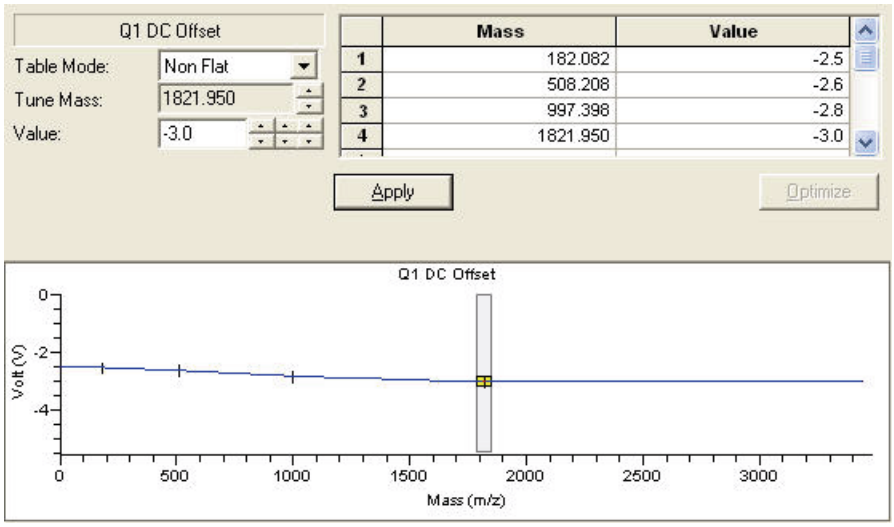
8. Select **Lens 1-2 Offset** and click **Optimize**. After the optimization procedure is complete, click **Accept**.

9. Select **Lens 2-1 Offset** and click **Optimize**. After the optimization procedure is complete, click **Accept**.

10. Click the **Q3** tab of the Device view.
11. Select **Tube Lens Offset** and click **Optimize**. After the optimization procedure is complete, click **Accept**.
12. Select **Lens 3-3 Offset** and click **Optimize**. After the optimization procedure is complete, click **Accept**.

13. Click the **Instrument Method Development Workspace** button to display the Instrument Method Development Workspace.
14. In the Define Scan view (Figure 75), under Scan Range select **1821.950** in the Center Mass box. Click **Apply**.
15. Repeat step 5 to step 12 for the Ultramark peak at *m/z* 1821.950. The signal intensity must be greater than 1.5×10^5 counts.
16. Click the **Q1** tab of the Device view.
17. Select **Q1 DC Offset**. Add -0.2 to the value for mass 997.389 and enter the sum (-2.8 + -0.2 = **-3.0** in Figure 77) in the Value box for mass 1821.950. Click **Apply**.

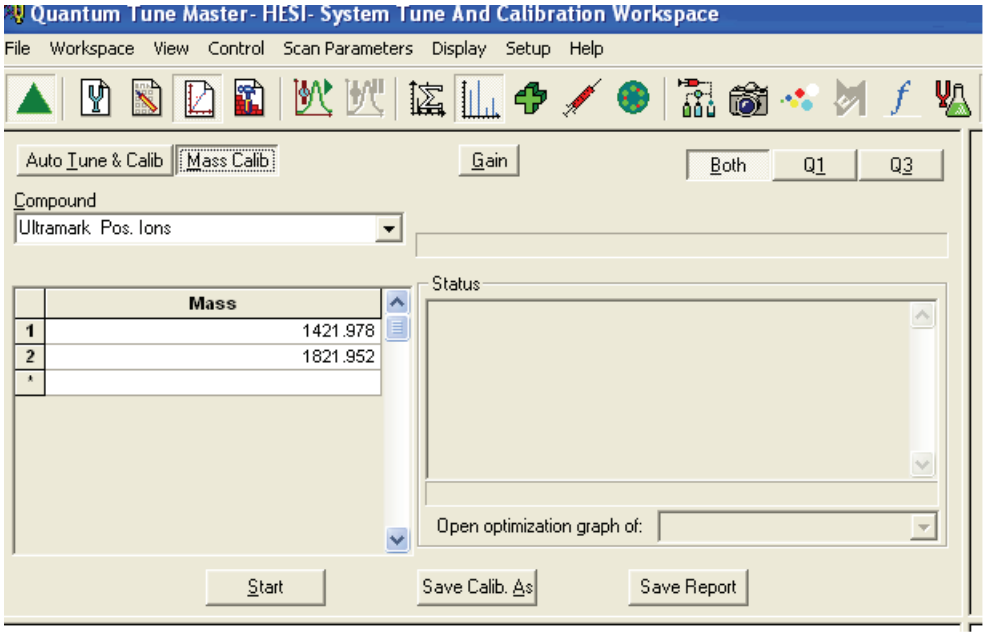
Figure 77. Optimization of the Q1 DC Offset in the Tune Table view



