

# LTQ Series

## Getting Started Guide

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DOCUMENTATION  
**SURVEY**

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Software version: Thermo LTQ Tune Plus version 2.7.0 or later; Microsoft Windows 7 Professional SP1—Thermo Foundation version 2.0 or later, and Thermo Xcalibur version 2.2 or later; Windows XP Workstation SP3—Foundation version 1.0.2 SP2 or earlier, and Xcalibur version 2.1 SP1 or earlier

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## Regulatory Compliance

Thermo Fisher Scientific performs complete testing and evaluation of its products to ensure full compliance with applicable domestic and international regulations. When the system is delivered to you, it meets all pertinent electromagnetic compatibility (EMC) and safety standards as described in the next section or sections by product name.

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Regulatory compliance results for the following Thermo Scientific products:

- [LXQ Mass Spectrometer \(February 2005\)](#)
- [LTQ XL Mass Spectrometer \(September 2006\)](#)
- [LTQ XL/ETD System \(January 2007\)](#)
- [MALDI LTQ XL System \(August 2007\)](#)
- [LTQ Velos Mass Spectrometer \(August 2008\)](#)
- [LTQ Velos/ETD System \(November 2008\)](#)
- [Velos Pro Mass Spectrometer \(April 2011\)](#)
- [Velos Pro/ETD System \(April 2011\)](#)

### LXQ Mass Spectrometer (February 2005)

#### **EMC Directive 89/336/EEC as amended by 92/31/EEC and 93/68/EEC**

EMC compliance has been evaluated by Underwriters Laboratories, Inc.

EN 55011: 1998	EN 61000-4-3: 2002, A1: 2002
EN 61000-3-2: 1995, A1: 1998, A2: 1998, A14: 2000	EN 61000-4-4: 1995, A1: 2001, A2: 2001
EN 61000-3-3: 1995	EN 61000-4-5: 1995, A1: 2001
EN 61326-1: 1997	EN 61000-4-6: 1996, A1: 2001
EN 61000-4-2: 1995, A1: 1998, A2: 2001	EN 61000-4-11: 1994, A1: 2001
FCC Class A, CFR 47 Part 15, Subpart B: 2004	CISPR 11: 1999, A1: 1999, A2: 2002

#### **Low Voltage Safety Compliance**

This device complies with Low Voltage Directive 73/23/EEC and harmonized standard EN 61010-1:2001.

## LTQ XL Mass Spectrometer (September 2006)

### EMC Directive 89/336/EEC

EMC compliance has been evaluated by TUV Rheinland of North America, Inc.

EN 55011: 1998, A1: 1999, A2: 2002	EN 61000-4-3: 2002
EN 61000-3-2: 1995, A1: 1998, A2: 1998, A14: 2000	EN 61000-4-4: 1995, A1: 2001, A2: 2001
EN 61000-3-3: 1995, A1:2001	EN 61000-4-5:1995, A1: 2001
EN 61326-1: 1997, A1: 1998, A2: 2001, A3: 2003	EN 61000-4-6: 2003
EN 61000-4-2: 2001	EN 61000-4-11: 2001
FCC Class A, CFR 47 Part 15: 2005	CISPR 11: 1999, A1: 1999, A2: 2002

### Low Voltage Safety Compliance

This device complies with Low Voltage Directive 73/23/EEC and harmonized standard EN 61010-1:2001.

## LTQ XL/ETD System (January 2007)

### EMC Directive 89/336/EEC

EMC compliance has been evaluated by TUV Rheinland of North America, Inc.

EN 61000-3-2: 1995, A1: 1998, A2: 1998, A14: 2000	EN 61000-4-4:1995, A1: 2000, A2:2001
EN 61000-3-3: 1995, A1:2001	EN 61000-4-5: 1995, A1: 2001
EN 61326-1: 1997, A1:1998, A2:2001, A3:2003	EN 61000-4-6: 2003
EN 61000-4-2: 2001	EN 61000-4-11: 1994, A1: 2001
EN 61000-4-3: 2002	CISPR 11: 1999, A1: 1999, A2: 2002
FCC Class A, CFR 47 Part 15: 2005	

### Low Voltage Safety Compliance

This device complies with Low Voltage Directive 73/23/EEC and harmonized standard EN 61010-1:2001.



## MALDI LTQ XL System (August 2007)

### EMC Directive 2004/108/EC

EMC compliance has been evaluated by TUV Rheinland of North America, Inc.

EN 55011: 1998, A1: 1999, A2: 2002	EN 61000-4-3: 2002
EN 61000-3-2: 2000	EN 61000-4-4: 1995, A1: 2000, A2: 2001
EN 61000-3-3: 1995, A1: 2001	EN 61000-4-5: 2001
EN 61326-1: 1998, A2: 2001, A3: 2003	EN 61000-4-6: 2003
EN 61000-4-2: 2001	EN 61000-4-11: 2001
FCC Class A, CFR 47 Part 15: 2006	CISPR 11: 1998, A1:1999, A2: 2002

### Low Voltage Safety Compliance

This device complies with Low Voltage Directive 2006/95/EC and harmonized standard EN 61010-1:2001.

### Safety of Laser Products

Compliance with safety of laser products is declared under Thermo Fisher Scientific sole responsibility. This device complies with the harmonized standard IEC/EN 60825-1/A2: 2001.

## LTQ Velos Mass Spectrometer (August 2008)

### EMC Directive 2004/108/EEC

EMC compliance has been evaluated by TUV Rheinland of North America, Inc.

EN 55011: 2007, A2: 2007	EN 61000-4-3: 2006
EN 61000-3-2: 2006	EN 61000-4-4: 2004
EN 61000-3-3: 1995, A1: 2001, A2: 2005	EN 61000-4-5: 2005
EN 61326-1: 2006	EN 61000-4-6: 2007
EN 61000-4-2: 1995, A1: 1999, A2: 2001	EN 61000-4-11: 2004
FCC Class A, CFR 47 Part 15: 2007	

### Low Voltage Safety Compliance

This device complies with Low Voltage Directive 2006/95/EEC and harmonized standard EN 61010-1:2001.

## LTQ Velos/ETD System (November 2008)

### EMC Directive 2004/108/EEC

EMC compliance has been evaluated by TUV Rheinland of North America, Inc.

EN 61326-1: 2006	EN 61000-4-4: 2004
EN 55011: 2007	EN 61000-4-5: 2005
EN 61000-3-2: 2006	EN 61000-4-6: 2007
EN 61000-3-3: 2005	EN 61000-4-11: 2004
EN 61000-4-2: 2001	FCC Part 15: 2007
EN 61000-4-3: 2006	

## Velos Pro Mass Spectrometer (April 2011)

### EMC Directive 2004/108/EEC

EMC compliance has been evaluated by TUV Rheinland of North America, Inc.

EN 61326-1: 2006	EN 61000-4-3: 2006
EN 55011: 2007, A2: 2007	EN 61000-4-4: 2004
CFR 47, FCC Part 15, Subpart B, Class A: 2009	EN 61000-4-5: 2005
EN 61000-3-2: 2006	EN 61000-4-6: 2007
EN 61000-3-3: 1995, A1: 2001, A2: 2005	EN 61000-4-11: 2004
EN 61000-4-2: 1995, A1: 1999, A2: 2001	

### Low Voltage Safety Compliance

This device complies with Low Voltage Directive 2006/95/EEC and harmonized standard EN 61010-1:2001.

## Velos Pro/ETD System (April 2011)

### EMC Directive 2004/108/EEC

EMC compliance has been evaluated by TUV Rheinland of North America, Inc.

EN 61326-1: 2006	EN 61000-4-3: 2006
EN 55011: 2007, A2: 2007	EN 61000-4-4: 2004
CFR 47, FCC Part 15, Subpart B, Class A: 2009	EN 61000-4-5: 2005
EN 61000-3-2: 2006	EN 61000-4-6: 2007
EN 61000-3-3: 1995, A1: 2001, A2: 2005	EN 61000-4-11: 2004
EN 61000-4-2: 1995, A1: 1999, A2: 2001	

### Low Voltage Safety Compliance

This device complies with Low Voltage Directive 2006/95/EEC and harmonized standard EN 61010-1:2001.

## FCC Compliance Statement

THIS DEVICE COMPLIES WITH PART 15 OF THE FCC RULES. OPERATION IS SUBJECT TO THE FOLLOWING TWO CONDITIONS: (1) THIS DEVICE MAY NOT CAUSE HARMFUL INTERFERENCE, AND (2) THIS DEVICE MUST ACCEPT ANY INTERFERENCE RECEIVED, INCLUDING INTERFERENCE THAT MAY CAUSE UNDESIRE OPERATION.



**CAUTION** Read and understand the various precautionary notes, signs, and symbols contained inside this manual pertaining to the safe use and operation of this product before using the device.

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## Preface

The *LTQ Series Getting Started Guide* describes how to set up, calibrate, and tune the following LTQ™ Series mass spectrometers (MSs):

- LXQ™, a single-segment 2D linear ion trap mass spectrometer
- LTQ XL™, a three-segment 2D linear ion trap mass spectrometer
- LTQ Velos™, a dual-cell 2D linear ion trap mass spectrometer
- Velos Pro™, a dual-cell 2D linear ion trap mass spectrometer

**Note** Unless otherwise noted:

- For the LTQ mass spectrometer, follow the LTQ XL information.
- For the LTQ Velos mass spectrometer, follow the Velos Pro information.

### Contents

- [Related Documentation](#)
- [Getting a Trap-HCD License](#)
- [Safety and Special Notices](#)
- [Contacting Us](#)

### ❖ To suggest changes to documentation or to Help

Complete a brief survey about this document by clicking the button below. Thank you in advance for your help.



## Related Documentation

In addition to this guide, Thermo Fisher Scientific provides the documentation listed in [Table 1](#) for the LTQ Series mass spectrometers. These PDF files are accessible from the data system computer.

**Table 1.** LTQ Series mass spectrometers documentation

Model	Related documents
LXQ, LTQ XL, Velos Pro	<i>LTQ Series Hardware Manual</i>
LXQ, LTQ XL, Velos Pro, LTQ XL/ETD system, Velos Pro/ETD system, MALDI LTQ XL system	<i>LTQ Series Preinstallation Requirements Guide</i> <i>LTQ Series Getting Connected Guide</i>
ETD module	<i>ETD Module Getting Started Guide</i> <i>ETD Module Hardware Manual</i>
MALDI source	<i>MALDI Source Getting Started Guide</i> <i>MALDI Source Hardware Manual</i>

To access the manuals for the mass spectrometer, from the Microsoft™ Windows™ taskbar, choose **Start > Programs > Thermo Instruments > Manuals > model**, where *model* is your specific model, and then click the PDF file you want to view.

**Note** For Xcalibur data system version 2.0.7 or earlier, choose **Start > Programs > Xcalibur > Manuals > LTQ > model**.

The software also provides Help. To access the Help, choose **Help** from the menu bar.



## Getting a Trap-HCD License

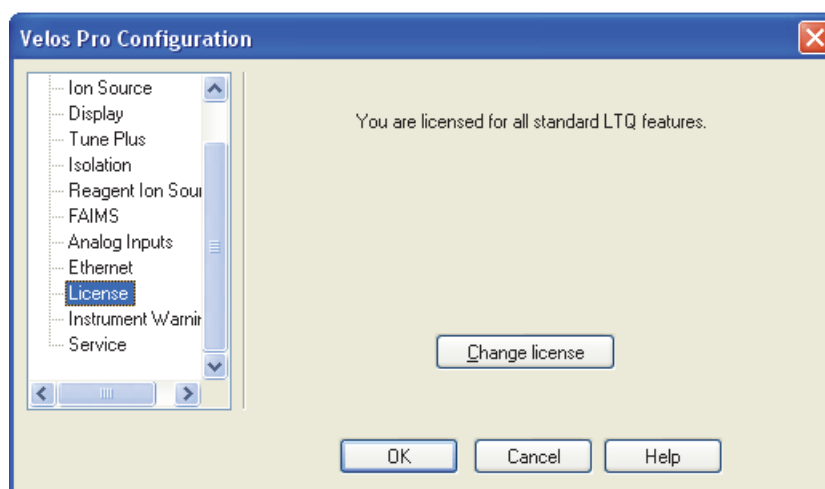
Ion trap higher energy collision-induced dissociation (Trap-HCD) fragmentation is an optional feature for the Velos Pro mass spectrometer. If you purchased this option, you must obtain a new license from Thermo Fisher Scientific and install it in your system before you can use this feature.

## Getting a New License Code

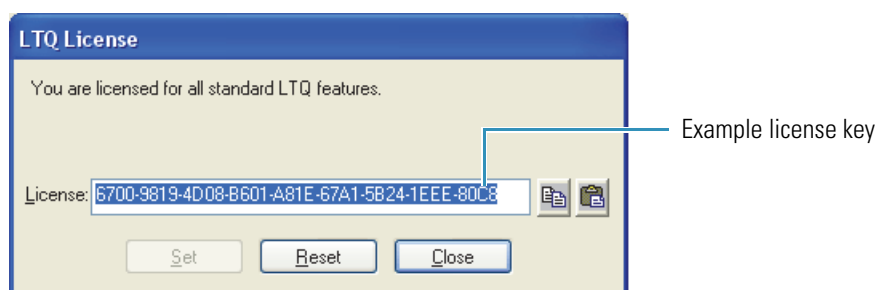
You can request a license code through e-mail or fax (see [page xxiv](#)). These instructions cover e-mail requests only that you send to a specific Thermo Fisher Scientific e-mail address.

### ❖ To get a Trap-HCD license code

1. Choose **Start > Programs > Thermo Foundation > Instrument Configuration** to open the Thermo Foundation Instrument Configuration window.
2. Under Available Devices, select the **Velos Pro MS** icon and click **Add**.
3. Under Configured Devices, select the **Velos Pro MS** icon and click **Configure** to open the Velos Pro Configuration dialog box.
4. Select License and click **Change License** to open the LTQ License dialog box.



5. Highlight the license key in the License box.



6. Press CTRL+C to copy the license key to the Windows Clipboard.

7. Send an e-mail message to [ThermoMSLicensing@thermofisher.com](mailto:ThermoMSLicensing@thermofisher.com):
  - In the Subject line, type **License Request**.
  - In the body of the e-mail message, paste the license key and type your name, company name, and phone number.
  - If you purchased the Trap-HCD option, locate the bar code on the *License Trap-HCD for Velos Pro* card that came with the instrument. In the body of the e-mail message, type the product key that appears below the bar code.
  - If you did not purchase the Trap-HCD option with the instrument, contact your local Thermo Fisher Scientific Technical Sales representative.

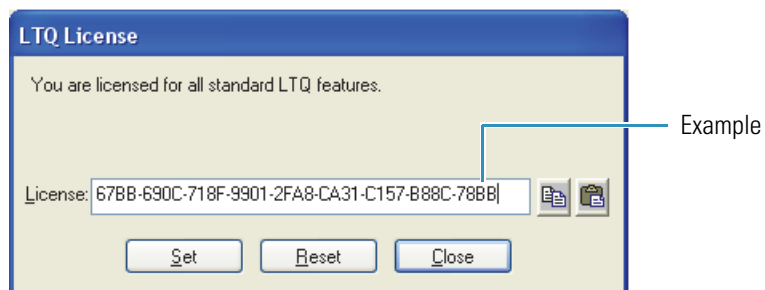
When Thermo Fisher Scientific Customer Support sends you a new license code, see “[Installing a New License Code](#).”

## Installing a New License Code

After you receive your new license code, follow this procedure.

### ❖ To install the HCD license number

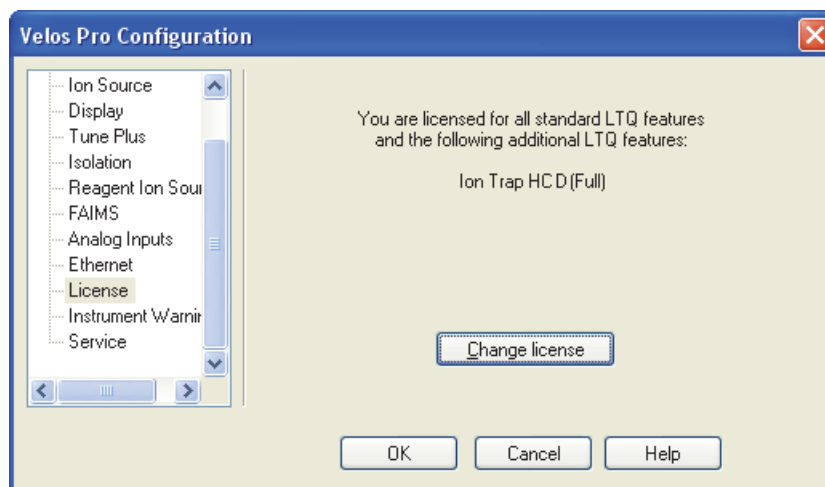
1. Open the LTQ License dialog box (see [step 1](#) through [step 4](#) on [page xxi](#)).
2. In the License box, paste a copy of the new license number from the e-mail message and click **Set**.



3. Click **OK** when the following message appears:

The new license number has been set.

- In the Velos Pro Configuration dialog box, verify the addition of the Ion Trap HCD (Full) feature and click **OK**.



- Reboot the data system and then the Velos Pro mass spectrometer.

## Safety and Special Notices

Ensure that 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.





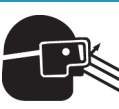
**IMPORTANT** Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.

**Note** Highlights information of general interest.

**Tip** Highlights helpful information that can make a task easier.

Table 2 lists additional caution-specific symbols that appear in the *LTQ Series Getting Started Guide*.

**Table 2.** Caution-specific symbols and their meanings

Symbol	Meaning
	<b>Electric Shock:</b> An electric shock hazard is present in the instrument. Proceed with caution.
	<b>Hot Surface:</b> Allow heated components to cool before touching or servicing the instrument.
	<b>Sharp Object:</b> A sharp object is present in the instrument. Proceed with caution.
	<b>Chemical:</b> Hazardous chemicals might be present in the instrument. Wear gloves when handling carcinogenic, corrosive, irritant, mutagenic, or toxic chemicals. Use only approved containers and procedures for disposing of waste oil.
	<b>Eye Hazard:</b> Eye damage could occur from splattered chemicals or airborne particles. Wear safety glasses when handling chemicals or servicing the instrument.

## Contacting Us

There are several ways to contact Thermo Fisher Scientific for the information you need.

### ❖ To contact Technical Support

Phone	800-532-4752
Fax	561-688-8736
E-mail	<a href="mailto:us.techsupport.analyze@thermofisher.com">us.techsupport.analyze@thermofisher.com</a>
Knowledge base	<a href="http://www.thermokb.com">www.thermokb.com</a>

Find software updates and utilities to download at [mssupport.thermo.com](http://mssupport.thermo.com).

### ❖ To contact Customer Service for ordering information

Phone	800-532-4752
Fax	561-688-8731
E-mail	<a href="mailto:us.customer-support.analyze@thermofisher.com">us.customer-support.analyze@thermofisher.com</a>
Web site	<a href="http://www.thermo.com/ms">www.thermo.com/ms</a>

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Go to [www.thermoscientific.com/wps/portal/ts/contactus](http://www.thermoscientific.com/wps/portal/ts/contactus).

❖ **To copy manuals from the Internet**

Go to [mssupport.thermo.com](http://mssupport.thermo.com), agree to the Terms and Conditions, and then click **Customer Manuals** in the left margin of the window.

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- Fill out a reader survey online at [www.surveymonkey.com/s/PQM6P62](http://www.surveymonkey.com/s/PQM6P62).
- Send an e-mail message to the Technical Publications Editor at [techpubs-lcms@thermofisher.com](mailto:techpubs-lcms@thermofisher.com).



# Introduction

This chapter provides general information about the LTQ Series mass spectrometer. For additional information, such as procedures for daily operation, maintenance, and system startup and shutdown, refer to the *LTQ Series Hardware Manual*.

**Note** Unless otherwise noted:

- For the LTQ mass spectrometer, follow the LTQ XL information.
- For the LTQ Velos mass spectrometer, follow the Velos Pro information.

The “[Glossary](#)” on [page 173](#) defines some of the terms used in this guide.

## Contents

- [Features of the LTQ Series Mass Spectrometer](#)
- [Sheath, Auxiliary, and Sweep Gases](#)
- [Ionization Techniques](#)
- [Sample Introduction Techniques](#)
- [Types of Buffers](#)
- [LC Flow Rate Ranges](#)
- [Tuning and Calibrating the Mass Spectrometer](#)
- [Ion Polarity Modes](#)
- [Data Types](#)
- [Scan Power and Scan Modes](#)
- [Scan Rates](#)
- [Scan Types](#)
- [Types of Experiments](#)
- [Mass/Charge Range](#)

## Features of the LTQ Series Mass Spectrometer

The LTQ Series mass spectrometer has the following features:

- [Linear Ion Trap Mass Analyzer](#)
- [ZoomScan Analysis for Charge State Determination](#)
- [Wideband Activation](#)
- [Switchable Ion Source](#)
- [Optional Ion Sweep Cone and Ion Sweep Gas for Complex Sample Matrices](#)

### Linear Ion Trap Mass Analyzer

All [high performance liquid chromatography \(HPLC\)](#) systems can provide chromatographic separation and compound detection based on retention time. Retention time alone, however, does not positively identify a compound because many compounds can have the same retention time under the same experimental conditions. In addition, other compounds in the sample can coelute with the compound of interest. The coelution of compounds causes quantitation errors.

The attribute that sets the LTQ Series mass spectrometer apart from other detectors used with LC systems, such as UV-Vis detectors or single-stage [mass analyzer](#) mass spectrometers, is the high level of analytical specificity that it provides. In addition to [mass-to-charge ratio \(m/z\)](#), the LTQ Series mass spectrometer can provide multiple levels of [mass analysis](#). Each level of mass analysis adds a new dimension of specificity for unequivocal compound identification. The LTQ Series mass spectrometer provides the following levels of mass analysis:

- Single-stage mass analysis (provides molecular mass information)
- Two-stage mass analysis, MS/MS (provides structural information)
- Multi-stage mass analysis, MS<sup>n</sup> (provides structural information)

Single-stage mass analysis identifies analytes based on molecular mass information. Typically, [atmospheric pressure ionization \(API\)](#) produces mass spectra that provide molecular mass information.

Two-stage mass analysis can provide even more certainty in compound identification. MS/MS analysis monitors how a [parent ion](#) fragments when exposed to an additional stage of excitation. There are two types of MS/MS analysis:

- Full-scan MS/MS monitors the production of all [product ions](#) from a specific parent ion.
- Selected reaction monitoring (SRM) MS/MS analysis monitors a specific reaction path, which is the production of a specific product ion from a specific parent ion.



Using either type of MS/MS analysis, you can quantify target analytes in complex matrices such as plant or animal tissue, plasma, urine, groundwater, or soil. Because of the specificity of MS/MS measurements and the ability to eliminate interferences by an initial mass selection stage, the LTQ Series mass spectrometer easily accomplishes [quantitative analysis](#) of target compounds.

Multi-stage mass analysis provides a unique capability to obtain structural information that can be useful in structure elucidation of metabolites, natural products, and sugars. MS<sup>n</sup> techniques on the LTQ Series mass spectrometer allow for stepwise fragmentation pathways, making interpretation of MS<sup>n</sup> spectra relatively straightforward. The LTQ Series mass spectrometer has several advanced features that make its MS<sup>n</sup> capabilities extremely powerful for [qualitative analysis](#).

For additional information, see [“Types of Experiments”](#) on [page 20](#).

## ZoomScan Analysis for Charge State Determination

The [ZoomScan scan type](#) analysis provides information about the charge state of one or more mass ions. ZoomScan™ data is collected by using slower scan rates that give higher resolution. This level of resolution can determine the charge state of an ion without ambiguity.

For additional information, see [“ZoomScan and UltraZoom”](#) on [page 19](#).

## Wideband Activation

Use the [WideBand Activation™](#) option for the LTQ Series mass spectrometer to apply [collision energy](#) to ions during MS/MS fragmentation over a fixed mass range of 20 Da. With this option, you can apply collision energy to both the parent ion and the product ions created as a result of non-specific losses of water (18 Da) or ammonia (17 Da), or to product ions formed from the loss of fragments smaller than 20 Da. For enhanced structural information without resorting to MS<sup>3</sup> analysis, choose the Wideband Activation option for qualitative MS/MS analysis. Because the fragmentation efficiency is somewhat reduced with Wideband Activation, you must increase the collision energy.

For an example procedure, see [“Optimizing the Collision Energy Automatically for an MS/MS Experiment”](#) on [page 103](#).

## Switchable Ion Source

You can configure the LTQ Series mass spectrometer with a standard [electrospray ionization \(ESI\)](#) probe (see [Figure 16](#) on [page 42](#)), a [heated-electrospray ionization \(H-ESI\)](#) probe (see [Figure 17](#) on [page 42](#)), or an [atmospheric pressure chemical ionization \(APCI\)](#) probe (see [Figure 31](#) on [page 56](#)). By using additional ion source kits, you can operate the mass spectrometer in the [atmospheric pressure photoionization \(APPI\)](#) mode and the [nanospray ionization \(NSI\)](#) mode.

For information about choosing the appropriate ionization source for the analytes, see [“Ionization Techniques”](#) on [page 5](#). For instructions about installing the ESI or APCI probe, see [Chapter 2](#), [“Setting Up the API Source.”](#)

## Optional Ion Sweep Cone and Ion Sweep Gas for Complex Sample Matrices

The [ion sweep cone](#) is a metal cone installed over the ion transfer tube. The ion sweep cone channels the sweep gas toward the entrance of the [API 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 to analyze without a loss of signal intensity. In addition, keeping the ion transfer tube entrance as clean as possible reduces the need for frequent maintenance.

For instructions about installing the ion sweep cone, refer to the *LTQ Series Hardware Manual*.

## Sheath, Auxiliary, and Sweep Gases

The LTQ Series mass spectrometer uses nitrogen as the sheath, auxiliary, and sweep gases. The ESI and APCI probes have inlets for the sheath (S) gas and auxiliary (A) gas. The optional ion sweep cone has an inlet for the sweep gas. The NSI probe does not use desolvation gases.

The descriptions of these gases are as follows:

- Sheath gas—An inner-coaxial gas that helps nebulize the sample solution into a fine mist as the solution exits the probe nozzle.
- Auxiliary gas—An outer-coaxial gas that helps the sheath gas in the nebulization and evaporation of the sample solution by focusing the vapor plume and lowering the humidity in the ion source.
- Sweep gas—An off-axis gas that flows out from behind the optional ion sweep cone.

Install the ion sweep cone to improve ruggedness when analyzing complex matrices such as plasma or nonvolatile salt buffers.

Auxiliary gas is not required for low-solvent flow applications. For high-solvent flow applications, first optimize the sheath gas flow, then the auxiliary gas flow, and then the sweep gas flow, if provided.

[Table 3](#) on [page 12](#) and [Table 4](#) on [page 13](#) list the guidelines for the operating parameters.

## Ionization Techniques

Typically, ESI is the preferred ionization mode for more polar compounds such as amines, peptides, and proteins. APCI is the preferred ionization mode for non-polar compounds such as steroids.

### Using ESI or H-ESI

ESI is a soft ionization technique. The ESI source transfers ions in solution to the gas phase. ESI can analyze many samples that previously were not suitable for mass analysis (for example, heat-labile compounds or high molecular mass compounds). You can use ESI to analyze any polar compound that is an ion in solution, including adduct ions. Included in this class of compounds are biological polymers (such as proteins, peptides, glycoproteins, and nucleotides), pharmaceuticals and their metabolites, and industrial polymers. For example, you might analyze polyethylene glycols from a solution containing ammonium acetate because of adduct formation between  $\text{NH}_4^+$  ions in the solution and oxygen atoms in the polymer. With ESI, the range of molecular masses that the LTQ Series mass spectrometer can analyze can be greater than 50 000 Da if there is multiple charging.

The ESI source can produce multiply-charged ions, depending on the structure of the analyte and the solvent. For example, the mass spectrum of a protein or peptide typically consists of a distribution of multiply-charged analyte ions. You can mathematically manipulate this mass spectrum to determine the molecular mass of the sample.

Use ESI in either positive or negative ion polarity mode. The polarity of the 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. The ESI needle can be either positively or negatively charged. When it is positively charged, it generates positive ions. When it is negatively charged, it generates negative ions.

Vary the flow rate into the mass spectrometer over a range of 0.1–1000  $\mu\text{L}/\text{min}$ . See [Table 3](#) on [page 12](#) for guidelines.

In ESI, because both the buffer type and buffer concentration have a noticeable effect on sensitivity, you must choose these variables correctly.

Large droplets with high surface tension, low volatility, low surface charge, strong ion solvation, and high conductivity negatively affect the ESI process. Conversely, ESI favors small droplets with low surface tension, high volatility, high surface charge, weak ion solvation, and low conductivity.

To obtain good ESI results, follow these guidelines:

- Keep nonvolatile salts and buffers out of the solvent system. For example, avoid the use of salts containing phosphate, potassium, or sodium. Use acetate salts or ammonium instead. Do not use strong mineral acids and bases—they can damage the instrument.
- Use organic/aqueous solvent systems and volatile acids and bases. Try to avoid the use of 100 percent aqueous solvents.
- If possible, optimize the pH of the solvent system for the analyte. For example, if the analyte contains a primary or secondary amine, the mobile phase should be slightly acidic (pH 2–5).

## Using APCI

Like ESI, APCI is a soft gas phase ionization technique. Therefore, the gas phase acidities and basicities of the analyte and solvent vapor play an important role in the APCI process. APCI provides molecular mass information for compounds of medium polarity that have some volatility. APCI is typically used to analyze small molecules with molecular masses up to about 2000 Da.

Use APCI in either positive or negative ion polarity mode. For most molecules, the positive ion mode produces a stronger ion current. This is especially true for molecules with one or more basic nitrogen (or other basic) atoms. Molecules that generally produce strong negative ions, with acidic sites such as carboxylic acids and acid alcohols, are an exception to this general rule.

In general, APCI produces fewer negative ions than positive ions. However, the negative ion polarity mode can be more specific because it generates less chemical noise than does the positive mode. Consequently, the [signal-to-noise ratio \(S/N\)](#) might be better in the negative ion mode.