18. Click the **Q3** tab of the Device view.
19. Select **Q3 DC Offset**. Add -0.2 to the value for mass 997.389, and enter the sum in the Value box for mass 1821.950. Click **Apply**.

20. Click the **System Tune and Calibration Workspace** button to open the Tuning and Calibration Workspace.
21. Select **Ultramark Pos. Ions** in the Compound list. See Figure 78.
22. Click **Mass Calib** and **Both**, and then click **Start**.

Figure 78. Selecting Ultramark positive ions in the System Tune and Calibration Workspace



23. After the calibration is complete, choose **File > Save Tune As** to open the Save Tune File dialog box. Name the tune file **Ultramark Pos Ions.TSQTune**. Click **Save**.

24. Choose **File > Save Calibration As** to open the Save Calibration File dialog box. Name the calibration file **Ultramark Pos Ions.TSQCalib**. Click **Save**.

❖ To perform high-mass calibration in the negative polarity mode

Positive Polarity 

Negative Polarity 

1. Place the mass spectrometer in the negative ion polarity mode. (Ensure that the Polarity button is in the positive polarity state.)

2. Infuse Ultramark at a flow rate of 1 to 3 µL/min.

   In Full Scan mode your spectrum should look similar to the spectrum shown in Figure 79.

Figure 79. Ultramark 1621 negative ion spectrum

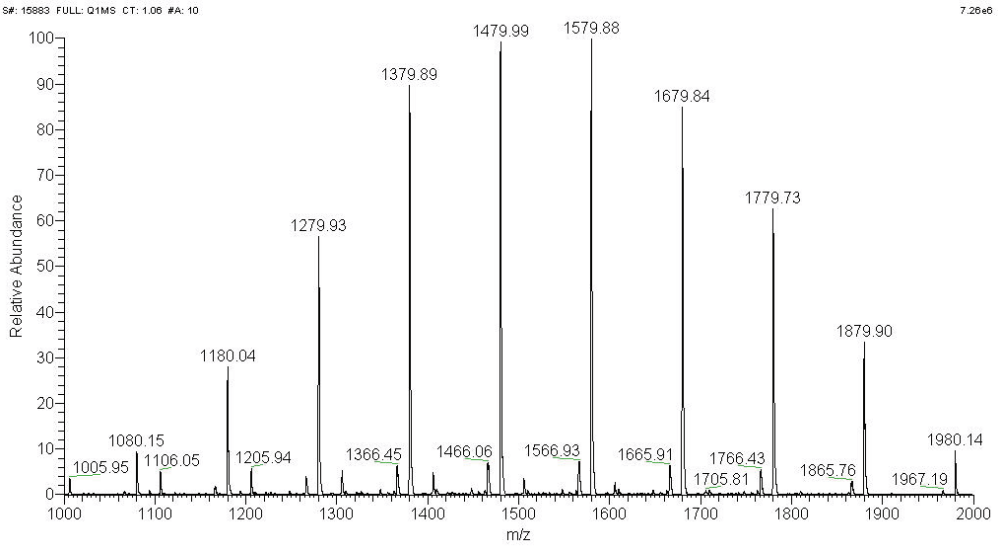


Table 7 lists the Ultramark 1621 negative ions.

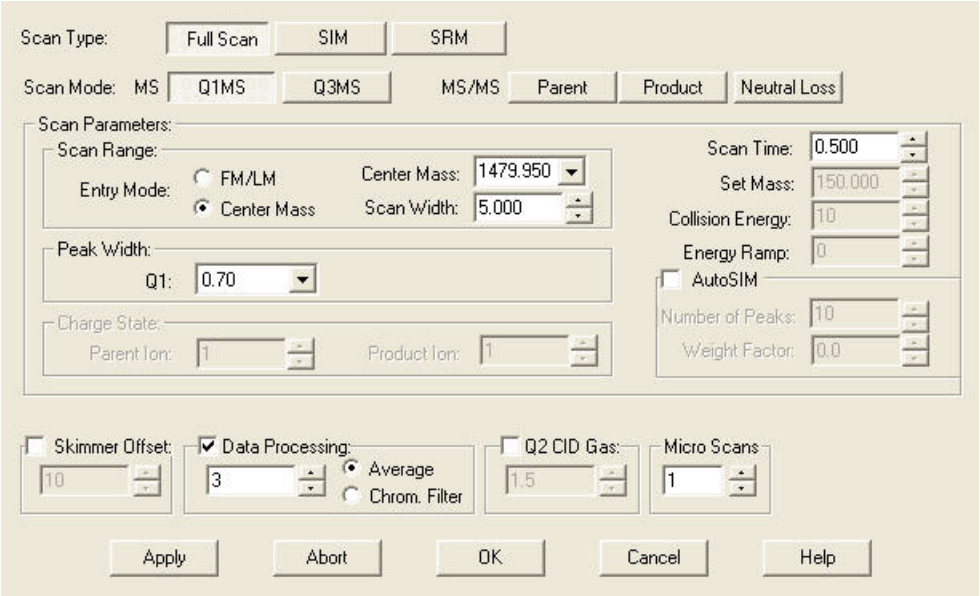
Table 7. Mass-to-charge ratios of Ultramark 1621 negative ions

Ultramark negative ions (*m/z*)
880.0228
980.0164
1080.0100
1180.0036
1279.9972
1379.9908
1479.9844
1579.9781
1679.9717
1779.9653
1879.9589
1979.9525
2079.9461
2179.9397

3. Click the **Instrument Method Development Workspace** button to display the Instrument Method Development Workspace.

4. In the Define Scan view, set the parameter values as shown in Figure 80 and click **Apply**.

Figure 80. Settings for high mass calibration in negative polarity mode in the Define Scan view



5. Click the **Full Instrument Control** button to display the Full Instrument Control Workspace.

6. Click the **Q1** tab of the Device view.

7. Select **Tube Lens Offset** and click **Optimize**. After the optimization procedure is complete, click **Accept**.

8. Select **Lens 1-2 Offset** and click **Optimize**. After the optimization procedure is complete, click **Accept**.

9. Select **Lens 2-1 Offset** and click **Optimize**. After the optimization procedure is complete, click **Accept**.

10. Click the **Q3** tab of the Device view.

11. Select **Tube Lens Offset** and click **Optimize**. After the optimization procedure is complete, click **Accept**.

12. Select **Lens 3-3 Offset** and click **Optimize**. After the optimization procedure is complete, click **Accept**.

13. Click the **Instrument Method Development Workspace** button to display the Instrument Method Development Workspace.

14. In the Define Scan view (Figure 75), under Scan Range select **1879.960** in the Center Mass box. Click **Apply**.
15. Repeat step 5 to step 12 for the Ultramark peak at *m/z* 1879.960. The signal intensity must be greater than 1.5×10^5 counts.
16. Click the **Q1** tab of the Device view.
17. Select **Q1 DC Offset**. Add +0.2 to the value for mass 995.383 and enter the sum in the Value box for mass 1879.960. Click **Apply**.
18. Click the **Q3** tab of the Device view.
19. Select **Q3 DC Offset**. Add +0.2 to the value for mass 995.383 and enter the sum in the Value box for mass 1879.960. Click **Apply**.