The rate of solvent flowing from the LC into the LTQ Series mass spectrometer in APCI mode is typically high (0.2–2 mL/min). See [Table 4](#) on [page 13](#) for guidelines.

APCI is a very robust ionization technique. It is not affected by minor changes in most variables, such as changes in buffer type or buffer strength.

## Sample Introduction Techniques

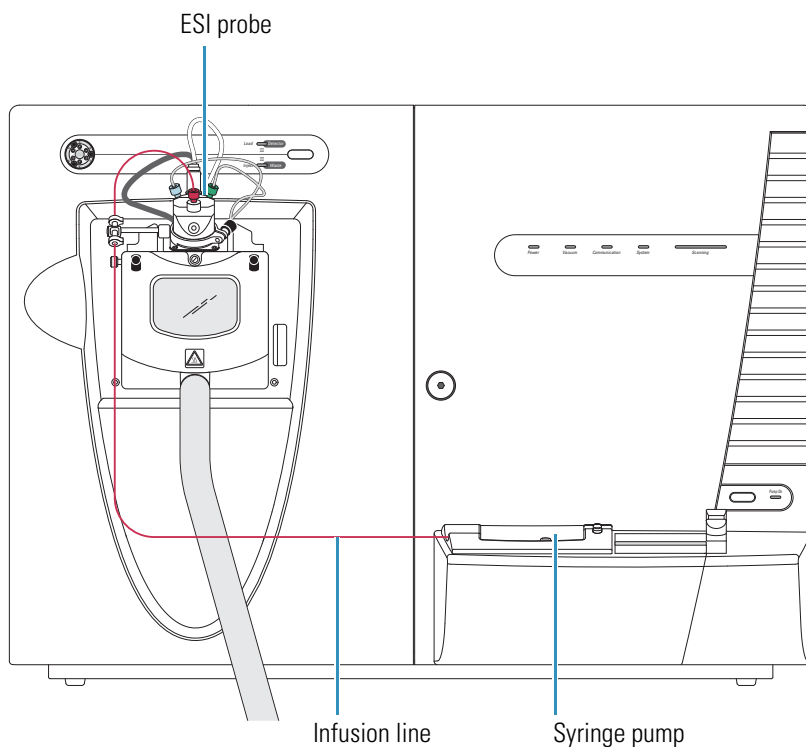
The LTQ Series mass spectrometer has a [divert/inject valve](#) and a [syringe pump](#). The following techniques are available to introduce samples into the [API source](#):

- [Direct Infusion](#)
- [High-Flow Infusion](#)
- [Loop Injection \(Flow-Injection Analysis\)](#)
- [Liquid Chromatography](#)

### Direct Infusion

The direct infusion technique uses the syringe pump to infuse sample directly into the ion source, as shown in [Figure 1](#). Use this technique to introduce a calibration solution for automatic tuning and calibrating in ESI mode. You can also use this technique to introduce a solution of pure analyte at a steady rate in ESI mode for qualitative analyses, and perform experiments at a low flow rate with the syringe pump.

**Figure 1.** Sample introduction by using direct infusion with a syringe pump



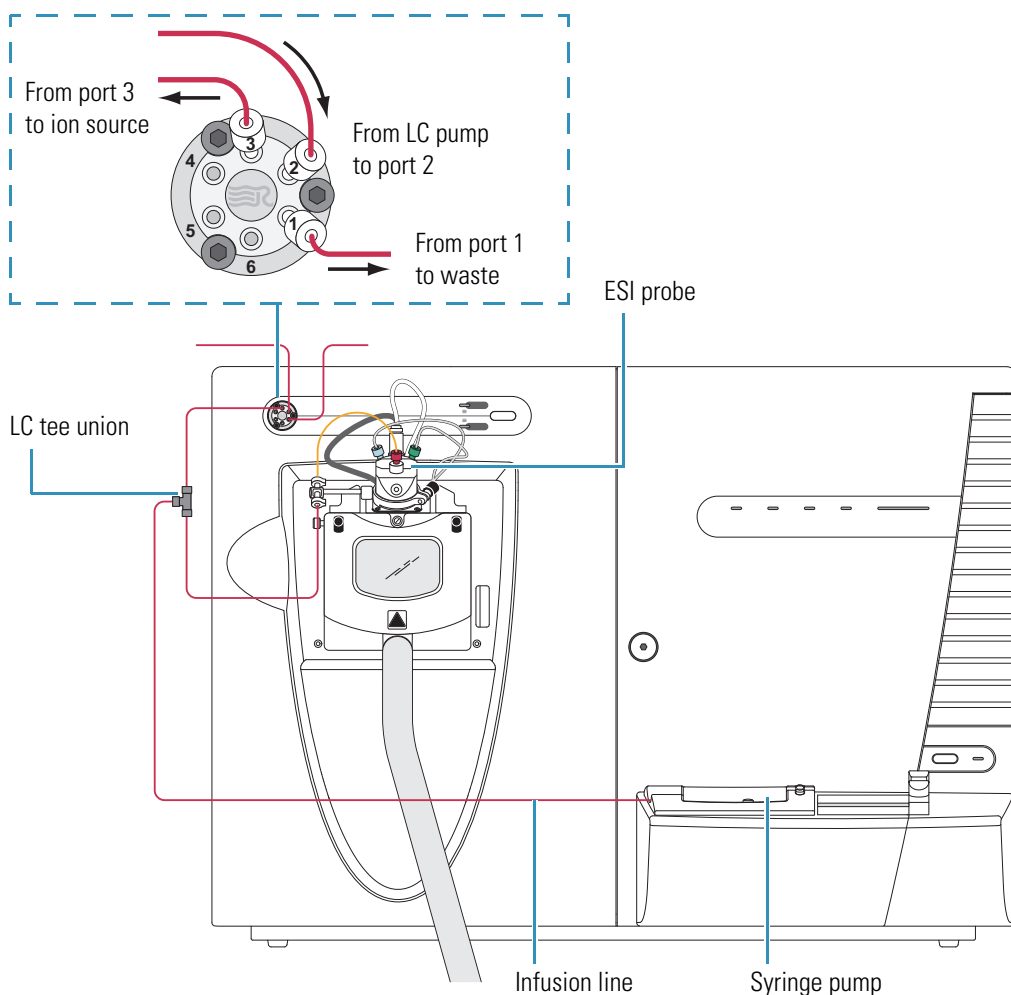
## High-Flow Infusion

The high-flow infusion technique uses an LC tee union to direct the solvent flow from the syringe pump into the solvent flow produced by an LC pump. The combined solvent flow goes through the divert/inject valve into the ion source. Use this technique to tune on an analyte (create a tune method for an analyte) in either ESI or APCI mode, using the same flow rate and mobile phase composition that you plan to use for the LC/MS experiments.

Use the high-flow infusion method for performing experiments at a higher flow rate with an LC system. The high-flow infusion method puts a comparatively large amount of solvent into the mass spectrometer, which means you might need to clean the API spray cone more frequently.

Figure 2 shows the inlet connections for high-flow infusion in ESI mode. When the divert/inject valve is in the Load position, solvent flow from the LC pump enters the valve through port 2 and exits the valve through port 3, which connects to the ion source. When the divert/inject valve is in the Inject position, solvent flow from the LC pump enters the valve through port 2 and exits the valve through port 1 to waste.

**Figure 2.** Sample introduction by using high-flow infusion in ESI mode

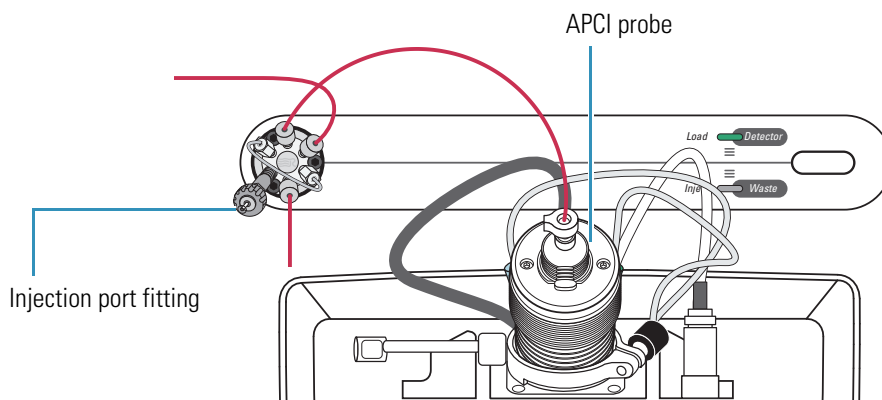


For instructions about setting up the inlet to perform high-flow infusion, see “[Setting Up the Inlet for High-Flow Infusion in ESI Mode](#)” on page 85.

## Loop Injection (Flow-Injection Analysis)

Use the loop injection technique when there is a limited amount of sample. To use this technique, attach a [sample loop](#), an injection port fitting, and an LC pump to the divert/inject valve, and then connect the divert/inject valve to the ion source ([Figure 3](#)). With the valve in the Load position, use a syringe to load sample through the injection port fitting into the sample loop, and then switch the position of the inject valve to the Inject position. Switching the valve to the Inject position allows the solvent flow from the LC pump to backflush the sample out of the loop and into the ion source. Use this technique in either ESI or APCI mode.

**Figure 3.** Sample introduction by using loop injection in APCI mode

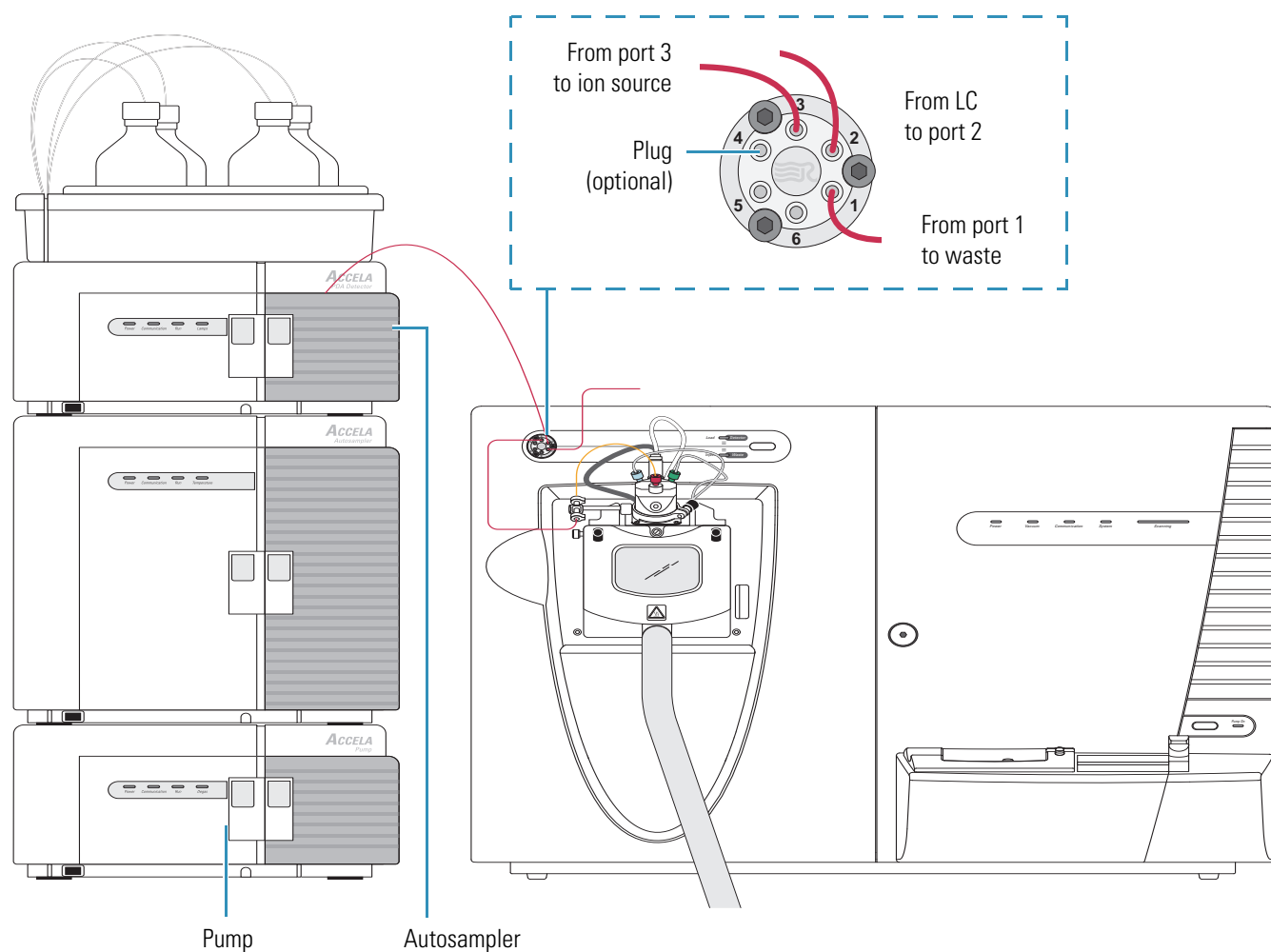


## Liquid Chromatography

To perform loop injection by using the liquid chromatography (LC) technique, install an LC column between the sample inlet of the ion source and port 3 of the divert/inject valve, or connect an LC system with an [autosampler](#) to the LTQ Series mass spectrometer.

To automatically inject a set of samples, connect an LC system with an autosampler to the divert/inject valve and connect the divert/inject valve to the ion source, as shown in [Figure 4](#). Use the autosampler to inject sample solution into the flow from an LC pump. In a typical LC/MS experiment, direct the solvent flow through an LC column to separate the compounds of a mixture before they are directed into the ion source. Use this technique in either ESI or APCI mode.

**Figure 4.** Sample introduction by using direct injection with an autosampler in ESI mode



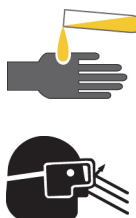


## Types of Buffers

Many LC applications use nonvolatile buffers such as phosphate and borate. Avoid using nonvolatile buffers because they can cause salt buildup in parts of the ion source, such as the transfer tube and spray nozzle of the ionization probe. Using nonvolatile buffers without also cleaning the ion source to remove salt deposits might compromise the integrity of the spray.

For LC/MS experiments, replace nonvolatile buffers with the following volatile buffers:

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



### CAUTION AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS

By law, producers and suppliers of chemical compounds are required to provide their customers with the most current health and safety information in the form of Material Safety Data Sheets (MSDSs). The MSDSs describe the chemicals and must be freely available to lab personnel to examine at any time. MSDSs provide summarized information on the hazard 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.

Read the MSDS for each chemical you use. Store and handle all chemicals in accordance with standard safety procedures. Always wear protective gloves and safety glasses when you use solvents or corrosives. Also, contain waste streams, use proper ventilation, and dispose of all laboratory reagents according to the directions in the MSDS.

## LC Flow Rate Ranges

The ESI probe can volatilize ions from liquid flows<sup>1</sup> of 1–1000  $\mu\text{L}/\text{min}$ . This flow rate range provides for a wide range of separation techniques: CE, CEC, analytical LC, capillary LC, and microbore LC.

The APCI probe can volatilize ions from liquid flows<sup>2</sup> of 200–2000  $\mu\text{L}/\text{min}$ . This flow range provides for the use of separation techniques: analytical LC, microbore LC, and semi-preparative LC.

<sup>1</sup> The ESI probe can generate ions from liquid flows as low as 1  $\mu\text{L}/\text{min}$ . However, flows below 5  $\mu\text{L}/\text{min}$  require more care, especially with the position of the fused silica sample tube within the ESI probe.

<sup>2</sup> For the APCI probe, flows below 200  $\mu\text{L}/\text{min}$  require more care to maintain a stable spray.

While changing the flow rate of solvents entering the mass spectrometer, adjust the following parameters:

- For ESI mode, adjust the ion transfer tube temperature and the flow rates for the sheath, auxiliary, and sweep gases.
- For APCI mode, adjust the ion transfer tube and vaporizer temperatures, and adjust the flow rates for the sheath, auxiliary, and sweep gases.
- For both ionization modes, increase the temperature of the ion transfer tube and the gas flow rates while increasing the flow rate of liquid into the mass spectrometer.

[Table 3](#) lists the guidelines for ESI operation for ion transfer tube temperatures and gas flow rates for various LC solvent flow rates.

[Table 4](#) lists the guidelines for APCI operation for the ion transfer tube temperature, vaporizer temperature, and gas flow rate for a range of LC solvent flow rates.

**Table 3.** Guidelines for setting operating parameters for LC/ESI/MS <sup>a</sup>

LC flow rate	Suggested column size	Ion transfer tube temperature (typical)	Sheath gas	Auxiliary and/or sweep gas
Infusion or LC, < 10 µL/min	Capillary (0.1–0.3 mm)	150–200 °C (302–392 °F)	Not required 5–15 units, typical	Not required 0 units, typical
LC, 50–200 µL/min	1 mm ID (microbore column)	200–275 °C (392–527 °F)	Required 20–40 units, typical	Not required, but might help depending on conditions 0–20 units, typical
LC, 100–500 µL/min	2–3 mm ID (narrow bore column)	250–350 °C (482–662 °F)	Required 30–60 units, typical	Not required, but usually helps to reduce solvent background ions 0–20 units, typical
LC, 0.4–1 mL/min	4.6 mm ID (standard column)	300–400 °C (572–752 °F)	Required 60–100 units, typical	Required 10–40 units, typical

<sup>a</sup> Choose auxiliary gas, sweep gas, or both, according to the suggestions in “[Sheath, Auxiliary, and Sweep Gases](#)” on [page 4](#).

**Table 4.** Guidelines for setting operating parameters for LC/APCI/MS<sup>a</sup>

LC flow rate	Vaporizer temperature (typical)	Ion transfer tube temperature (typical)	Sheath gas	Auxiliary gas	Sweep gas
LC, 200–2000 µL/min	LXQ: 350–500 °C	LXQ: 150–350 °C	Required 40–100 units	5–20 units	0–5 units
	LTQ XL and Velos Pro: 350–500 °C	LTQ XL and Velos Pro: 250–350°C			

<sup>a</sup> Choose auxiliary gas, sweep gas, or both, according to the suggestions in “Sheath, Auxiliary, and Sweep Gases” on page 4.

## Tuning and Calibrating the Mass Spectrometer

Calibrate the LTQ Series mass spectrometer to ensure its mass accuracy. Before calibrating the mass accuracy, tune the mass spectrometer with the ESI calibration solution to optimize the transmission of ions.

**IMPORTANT** Thermo Fisher Scientific recommends that you check the calibration once a week and calibrate as needed.

Calibration parameters are instrument parameters whose values do not vary with the type of experiment. Automatic and semi-automatic calibration (including checking the calibration) require introducing the calibration solution into the mass spectrometer at a steady flow rate while the calibration is running. Introduce the solution directly from the syringe pump into the mass spectrometer in ESI/MS mode.

Tune parameters are instrument parameters whose values can vary with the type of experiment. For example, if the experiment requires quantitative data on one or more particular ions, you must tune the mass spectrometer with the analyte. You must also tune the mass spectrometer if you change any parameter that is specific to the experiment or analyte.

Create tune methods for analytes by using the same solvent composition and flow rate that you plan to use in the chromatographic method for the LC/MS experiments. Tune on a particular peak in the mass spectrum of the analyte, or choose an ion in the calibration solution that is closest to the mass-to-charge ratio for the target ion.

Automatic and semi-automatic tuning procedures (including optimizing the collision energy) require introducing the analyte into the mass spectrometer at a steady rate in one of two ways:

- Direct infusion (see [page 7](#))
- High-flow infusion (see [page 8](#))

In most cases, you can use the tune file obtained from the automatic or semi-automatic tuning procedures for the analytical experiments. However, for some applications, you might need to tune several parameters. In that case, tune manually. With manual tuning, introduce a tuning solution at a steady flow rate.

**Note** For ESI operation, the most important parameters that affect the signal quality are the ion transfer tube temperature, [API tube lens offset voltage](#) (except for the LTQ Velos and Velos Pro mass spectrometers), gases, and solvent flow rate. For optimum sensitivity, tune the mass spectrometer in the operational mode that you plan to use for the experiments.

The LTQ Velos and Velos Pro mass spectrometers do not have a [API tube lens](#).

After creating a tune method for a particular application, add the tune method to the instrument method. You can add a different tune method for each segment of the instrument method. For example, if analyte A and analyte B elute at 2 and 4 minutes, respectively, create an instrument method with two segments: Specify a tune method optimized for analyte A in segment one and a tune method optimized for analyte B in segment two.

[Table 5](#) lists the methods of sample introduction for each of the calibration and tuning procedures.

**Table 5.** Summary of methods of sample introduction for calibration and tuning

Sample/ Sample introduction	Calibration			Tuning			
	Check	Auto	Semi-auto	Auto	Semi-auto	Manual	Collision energy
Calibration solution/ Direct infusion	✓	✓	✓	✓	✓	✓	✓
Your tune solution/ Direct infusion				✓	✓	✓	✓
Your tune solution/ High-flow infusion				✓	✓	✓	✓

## Ion Polarity Modes

The LTQ Series mass spectrometer can operate in either positive or negative ion polarity modes. The mass spectrometer controls whether positive ions or negative ions are transmitted to the mass analyzer for mass analysis by changing the polarity of the voltage potentials applied to the API source, [ion optics](#), and [ion detection system](#). The ion optics are located between the API source and the mass analyzer.

The information obtained from a positive-ion mass spectrum is different from and complementary to that obtained from a negative-ion mass spectrum. “[Ionization Techniques](#)” on [page 5](#) describes the spectral characteristics for these two polarity modes. The ability to obtain both positive-ion and negative-ion mass spectra during a single scan run reduces the time required to obtain a complete qualitative analysis of the sample.

## Data Types

With the LTQ Series mass spectrometer you can acquire and display mass spectral data (intensity versus mass-to-charge ratio) in one of two data types:

- Profile data

With profile data you can see the inherent shape of the peaks in the mass spectrum. The mass spectrum divides each atomic mass unit into several sampling intervals. The intensity of the ion current is determined at each sampling interval. The intensity at each sampling interval is displayed with the intensities connected by a continuous line. In general, use the profile scan data type when you tune and calibrate the mass spectrometer so that you can easily see and measure mass resolution.

- Centroid data

Centroid data displays the mass spectrum as a bar graph. This scan data type sums the intensities of each set of sampling intervals. This sum is displayed versus the integral center of mass of the many sampling intervals. The disk space requirements for centroid data are about one-tenth of what is required for profile data. Consequently, data processing for centroid data is faster than that for profile data.

## Scan Power and Scan Modes

Ions produced in the ion source are often referred to as parent ions or precursor ions. To produce a mass spectrum, the mass analyzer scans its dc and rf voltages to sequentially eject ions from the trap based on their  $m/z$  values. Or, by varying the rf voltages and applying an additional isolation waveform voltage to the mass analyzer, the LTQ Series mass spectrometer can first eject all ions, except for several selected parent ions, and then collide these ions with the helium that is present in the mass analyzer. This helium is known as a buffer gas. The collisions can cause the selected parent or precursor ions to fragment into product ions. The ion trap can then sequentially eject these product ions based on their  $m/z$  values to produce a mass spectrum of the product ions.

$MS^n$  represents the number of stages of mass analysis where  $n$  is the scan power. Each stage of mass analysis where  $n > 1$  includes an ion selection step. The mass spectrometer supports scan powers of  $n = 1$  to  $n = 10$ . As you raise the scan power, you can obtain more structural information about the analyte.

The standard configurations of the mass spectrometer support several scan powers.

- MS scan mode

The mass spectrometer (MS) scan mode corresponds to a single stage of mass analysis—that is, a scan power of  $n = 1$ . The [MS scan modes](#) only involve parent ions, and no fragmentation of the parent ions occurs. The MS scan mode can be a full-scan experiment or a [selected ion monitoring \(SIM\) scan type](#) experiment (see “[Selected Ion Monitoring](#)” on page 18).

- MS/MS scan mode

The MS/MS scan corresponds to two stages of mass analysis (scan power of  $n = 2$ ). In an MS/MS scan, parent ions fragment into product ions. An MS/MS scan can be a full-scan experiment or a [selected reaction monitoring \(SRM\) scan type](#) experiment (see “[Selected Reaction Monitoring](#)” on page 18).

- MS<sup>n</sup> scan mode

An MS<sup>n</sup> scan involves three to 10 stages of mass analysis (scan power of  $n = 3$  to  $n = 10$ ). However, the term can also apply to one stage of mass analysis (with  $n = 1$ ) or to two stages of mass analysis (with  $n = 2$ ). An MS<sup>n</sup> scan can be either a full-scan experiment or a [consecutive reaction monitoring \(CRM\) scan type](#) experiment (see “[Consecutive Reaction Monitoring](#)” on page 19).

## Scan Rates

The LTQ Series mass spectrometer can operate in the following scan rates: Normal, Rapid (for the Velos Pro only), Enhanced, Turbo, Zoom, and UltraZoom. To use the Rapid scan rate on the Velos Pro mass spectrometer, you must activate the Trap-HCD license; see “[Getting a Trap-HCD License](#)” on page xxi. For information about these scan rates, refer to the data system Help.

## Scan Types

The LTQ Series mass spectrometer can operate in the following scan types:

- [Full Scan](#)
- [Selected Ion Monitoring](#)
- [Selected Reaction Monitoring](#)
- [Consecutive Reaction Monitoring](#)
- [ZoomScan and UltraZoom](#)

## Full Scan

A [full-scan type](#) provides an entire or wide range mass spectrum of the analyte in a particular scan time. With a full scan, the mass analyzer scans from the first mass to the last mass without interruption in the last step of mass analysis (ion scan-out).

A full scan provides more information about an analyte than does SIM or SRM. But because a full scan scans an entire mass range during a particular scan time, it does not provide the sensitivity that the other scan types can achieve.

- Single-stage full scan ( $MS^1$ )

The single-stage full scan has one stage of mass analysis (scan power of  $n = 1$ ). With the single-stage full scan, the mass analyzer stores ions formed in the ion source. These ions are then sequentially scanned out of the mass analyzer to produce a full mass spectrum (a mass spectrum of the observable ions in the specified mass range at a specific time point in the analysis).

Single-stage full-scan analysis is a useful tool for qualitative analysis. Use single-stage full-scan experiments to determine the molecular weight of unknown compounds or the molecular weight of each component in a mixture of unknown compounds.

After you determine the molecular weight of a target compound, you can use the SIM or SRM scan type to perform routine quantitative analyses of the compound (see [“Selected Ion Monitoring”](#) on page 18 and [“Selected Reaction Monitoring”](#) on page 18).

- Two-stage full scan ( $MS/MS$ )

The two-stage full scan has two stages of mass analysis (scan power of  $n = 2$ ). In the first stage, the mass analyzer stores ions formed in the ion source. Then ions of one mass-to-charge ratio (the parent ions) are selected, and all other ions are ejected from the mass analyzer. The parent ions are excited and collide with background gas that is present in the mass analyzer. The collisions of the parent ions cause them to fragment to produce one or more product ions.

In the second stage of mass analysis, the mass analyzer stores the product ions. Then they are sequentially scanned out of the mass analyzer to produce a full-product ion mass spectrum.

A two-stage full scan gives you more information about a sample than SRM does but at a lower speed.

To use the SRM scan you must know which parent and product ions to observe. To obtain this information you could use a one-stage full scan to determine the parent mass spectrum and a two-stage full scan to determine the [product mass](#) spectrum for the parent ions of interest. For subsequent routine quantitative analysis you would use an SRM scan type based on the one-stage and two-stage full-scan results.

## Selected Ion Monitoring

SIM is a single-stage (scan power of  $n = 1$ ) technique that monitors a particular ion or set of ions. In a SIM scan, the mass analyzer stores ions formed in the ion source. The mass spectrometer then selects ions of one or more mass-to-charge ratios, and ejects all other ions from the mass analyzer. The selected ions are then sequentially scanned out of the mass analyzer to produce a SIM mass spectrum.

Use SIM experiments to detect small quantities of a target compound in a complex mixture when you know the mass spectrum of the target compound. SIM is useful in trace analysis and in the rapid screening of a large number of samples for a target compound.

Because a SIM scan monitors only a few ions, SIM provides lower detection limits and greater speed than a single-stage full-scan analysis. SIM achieves lower detection limits because more time is spent monitoring significant ions that are known to occur in the mass spectrum of the target sample. SIM achieves greater speed because it only monitors a few ions of interest while ignoring regions of the spectrum that are empty or have no ions of interest.

SIM can improve the detection limit and decrease analysis time, but it can also reduce target compound specificity compared to an MS/MS scan. SIM analysis decreases the analysis time because it only monitors particular ions. SIM analysis reduces specificity because any compound that produces ions of the  $m/z$  being monitored would appear to be the target compound. To avoid false positive results when using SIM for routine analyses, first verify that SIM is monitoring ions from the target compound and nothing else.

## Selected Reaction Monitoring

SRM is a two-stage (scan power of  $n = 2$ ) technique that monitors parent ion and product ion pairs.

In the first stage of mass analysis, the mass analyzer stores the ions formed in the ion source. The mass spectrometer selects ions of one mass-to-charge ratio (the parent ions) and ejects all other ions from the mass analyzer. The parent ions are excited and collide with background gas that is present in the mass analyzer. The collisions of the parent ions cause them to fragment to produce one or more product ions.

In the second stage of mass analysis, the mass analyzer stores the product ions. The selected  $m/z$  range of ions are then sequentially scanned out of the mass analyzer to produce an SRM product ion mass spectrum.

Like SIM, SRM provides very rapid analysis of trace components in complex mixtures. However, because you are monitoring pairs of ions (one product ion for each parent ion), the specificity obtained in SRM can be much greater than that obtained in SIM. You are very unlikely to get a false positive result with SRM. To get a false positive result, the interfering compound must form a parent ion of the same mass-to-charge ratio as the selected parent ion from the target compound. The compound must also fragment to form a product ion of the same mass-to-charge ratio as the selected product ion from the target compound.