20. Click the **System Tune and Calibration Workspace** button to open the Tuning and Calibration Workspace.
21. In the System Tune and Calibration Workspace, select **Ultramark Neg. Ions** in the Compound list.
22. Click **Mass Calib** and **Both**, and then click **Start**.
23. After the calibration completes, choose **File > Save Tune As** to open the Save Tune File dialog box. Type the file name **Ultramark Neg Ions.TSQTune** and click **Save**.
24. Choose **File > Save Calibration As** to open the Save Calibration File dialog box. Select the file **ESI Polytyrosine Calibration.TSQCalib**. Type the file name **Ultramark Neg Ions.TSQCalib.** and click **Save**.

Accurate Mass Calibration

In addition to the standard tuning and calibrating procedures that you perform for all TSQ Series instruments, you must also perform an accurate mass calibration for the TSQ Quantum Ultra AM and TSQ Vantage AM instruments to complete the linearization of the mass axis. The tuning and calibration procedures for the TSQ Quantum Ultra AM and TSQ Vantage AM also include a high resolution calibration procedure to aid in peak shape optimization. You only need to perform the standard tuning and calibrating and high resolution (Hi Res) calibration after system shutdown or maintenance. You must perform mass calibration and the accurate mass calibration procedure as needed, however, to ensure accuracy of the mass axis.

Contents

High Resolution Calibration

To achieve the best results from your accurate mass experiments, optimize the peak shape. The more symmetrical the peak, the better the system is able to determine the centroid of the peak. The TSQ Quantum Ultra AM and TSQ Vantage AM systems have a calibration procedure built into the application to ensure that the system is attaining optimum peak shape and width.

❖ To perform a high resolution calibration

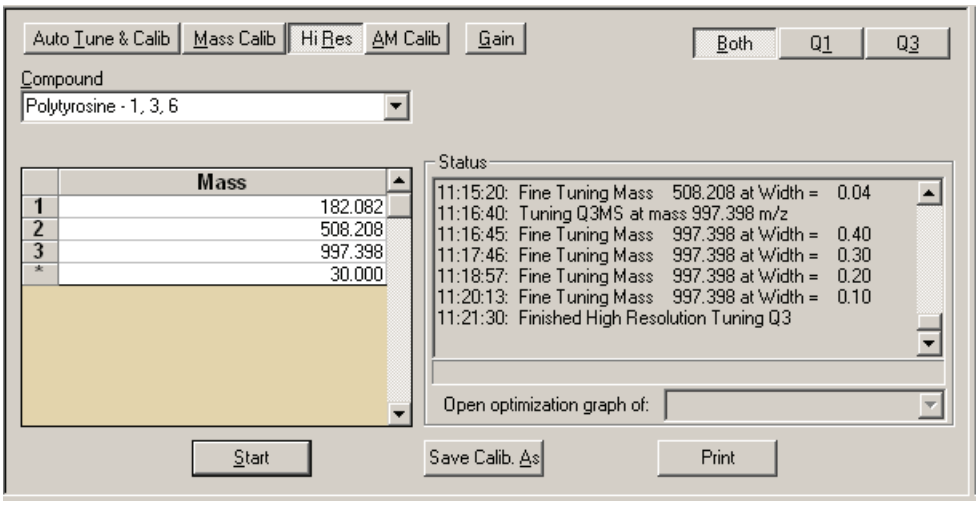
1. Using Tune Master, complete the standard automatic tuning and calibration procedure, detailed in Chapter 3, "Tuning and Calibrating the Mass Spectrometer."

2. With the polytyrosine 1, 3, 6 solution still in the syringe pump, click the **System Tune and Calibration Workspace** button to open the Tuning and Calibration Workspace.
3. Click **Hi Res** to open the High Resolution Calibration view of the workspace.
4. Click **Start**.
5. Monitor the status of the tuning procedure using the Status view. The procedure takes about 30 minutes to complete.

Note The Hi Res calibration is an algorithm that automatically optimizes Quad DC offset voltages to achieve the optimum peak shape for resolutions below 0.5 FWHM (full width half maximum). In some cases, the algorithm is unable to solve certain issues. For this reason, to achieve the optimum peak shape, you must complete the tuning procedure and then review each of the instrument parameters in the Full Instrument Control Workspace.

6. When the procedure completes successfully, click **Accept** to apply the settings to the mass spectrometer.
7. Click **Save Calib As** to save the calibration file. Save the file with a unique name so that you can recall calibration files for before and after tuning to observe the changes that the Hi Res calibration has made to the instrument calibration. See Figure 81.

Figure 81. A successfully completed high resolution calibration in the High Resolution Calibration view of the System Tune and Calibration Workspace



Accurate Mass Calibration

Before you begin accurate mass experiments, calibrate the mass axis. You have already performed the automatic tuning and calibration as described in Chapter 3, "Tuning and Calibrating the Mass Spectrometer," and also a high resolution calibration as described in the section "High Resolution Calibration." Now you must perform an accurate mass calibration to complete the mass axis linearization. It is not necessary to perform the accurate mass calibration before each experiment, because in a stable environment the drift in the mass axis might not be significant over moderate periods of time. To ensure the quality of your data, establish several QC samples across the mass range of interest that you can use to determine when recalibration is required.

❖ To perform accurate mass calibration

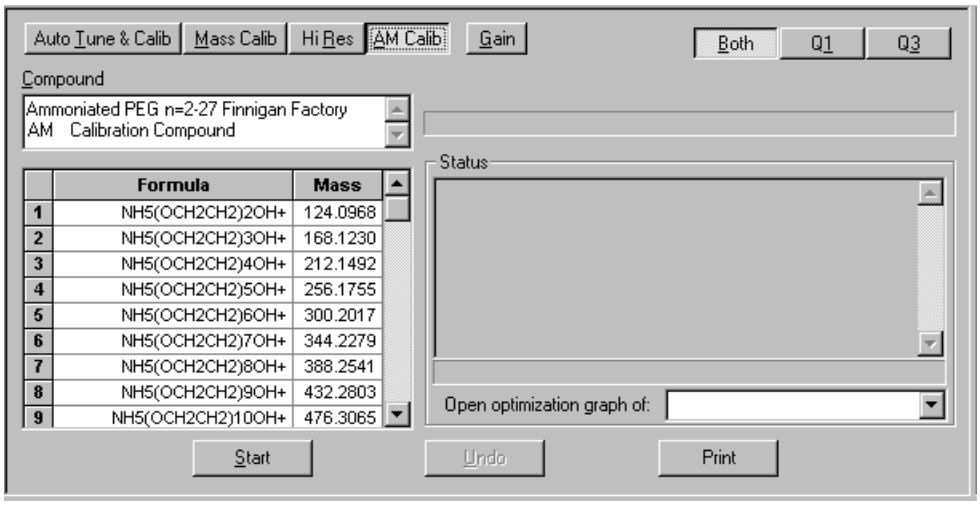
1. Using Tune Master, complete the automatic tuning and calibration procedure as described in Chapter 3, "Tuning and Calibrating the Mass Spectrometer," if you have not already done so.
2. Complete the high resolution calibration procedure as described in "High Resolution Calibration," if you have not already done so.
3. Remove the syringe of polytyrosine tuning and calibration solution from the syringe pump.
4. Remove the sample transfer line and install a new length of tubing.

   Note To minimize the possibility of cross-contamination of calibration solutions, for your accurate mass calibration solution use a different syringe and a different sample transfer line from ones you use for your samples and tuning and calibration solutions.

5. Run a small amount of 50:50 methanol/water solution through the TSQ Quantum Ultra AM or TSQ Vantage AM instrument.
6. Prepare the accurate mass calibration solution as described in "Accurate Mass Calibration Solution" on page 121.
7. Fill a clean, 500-µL Unimetrics syringe with the accurate mass calibration solution.
8. Begin infusing the accurate mass calibration solution. Allow several minutes for the flow (and spray) to stabilize. You might have make manual adjustments at the high and low masses (*m/z* 124 and *m/z* 1268) to obtain a stable spray and adequate signal.

9. Click the **System Tune and Calibration** button to display the System Tune and Calibration Workspace. See Figure 82.
10. To show the AM Calibration view of the workspace, click **AM Calib**.
11. To select calibration of the mass axis for both Q1 and Q3, click **Both**.
12. If the Ammoniated PEG list does not appear in the Calibration Mass table, import it.
13. In the Accurate Mass Calibration view, click **Start** to begin the accurate mass calibration.