## Consecutive Reaction Monitoring

CRM is the multi-stage (scan power of  $n = 3$  to  $n = 10$ ) analog of SIM ( $n = 1$ ) and SRM ( $n = 2$ ) that monitors a multi-step reaction path. In the first stage of mass analysis, the mass analyzer stores the ions formed in the ion source. The mass spectrometer selects ions of one mass-to-charge ratio (the parent ions) and ejects all other ions from the mass analyzer. The parent ions are excited and collide with background gas that is present in the mass analyzer. The collisions of the parent ions cause them to fragment to produce one or more product ions.

In the second stage of mass analysis, the mass analyzer stores the product ions. The mass spectrometer selects product ions of one mass-to-charge ratio and ejects all other ions from the mass analyzer. The selected product ions now become the new parent ions for the next stage of mass analysis. The new parent ions are excited and collide with background gas. The collisions of the new parent ions cause them to fragment to produce one or more new product ions.

In the third stage of mass analysis, the mass analyzer stores the new product ions. This process repeats up to seven more times until the mass spectrometer produces the final product ions of interest.

In the  $n^{\text{th}}$  stage of mass analysis, the mass analyzer stores the final product. The mass spectrometer then sequentially scans the  $m/z$  range of the selected ions out of the mass analyzer to produce a CRM final product ion mass spectrum.

In CRM, the specificity increases as you increase the number of monitored consecutive reactions. However, the sensitivity decreases as you increase the number of monitored consecutive reactions—especially if many fragmentation pathways are available to the parent ion.

## ZoomScan and UltraZoom

Determining the mass of an ion from its mass-to-charge ratio can be complicated if the charge state of the ion is unknown. ZoomScan and UltraZoom are scan modes in which the mass spectrometer achieves a higher resolution. The resolution is sufficient to determine the  $^{12}\text{C}/^{13}\text{C}$  isotopic separation that then allows the system to determine the charge state by using the following:

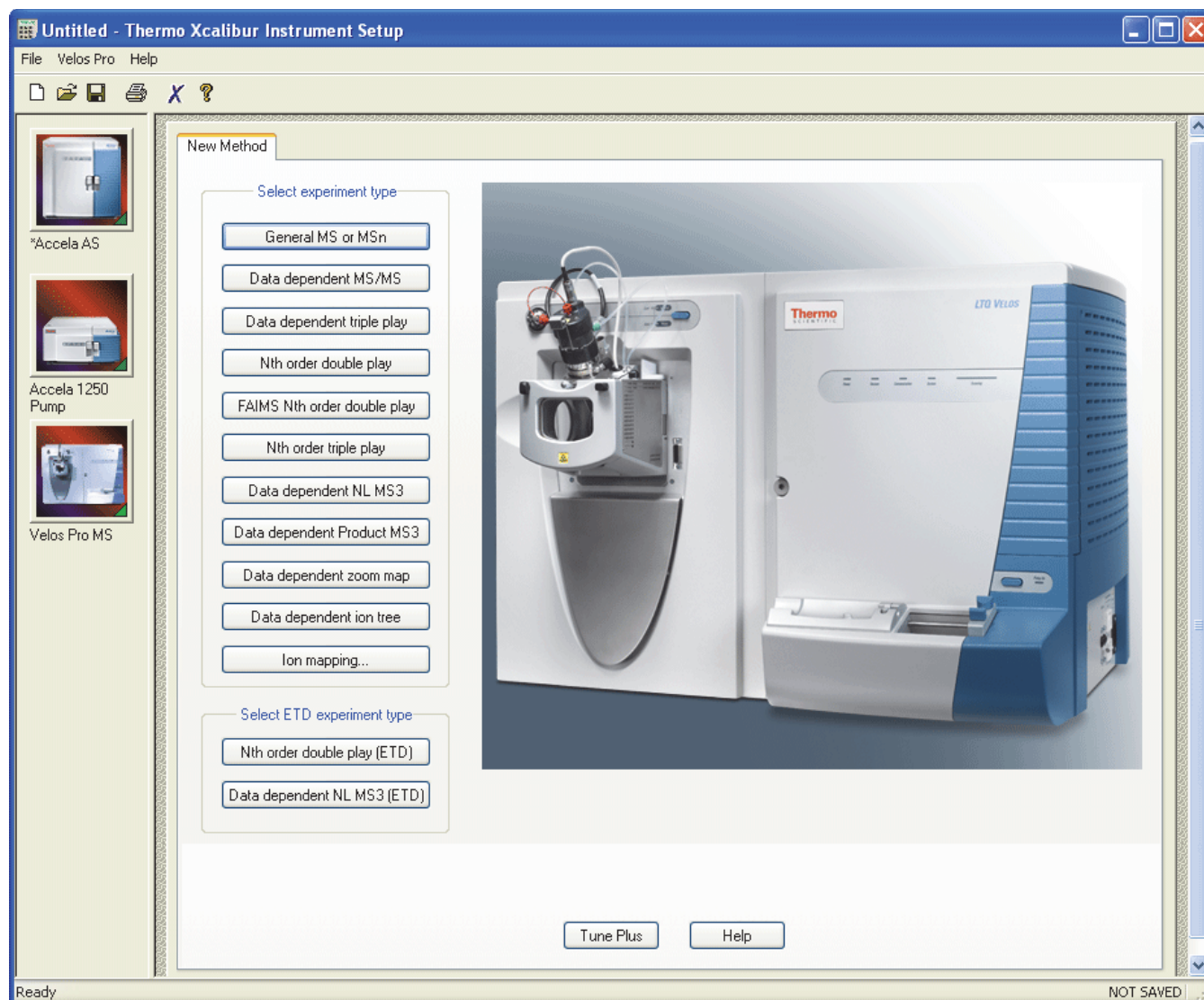
- If the isotopic peaks are 1 Da apart, the ion has a charge state of  $\pm 1$ .
- If the isotopic peaks are 0.5 Da apart, the ion has a charge state of  $\pm 2$ .
- If the isotopic peaks are 0.33 Da apart, the ion has a charge state of  $\pm 3$ .
- If the isotopic peaks are 0.25 Da apart, the ion has a charge state of  $\pm 4$ .
- If the isotopic peaks are 0.2 Da apart, the ion has a charge state of  $\pm 5$ .

With the UltraZoom scan mode, the preceding list can continue up to a charge state of  $\pm 10$ . You can then determine the molecular weight of the ion from the knowledge of the charge state and mass-to-charge ratio of the ion.

## Types of Experiments

The New Method page of the Thermo Xcalibur Instrument Setup window for the LTQ Series mass spectrometer (Figure 5) contains links to templates for various types of experiments. To save time entering the parameters for the instrument method, open the template designed for the experiment type that you want to perform, enter the parameters specific to the experiment, and then save the entries as part of an Xcalibur instrument method (.meth file).

**Figure 5.** Thermo Xcalibur Instrument Setup window showing the New Method page (Velos Pro with ETD experiment types)



This section describes the types of experiments for the LTQ Series mass spectrometer. The experiments are grouped into the following categories:

- [General MS or MS<sub>n</sub> Experiments](#)
- [Data Dependent Experiments](#)
- [Ion Mapping Experiments](#)
- [Ion Tree Experiments](#)

## General MS or MS<sup>n</sup> Experiments

A General MS or MS<sup>n</sup> experiment is best used to collect qualitative data for structural analysis. However, you can also use a General experiment for the quantitative analysis of known compounds.

The Xcalibur data system includes an Instrument Method template in the Instrument Setup window for a General MS or MS<sup>n</sup> experiment; click **General MS or MS<sub>n</sub>** on the New Method page in the Xcalibur application ([Figure 5](#) on [page 20](#)). [Figure 6](#) shows an example of a General MS or MS<sup>n</sup> experiment template.

In a General MS quantitation experiment, specify the following:

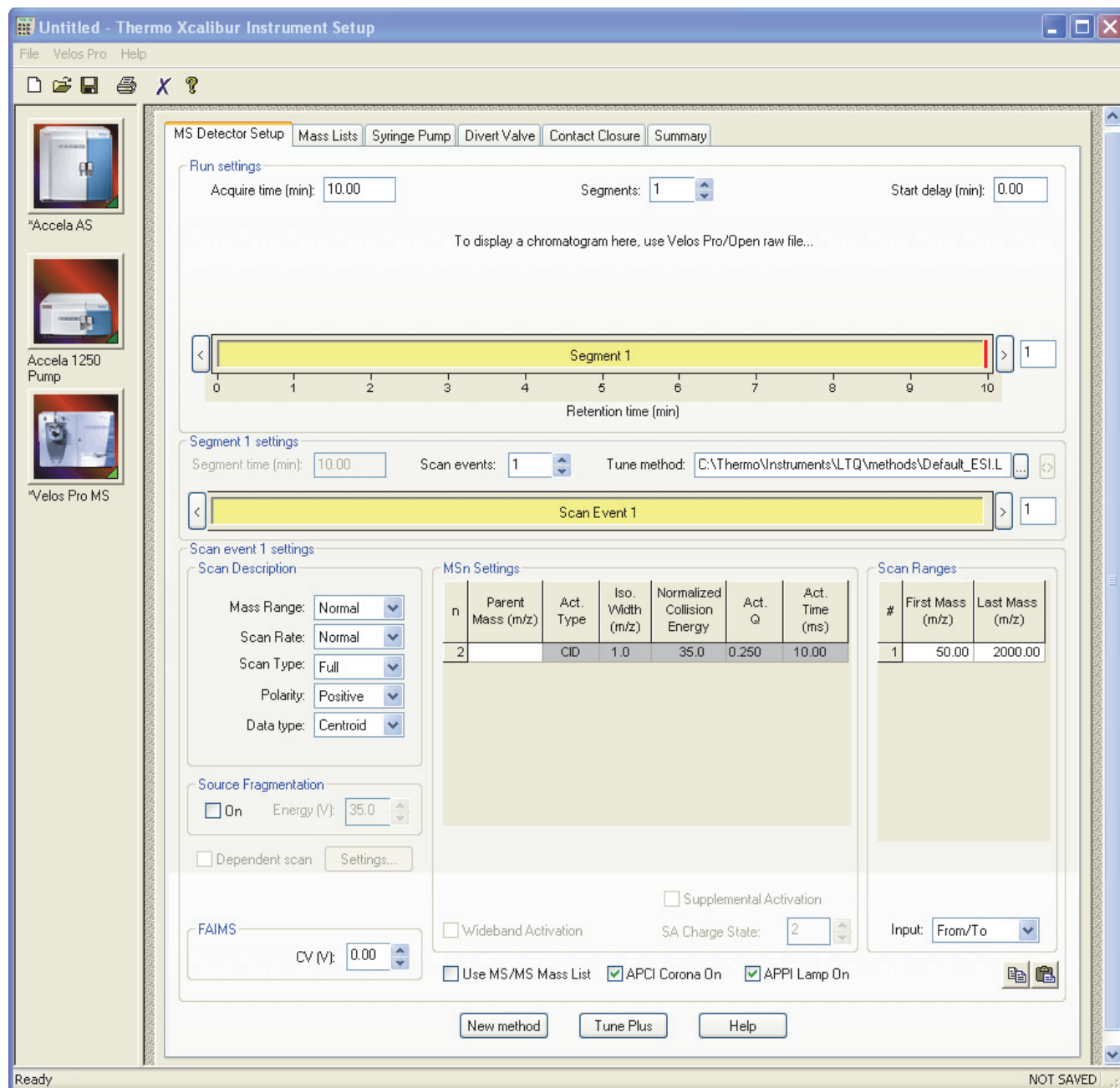
- Mass range of the analyte or analytes
- Parent (precursor) ion that fragments into distinctive product ions
- Mass-to-charge ratios of all the product ions

The LTQ Series mass spectrometer can then collect data on the ions in the mass range or on the product ions of the parent ion or ions that you specify.

If using a General experiment to collect data for qualitative (structural) analysis, specify the scan mode (MS through MS<sup>n</sup>) that you want data for on the MS Detector Setup page under Scan Event 1 Settings ([Figure 6](#)). If specifying MS/MS or MS<sup>n</sup>, choose the parent ion or ions that you want data for on the MS Detector Setup page under MS<sup>n</sup> Settings. Under MS<sup>n</sup> Settings, you can specify one of these fragmentation methods: [collision-induced dissociation \(CID\)](#), [pulsed Q collision-induced dissociation \(PQD\)](#), and for the Velos Pro there is also [higher energy collision-induced dissociation \(HCD\)](#) (requires an activated Trap-HCD license; see [“Getting a Trap-HCD License”](#) on [page xxi](#)). The LTQ Series mass spectrometer then collects distinct qualitative information for structural analysis or for spectral reference.

The mass spectrometer can generate reproducible, analyte-specific spectra. Consequently, you can use the reference spectra generated with one LTQ Series mass spectrometer to confirm structures of compounds generated with another LTQ Series mass spectrometer.

Figure 6. General MS experiment template on the MS Detector Setup page



## Data Dependent Experiments

A Data Dependent™ experiment is best used for the qualitative analysis of unknown compounds for structure elucidation or confirmation. These experiments maximize the data obtained while requiring minimal user input. The LTQ Series mass spectrometer uses the information in a Data Dependent experiment to make decisions automatically about subsequent experiments. The Xcalibur Instrument Setup window ([Figure 5](#) on [page 20](#)) contains the Instrument Method templates for Data Dependent experiments.

A Data Dependent experiment produces a large amount of data from a single sample analysis. In a Data Dependent experiment, specify parent ions for fragmentation or let the mass spectrometer automatically select the ions for fragmentation. The mass spectrometer can automatically collect the structural information for every parent ion in the sample, even if the sample is a mixture of compounds.

Find useful structural information about a compound automatically with the simplest Data Dependent experiment, Data Dependent MS/MS. In this experiment, specify only the MS scan range—you do not need to specify a parent ion. The mass spectrometer then collects full-scan MS data, picks the most intense parent ion in the spectrum, and then fragments the ion to generate product ions.

A Data Dependent Triple-Play experiment is the same as Data Dependent MS/MS but includes the identification of the charge state of the parent with the ZoomScan feature. A Data Dependent Triple-Play experiment collects full-scan MS data, and then uses ZoomScan to determine the charge state of the parent ion and calculate the molecular weight. The parent ion is then fragmented into product ions (MS/MS). For example, if the mass spectrometer determines a charge state equal to two, and if the mass-to-charge ratio of the parent ion is  $m/z$  500, then the mass-to-charge ratios of the product ions can be up to and including  $m/z$  1000 (or  $2 \times 500$ ). [Figure 7](#) shows an example of a Data Dependent Triple-Play experiment template.

Ion Mapping experiments can be Data Dependent. However, the Total Ion Map, Neutral Loss Ion Map, and Parent Ion Map experiments are not Data Dependent. The Data Dependent Zoom Map experiment collects ZoomScan data on every scan interval in a specified mass range. An MS/MS experiment on the largest ion in each ZoomScan interval is automatically performed. For additional information, see [“Ion Mapping Experiments”](#) on [page 26](#).

Ion Tree experiments are types of Data Dependent experiments. These experiments provide methods for automatically interpreting MS<sup>n</sup> data and arranging the data in formats that are easy to manipulate. For additional information, see [“Ion Tree Experiments”](#) on [page 28](#).

Set up the Data Dependent experiments in one of two ways:

- If you have some idea of the parent ion, or if you expect a certain kind of parent, set up a list of possible parent ions. After detecting one of the specified parent ions, you can acquire product spectra and analyze the information. Conversely, you can set up a list of ions that you do not want to be selected for fragmentation.
- If you have little information about the compound, you can set up the parameters of a Data Dependent experiment so that if the intensity of the ion signal is above a specified threshold, the mass spectrometer generates product spectra. (Decide later if the information is useful.) Parameters might include threshold values for the intensity of the MS or MS<sup>n</sup> ion signal. Whatever threshold values you choose should isolate the parent ions of interest.

Use a Data Dependent experiment to do the following:

- Identify low-level impurities in high-purity compounds (Data Dependent MS/MS).

A Data Dependent MS<sup>n</sup> experiment can identify process impurities. In the quality assurance process for aspirin, for example, the mass spectrometer can identify impurities of less than 0.1 percent.

- Identify metabolites in a complex mixture (chromatographic separation with Data Dependent MS/MS).

A Data Dependent MS/MS experiment of a complex mixture can provide highly specific structural information. For example, characteristic masses along the metabolic pathways of a drug can produce MS<sup>n</sup> spectra that are specific to the structure of the drug and its metabolites. These spectra are essential in metabolite identification.

- Build a custom library of composite MS<sup>n</sup> spectra (Ion Tree).

A Data Dependent experiment can produce a composite spectrum of, for example, MS/MS, MS<sup>3</sup>, and MS<sup>4</sup> data. The mass spectrometer can store the MS<sup>n</sup> fingerprint data in a custom MS<sup>n</sup> library spectrum. The data is valuable for use in process control, quality assurance, or research.



Figure 7. Data Dependent Triple-Play experiment template on the MS Detector Setup page

The screenshot displays the 'MS Detector Setup' window in Thermo Xcalibur Instrument Setup. The window title is 'Untitled - Thermo Xcalibur Instrument Setup'. The interface includes a menu bar (File, Velos Pro, Help), a toolbar, and a sidebar on the left with icons for 'Accela AS', 'Accela 1250 Pump', and 'Velos Pro MS'. The main area is divided into several sections:

- MS Detector Setup** (selected tab): Includes sub-tabs for Mass Lists, Syringe Pump, Divert Valve, Contact Closure, and Summary.
- Run settings:** Acquire time (min): 10.00, Segments: 1, Start delay (min): 0.00. A note states: 'To display a chromatogram here, use Velos Pro/Open raw file...'. A timeline shows 'Segment 1' from 0 to 10 minutes.
- Segment 1 settings:** Segment time (min): 10.00, Scan events: 3, Tune method: C:\Thermo\Instruments\LTQ\methods\Default\_ESI.L.
- Scan Event 1 settings:** Includes a table for MSn Settings and a table for Scan Ranges.

**Scan Event 1 settings:**

Scan Description:

- Mass Range: Normal
- Scan Rate: Normal
- Scan Type: Full
- Polarity: Positive
- Data type: Centroid

Source Fragmentation:

- On Energy (V): 35.0
- Dependent scan Settings...

FAIMS:

- CV (V): 0.00

**MSn Settings Table:**

n	Parent Mass (m/z)	Act. Type	Iso. Width (m/z)	Normalized Collision Energy	Act. Q	Act. Time (ms)
2		CID	1.0	35.0	0.250	10.00

**Scan Ranges Table:**

#	First Mass (m/z)	Last Mass (m/z)
1	50.00	2000.00

Additional settings include Supplemental Activation, Wideband Activation, SA Charge State (2), and checkboxes for Use MS/MS Mass List, APCI Corona On, and APPI Lamp On. Buttons for 'New method', 'Tune Plus', and 'Help' are at the bottom.

## **Ion Mapping Experiments**

An Ion Mapping experiment is best used to get full structural characterization of unknown molecules in complex mixtures. In an Ion Mapping experiment, you can get product ion scans on every parent ion over a specified mass range. An Ion Mapping experiment can help to identify automatically which parent ions were fragmented to yield a specified product ion. The experiment “maps” one or more parent ions by using the information from product ion scans.

These experiments require that sample solution enter the mass spectrometer at a composition that is constant throughout. Therefore, use an infusion technique to introduce the sample for these experiments. [Figure 8](#) shows an example of an Ion Mapping experiment template.

The LTQ Series mass spectrometer includes the following Ion Mapping templates on the Instrument Setup window for an Ion Mapping experiment:

- Total (or Full-scan) Ion Map

A Total (or Full-scan) Ion Map experiment produces product ion scans for every possible parent ion in a specified mass range to determine which parent ions lost a particular fragment to yield a particular product ion (neutral loss scan information). Furthermore, you can use the data results to determine which parent ions are related to specific product ions (parent scan information). For example, map all masses from  $m/z$  400–2000 and specify all possible scans for MS/MS product ions in incremental steps of every mass-to-charge ratio, every fifth mass-to-charge ratio, or every tenth mass-to-charge ratio.

- Neutral Loss Ion Map

A Neutral Loss Ion Map experiment collects scans for masses and looks for the possible loss of a particular neutral fragment. The Neutral Loss Ion Map identifies which parent ions lost a neutral fragment of a particular mass. For example, specify a neutral loss of 80 Da (as in the case of a phosphorylated peptide). A Neutral Loss Ion Map experiment can step through every product mass in the mixture. The experiment searches for evidence of the loss of a neutral moiety of mass 80 Da. The data produced by a Neutral Loss Ion Map is a subset of the data produced by the Total Ion Map but can be obtained in a shorter time.

- Parent Ion Map

A Parent Ion Map experiment identifies all parent ions that produce a particular product ion that you specify. For example, if you specify a product ion mass of  $m/z$  250, a Parent Ion Map includes all the parent ions that yielded the specified product ion,  $m/z$  250. The data produced by a Parent Ion Map is a subset of the data produced by the Total Ion Map but can be obtained in a shorter time.

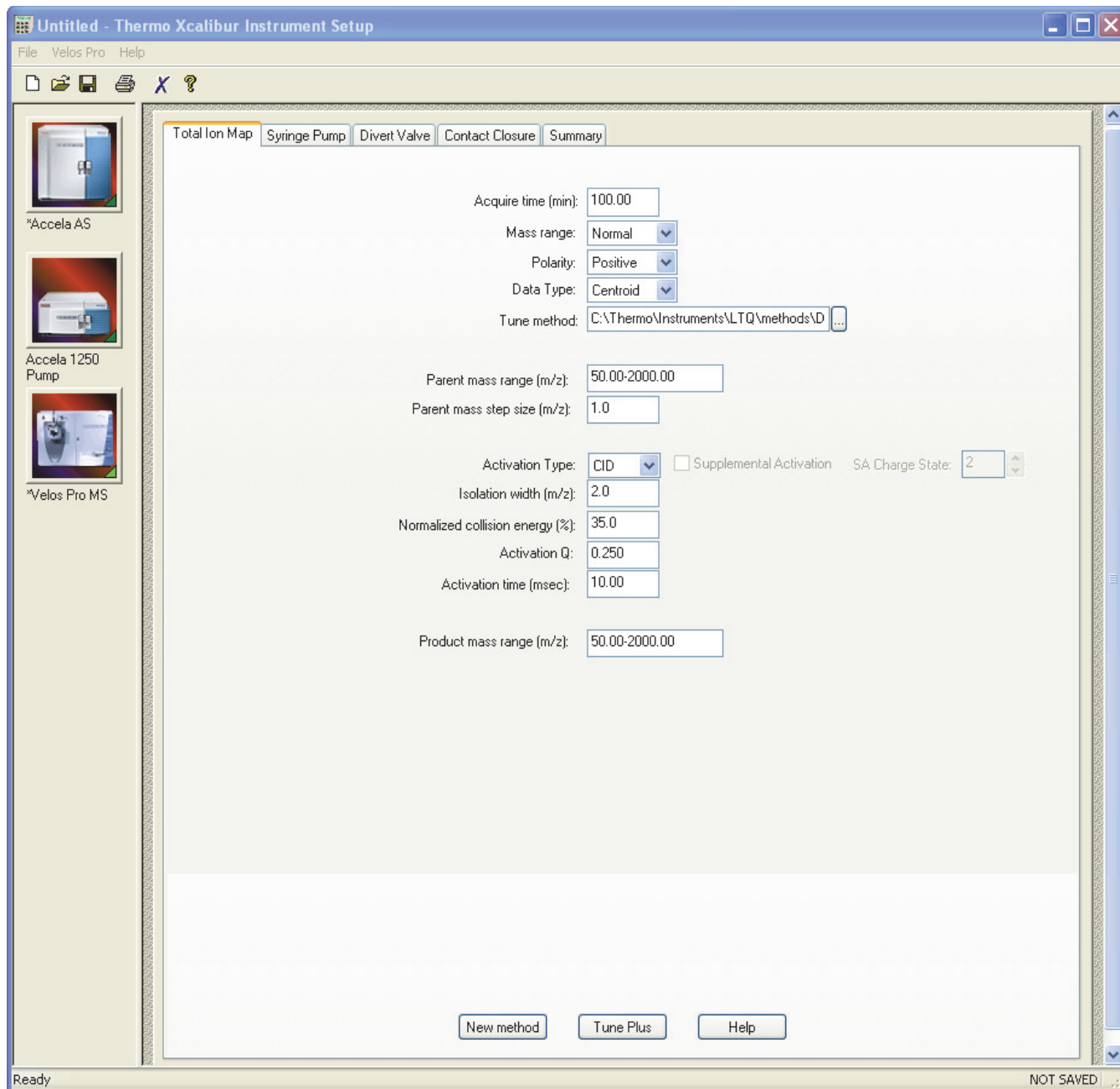
- Data Dependent Zoom Map

A Data Dependent Zoom Map is an Ion Map experiment that collects ZoomScan data on every scan interval in a specified mass range and Data Dependent MS/MS product spectra on the most intense mass peak above an intensity threshold with each ZoomScan.



You can view the results of any of the Ion Mapping experiments in the Xcalibur Qual Browser window.

**Figure 8.** Ion Mapping experiment template on the Total Ion Map page



## Ion Tree Experiments

In an Ion Tree experiment, the LTQ Series mass spectrometer can collect MS<sup>n</sup> data automatically. To maximize the structural information acquired for a particular sample, perform an Ion Tree experiment. Specify a particular parent ion for MS<sup>n</sup> fragmentation or let the mass spectrometer find the parent ions automatically and fragment them to any level between MS/MS and MS<sup>10</sup>. The mass spectrometer automates the collection of data by deciding what actions must occur next for the experiment to progress. [Figure 9](#) shows an example of an Ion Tree experiment template.

In an Ion Tree experiment, to prioritize how the mass spectrometer gathers information in the allowed time, choose one of two options:

- Depth focus  
Characterizes an ion by performing a series of MS<sup>n</sup>-level fragmentations (for example, MS/MS, MS<sup>3</sup>, MS<sup>4</sup>, and so on) before characterizing the next most intense ion in the MS<sup>n</sup> series.
- Breadth focus  
Characterizes all ions to the same MS<sup>n</sup> level before advancing to the next MS<sup>n</sup> level.

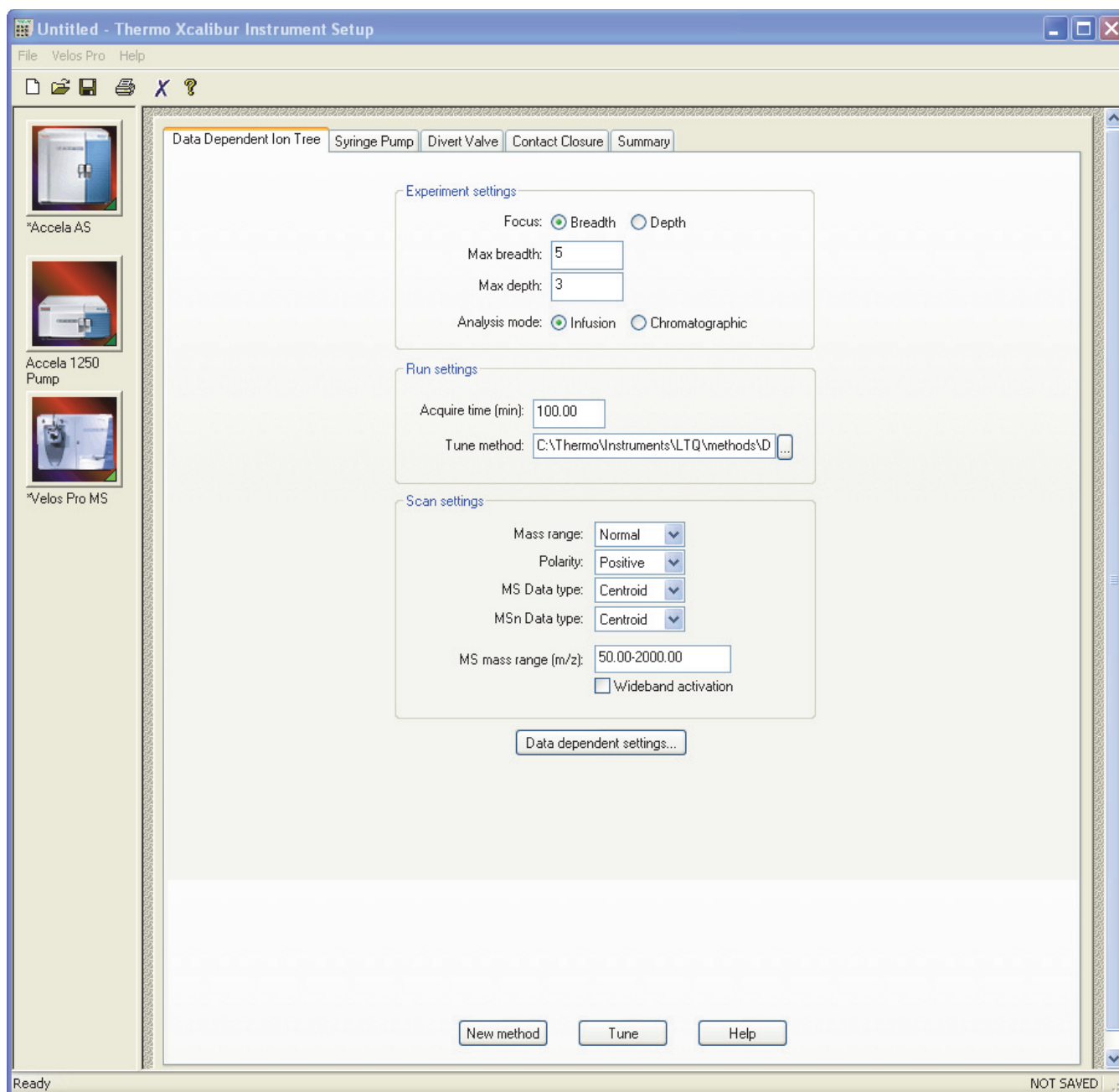
For example, if you specify a Maximum Depth of 3 and a Maximum Breadth of 2 in an Ion Tree experiment, the following occurs:

In either case, the mass spectrometer first scans for parent ions (MS) over the specified mass range. Next, it selects the first most intense ion of the MS spectrum for fragmentation (MS/MS).

- For depth focus, after fragmenting the most intense ion of the MS spectrum—producing an MS/MS spectrum—the mass spectrometer selects and fragments the most intense ion of the MS/MS spectrum. This results in an MS<sup>3</sup> spectrum, the level specified as the maximum depth for this example. The mass spectrometer then backs up one level and fragments the second most intense ion of the MS/MS spectrum, creating more product ions on the level of MS<sup>3</sup> from this parent ion. This process then repeats for the second most intense ion in the MS spectrum.
- For breadth focus, after fragmenting the most intense ion of the MS spectrum—producing an MS/MS spectrum—the mass spectrometer selects and fragments the second-most intense ion in the same MS spectrum. The fragmentation of parent ions continues to the Max Breadth level (2, for this example). After the two most intense peaks on the MS level are fragmented, the mass spectrometer selects and fragments the two most intense ions in the first MS/MS spectrum. This results in product ions on the level of MS<sup>3</sup>, the level specified as the maximum depth for this example. This process then repeats for the second most intense ions in the second MS/MS spectrum.

You can view the results of a Data Dependent Ion Tree experiment in the Xcalibur Qual Browser window. The results appear as a structure tree that originates from a particular parent ion.

**Figure 9.** Ion Tree experiment template on the Data Dependent Ion Tree page



## Mass/Charge Range

The LTQ Series mass spectrometer has three mass/charge range modes:

- Low: 15 to 200 Da
- Normal: 50 to 2000 Da
- High: 100 to 4000 Da



## Setting Up the API Source

This chapter provides information about setting up the API source for the ESI, HESI-II, or APCI modes. The API source consists of the Ion Max™ or Ion Max-S™ API source housing and an ESI, HESI-II, or APCI probe.

**Note** Tune and calibrate the LTQ Series mass spectrometer in ESI or HESI-II mode before acquiring data in ESI, HESI-II, or APCI modes.

### Contents

- [Opening the Tune Plus Window](#)
- [Placing the Mass Spectrometer in Standby Mode](#)
- [API Source Housing Installation and Removal](#)
- [API Source Housing Drain](#)
- [ESI or HESI-II Probe Installation and Removal](#)
- [APCI Probe Installation and Removal](#)
- [Adjusting the Probe Position on the Ion Max API Source Housing](#)

## Opening the Tune Plus Window

There are several methods for opening the Tune Plus window.

### ❖ To open the Tune Plus window

Do one of the following to open the Tune Plus window (Figure 10):

- On the Windows taskbar, choose **Start > Programs > Thermo Instruments > LTQ > *model* Tune**, where *model* is your specific LTQ Series model.

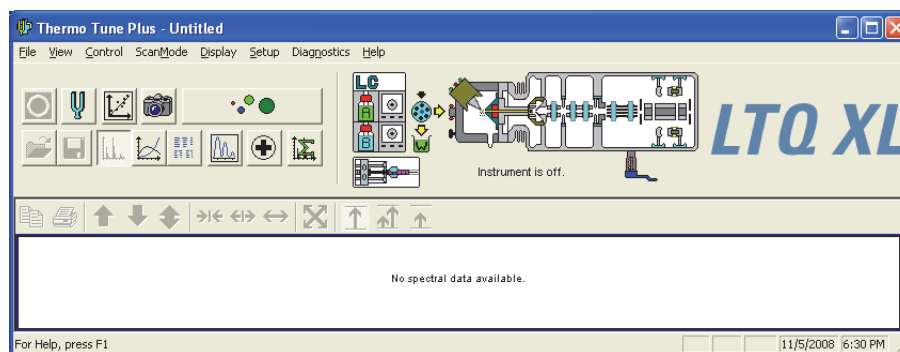
**Note** For LTQ Series version 2.5.0 or earlier, choose **Start > Programs > Xcalibur > *model* Tune**.

- In the Xcalibur application, choose **Roadmap view > Instrument Setup > *model* (left pane) > Tune Plus**.

—OR—

- In the Xcalibur application, choose **Roadmap view > Instrument Setup > *model* (menu toolbar) > Start Tune Plus**.

**Figure 10.** Tune Plus window for the LTQ XL



## Placing the Mass Spectrometer in Standby Mode

Always place the LTQ Series mass spectrometer in standby mode before removing the ion source probe or API source housing.

**Note** When the mass spectrometer is in standby mode, the API gases, high voltage, and syringe pump are off.

### ❖ To place the mass spectrometer in Standby mode

1. Complete all data acquisition, if any.
2. Open the Tune Plus window (see [page 32](#)).
3. If an LC pump is provided, turn off the solvent flow to the API source.

When controlling the LC pump through the Xcalibur data system, you can turn off the solvent flow from the Inlet Direct Control dialog box. For example, to turn off the solvent flow from an Accela™ pump, do the following:

- a. Choose **Setup > Inlet Direct Control**, and then click the tab for the LC pump.
- b. Select the **Take Pump Under Control** check box, and then click the **Stop** button.

4. In the Tune Plus window, do one of the following:

- If the mass spectrometer is off, choose **Control > Standby**.

–or–

- If the mass spectrometer is on, click the **On/Standby** button to select the **Standby** mode.

When clicked, this button cycles through the power modes shown in the left margin.

The LC/MS system is now in standby mode and you can safely remove the ion source probe or API source housing after it has cooled to room temperature.



**CAUTION** If you are using APPI, do not leave the LC or other liquid delivery device on while the mass spectrometer is in standby mode. The absence of sheath and auxiliary gas can cause the hot VUV vacuum lamp to break upon contact with liquids.

The mass spectrometer turns off the [electron multipliers](#), [consecutive reaction monitoring \(CRM\) scan types](#), 8 kV power to the API source, main rf voltage, and ion optic rf voltages. The mass spectrometer also turns off the auxiliary and sheath gas flows.

Refer to Chapter 3 in the *LTQ Series Hardware Manual* for the On/Off status of the mass spectrometer components when the mass spectrometer is in standby mode. The System LED on the front panel turns yellow when the system is in standby mode.

# API Source Housing Installation and Removal

The Ion Max or Ion Max-S API source housing holds the ESI, HESI-II, or APCI probe. The Ion Max has two features that the Ion Max-S does not have: an adjustable probe port and a front door with a window. Aside from these two features, these two source housings have the same functionality and mount to the LTQ Series mass spectrometer in the same way. No tools are needed to remove or install the API source housing or source drain.

**Note** These instructions apply to both the Ion Max and Ion Max-S API source housing, unless otherwise noted.

This section provides the following procedures:

- “Installing the API Source Housing” on page 34
- “Removing the API Source Housing” on page 36

## Installing the API Source Housing

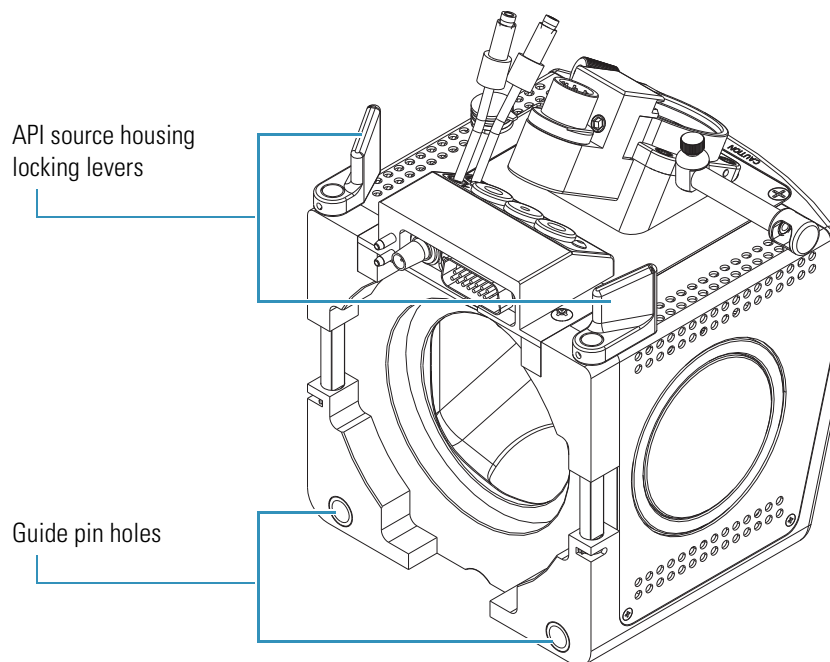
### ❖ To install the API source housing

1. Place the mass spectrometer in **Standby** mode (see page 33).
2. Place the locking levers on the API source housing in the unlocked position (Figure 11).
3. Align the two guide pin holes on the back of the API source housing (Figure 11) with the API source housing guide pins on the API source mount on the front of the mass spectrometer (Figure 12), and carefully press the housing until it is flush with the mount.

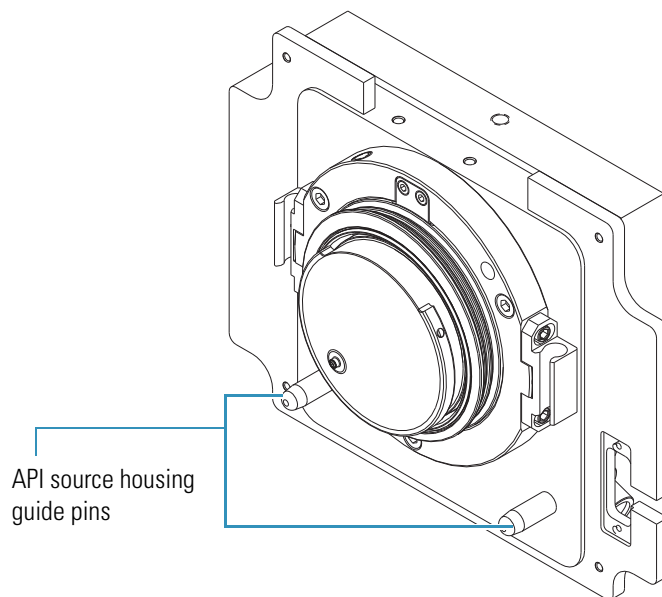
**Note** From the back view, the Ion Max looks the same as the Ion Max-S.



**Figure 11.** Ion Max-S API source housing (back view)



**Figure 12.** API ion source mount assembly showing the guide pins



4. Rotate the API source housing locking levers 90 degrees toward the housing to lock them.

## 2 Setting Up the API Source

### API Source Housing Installation and Removal

5. Install the source drain assembly as follows:



**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.

- a. Connect the source drain assembly to the API source housing drain fitting (see [page 38](#)).
- b. Attach the free end of the hose to a waste container, and vent the waste container to a fume exhaust system.

## Removing the API Source Housing

To access the APCI corona needle or the ion source interface, you must remove the API source housing.



**CAUTION HOT SURFACE** At operating temperatures above 350 °C (662 °F), the probe and API source housing can severely burn you.

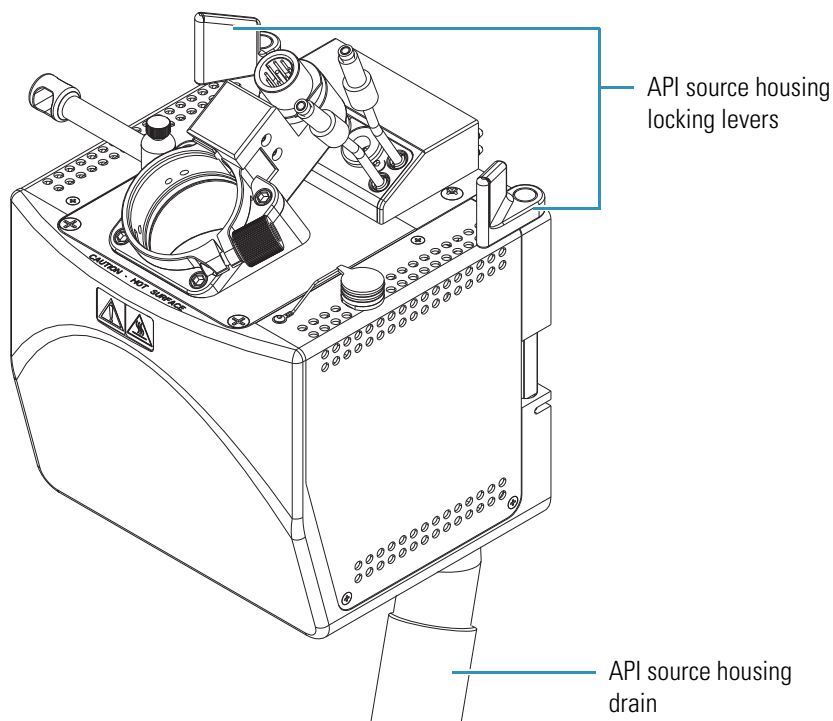
- Before removing the probe or API source housing, allow the part to cool to room temperature (approximately 20 minutes) before touching it.
- If the mass spectrometer connects to an LC system, leave the solvent flow from the LC pump on while the probe cools to room temperature.

### ❖ To remove the API source housing

1. Place the mass spectrometer in **Standby** mode ([page 33](#)) and let it cool to room temperature. See the preceding CAUTION statement.
2. If there is a probe connected to the API source housing, disconnect the external liquid lines before removing the API source housing.

3. Remove the source drain tube from the API source housing drain (Figure 13).

**Figure 13.** Ion Max-S API source housing showing the locking levers and drain (front view)



4. Rotate the API source housing locking levers 90 degrees away from the housing to unlock it.
5. Pull the API source housing straight off of the API source mount assembly.
6. Place the housing in a safe location for temporary storage.

## API Source Housing Drain

When installing the API source, connect the drain at the bottom of the API source housing to the solvent waste container (Figure 14).

**Figure 14.** API source drain assembly and waste container

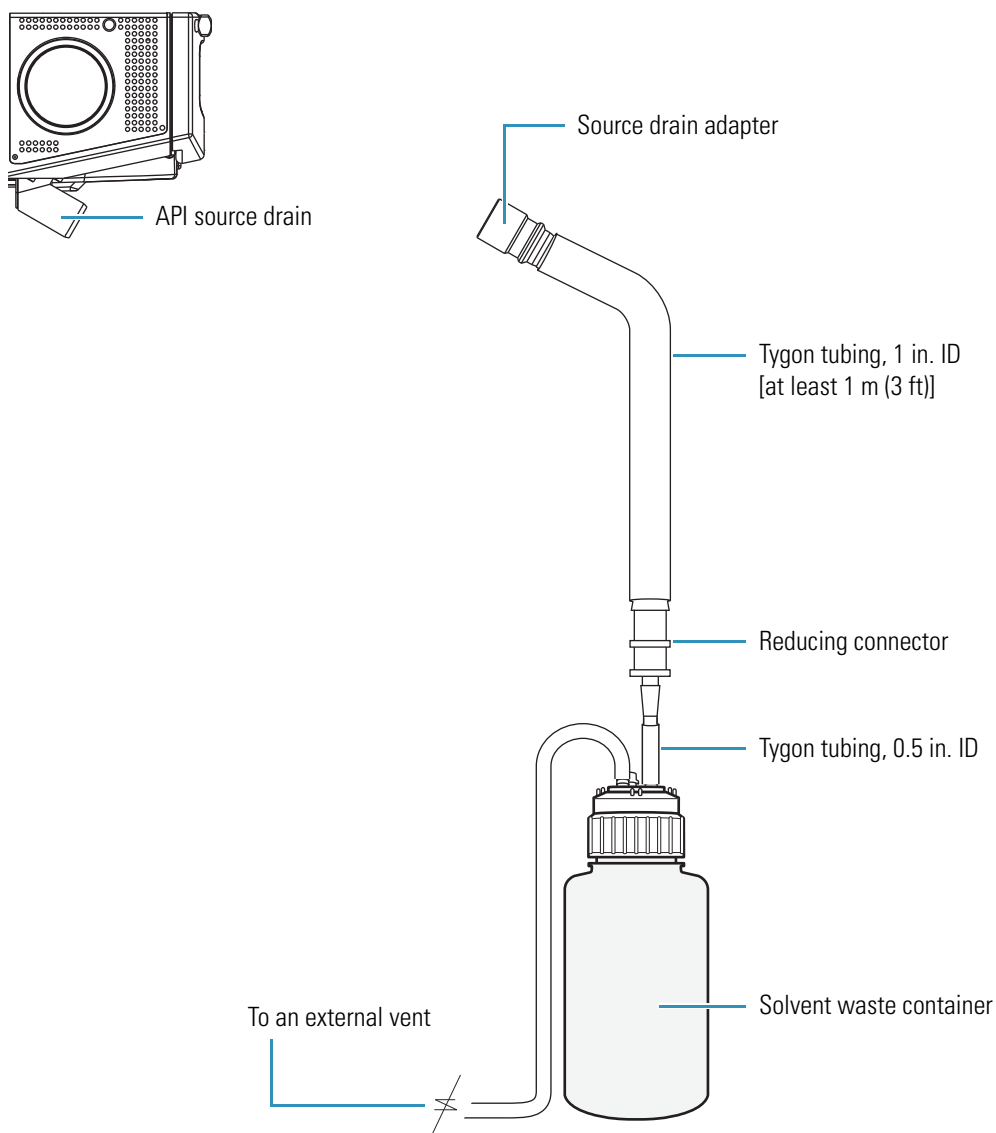


Table 6 lists the components of the solvent waste system. During the initial installation of the mass spectrometer, a Thermo Fisher Scientific field service engineer installs the solvent waste system.

**Table 6.** Solvent waste system parts

Description	Part Number	Kit
Cap, filling/venting	00301-57022	MS Ship Kit
Heavy-duty, 4 L Nalgene™ container	00301-57020	MS Ship Kit
Reducing connector, single barbed fitting, 1 in. ID to 0.5 in. ID	00101-03-00001	MS Ship Kit
Source drain adapter, Teflon™	70111-20971	MS Accessory Kit
Tubing, Tygon™, 0.5 in. ID, 3/4 in. OD	00301-22920	MS Ship Kit
Tubing, Tygon PVC, 1 in. ID, 1-3/8 in. OD	00301-22922	MS Ship Kit

When reconnecting the API source drain tubing to the drain at the bottom of the API source housing, ensure that you first connect the Teflon source drain adapter. This adapter can withstand the high temperatures produced by the H-ESI or APCI source.

**CAUTION**

Follow these guidelines for the API source drain:

- Do not connect silicone tubing to the API source drain. If silicone tubing connects to the outlet drain, you might observe background ions at  $m/z$  536, 610, and 684. Use the PVC tubing provided with the solvent waste container to connect the solvent waste container to a fume exhaust system.
- Do not connect Tygon tubing directly to the API source drain. At high temperatures, Tygon releases volatile contaminants. Use the Teflon Source Drain adapter as described in the *LTQ Series Getting Connected Guide*.
- Prevent solvent waste from backing up into the mass spectrometer. Always ensure that the Tygon line from the mass spectrometer to the solvent waste container and the PVC line from the waste container to the exhaust are above the level of liquid in the waste container.

Equip your lab with at least two fume exhaust systems:

- The analyzer optics can become contaminated if the API source drain tube and the (blue) exhaust tubing from the forepumps connect to the same fume exhaust system. Route the (blue) exhaust tubing from the forepumps to a dedicated fume exhaust system.
- Do not vent the PVC drain tube (or any vent tubing connected to the waste container) to the same fume exhaust system that the forepumps connect to. Vent the waste container to a dedicated fume exhaust system. The exhaust system for the Ion Max or Ion Max-S API source must accommodate a flow rate of up to 30 L/min (64 ft<sup>3</sup>/h).

# ESI or HESI-II Probe Installation and Removal

The Ion Max and Ion Max-S API source housings have the same interlock socket, probe interlock block, probe locking knob, and probe locking ring. No tools are needed to remove or install the probe.



**CAUTION HOT SURFACE** At operating temperatures above 350 °C (662 °F), the probe and API source housing can severely burn you.

- Before removing the probe or API source housing, allow the part to cool to room temperature (approximately 20 minutes) before touching it.
- If the mass spectrometer connects to an LC system, leave the solvent flow from the LC pump on while the probe cools to room temperature.

This section provides the following procedures:

- “[Installing the ESI or HESI-II Probe](#)” on [page 40](#)
- “[Removing the ESI or HESI-II Probe](#)” on [page 47](#)

## Installing the ESI or HESI-II Probe

These instructions are for the ESI and HESI-II probes. All steps are for both probe types, unless otherwise noted.

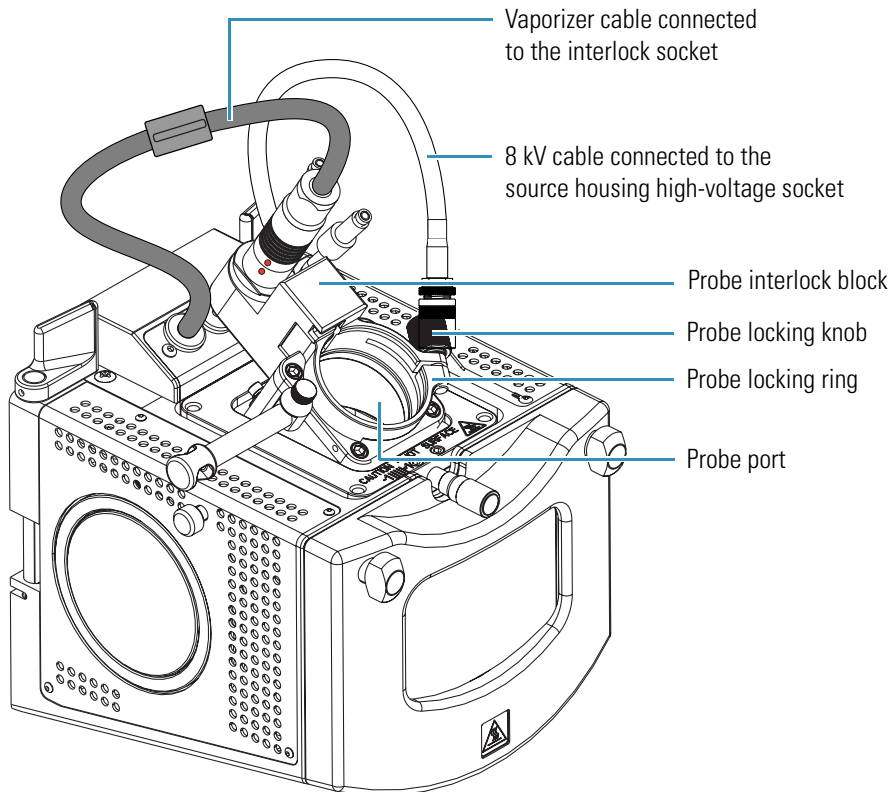
### ❖ To install the ESI or HESI-II probe

1. Place the mass spectrometer in **Standby** mode (see [page 33](#)) and let it cool to room temperature. See the preceding CAUTION statement.
2. If the mass spectrometer is set up for APCI mode, follow the procedure “[Removing the APCI Probe and the Corona Needle](#)” on [page 59](#).
3. If necessary, inspect and clean the selected probe before installing it.
4. If installing the ESI probe, ensure that it has a fused-silica or metal needle sample tube installed ([Figure 23](#) on [page 48](#)).

Refer to the *LTQ Series Hardware Manual* or the installation guide provided in the Metal Needle Kit.

5. Turn the probe locking knob counterclockwise until the probe locking ring opens to its widest position (Figure 15).

**Figure 15.** Ion Max API source housing (left side view)



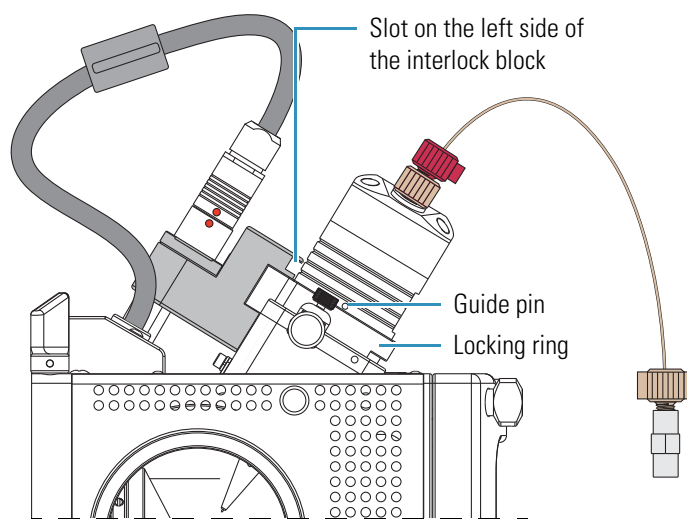
## 2 Setting Up the API Source

ESI or HESI-II Probe Installation and Removal

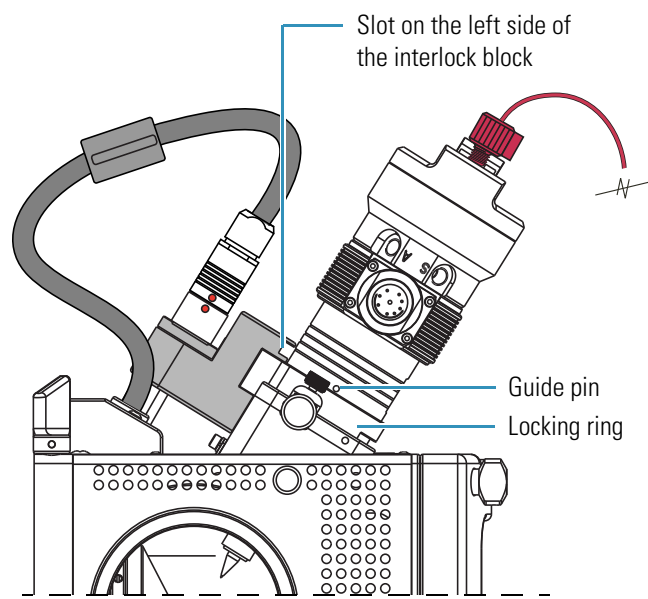
6. Position the probe in the API source housing probe port as follows:
  - a. Hold the probe with the nozzle facing down and the guide pin facing toward the left, and then slowly insert the probe into the port until the guide pin meets the locking ring on the source housing.

Figure 16 shows the ESI probe, and Figure 17 shows the HESI-II probe.

**Figure 16.** ESI probe showing the guide pin and locking ring



**Figure 17.** HESI-II probe showing the guide pin and locking ring

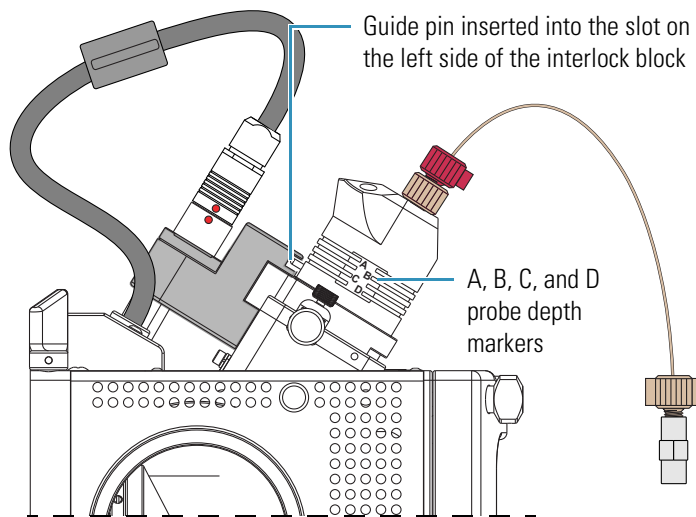




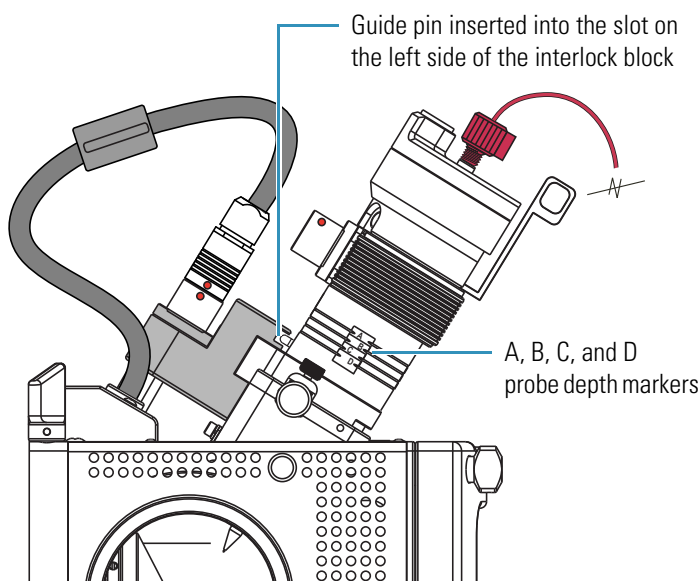
- b. Pull the probe slightly upward until the guide pin is level with the slot on the left side of the interlock block, and then turn the probe clockwise until the guide pin meets resistance from the interlock block.

Figure 18 shows the ESI probe, and Figure 19 shows the HESI-II probe.

**Figure 18.** ESI probe showing the guide pin inserted into the interlock block slot



**Figure 19.** HESI-II probe showing the guide pin inserted into the interlock block slot



## 2 Setting Up the API Source

### ESI or HESI-II Probe Installation and Removal

- c. Push the probe down into the port to the appropriate depth indicated by the A, B, C, and D depth markers on the probe.

Insert the probe to a depth of B, C, or D. For high solvent flow rates, adjust the probe depth so that the nozzle is farther away from the ion interface (depth C or D). Conversely, for low solvent flow rates, adjust the probe depth so that the nozzle is closer to the ion interface (depth B or C).

**Note** Probe position A is used only for instrument calibration (flow rates less than 10  $\mu\text{L}/\text{min}$ ).

7. Lock the probe in place by turning the probe locking knob clockwise until you feel resistance.
8. Connect the nitrogen gas lines to the probe as follows:
  - Connect the sheath gas fitting (blue) to the sheath gas inlet (S).
  - Connect the auxiliary gas fitting (green) to the auxiliary gas inlet (A).
9. Connect the 8 kV cable connector to the 8 kV cable socket on the probe, and then tighten the locking ring by turning it clockwise.