Figure 82. AM Calibration view in the System Tune and Calibration Workspace



14. Monitor the results in the Status view.

> **Tip** At the start of the accurate mass calibration, each mass in the Calibration Mass table is scanned briefly to ensure that there is sufficient signal. While this is done, inspect each mass for proper peak shape. If the peak shape of any mass is not ideal, stop the calibration and perform a closer inspection of the ion.

15. When the calibration has completed successfully, view the results of the calibration by selecting an optimization graph from the list below the Status box.
16. Click **Accept** to accept the calibration, and then save the calibration file with a unique name.
17. Check the calibration tables to verify the success of the calibration.

You are ready to proceed with your accurate mass experiments.

Solution Formulations

This appendix provides instructions for preparing tuning and calibration solutions for normal mass range, high mass range, and accurate mass operation. It also provides instructions for preparing reserpine solutions that you can use to perform the compound optimization examples in Chapters 4, 5, 7, and 8.

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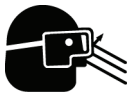

CAUTION AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS. Always wear protective gloves and safety glasses when you use solvents or corrosives. Also, contain waste streams and use proper ventilation. Refer to your supplier's Material Safety Data Sheet (MSDS) for the proper handling of a particular solvent.

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 acetic acid, methanol, and reserpine.

Polytyrosine – 1, 3, 6 Tuning and Calibration Solution

Two procedures for preparing solutions of polytyrosine – 1, 3, 6 are suitable for tuning and calibrating the mass spectrometer.

The first procedure describes how to reconstitute the tuning and calibration solution by using the 20 mL vial (P/N 00301-22925) 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 8 provides a summary of the polytyrosine standards supplied in the accessory kit.

Table 8. Polytyrosine standards supplied in the accessory kit

Standard description (on label)	Thermo Fisher Scientific 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 Solid

❖ **To reconstitute the polytyrosine – 1, 3, 6 tuning and calibration solution from the vial of polytyrosine solid**

1. Obtain the vial of premixed polytyrosine chemicals (P/N 00301-22925) 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

❖ **To prepare 250 mL of the polytyrosine – 1, 3, 6 tuning and calibration solution from your stock of dry chemicals**

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 9 provides a summary of the compounds used in the preparation of the tuning and calibration solution.

Table 9. 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 form Thermo Fisher Scientific (visit www.FisherLCMS.com), 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)

Ultramark 1621 High Mass Calibration Solution

Follow this next procedure to prepare a solution of Ultramark 1621 for calibrating the TSQ Quantum Access, TSQ Quantum Access MAX, TSQ Quantum Ultra EMR, or TSQ Vantage EMR for high mass range (*m/z* 1500 to 3000) operation. The high mass calibration procedure involves optimizing the lens parameters for the high mass peaks, performing a mass calibration, and then saving the calibration file. See "High Mass Calibration" on page 103. Refrigerated Ultramark 1621 calibration solution is good for two months.

❖ To prepare the Ultramark 1621 stock solution

1. Obtain the vial of Ultramark 1621 from the Access MAXory Kit.
2. Use a pipette to add 100 µL of Ultramark 1621 to a 100 mL volumetric flask.
3. Fill the 100 mL volumetric flask to volume with acetonitrile.
4. Transfer the solution to a clean, dry bottle.
5. Close the bottle and shake to mix the solution.
6. Label the bottle *Ultramark 1621 Stock Solution.*
7. Store the stock solution in a refrigerator. The stock solution is good for six months.

❖ To prepare 500 mL of the Ultramark 1621 high mass calibration solution

1. Obtain a clean, 1 L glass bottle.
2. Add 250 mL of acetonitrile to the 1 L glass bottle.
3. Pipette 50 mL of the Ultramark 1621 stock solution into the 1 L glass bottle.
4. Use a 1 mL syringe to transfer 5 mL of glacial acetic acid to the 1 L glass bottle. Do not use a plastic pipette.
5. Add 200 mL of 50:50 methanol/water to the 1 L glass bottle.
6. Close the bottle and shake to mix the solution.
7. Label the bottle *Ultramark 1621 Calibration Solution.*
8. Store the calibration solution in a refrigerator. The calibration solution is good for two months.