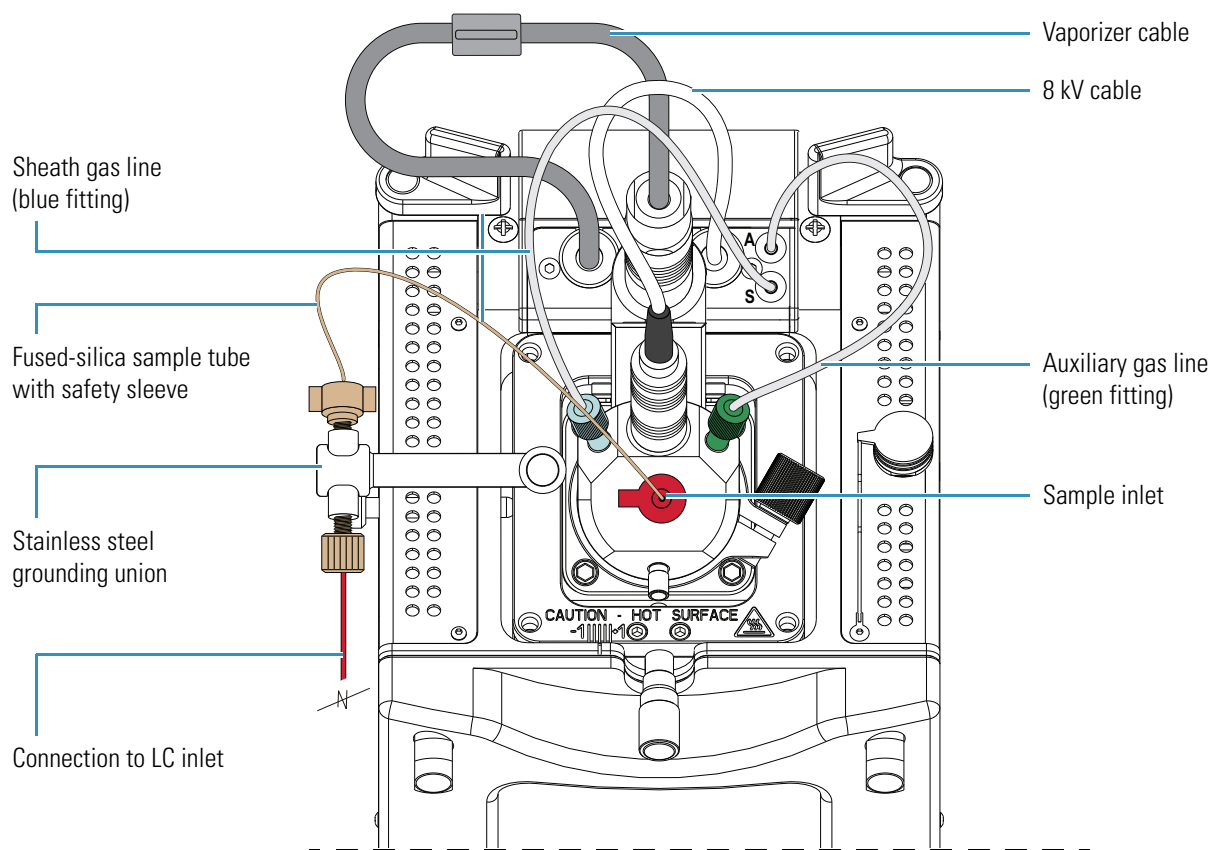
**CAUTION HIGH VOLTAGE** The mass spectrometer must be in standby mode before you disconnect or connect the 8 kV cable.

10. If installing the HESI-II probe, do the following:
  - Unplug the vaporizer cable connector from the interlock socket and connect it to the vaporizer cable connector socket on the probe (Figure 28 on page 52).
  - Align the socket pins with the socket by aligning the red dot on the vaporizer cable connector with the red dot on the interlock socket.
11. Ensure that the grounding union is positioned in the grounding union bar.
12. Use two fingertight fittings to connect a length of red PEEK™ tubing to the LC outlet and to the left side of the grounding union.
13. If installing the HESI-II probe, use two fingertight fittings to connect a short length of red PEEK tubing to the probe sample inlet and to the right side of the grounding union.

Figure 20 shows the ESI probe installed in the Ion Max API source housing. Figure 21 on page 46 shows the HESI-II probe installed in the Ion Max API source housing.

**Note** The LTQ Series mass spectrometer auto-senses the installed probe type.

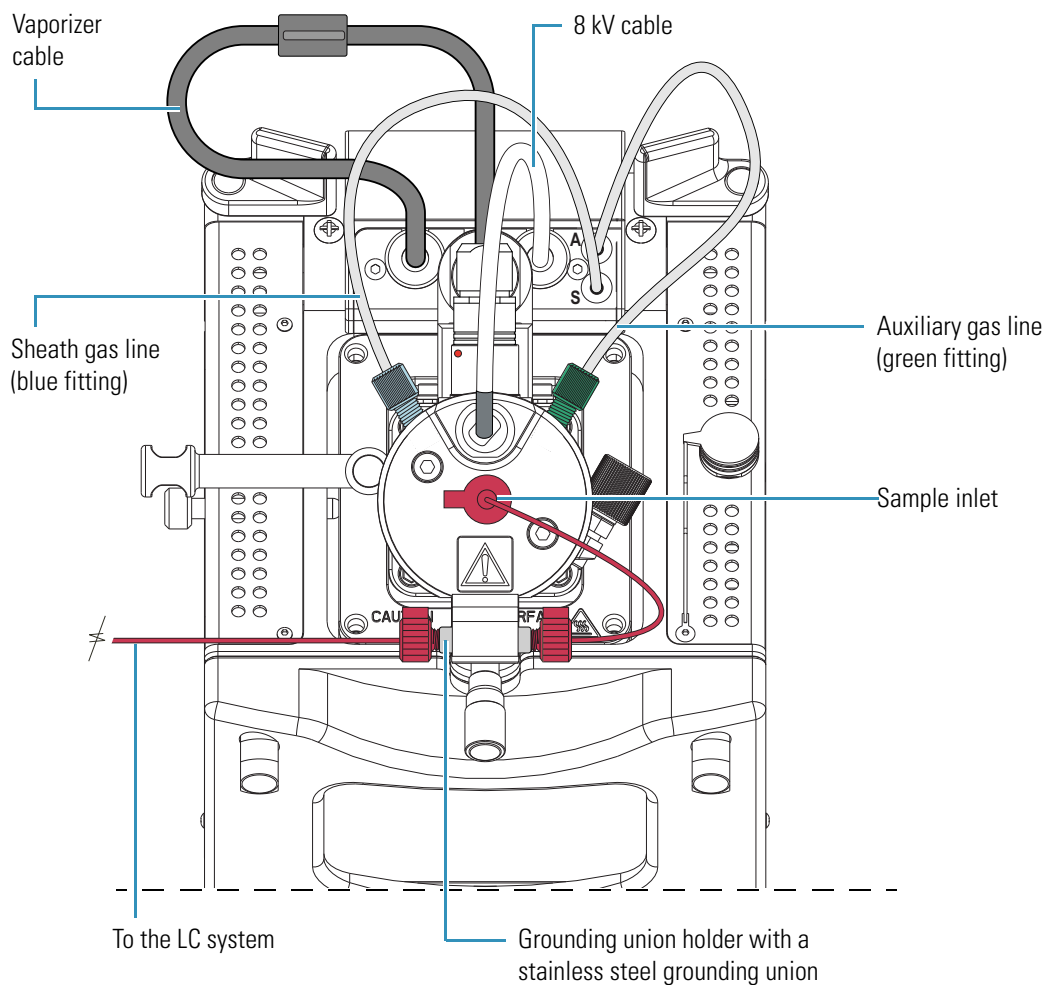
Figure 20. ESI probe installed in the Ion Max API source housing



## 2 Setting Up the API Source

ESI or HESI-II Probe Installation and Removal

**Figure 21.** HESI-II probe installed in the Ion Max API source housing



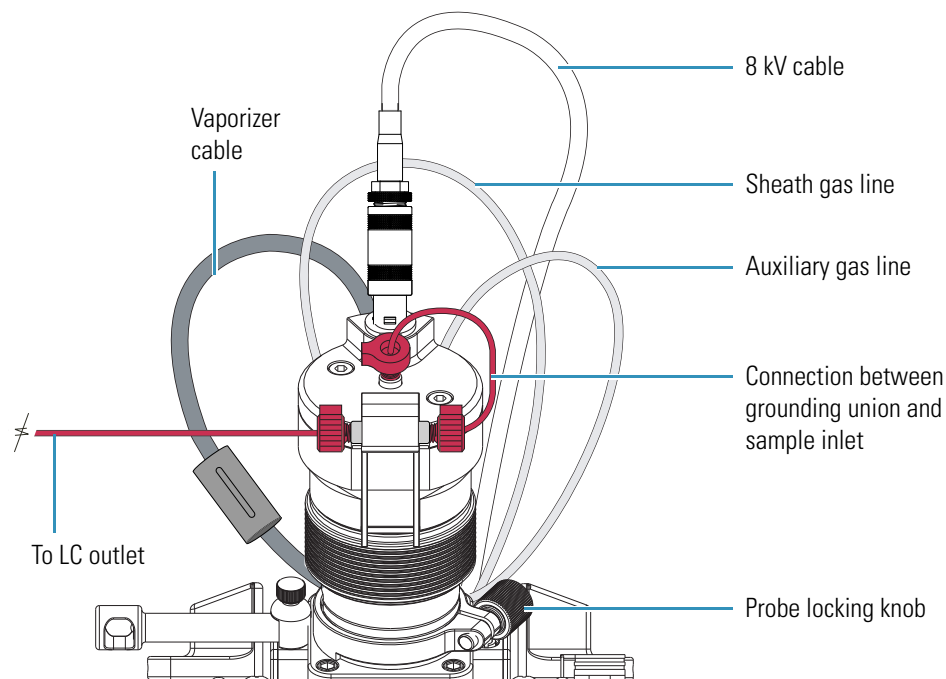
## Removing the ESI or HESI-II Probe

These instructions are for the ESI and HESI-II probes. All steps are for both probe types, unless otherwise noted.

### ❖ To remove the probe from the API source housing

1. Place the mass spectrometer in **Standby** mode (see [page 33](#)) and let it cool to room temperature. See the CAUTION statement on [page 40](#).
2. If provided, turn off the solvent flow from the optional LC pump to the API source (see [step 3](#) on [page 33](#)).
3. If removing the HESI-II probe, disconnect the tubing from the left side of the HESI-II probe grounding union (Figure 22).

**Figure 22.** HESI-II probe connections



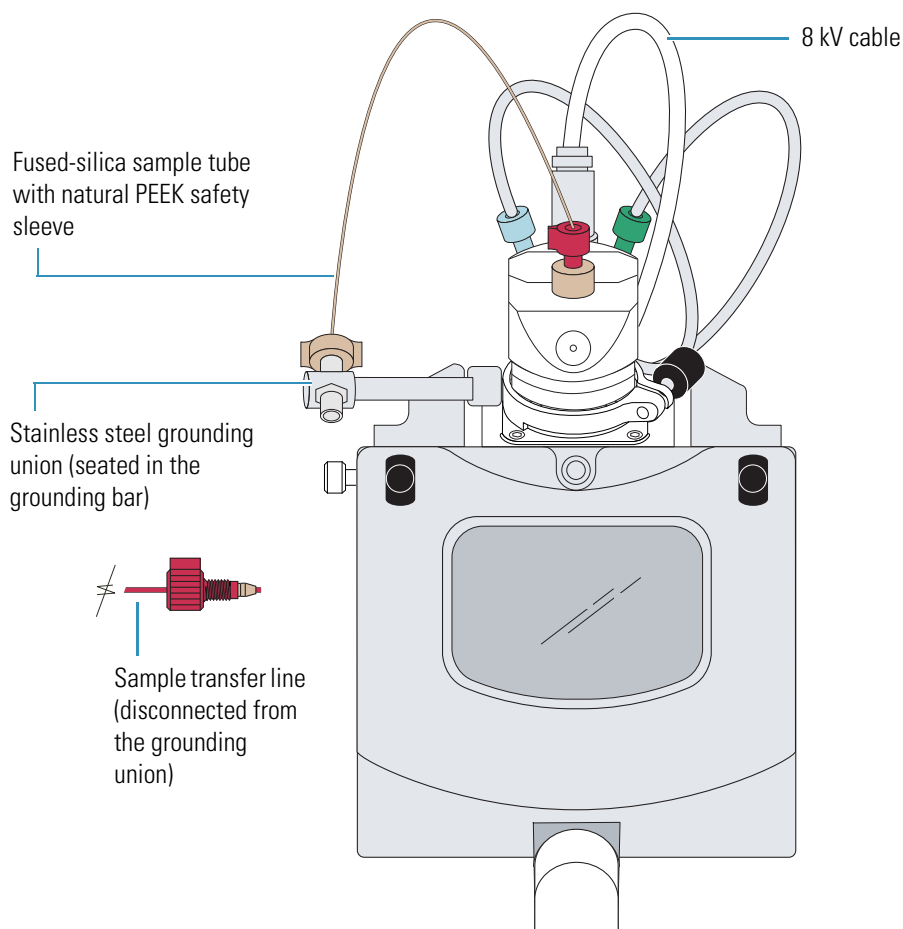
## 2 Setting Up the API Source

ESI or HESI-II Probe Installation and Removal

4. Disconnect the sample transfer line from the grounding union, which is positioned in the grounding bar (Figure 23).

**Tip** Unless you want to replace the sample tube, do not disconnect it from the probe or the stainless steel grounding union when removing the probe from the API source housing.

**Figure 23.** Sample transfer line disconnected from the grounding union



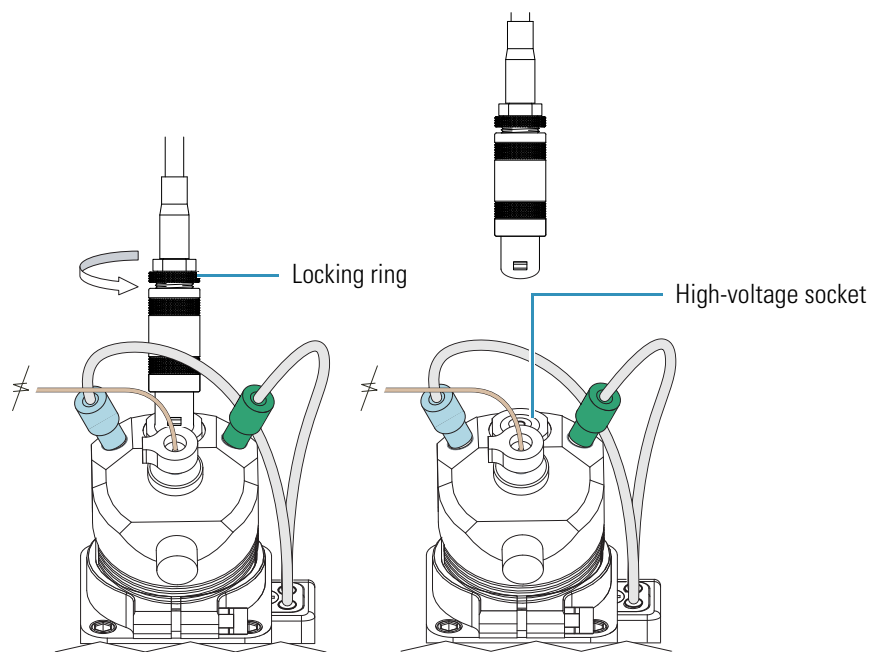
5. Disconnect the 8 kV cable from the probe (Figure 24 and Figure 25) as follows:



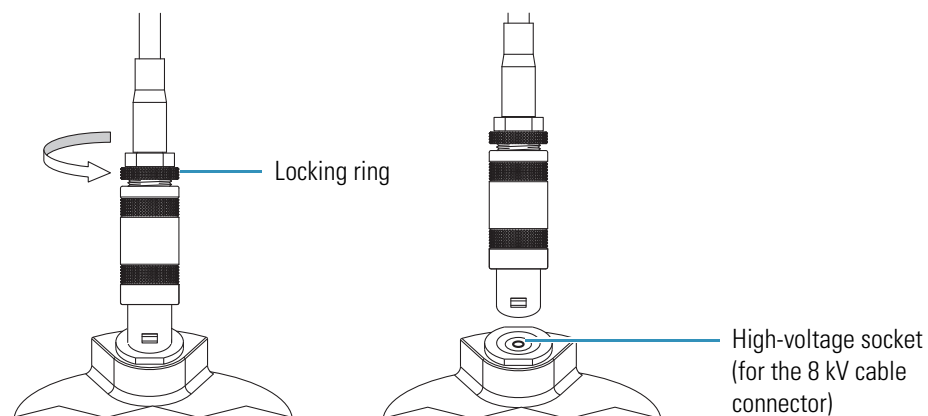
**CAUTION HIGH VOLTAGE** The mass spectrometer must be in standby mode before you disconnect or connect the 8 kV cable.

- Unlock the cable connector by turning the probe locking ring counterclockwise.
- Pull the 8 kV cable connector from the probe high-voltage socket.

**Figure 24.** 8 kV cable connector removed from the ESI probe



**Figure 25.** 8 kV cable connector removed from the HESI-II probe (enlarged view)

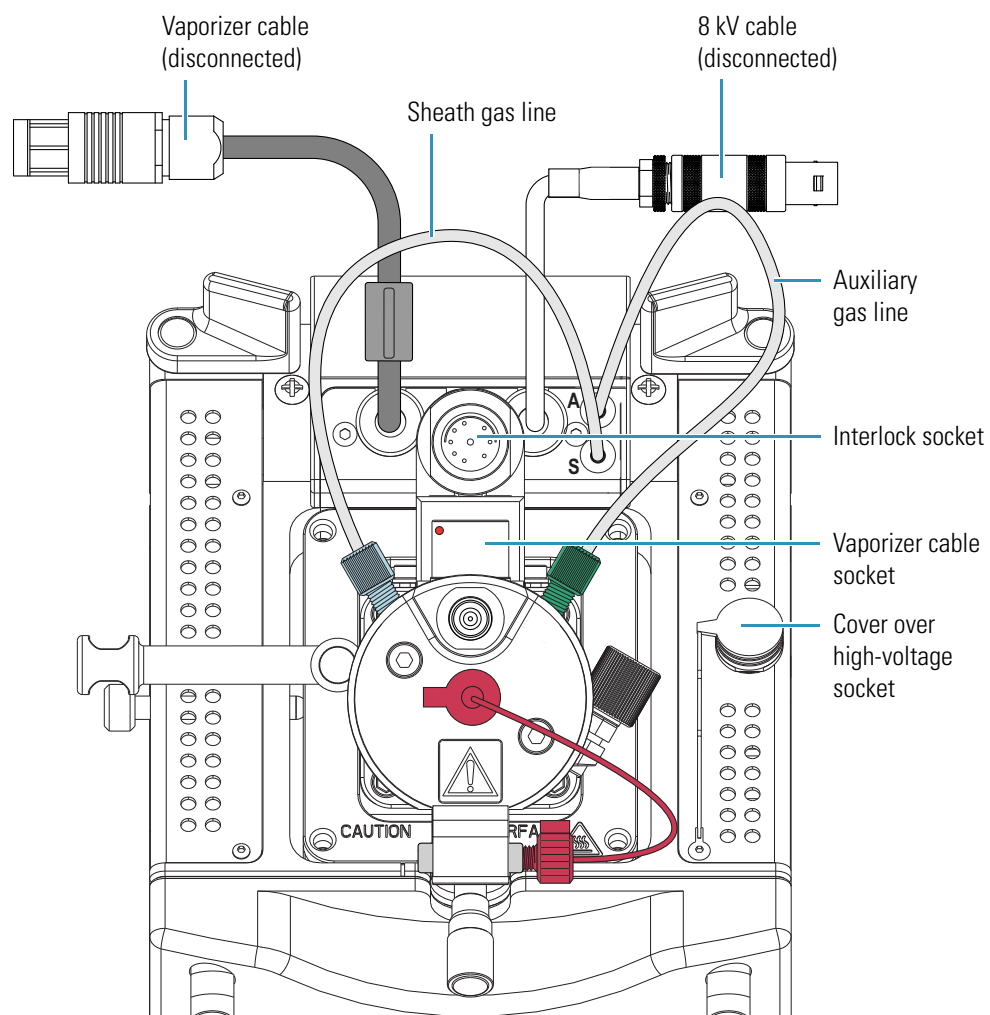


## 2 Setting Up the API Source

ESI or HESI-II Probe Installation and Removal

6. Disconnect the vaporizer cable from the probe vaporizer cable socket (Figure 26).

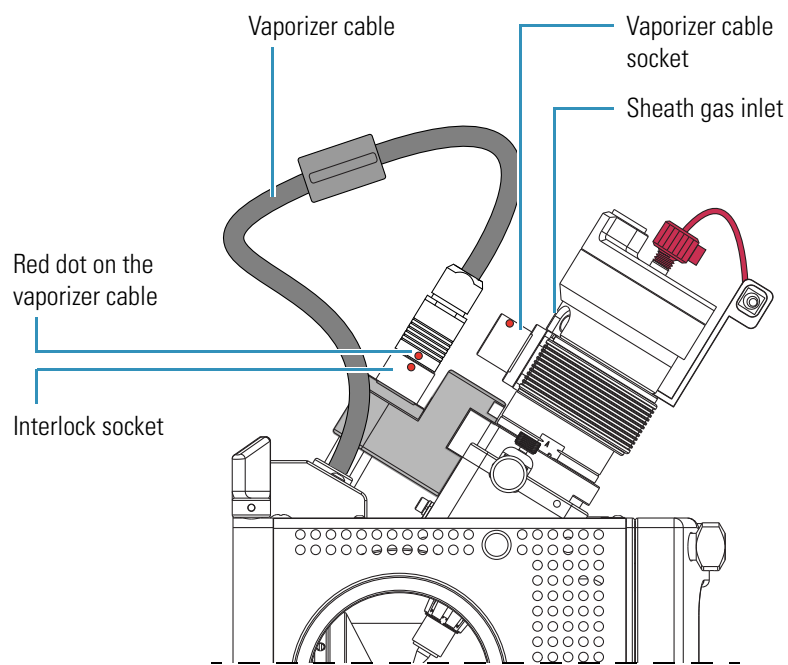
**Figure 26.** HESI-II probe showing the disconnected vaporizer and 8 kV cables (top view)





7. Connect the vaporizer cable connector to the interlock socket on the interlock block, and align the socket pins by aligning the red dot on the vaporizer cable connector with the red dot on the interlock socket (Figure 27).

**Figure 27.** API source housing with the HESI-II probe (left side view)



8. Disconnect the nitrogen gas lines from the probe as follows:
  - Disconnect the auxiliary gas fitting (green) from the auxiliary gas inlet (A).
  - Disconnect the sheath gas fitting (blue) from the sheath gas inlet (S).

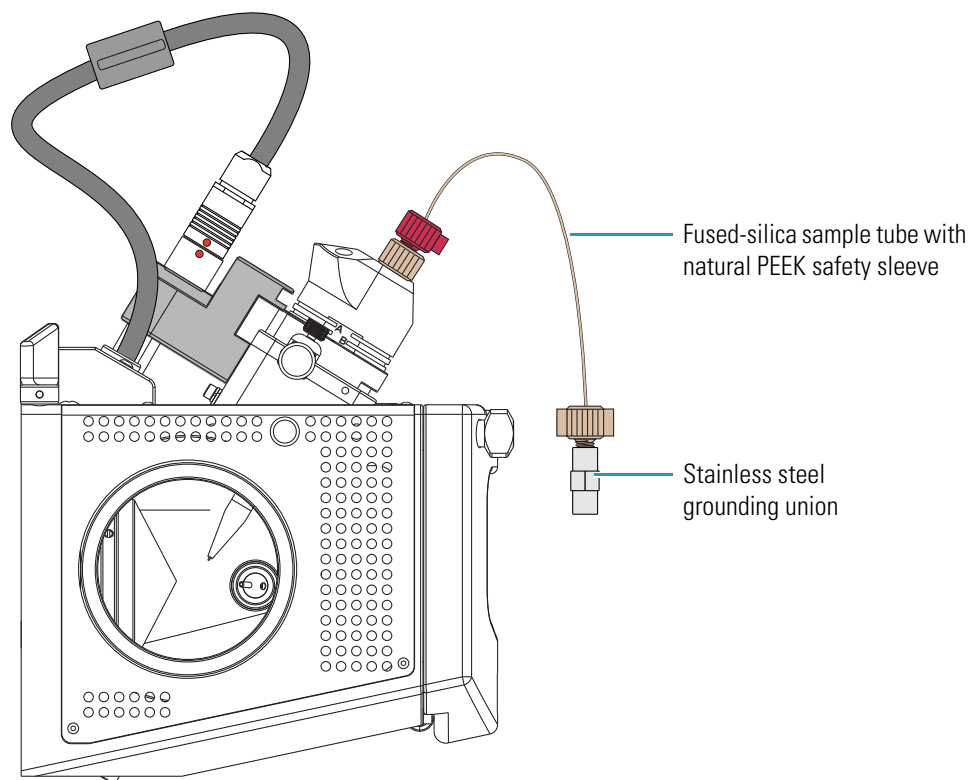
## 2 Setting Up the API Source

### ESI or HESI-II Probe Installation and Removal

9. If removing the ESI probe, remove the grounding union from the grounding bar.

Figure 28 shows the nitrogen lines and 8 kV cable disconnected from the ESI probe, and the grounding union removed from the grounding union bar.

**Figure 28.** API source housing with ESI probe (left side view)



10. Unlock the probe locking ring by turning the probe locking knob counterclockwise.
11. Remove the probe as follows:
  - a. Slowly pull the probe out of the port until you feel the resistance caused by the probe guide pin meeting the interlock block.
  - b. Turn the probe counterclockwise until the guide pin is free of the interlock block.
  - c. When the guide pin is free of the interlock block, pull the probe out of the port.
12. If needed, clean the probe before storing it in its original shipping container.

## APCI Probe Installation and Removal

The only tools needed to remove or install the corona needle is a set of pliers. This section provides the following procedures:

- “Installing the Corona Needle and APCI Probe” on page 53
- “Removing the APCI Probe and the Corona Needle” on page 59



**CAUTION HOT SURFACE** At operating temperatures above 350 °C (662 °F), the probe and API source housing can severely burn you.

- Before removing the probe or API source housing, allow the part to cool to room temperature (approximately 20 minutes) before touching it.
- If the mass spectrometer connects to an LC system, leave the solvent flow from the LC pump on while the probe cools to room temperature.

**Note** The figures in this section show the Ion Max API source housing.

### Installing the Corona Needle and APCI Probe

To operate the system in APCI mode, install the corona needle and APCI probe.

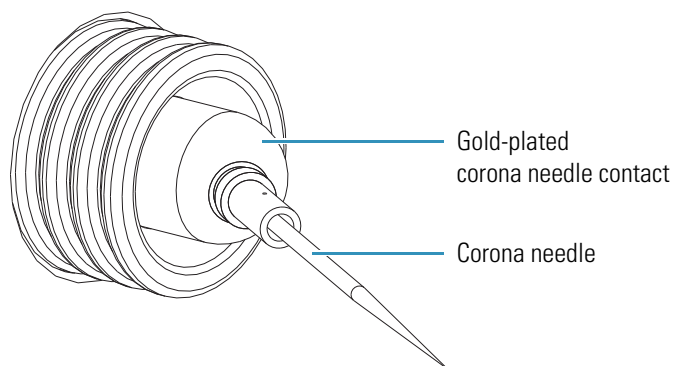
❖ **To install the corona needle**

1. Place the mass spectrometer in **Standby** mode (see page 33) and let it cool to room temperature. See the preceding CAUTION statement.
2. If the mass spectrometer is set up for ESI or H-ESI mode, follow the procedure “Removing the ESI or HESI-II Probe” on page 47.
3. Remove the API source housing (see page 36).
4. Grasp the clean corona needle by using the pliers and carefully push the larger end straight into the corona needle contact (Figure 29).



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

**Figure 29.** Corona needle

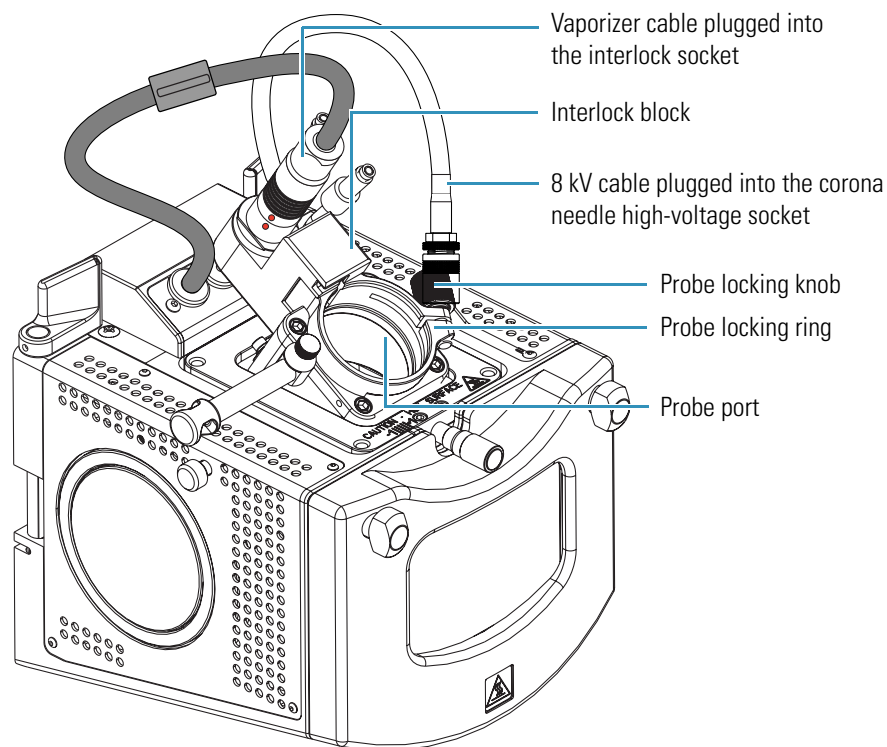


5. Ensure that the needle tip aligns with the travel path between the APCI probe and the ion source interface.
6. Reinstall the API source housing (see [page 34](#)).

❖ **To install the APCI probe**

1. Connect the 8 kV cable connector to the high-voltage socket on the API source housing as follows:
  - a. On the right side of the API source housing, remove the cover over the high-voltage socket, which is for the corona needle.
  - b. Connect the 8 kV cable connector into the high-voltage socket on the API source housing ([Figure 30](#)).
  - c. Lock the 8 kV cable by turning the locking ring clockwise.

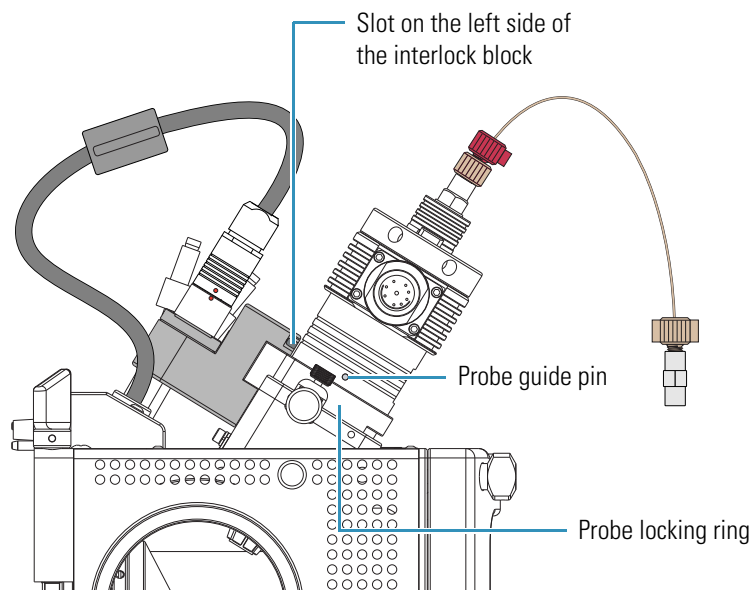
**Figure 30.** Ion Max API source housing



2. Turn the probe locking knob counterclockwise until the probe locking ring opens to its widest position.

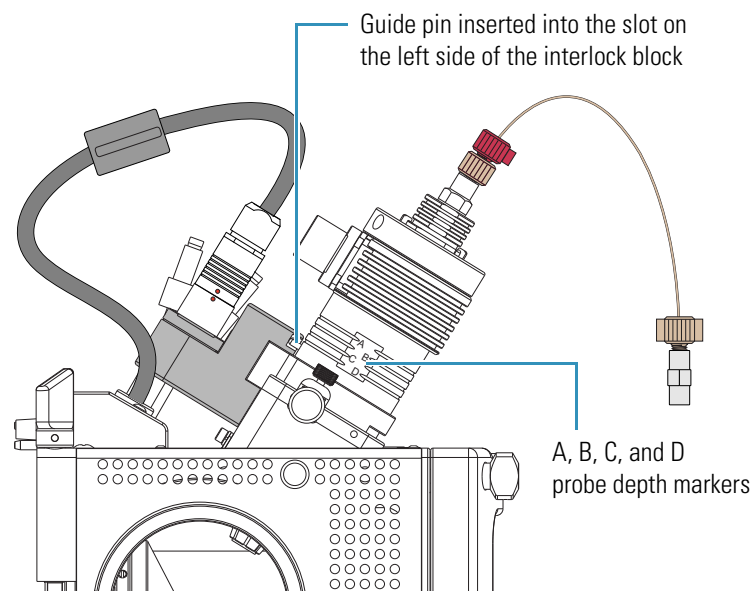
3. Position the probe in the API source housing probe port as follows:
  - a. Hold the probe with the nozzle facing down and the guide pin facing toward the left, and then slowly insert the probe into the port until the guide pin meets the locking ring on the API source housing (Figure 31).

**Figure 31.** APCI probe guide pin touching the locking ring



- b. Pull the probe slightly upward until the guide pin is level with the slot on the left side of the interlock block, and then turn the probe clockwise until the guide pin meets resistance from the interlock block (Figure 32).

**Figure 32.** APCI probe guide pin inserted into the interlock block slot



- c. Push the probe down into the port to the appropriate depth indicated by the A, B, C, and D depth markers on the probe.

Insert the probe to a depth of B, C, or D. For high solvent flow rates, adjust the probe depth so that the nozzle is farther away from the ion interface (depth C or D). Conversely, for low solvent flow rates, adjust the probe depth so that the nozzle is closer to the ion interface (depth B or C).

**Note** Probe position A is used only for instrument calibration (flow rates less than 10  $\mu\text{L}/\text{min}$ ).

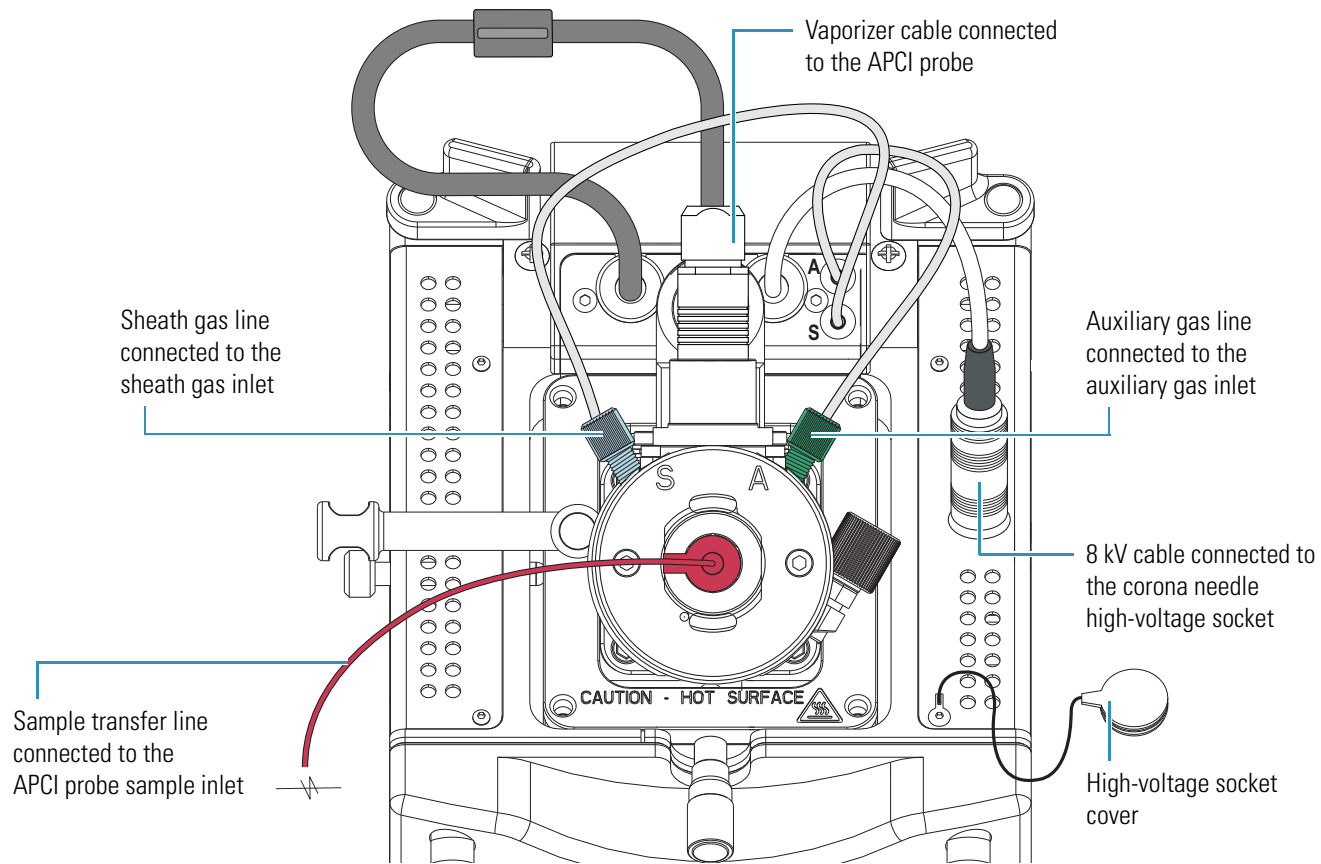
4. Lock the probe in place by turning the probe locking knob clockwise until you feel resistance.
5. Connect the nitrogen gas lines to the probe as follows:
  - Connect the sheath gas fitting (blue) to the sheath gas inlet (S).
  - Connect the auxiliary gas fitting (green) to the auxiliary gas inlet (A).
6. Unplug the vaporizer cable connector from the interlock socket and connect it to the vaporizer cable connector socket on the APCI probe (Figure 33).
7. Align the socket pins with the socket by aligning the red dot on the vaporizer cable connector with the red dot on the interlock socket.
8. Connect the sample transfer line to the probe sample inlet.

Figure 33 shows the APCI probe installed in the Ion Max API source housing.

## 2 Setting Up the API Source

APCI Probe Installation and Removal

**Figure 33.** APCI probe installed in the Ion Max API source housing





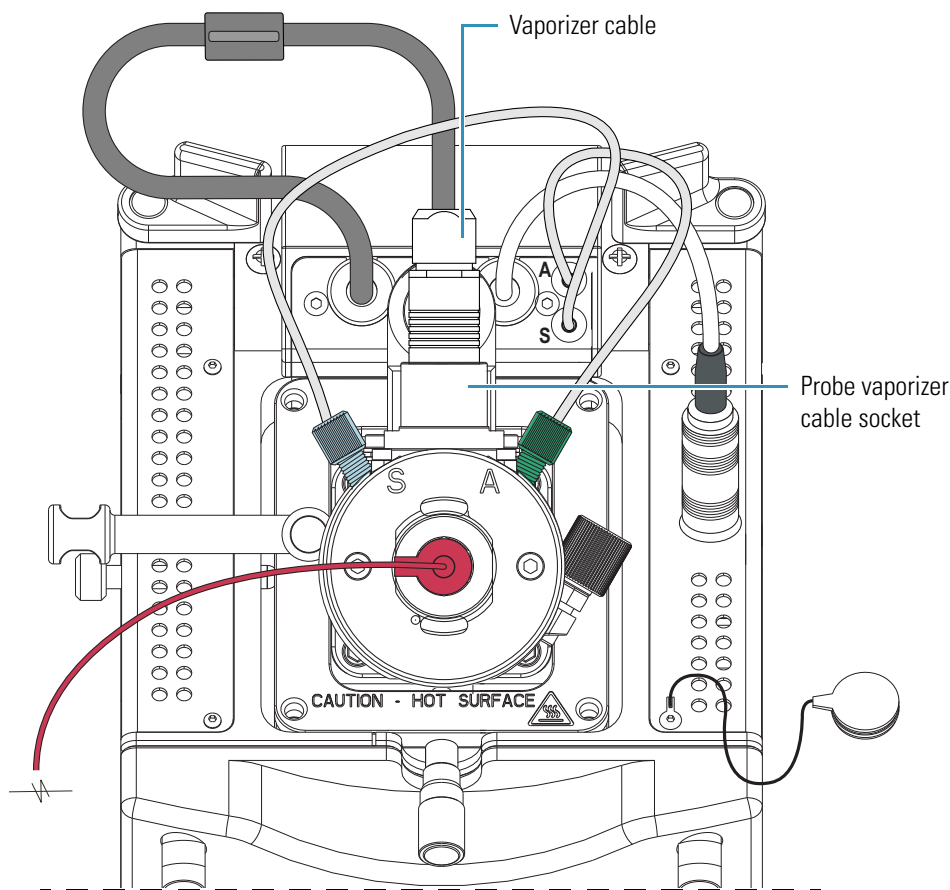
## Removing the APCI Probe and the Corona Needle

This section describes how to remove the APCI probe and the corona needle.

### ❖ To remove the APCI probe

1. Place the mass spectrometer in **Standby** mode (see [page 33](#)) and let it cool to room temperature. See the CAUTION statement on [page 53](#).
2. If an LC pump is provided, turn off the solvent flow to the API source (see [step 3](#) on [page 33](#)).
3. Unplug the vaporizer cable from the probe vaporizer cable socket ([Figure 34](#)).

**Figure 34.** APCI probe connections



4. Disconnect the nitrogen gas lines from the probe as follows:
  - Disconnect the auxiliary gas fitting (green) from the auxiliary gas inlet (A).
  - Disconnect the sheath gas fitting (blue) from the sheath gas inlet (S).
5. Connect the vaporizer cable connector to the interlock socket on the interlock block.

6. Align the socket pins with the socket by aligning the red dot on the vaporizer cable connector with the red dot on the interlock socket (Figure 30 on page 55).
7. Unlock the probe locking ring by turning the probe locking knob counterclockwise.
8. Remove the probe from the probe port in the API source housing as follows:
  - a. Slowly pull the probe out of the port until you feel the resistance caused by the probe guide pin meeting the interlock block.
  - b. Turn the probe counterclockwise until the guide pin is free of the interlock block.
  - c. When the guide pin is free of the interlock block, pull the probe out of the port.
9. If needed, clean the probe before storing it in its original shipping container.

❖ **To remove the corona needle**

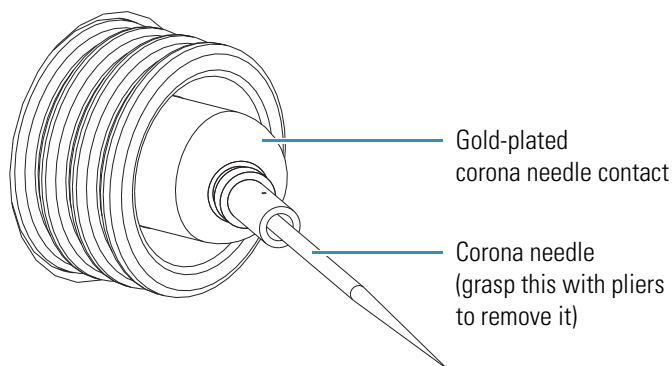


**CAUTION SHARP OBJECT** The corona needle is very sharp and can puncture your skin. Handle it with care.

1. Place the mass spectrometer in **Standby** mode (see page 33) and let it cool to room temperature. See the CAUTION statement on page 53.
2. Remove the APCI probe (see page 59).
3. Disconnect the 8 kV cable from the high-voltage socket on the API source housing.
4. Insert the high-voltage socket cover into the high-voltage socket on the API source housing.
5. Remove the API source housing (see page 36).
6. Grasp the corona needle by using the pliers and carefully pull it straight out of the corona needle contact (Figure 35).

The corona needle is in the corona assembly inside of the API source housing across from the window.

**Figure 35.** Corona needle



7. Reinstall the API source housing or place it in a safe location for temporary storage.
8. If needed, clean the corona needle before storing it in its original shipping container.

## Adjusting the Probe Position on the Ion Max API Source Housing

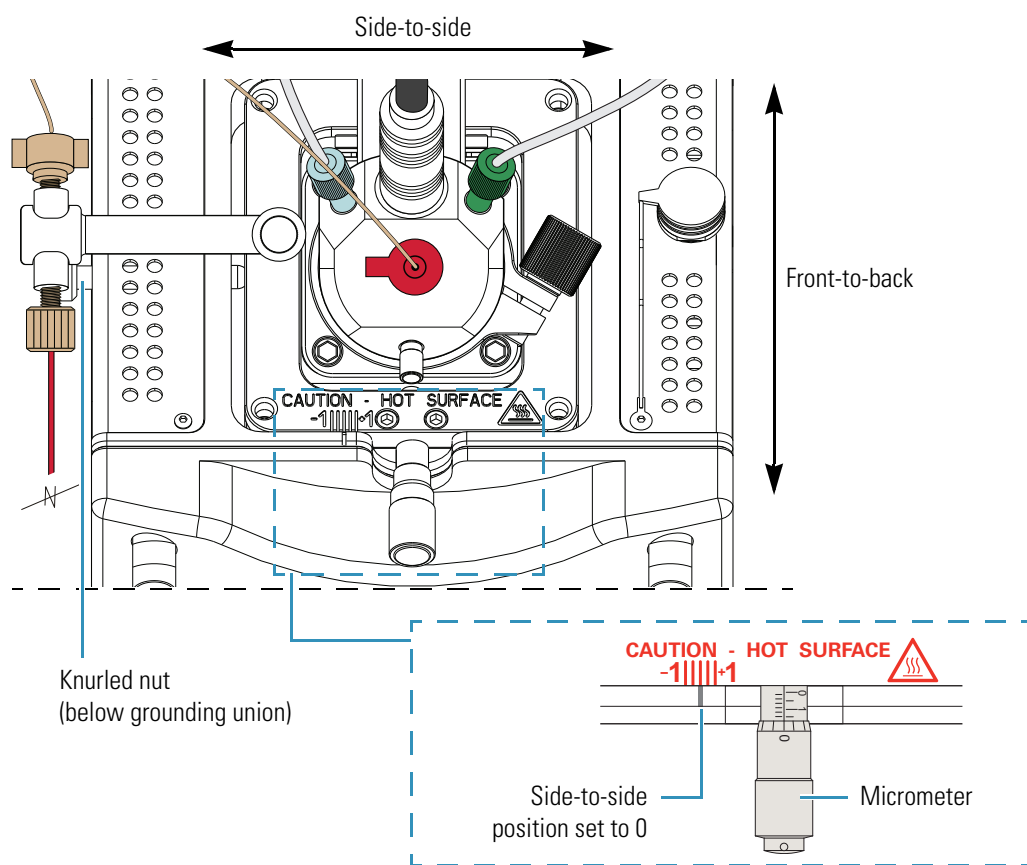
If the LTQ Series mass spectrometer has an Ion Max API source housing, you can maximize sensitivity by adjusting the side-to-side and front-to-back probe position by a few millimeters (Figure 36).

**Note** The probe position on the Ion Max-S API source housing is not adjustable.

### ❖ To adjust the probe position

- To adjust the front-to-back probe position, use the micrometer on the front of the Ion Max source housing.
- To adjust the side-to-side probe position, use the knurled nut on the left side of the housing and the -1 to +1 markers on the top front of the Ion Max source housing.
- To adjust the probe depth, use the A, B, C, and D markers on the probe as a guide.

**Figure 36.** Ion Max API source housing showing probe adjustment controls





## Automatic Tuning and Calibration in ESI Mode

This chapter provides information about how to tune and calibrate the LTQ Series mass spectrometer. First, tune and calibrate the mass spectrometer in ESI mode—that is, set up the mass spectrometer for ESI mode (see “[Installing the ESI or HESI-II Probe](#)” on [page 40](#)). Then, set up the scan parameters for a full-scan, single-stage mass analysis. For most applications, you infuse a calibration solution directly into the ion source while running the automatic tune and automatic calibration options provided within the data system.

You must calibrate the mass spectrometer every one to three months of operation for optimum performance over the entire mass range of the detector.

### Contents

- [Setting Up the Syringe Pump for Tuning and Calibration](#)
- [Setting Up the Mass Spectrometer for Tuning and Calibration](#)
- [Testing the Mass Spectrometer in ESI Mode](#)
- [Tuning the Mass Spectrometer Automatically in ESI Mode](#)
- [Saving the ESI Tune Method](#)
- [Calibrating Automatically in the Normal Mass Range](#)

## Setting Up the Syringe Pump for Tuning and Calibration

Use the syringe pump, located on the front of the LTQ Series mass spectrometer, to infuse solution for tuning and calibration.

**IMPORTANT** To minimize the possibility of cross-contamination, use a different syringe and length of PEEK tubing for each type of solution.

### ❖ To set up the syringe pump for infusion

1. Load a clean, 500  $\mu\text{L}$  Unimetrics™ syringe with 450  $\mu\text{L}$  of the ESI calibration solution.

To prepare the ESI calibration solution, follow one of these procedures:

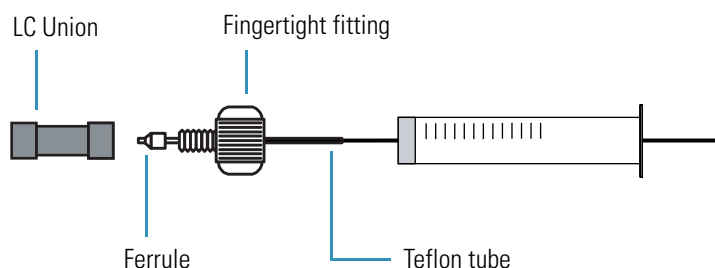
- For the LXQ and LTQ XL, see “Preparing the Normal Mass Range Calibration Solution for ESI Mode” on page 140.
- For the Velos Pro, see “Preparing the Normal Mass Range Calibration Solution for ESI Mode” on page 148.

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

2. Hold the plunger of the syringe in place and carefully insert the tip of the syringe needle into the end of a 4 cm (1.5 in.) Teflon tube with a fingertight fitting and a ferrule to the (black) LC union (Figure 37).

The LC union has a 10-32, coned-bottom receiving port.

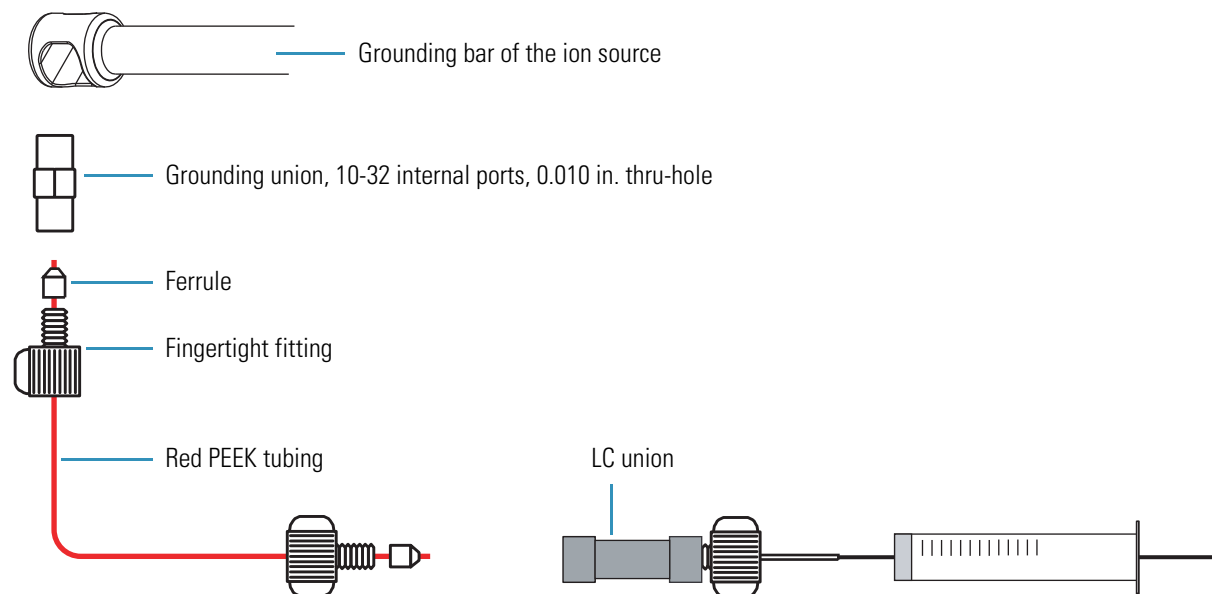
**Figure 37.** Plumbing connection for the syringe



3. Place the syringe into the syringe holder of the syringe pump.
4. Use a fingertight fitting and ferrule to connect an infusion line of red PEEK tubing (0.005 in. ID, 1/16 in. OD) between the LC union and the grounding union held by the grounding bar of the ion source.

Figure 38 shows the suggested fittings and ferrules for connecting the LC union to the grounding union. Both the LC union and the grounding union have 10-32, coned-bottom receiving ports.

**Figure 38.** Plumbing connection between the LC union and the grounding union



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

## Setting Up the Mass Spectrometer for Tuning and Calibration

Before running the automatic calibration procedure on the LTQ Series mass spectrometer, manually tune with calibration solution to establish a stable spray of solution and to ensure that the mass analyzer is transmitting a sufficient level of ions to the mass detector. After establishing these conditions, calibrate the mass spectrometer automatically to optimize the parameters that affect ion detection.



**CAUTION** Before beginning normal operation each day, ensure that there is sufficient nitrogen for the ESI source. If you run out of nitrogen, the mass spectrometer automatically turns off to prevent atmospheric oxygen from damaging the ion source. The presence of oxygen in the ion source when the mass spectrometer is on could be unsafe. In addition, if the mass spectrometer turns off during an analytical run, you could lose data.

To set up the mass spectrometer for tuning and calibration in ESI mode, follow these procedures:

1. [To open a tune method for ESI mode](#)
2. [To view the pre-tune ESI source settings](#)
3. [To define the scan parameters](#)
4. [To set the data type and ion polarity mode on page 67](#)

### 3 Automatic Tuning and Calibration in ESI Mode

Setting Up the Mass Spectrometer for Tuning and Calibration

#### ❖ To open a tune method for ESI mode

1. Open the Tune Plus window (see [page 32](#)).



2. Click the **Open** button.

3. Browse to the *drive:\Thermo\Instruments\LTQ\methods* folder, and then select either a recently saved tune file that worked on the instrument (preferred), or use the default tune file, **Default\_ESI**.

4. Click **Open**.

The tune method parameters download to the mass spectrometer.

#### ❖ To view the pre-tune ESI source settings

1. In the Tune Plus window, choose **Setup > ESI Source**.

2. Observe the settings.

3. Click **OK** when finished.

#### ❖ To define the scan parameters

1. In the Tune Plus window, click the **Define Scan** button to open the Define Scan dialog box ([Figure 39](#)).



**Figure 39.** Define Scan dialog box

n	Parent Mass (m/z)	Act. Type	Iso. Width (m/z)	Normalized Collision Energy	Act. Q	Act. Time (ms)
2		CID	1.0	0.0	0.250	30.000

#	First Mass (m/z)	Last Mass (m/z)
1	150.00	2000.00

2. Under Scan Description, do the following:

- In the Mass Range list, select **Normal**.
- In the Scan Rate list, select **Normal**.
- In the Scan Type list, select **Full**.



3. Under Scan Time, do the following:
  - In the Microscans box, enter **1**.
  - In the Max. Inject Time (ms) box, enter **10.000**.
4. Under Source Fragmentation, do one of the following:
  - For the LXQ and LTQ XL, clear the **On** check box.

**Note** Selecting the Source Fragmentation check box allows collision-induced fragmentation to occur in the ion source. Because collision-induced fragmentation performed in the ion source is not very specific, this feature is rarely used.

- For the Velos Pro, select the **On** check box.

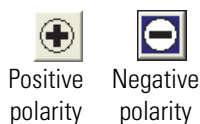
**Note** You must select the Source Fragmentation check box to produce the calibration peak at  $m/z$  138, which is a fragment of caffeine  $m/z$  195.

5. Under Scan Ranges, do the following:
  - a. In the Input list, select **From/To**.
  - b. In the First Mass ( $m/z$ ) column, type **150.00**.
  - c. In the Last Mass ( $m/z$ ) column, type **2000.00**.
6. Ensure the settings in the Define Scan dialog box are the same as those shown in [Figure 39](#) on page 66.
7. Click **OK**.

❖ **To set the data type and ion polarity mode**

1. In the Tune Plus window, click the **Centroid/Profile** button to select the **profile** data type.
2. Click the **Positive/Negative** button to select the **positive** ion polarity mode.

This completes the setup to tune with the calibration solution in ESI mode.



## Testing the Mass Spectrometer in ESI Mode

To test the operation of the LTQ Series mass spectrometer, infuse the calibration solution into the ESI source and monitor the real-time display of the mass spectrum of the calibration solution.



**CAUTION** Do not infuse calibration solution at syringe pump flow rates above 10  $\mu\text{L}/\text{min}$ . You can contaminate the sample with high concentrations of the Ultramark 1621 component of the calibration solution.

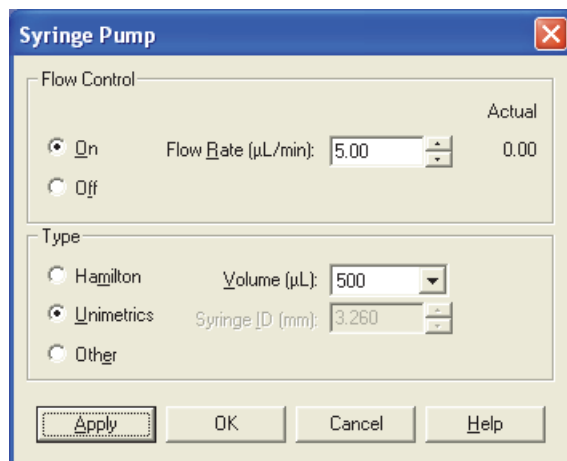
To test the operation of the mass spectrometer in ESI mode, follow these procedures:

1. To infuse the calibration solution into the ion source
2. To monitor the mass spectrum and the spray current

#### ❖ To infuse the calibration solution into the ion source

1. In the Tune Plus window, choose **Setup > Syringe Pump** to open the Syringe Pump dialog box (Figure 40).

**Figure 40.** Syringe Pump dialog box



2. Under Flow Control, do the following:
  - a. Select the **On** option.
  - b. In the Flow Rate ( $\mu\text{L}/\text{min}$ ) box, enter **5.00**.

## 3. Under Type, do the following:

## a. Select the type of syringe.

A 500  $\mu\text{L}$  Unimetrics syringe is supplied with the LTQ Series mass spectrometer. If you select the Hamilton™ or Unimetrics option, the Syringe ID (mm) box remains unavailable; the data system uses a predefined syringe ID for these syringes. If you select the Other option, the Syringe ID (mm) box becomes available and the Volume ( $\mu\text{L}$ ) list becomes unavailable.

## b. Do one of the following:

- For a Hamilton or Unimetrics syringe, in the Volume ( $\mu\text{L}$ ) list, select the volume of the syringe.
- For another type of syringe, in the Syringe ID (mm) box, enter the ID of the syringe.

4. Click **OK**.

The syringe pump starts. Or, use the On/Off syringe pump button.

Before starting the next procedure, ensure that you have set up the syringe pump to infuse the ESI calibration mixture as described in “[To infuse the calibration solution into the ion source](#)” on [page 68](#).

❖ **To monitor the mass spectrum and the spray current**1. In the Tune Plus window, click the **On/Standby** button to select the **On** mode.