Accurate Mass Calibration Solution

For accurate mass calibration you must have compounds with well formed peaks spaced throughout your mass range of interest that you can use to create a calibration of the mass axis. The standard calibration utilizes ammoniated polyethylene glycols (ammoniated PEGs) with masses from 124 to 1268 u. You can alter this list with different compounds, or create custom lists to shorten the mass range to be calibrated.

Note The PEGs necessary for the calibration of your system are shipped in the TSQ Quantum Accurate Mass Calibration Compounds Kit (P/N 70111-62029). You must provide ammonium acetate and the required solvents to complete the necessary calibration during the demonstration of your system.

Procedures follow for the preparation of these solutions:

- PEG Stock Solution
- Accurate Mass Calibration Solution

PEG Stock Solution

❖ **To prepare a stock solution of 1000 pmol/µL PEG in 50:50 methanol/water**

Note All reagents must be of A.C.S. grade or better. The success of your experiments depends upon quality reagents.

1. Weigh out the following masses of PEG listed in Table 10.

   Table 10. PEGs used in stock solution

Average mass per mole	Thermo Fisher Scientific part number	Aldrich part number	Mass to add
200 g	00301-07712	20,236-3	0.020 g
400 g	00301-07714	20,239-8	0.040 g
600 g	00301-07716	20,240-1	0.060 g
1000 g	00301-07710	20,242-8	0.100 g

2. Add the PEG to a clean 100 mL volumetric flask.
3. Dissolve the PEG in 50:50 methanol/water to achieve a total volume of 100 mL.
4. Ensure that the PEG is thoroughly dissolved.
5. Label the vial bottle *PEG Stock Solution (1000 pmol/mL).*

Accurate Mass Calibration Solution

❖ **To prepare 100 mL of the 50 pmol/µL Ammoniated PEG stock solution in 50:50 methanol/water and 5 mM ammonium acetate**

1. Pipet 5 mL of the PEG stock solution into a clean 100 ml volumetric flask.
2. Add 0.04 g ammonium acetate (NH_4OAc).
3. Add 50:50 methanol/water to achieve a total volume of 100 ml.
4. Mix this solution thoroughly.
5. Label the vial *Accurate Mass Calibration Solution (50 pmol/mL)* and store it in a refrigerator until it is needed.

Reserpine Solutions

Follow these directions to prepare a 2 pg/µL stock solution of reserpine. (This can be used as the TSQ Quantum Access or TSQ Quantum Access MAX compound optimization solution.) Then, use serial dilutions of the stock solution to make the compound optimization solution for theTSQ Quantum Ultra (200 fg/µL) or the TSQ Vantage (100 fg/µL).

❖ **To prepare a 2 pg/µL reserpine solution**

1. Prepare a 1 mg/mL reserpine solution:
   a. Add 100 mg of reserpine to a 100 mL volumetric flask.
   b. Fill the 100 mL flask to volume with a solution of methanol containing 1% acetic acid. Mix the contents thoroughly.
2. Prepare a 10 ng/µL reserpine solution:
   a. Use a volumetric pipette to transfer 1 mL of the 1 mg/mL solution to a 100 mL volumetric flask.
   b. Fill the 100 mL flask to volume with a solution of methanol containing 1% acetic acid. Mix the contents thoroughly.
3. Prepare a 100 pg/µL reserpine solution:
   a. Use a volumetric pipette to transfer 1 mL of the 10 ng/mL solution to a 100 mL volumetric flask.
   b. Fill the 100 mL flask to volume with a solution of methanol containing 1% acetic acid. Mix the contents thoroughly.
4. Prepare a 2 pg/µL reserpine solution:
   a. Use a volumetric pipette to transfer 2 mL of the 100 pg/µL to a 100 mL volumetric flask.
   b. Fill the 100 mL flask to volume with a solution of methanol containing 1% acetic acid. Mix the contents thoroughly.
5. Transfer the solution to a clean, dry bottle and label it *Reserpine Stock Solution (2 pg/µL)* or *TSQ Quantum Access MAX Sample Solution (2 pg/µL).*