After you turn on the mass spectrometer, it begins scanning, nitrogen flows into the ESI ion source, high voltage is applied to the ESI ion source, and a real-time mass spectrum display appears in the Spectrum view of the Tune window. For information about spectrums, refer to the topic “Spectrum View” in the data system Help.

2. Click the **Display Spectrum View** button.3. Observe the mass spectra of the singly-charged ions in the calibration solution ([Figure 41](#), [Figure 42](#), and [Figure 43](#) on pages [70](#) to [72](#)).

The ions are as follows:

- Caffeine:  $m/z$  195 for the LXQ and LTQ XL;  $m/z$  138.1 and 195 for Velos Pro
- MRFA:  $m/z$  524
- Ultramark 1621:  $m/z$  1022, 1122, 1222, 1322, 1422, 1522, 1622, 1722, 1822, and 1922

### 3 Automatic Tuning and Calibration in ESI Mode

Testing the Mass Spectrometer in ESI Mode

**Figure 41.** Spectrum of the calibration solution for the LTQ XL

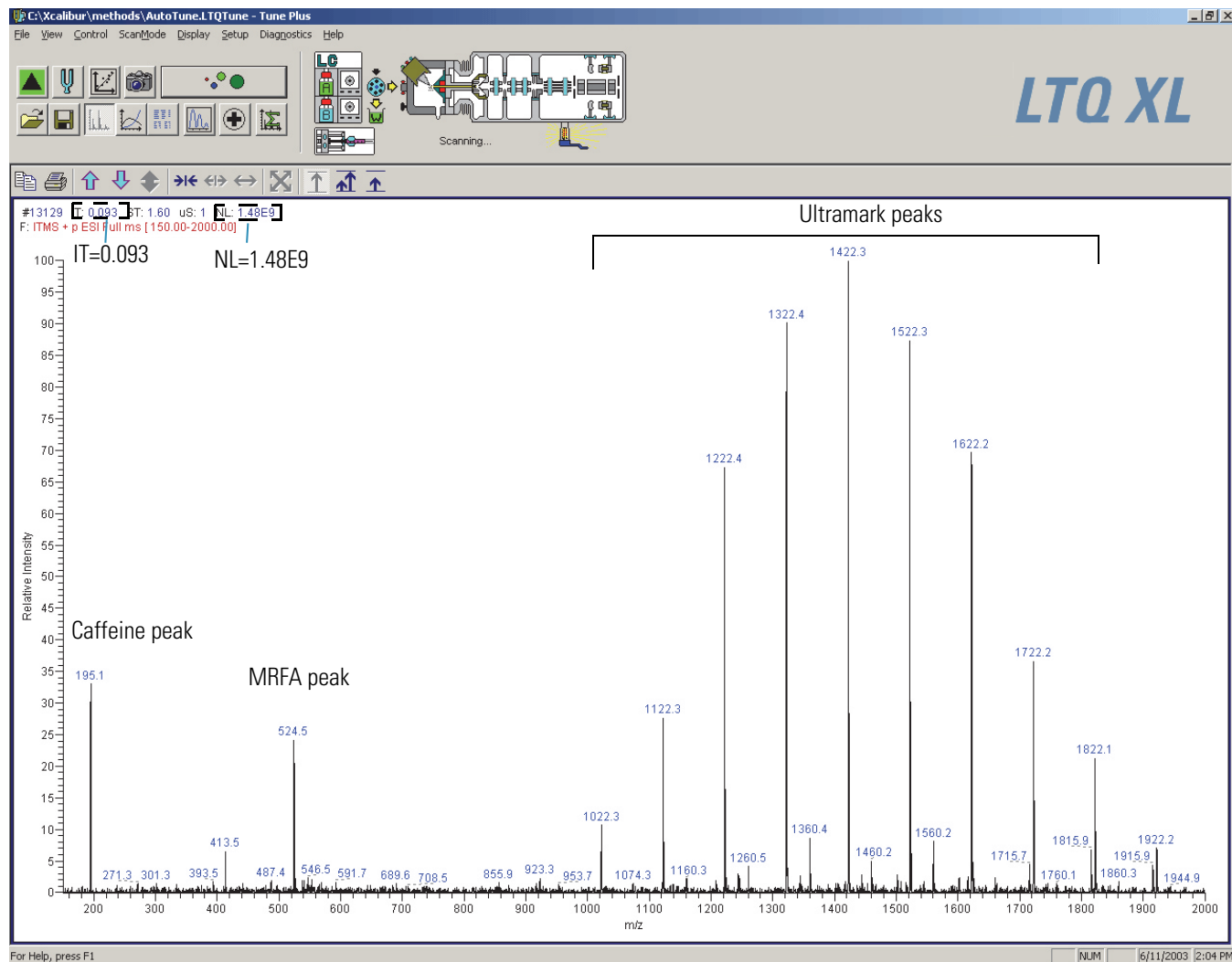
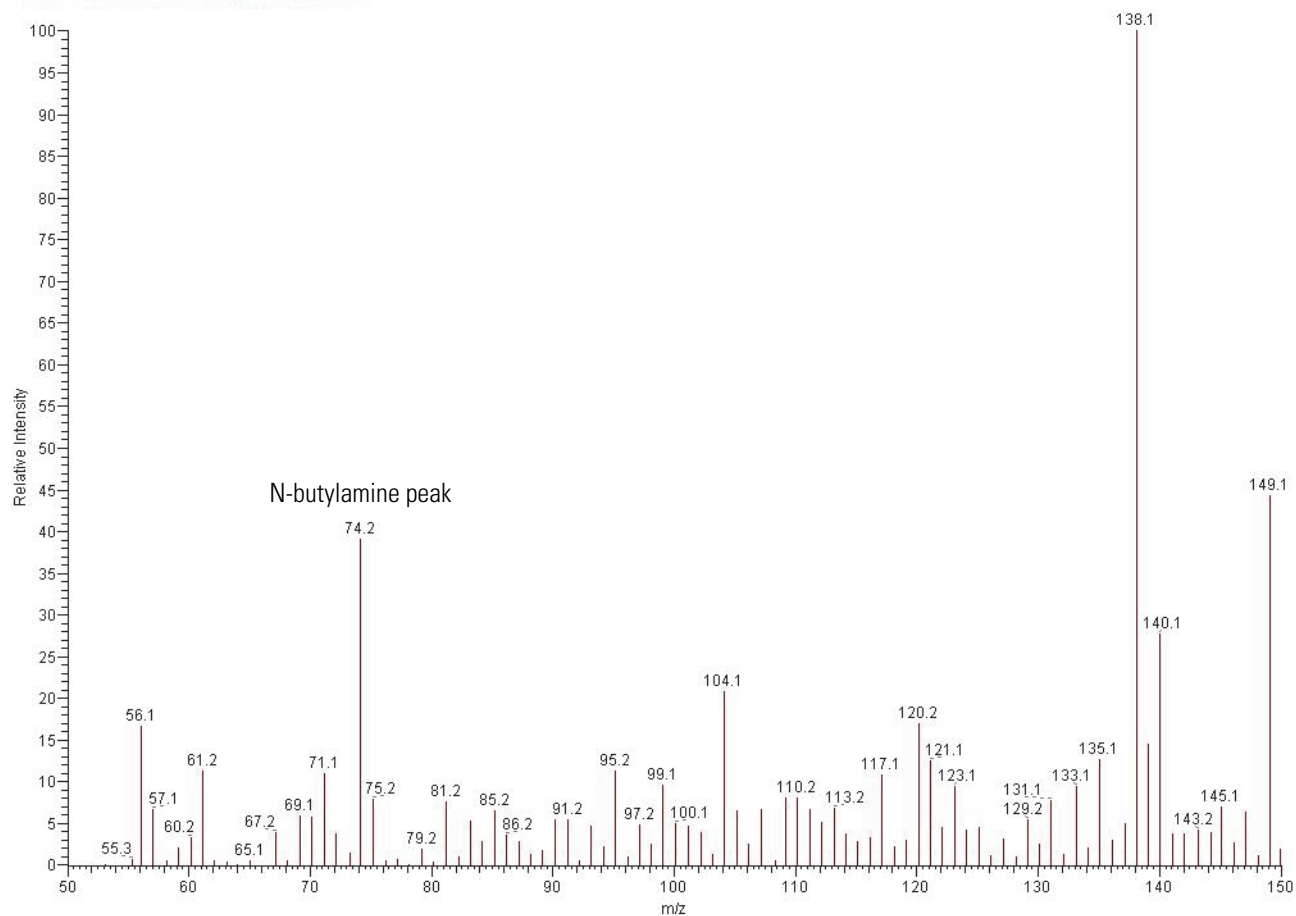


Figure 42. Spectrum of the calibration solution for the Velos Pro (lower range)

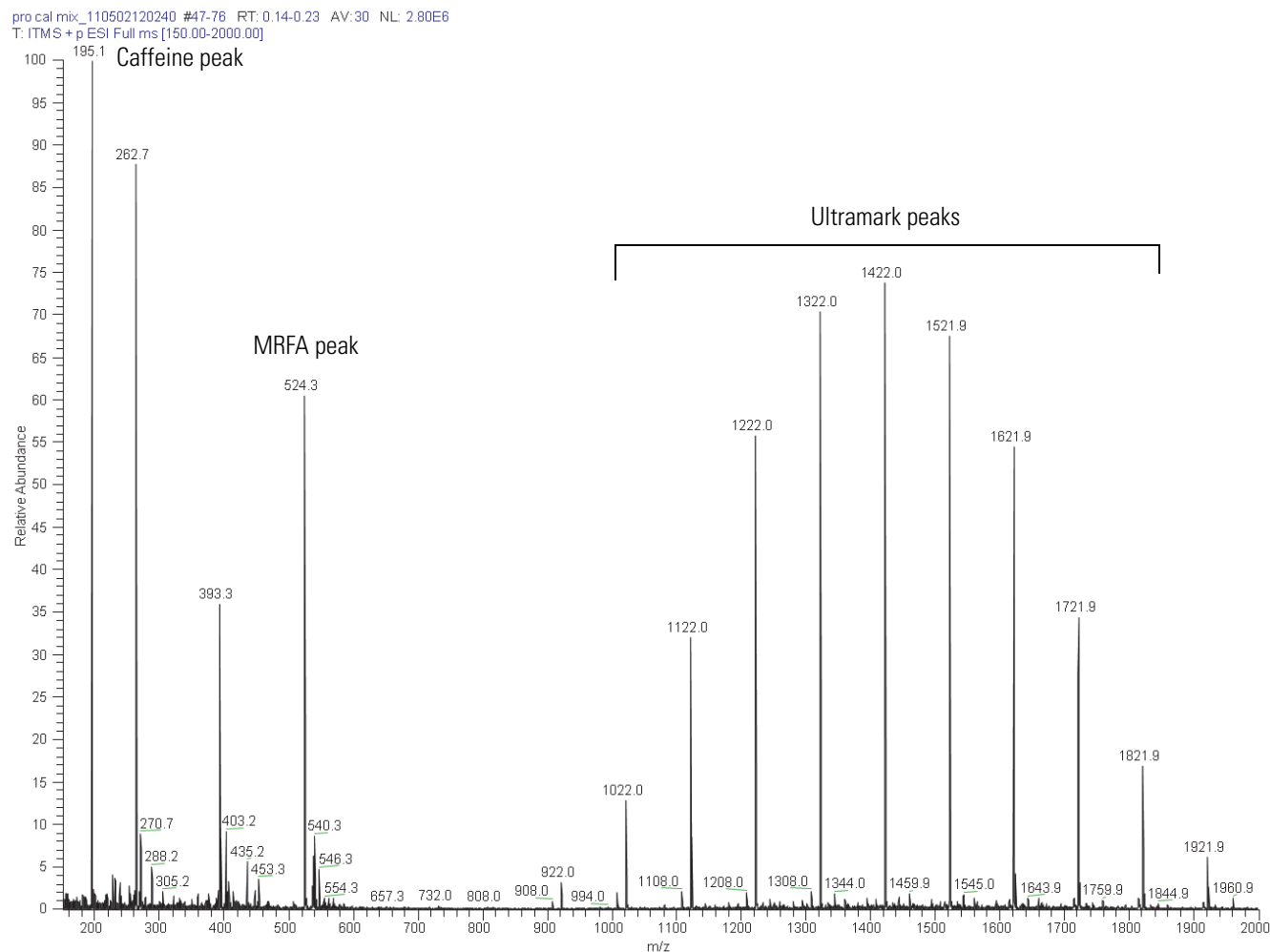
#6371 AV: 10 IT: 0.048 ST: 0.07  $\mu$ S: 1 CS: 1 AMWV: 137.08 NL: 3.13E6  
F: ITMS + c.NSI sid=25.00 Full ms [50.00-150.00]



### 3 Automatic Tuning and Calibration in ESI Mode

Testing the Mass Spectrometer in ESI Mode

**Figure 43.** Spectrum of the calibration solution for the Velos Pro (upper range)



4. At the top of the Spectrum view, check the values for the ionization time (IT) and normalization level (NL) (Figure 41 on page 70).
5. Choose **Setup > ESI Source**.
6. Check the Spray Current readback.
7. Observe the values for NL and IT in the Spectrum view.

As calibration solution infuses, and the readback values fluctuate, consider the following questions about the ion current signal:

- Is the signal present?
- Is the signal stable, varying by less than about 15% from scan to scan?

To assist in assessing the signal stability, a diagnostic tool is available. In Tune Plus, choose **Diagnostics > Diagnostics**. In the Tools list, select **System Evaluation**, and then click the **API Stability Evaluation** check box.

If you answered “yes” to the questions in [step 7](#), then the mass spectrometer is operating properly.

If you answered “no” to either of these questions, try the following troubleshooting measures:

- Ensure that the fused-silica sample tube on a ESI probe does not extend beyond the tip of the ESI needle. For instructions about inserting the fused-silica sample tube, refer to the *LTQ Series Hardware Manual*.
- Ensure that the entrance to the ion transfer tube is clean and is not blocked.
- Ensure that the solution entering the probe is free of air bubbles and that the tubing and connectors are free of leaks.
- Ensure that the flow rate of the sheath gas is 5–8 units.

If you have demonstrated that the mass spectrometer is operating properly in ESI mode, you are now ready to tune and calibrate the mass spectrometer. Leave the mass spectrometer as it is, and go to the next topic, [“Tuning the Mass Spectrometer Automatically in ESI Mode.”](#)

## Tuning the Mass Spectrometer Automatically in ESI Mode

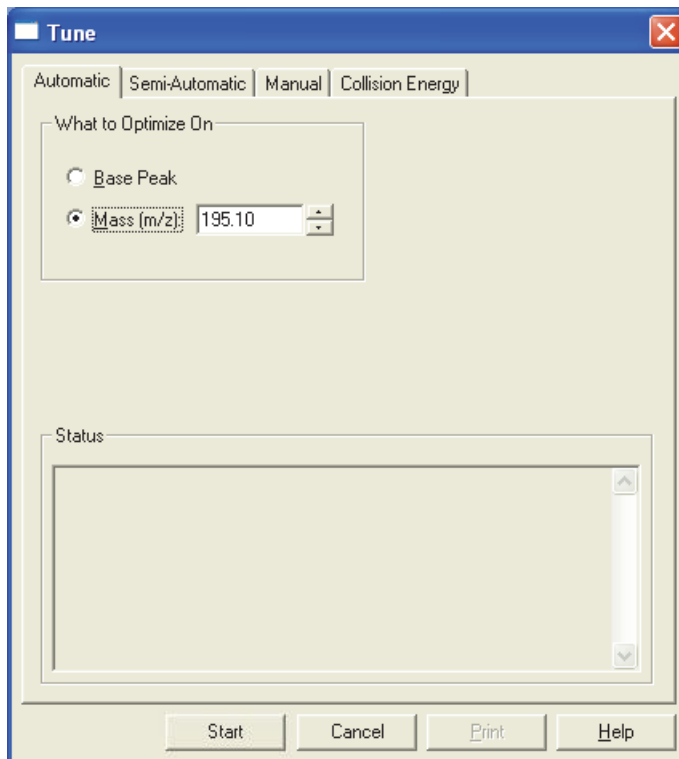
To optimize important parameters, tune the LTQ Series mass spectrometer automatically in ESI mode.

❖ **To automatically tune the mass spectrometer in ESI mode**



1. In the Tune Plus window, click the **Tune** button to open the Tune dialog box (Figure 44).

**Figure 44.** Tune dialog box showing the Automatic page



2. Under What to Optimize On, select the **Mass ( $m/z$ )** option.
3. To optimize the transmission of ions for a specific peak in the mass spectrum of the ESI calibration solution, enter, for example, **195.1** in the Mass ( $m/z$ ) box.

The mass spectrometer optimizes the tune on the peak at  $m/z$  195.1. However, you can optimize the tune on any peak in the mass spectrum of the ESI calibration solution.

4. Click **Start**.

A message appears:

Please ensure that the 500 microliter syringe is full.



5. Ensure that the syringe contains at least 450  $\mu\text{L}$  of calibration solution.

To prepare the ESI calibration solution, follow these procedures:

- For the LXQ and LTQ XL, see “[Preparing the Normal Mass Range Calibration Solution for ESI Mode](#)” on [page 140](#).
- For the Velos Pro, see “[Preparing the Normal Mass Range Calibration Solution for ESI Mode](#)” on [page 148](#).

6. Click **OK**.



7. In the Tune Plus window, click the **Graph View** button.

8. Observe the Tune Plus window and the Tune dialog box.

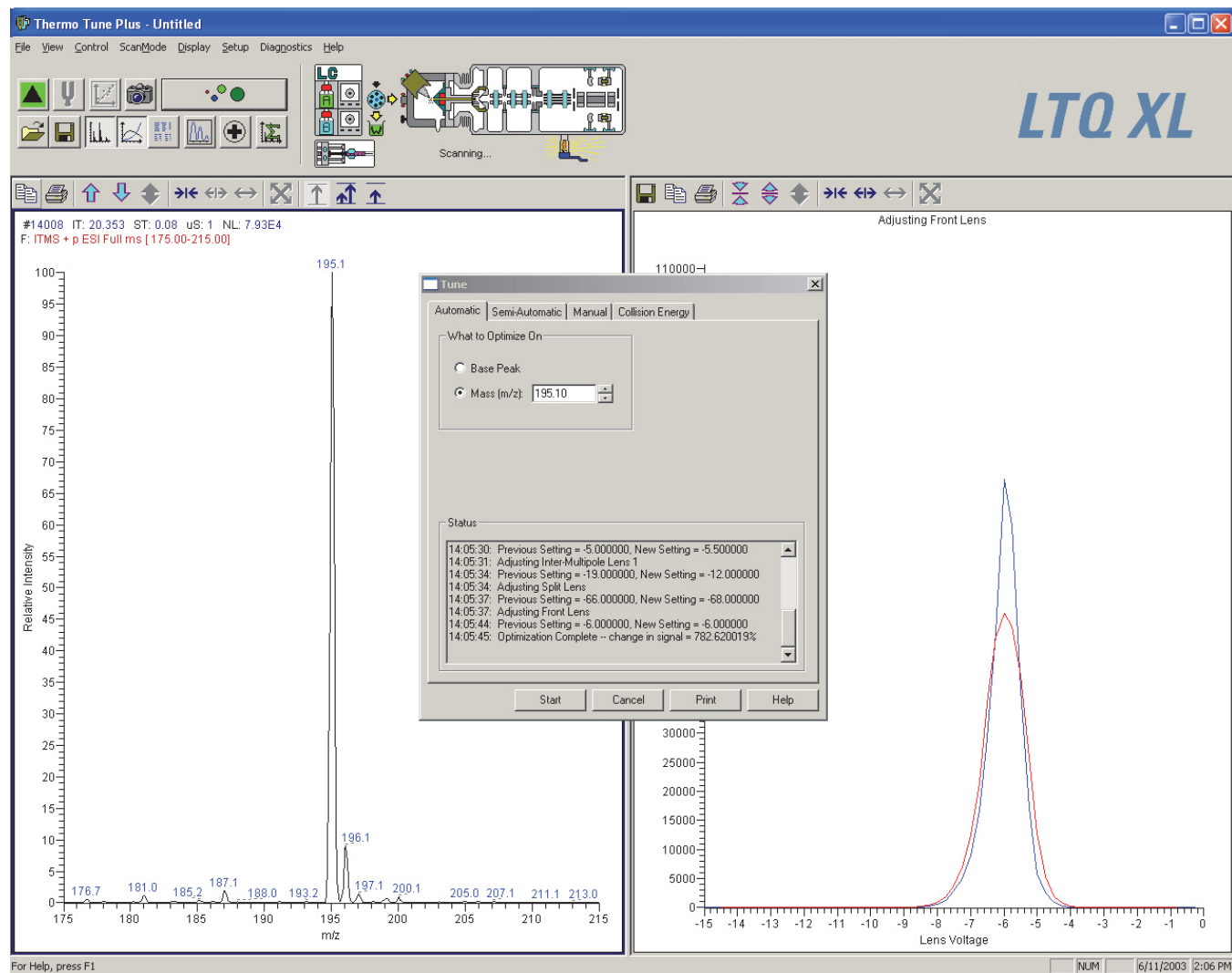
While automatic tuning is in progress, the mass spectrometer displays the results of various adjustments in the Spectrum and Graph views in the Tune Plus window and displays various messages under Status in the Tune dialog box.

For the LXQ and LTQ XL, check that the Tune Plus window looks similar to the one shown in [Figure 45](#). [Figure 46](#) shows the Velos Pro spectrum.

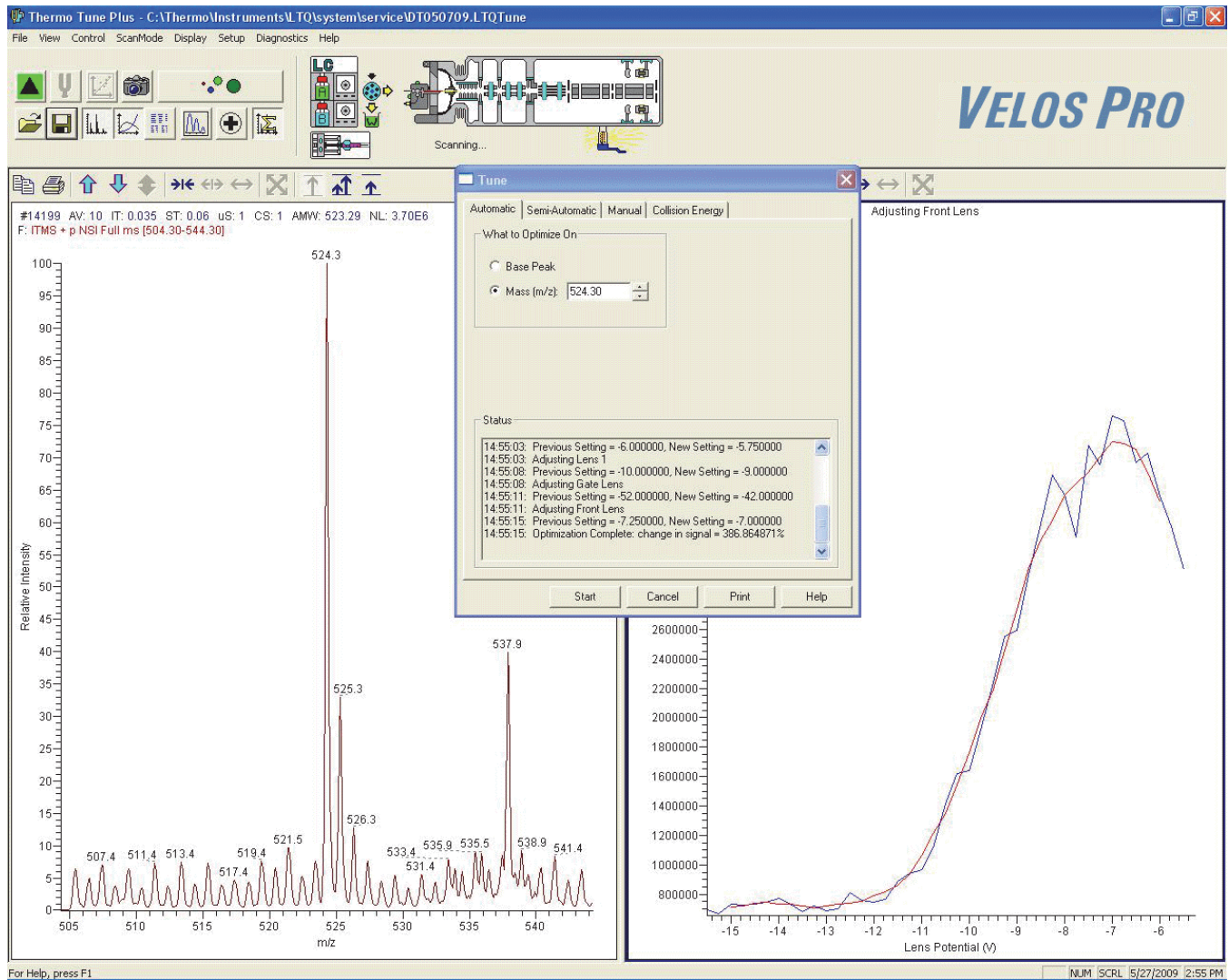
### 3 Automatic Tuning and Calibration in ESI Mode

Tuning the Mass Spectrometer Automatically in ESI Mode

**Figure 45.** Tune Plus window and Tune dialog box to set the LTO XL on  $m/z$  195.1



**Figure 46.** Tune Plus window and Tune dialog box to set the Velos Pro on  $m/z$  524.3



The Optimization Complete message in the Status box of the Tune dialog box should show a positive increase in the signal.

9. After the automatic tuning process ends, choose **Setup > ESI Source**.
10. Observe the post-tune settings for the ESI source.
11. Choose **Setup > Ion Optics**.
12. Observe the post-tune settings for the ion optics.

This completes the tuning in ESI mode by using the calibration solution.

## Saving the ESI Tune Method

You can save the parameters that you just set in a tune method specific to the particular analyte and solvent flow rate. Recall the tune method and use it as a starting point for optimizing the mass spectrometer on a different analyte or at a different flow rate.

**Note** You must save the tune method while the mass spectrometer is on.

### ❖ To save the ESI tune method



1. In the Tune Plus window, click the **Save** button.
2. Browse to the *drive:\Thermo\Instruments\LTQ\methods* folder.
3. In the File Name box, type a name, such as **ESImyTune**, to identify the tune method.
4. Click **Save**.

## Calibrating Automatically in the Normal Mass Range

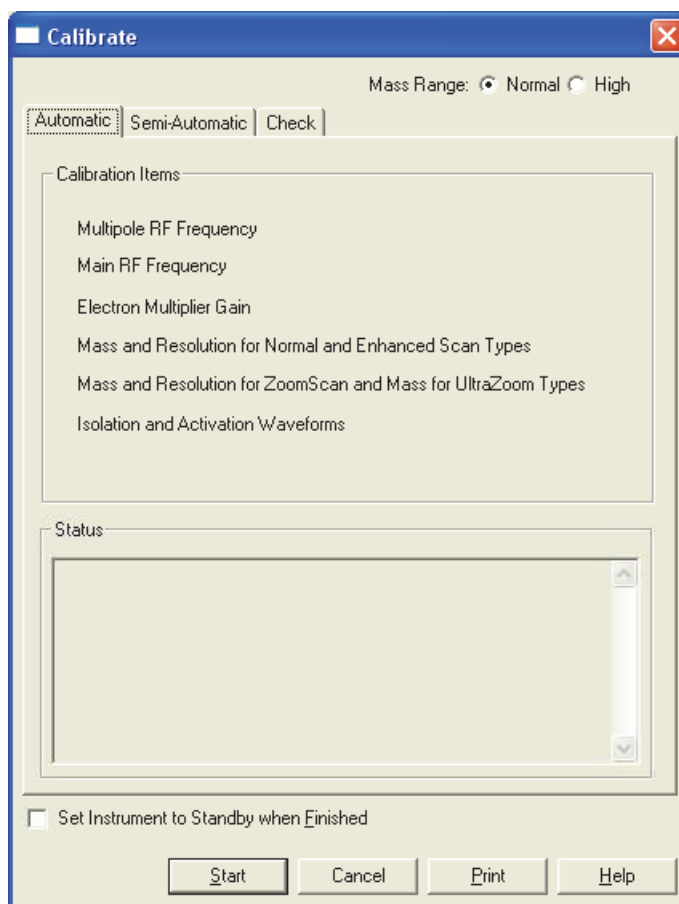
**Note** To calibrate the mass spectrometer in the high mass range, see [Appendix C, “High Mass Range Calibration.”](#)

❖ **To automatically calibrate the mass spectrometer in the normal mass range**



1. In the Tune Plus window, click the **Calibrate** button to open the Calibrate dialog box (Figure 47).

**Figure 47.** Calibrate dialog box showing the Automatic page



2. Specify the instrument state when the calibration is completed:
  - To have the mass spectrometer switch to Standby mode after the calibration, click the **Set Instrument to Standby when Finished** check box.
  - To have the mass spectrometer remain in the On mode after the calibration, clear the **Set Instrument to Standby when Finished** check box.

### 3 Automatic Tuning and Calibration in ESI Mode

Calibrating Automatically in the Normal Mass Range

3. Click **Start**.

A message appears:

Please ensure that the 500 microliter syringe is full.