❖ To prepare a 200 fg/µL reserpine solution

1. Use a volumetric pipette to transfer 10 mL of the 2 pg/µL reserpine stock solution to a 100 mL volumetric flask.
2. Fill the 100 mL flask to volume with a solution of methanol containing 1% acetic acid. Mix the contents thoroughly.
3. Transfer the solution to a clean, dry bottle and label it *TSQ Quantum Ultra Sample Solution (200 fg/µL).*

❖ To prepare a 100 fg/µL reserpine solution

1. Use a volumetric pipette to transfer 5 mL of the 2 pg/µL reserpine stock solution to a 100 mL volumetric flask.
2. Fill the 100 mL flask to volume with a solution of methanol containing 1% acetic acid. Mix the contents thoroughly.
3. Transfer the solution to a clean, dry bottle and label it *TSQ Vantage Sample Solution (100 fg/µL).*

Instrument Method Development Guidelines

This appendix provides guidelines for developing instrument methods. Note that the settings provided here might not be directly applicable to your application because they demonstrate various analytical techniques using a polytyrosine sample. When you develop an instrument method for your application, first fine tune the instrument by optimizing on your compound.

The following tables contain instrument settings that you can apply to your mass spectrometer using the Instrument Method Development Workspace of Tune Master. With these settings you can immediately assess instrument performance with your analyte before you build a method. Once you achieve the desired result using Tune Master, you can copy and paste the scan event into the Instrument Setup window.

❖ **To copy the scan event from Tune Master**

Right-click 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 the Scan Editor page of Instrument Setup, and select **Paste Scan Event** from the shortcut menu.

Table 11. Instrument parameters for full scan Q1MS

Entry mode	First mass (*m/z*)	Last mass (*m/z*)	Scan time (s)	Q1 Peak width (u)	Skimmer offset	Data processing	Q2 CID gas
FM/LM	150.000	1050.000	0.65	0.70	Off	10 spectrum avg.	Off

Table 12. Instrument parameters for full scan Q3MS

Entry mode	Center mass (*m/z*)	Scan width (u)	Scan time (s)	Skimmer offset	Data processing	Q2 CID gas
Center mass	182.082	6.000	0.20	Off	10 spectrum avg.	Off

Table 13. 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)	Skimmer offset	Data processing	Q2 CID gas (mTorr)
Center mass	182.082	10.000	0.20	136.076	20	0.70	0.70	Off	10 spectrum avg.	0.8*

* Q2 CID gas pressure is reduced in the parent MS/MS scan mode to preserve peak shape and peak resolution.

Table 14. 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)	Skimmer offset	Data processing	Q2 CID gas (mTorr)
Center mass	182.082	10.000	0.20	182.082	18	0.70	0.70	Off	10 spectrum avg.	1.5

Table 15. 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)	Charge state	Skimmer offset	Data processing	Q2 CID gas (mTorr)
Center mass	182.082	6.000	0.20	17.027	10	0.70	1 (for both Q1 & Q3)	Off	10 spectrum avg.	1.5

Table 16. 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	Skimmer offset	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 17. 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)	Use tuned lens value	Skimmer offset	Data processing	Q2 CID gas
182.082	6.000	0.20	136.076	20	0.70	On	Off	10 spectrum avg.	0.8*
508.208	6.000	0.20			0.70	On	Off	10 spectrum avg.	0.8*

*Q2 CID gas pressure is reduced in the parent MS/MS scan mode to preserve peak shape and peak resolution.

Table 18. 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)	Use tuned lens value	Skimmer offset	Data processing	Q2 CID gas
136.076	6.000	0.20	182.082	18	0.70	On	Off	10 spectrum avg.	1.5
165.055	6.000	0.20			0.70	On	Off	10 spectrum avg.	1.5

Table 19. 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	Skimmer offset	Data processing	Q2 CID gas
182.082	6.000	0.20	17.027	0.70	18	On	Off	10 spectrum avg.	1.5

Table 20. 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)	Skimmer offset	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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