4. Ensure that the syringe contains at least 450  $\mu\text{L}$  of the calibration solution.

To prepare the ESI calibration solution, follow these procedures:

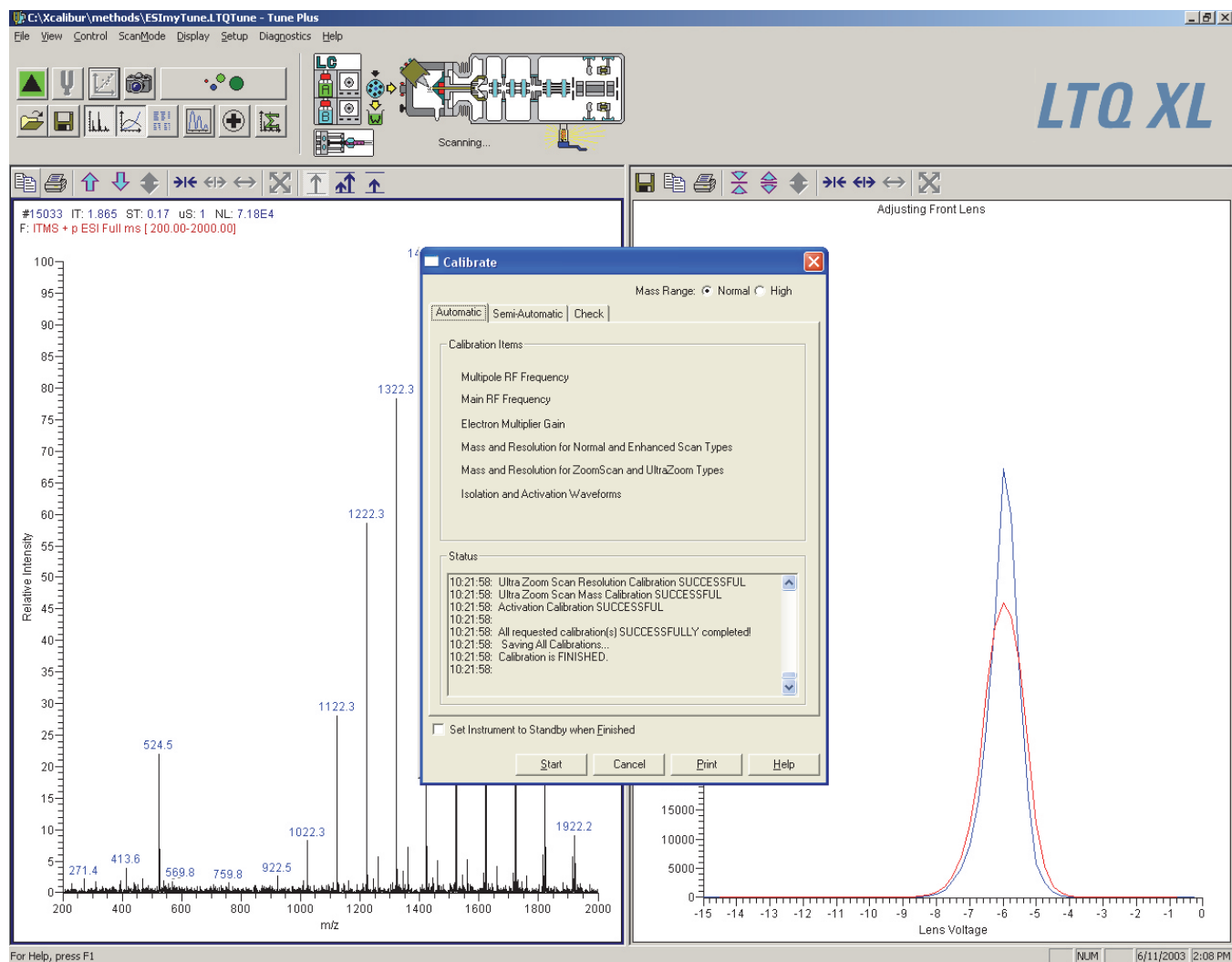
- For the LXQ and LTQ XL, see “[Preparing the Normal Mass Range Calibration Solution for ESI Mode](#)” on page 140.
- For the Velos Pro, see “[Preparing the Normal Mass Range Calibration Solution for ESI Mode](#)” on page 148.

5. Click **OK**.

6. Observe the Tune Plus window and the Calibrate dialog box.

While the automatic calibration is in progress, test results appear in the Spectrum and Graph views and messages appear in the Status box of the Calibrate dialog box ([Figure 48](#)).

**Figure 48.** Tune Plus window and Calibrate dialog box for the LTQ XL



### 3 Automatic Tuning and Calibration in ESI Mode

Calibrating Automatically in the Normal Mass Range

The automatic calibration procedure typically takes 20–30 minutes.

After the calibration procedure ends, the full-scan ESI mass spectrum appears in the Spectrum view (Figure 48 on page 81).

- If a calibration item is successful, the mass spectrometer saves the new calibration values automatically to the master calibration file on the hard disk drive.
- If a calibration item fails, try calibrating on that item again after ensuring that the spray is stable, the solution flow rate is sufficient, and all the ions in the calibration solution are present with adequate signal-to-noise ratios. Use the semi-automatic calibration page to calibrate the specific item that failed.

**Tip** After performing an automatic calibration, green checks ✓, red X marks ✗, or both appear in the results column of the Semi-Automatic page of the Calibration dialog box. A green check indicates a successful calibration, and a red X mark indicates a failed calibration for the corresponding item. Additional information is available in the Status box at the bottom of the Calibration dialog box.

- If repeated failures occur, consider clearing the ZoomScan Mode option.

After all the calibration items are successful, the mass spectrometer is properly tuned and calibrated for low-flow experiments. A successful calibration exhibits adequate intensities of the following calibrant ions at the correct masses:

- For the LXQ and LTQ XL:  $m/z$  195, 524, 1222, 1522, and 1822
- For the Velos Pro:  $m/z$  74, 138, 195, 524, 1222, 1522, and 1822

In many cases, fine tuning on the particular analyte is not necessary if the intensity of these ions is sufficient.

**Note** To calibrate the mass spectrometer in the high mass range, see [Appendix C, “High Mass Range Calibration.”](#)

Before tuning with an analyte, you must clean the mass spectrometer as described in [Chapter 8, “Cleaning the Mass Spectrometer After Tuning and Calibrating.”](#)

**Tip** If you plan to run analytical samples in high-flow ESI mode (by using flow rates of 50–1000  $\mu\text{L}/\text{min}$ ), optimize the tune further by following the procedures in [Chapter 4, “Tuning with an Analyte in ESI Mode,”](#) on page 83.



## Tuning with an Analyte in ESI Mode

This chapter describes how to tune the LTQ Series mass spectrometer in ESI mode by using an analyte. An automated procedure optimizes the sensitivity of the mass spectrometer to the specific analyte. You can often use the customized tune methods available for each instrument type without further tuning of the mass spectrometer, as they are optimized for a wide range of applications.

### Contents

- [Setting Up the Inlet for High-Flow Infusion in ESI Mode](#)
- [Setting Up the Mass Spectrometer to Tune with an Analyte in ESI Mode](#)
- [Tuning the Mass Spectrometer Automatically](#)
- [Saving the ESI Tune Method](#)

The following mass spectrometer parameters have the most important impact on signal quality in ESI mode:

- Ion transfer tube voltage (LXQ and LTQ XL only)
- Electrospray voltage
- Tube lens voltage (LXQ and LTQ XL only)
- Heated ion transfer tube temperature (voltage)
- Gas flow rates for the API gases
- S-lens rf level (Velos Pro only)

The settings for these parameters depend on the solvent flow rate and target analyte composition. In general, fine-tune the mass spectrometer whenever you change the solvent flow rate conditions of the particular application. In this procedure, you use a low-flow tune method (or the tune file that you created for the analyte by using direct infusion) as a starting point, and then further optimize the parameters by using an automated procedure. The automatic procedure adjusts the voltages applied to the ion optics until the ion transmission of the analyte is maximized.

**Note** Based on the LC flow rate of the experiment, specify the value of each of the following tuning parameters: the mass spectrometer's sheath, auxiliary, and sweep gas pressures; ESI needle (or "spray") voltage; ion transfer tube temperature; and probe position. [Table 3 on page 12](#) lists the recommended tuning parameters, with the exception of the ESI needle voltage and probe position. Automatic tuning sets the values of the parameters not mentioned here.

The ion transfer tube is heated to maximize the ion transmission to the mass spectrometer. For ESI only, set the ion transfer tube temperature proportional to the flow rate of the solution. See [Table 3 on page 12](#) for guidelines on setting the operating parameters for ESI mode.

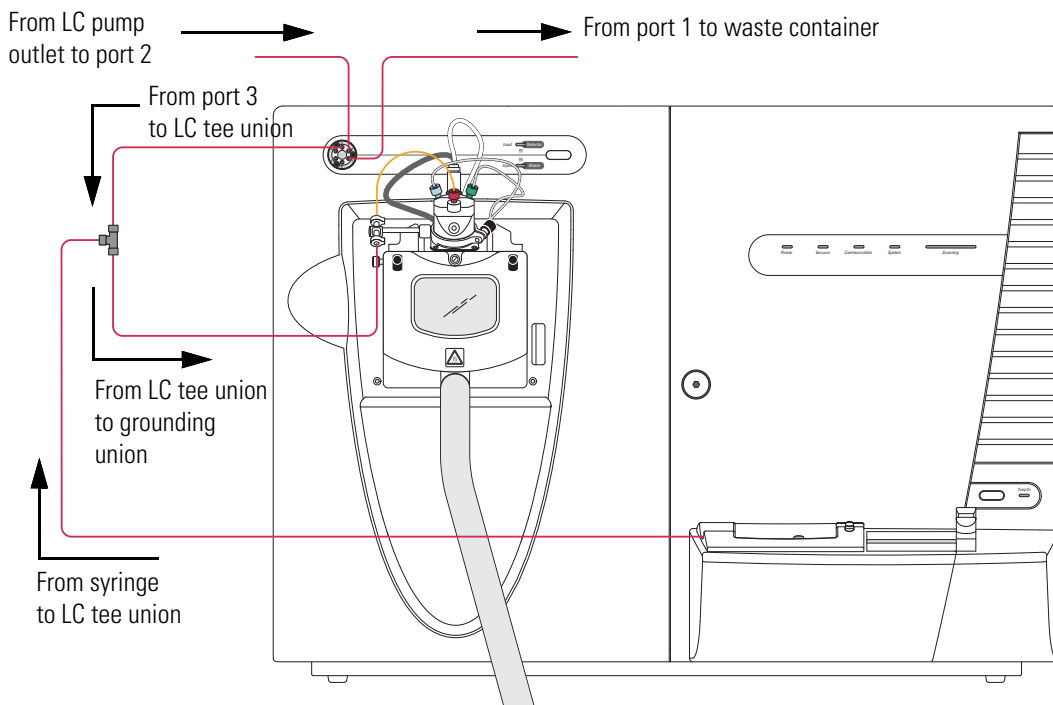
**Note** If performing the experiment at a flow rate less than 10  $\mu\text{L}/\text{min}$ , and you can obtain the results without optimizing the mass spectrometer on the particular analyte, see [Chapter 5, "Acquiring ESI Sample Data by Using Tune Plus,"](#) for instructions about acquiring sample data.

Before optimizing the tune for the analyte, ensure that the mass spectrometer has been calibrated within the last three months. If the system needs to be calibrated, follow the procedures in [Chapter 3, "Automatic Tuning and Calibration in ESI Mode."](#)

## Setting Up the Inlet for High-Flow Infusion in ESI Mode

This section describes how to set up the LTQ Series mass spectrometer to infuse a sample solution with the syringe pump into the solvent flow from an LC pump. Figure 49 shows the plumbing connections to introduce sample by high-flow infusion.

**Figure 49.** Plumbing setup for LC/ESI/MS infusion into the solvent flow from an LC pump



To connect the inlet plumbing for high-flow infusion analyses, use the parts listed in Table 7, which are in the instrument accessory kit.

**Table 7.** Inlet plumbing parts for high-flow infusion analysis (Sheet 1 of 2)

Description	Part number
Grounding union (ESI mode)	00101-18182
LC tee union	00101-18204
LC union	00101-18202
PEEK fingertight fittings and ferrules or stainless steel fittings and ferrules for conical 10-32 receiving ports and 1/16 in. OD tubing:	
• PEEK ferrule	00101-18196
• PEEK fitting	00101-18081
• Stainless steel nut	2522-0066
• Stainless steel ferrule	2522-3830

## 4 Tuning with an Analyte in ESI Mode

Setting Up the Inlet for High-Flow Infusion in ESI Mode

**Table 7.** Inlet plumbing parts for high-flow infusion analysis (Sheet 2 of 2)

Description	Part number
Red PEEK tubing (0.005 in. ID, 1/16 in. OD)	00301-22912
Teflon tubing or Injection Port filler	00301-22915
	00101-18206

To make the plumbing connections for ESI sample introduction from the syringe pump into solvent flow from an LC pump, follow these procedures:

- [Setting Up the Syringe Pump](#)
- [Connecting the Plumbing to Introduce Sample by High-Flow Infusion](#)

## Setting Up the Syringe Pump

### ❖ To set up the syringe pump



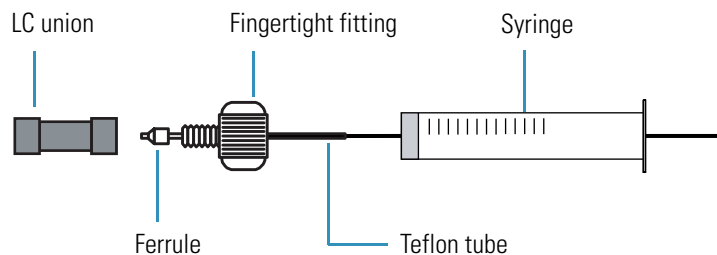
**CAUTION SHARP OBJECT** The syringe needle can puncture your skin. Handle it with care.

1. Load a clean, 500  $\mu\text{L}$  Unimetrics syringe with 100  $\text{pg}/\mu\text{L}$  solution of reserpine or the analyte.

To prepare the reserpine tuning solution, follow one of these procedures:

- For the LXQ and LTQ XL, see “[Preparing the Reserpine Tuning and Sample Solutions for the ESI and APCI Modes](#)” on page 144.
  - For the Velos Pro, see “[Preparing the Reserpine Tuning and Sample Solutions for ESI and APCI Modes](#)” on page 152.
2. Hold the plunger of the syringe in place and carefully insert the tip of the syringe needle into the end of a 4 cm (1.5 in.) Teflon tube with a fingertight fitting and a ferrule to the (black) LC union ([Figure 50](#)).

**Figure 50.** Plumbing connections for the syringe



3. Place the syringe into the syringe holder of the syringe pump.
4. Use a fingertight fitting and ferrule to connect an infusion line of red PEEK tubing between the LC union and the grounding union held by the grounding bar of the ion source (Figure 38 on page 65).
5. While squeezing the blue release buttons on the syringe pump handle, push the handle forward until it just contacts the syringe plunger.

## Connecting the Plumbing to Introduce Sample by High-Flow Infusion

To introduce sample by high-flow infusion, follow these procedures to connect solvent flow from the syringe pump and the LC pump to the ion source:

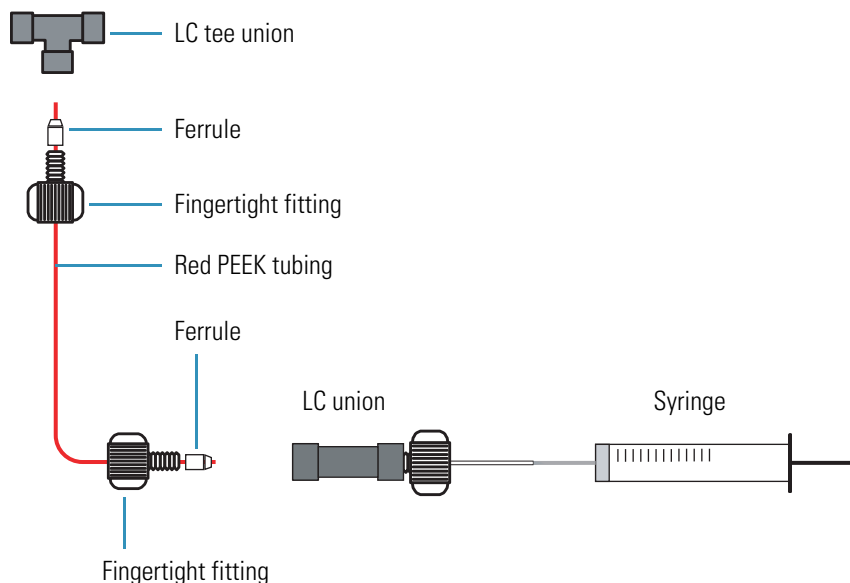
1. To connect the syringe to the LC tee union
2. To connect the LC tee union to the divert/Inject valve
3. To connect the LC pump to the divert/inject valve on page 89
4. To connect the divert/ inject valve to a waste container on page 89
5. To connect the LC tee union to the grounding union on page 90
6. To connect the Grounding Union to the ESI Probe Sample Inlet on page 91

### ❖ To connect the syringe to the LC tee union

1. Set up the syringe pump (see page 86).
2. Use a fingertight fitting and a ferrule to connect the red PEEK infusion line to the free end of the LC union that connects to the syringe.
3. Use a fingertight fitting and a ferrule to connect the other end of the red PEEK infusion line to the LC tee union.

Figure 51 shows the fittings required to connect the LC union to the union tee.

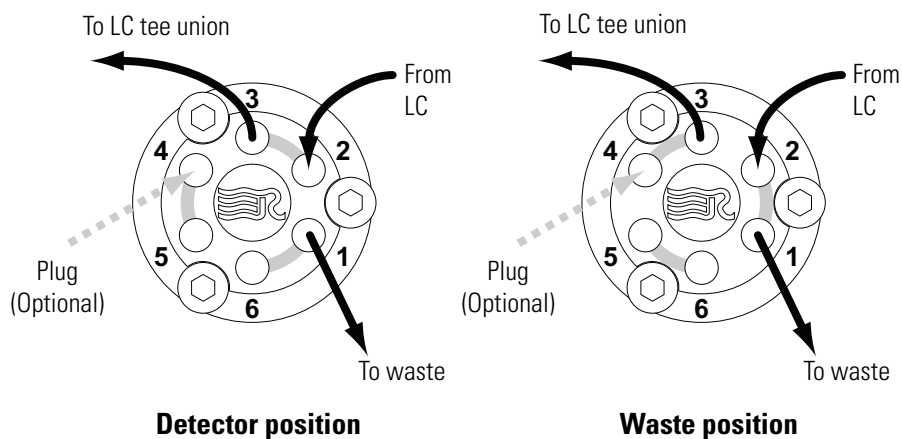
**Figure 51.** Plumbing connections between the LC tee union and the LC union



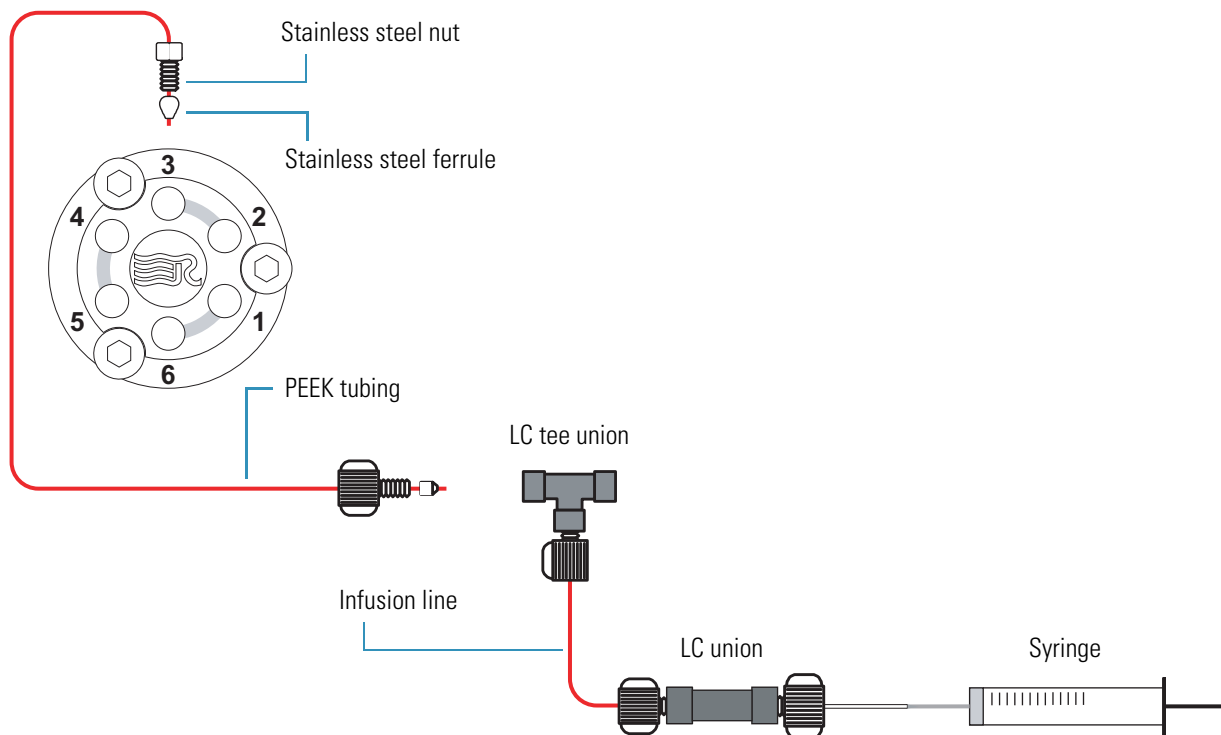
❖ **To connect the LC tee union to the divert/Inject valve**

1. Use a fingertight fitting and a ferrule to connect a length of red PEEK tubing to port 3 of the divert/inject valve. Or, use a stainless steel nut and ferrule to connect the tubing.
2. Use a fingertight fitting and a ferrule to connect the other end of the tubing to the free end of the LC tee union (Figure 52 and Figure 53).

**Figure 52.** Six-port divert/inject valve connections



**Figure 53.** Connections between the LC tee union and the divert/inject valve



❖ **To connect the LC pump to the divert/inject valve**

1. Use a fingertight fitting and a ferrule to connect a length of PEEK tubing to port 2 of the divert/inject valve (Figure 52 on page 88).
2. Use an appropriate fitting and ferrule to connect the other end of the tubing to the outlet of the LC.

❖ **To connect the divert/ inject valve to a waste container**

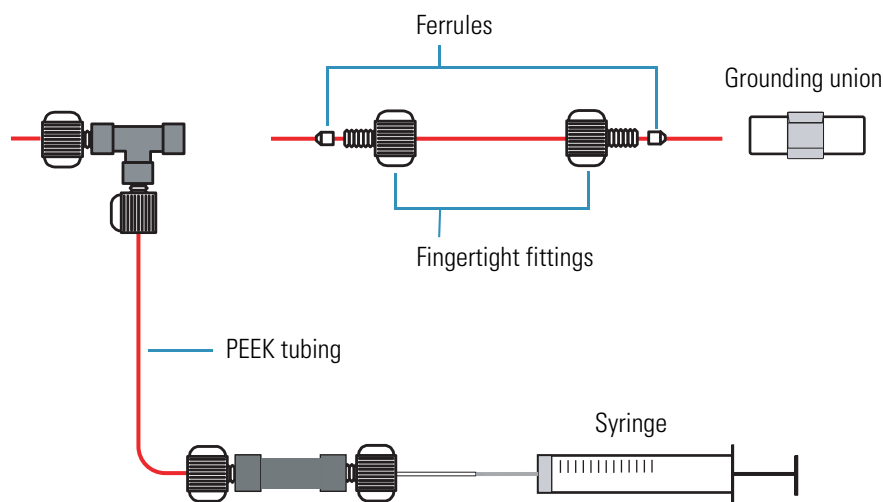
1. Use a fingertight fitting and a ferrule to connect a length of red PEEK tubing to port 1 of the divert/inject valve (Figure 52 on page 88).
2. Insert the other end of the tubing into a suitable waste container.

❖ **To connect the LC tee union to the grounding union**

1. Use a fingertight fitting and a ferrule to connect one end of a length of red PEEK tubing to the LC tee union (Figure 55).

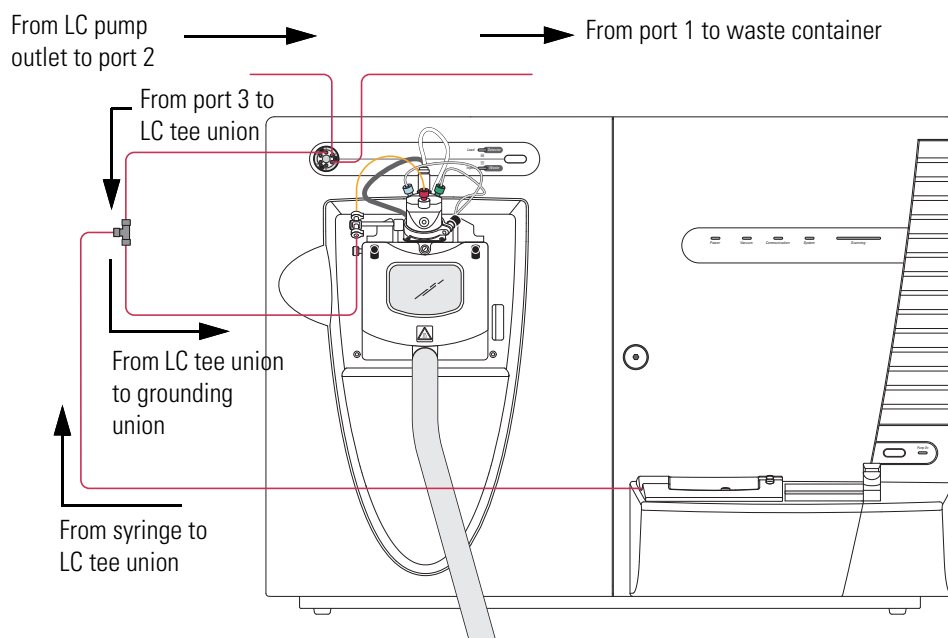
The grounding union slides into the grounding bar on the ion source as shown in Figure 54. For instructions about connecting the grounding union to the ESI probe sample inlet, refer to the *LTQ Series Hardware Manual*. Figure 56 shows the complete connection.

**Figure 54.** Connections between the LC tee union and the grounding union (ESI probe)



2. Use a fingertight fitting and a ferrule to connect the other end of the tubing to the grounding union that is held by the grounding bar of the API source (Figure 54 on page 90).

**Figure 55.** Plumbing setup for ESI/MS sample introduction with high-flow infusion

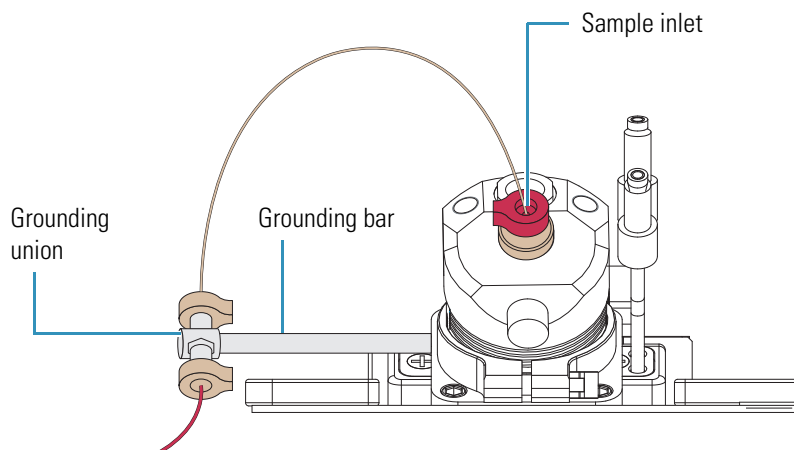




**❖ To connect the Grounding Union to the ESI Probe Sample Inlet**

For instructions about connecting the PEEK safety sleeve and fused-silica sample tube from the grounding union to the ESI probe sample inlet (Figure 56), refer to the *LTQ Series Hardware Manual*.

**Figure 56.** Grounding union connected to the sample inlet of the ESI probe



## Setting Up the Mass Spectrometer to Tune with an Analyte in ESI Mode

You can tune the LTQ Series mass spectrometer in ESI mode by using a high solvent flow: a 100 pg/ $\mu$ L solution of reserpine or a solution of the analyte. To prepare the 100 pg/ $\mu$ L reserpine tuning solution, follow one of these procedures:

- For the LXQ and LTQ XL, see “[Preparing the Reserpine Tuning and Sample Solutions for the ESI and APCI Modes](#)” on page 144.
- For the Velos Pro, see “[Preparing the Reserpine Tuning and Sample Solutions for ESI and APCI Modes](#)” on page 152.

To set up the mass spectrometer for tuning at the solvent flow rate for the experiments, follow these procedures:

1. [To open a stored tune method](#)
2. [To define the scan parameters, scan type, and scan polarity for the analyte](#)
3. [To set the data type and ion polarity mode on page 93](#)

## 4 Tuning with an Analyte in ESI Mode

Setting Up the Mass Spectrometer to Tune with an Analyte in ESI Mode

### ❖ To open a stored tune method

1. Open the Tune Plus window (see [page 32](#)).
2. Click the **On/Standby** button to select the **On** mode.



The mass spectrometer applies high voltage to the ESI probe and begins scanning. A real-time display begins in the Spectrum view.

3. Open **ESImyTune**, or the file name used for your saved tune method from Chapter 3, as follows:



- a. Click the **Open** button.
- b. Browse to the *drive:\Thermo\Instruments\LTQ\methods* folder, and then select the tune method you saved.
- c. Click **Open**.

Tune Plus downloads the tune method parameters to the mass spectrometer, and the title bar in the Tune Plus window lists the name of the current tune method, for example, *C:\Thermo\Instruments\LTQ\ESImyTune*.

### ❖ To define the scan parameters, scan type, and scan polarity for the analyte

1. In the Tune Plus window, click the **Define Scan** button to open the Define Scan dialog box.



[Figure 57](#) shows the typical settings for acquiring reserpine data by using the selected ion monitoring (SIM) scan type.

**Figure 57.** Define Scan dialog box for acquiring reserpine SIM data in ESI mode

The screenshot shows the "Define Scan" dialog box. It has a title bar "Define Scan" with a close button. The "Scan History" field contains "ITMS + c SIM ms [608.20-610.20]". The "Scan Description" section has "Mass Range: Normal", "Scan Rate: Normal", and "Scan Type: SIM". The "Scan Time" section has "Microscans: 1" and "Max. Inject Time (ms): 200.000". The "Source Fragmentation" section has "On" checked and "Energy (V): 20.0". The "MSn Settings" table has 10 rows with columns for n, Parent Mass (m/z), Isolation Width (m/z), Normalized Collision Energy, Activation Q, and Activation Time (ms). The "Scan Ranges" table has 10 rows with columns for #, Center Mass (m/z), and Width (m/z). The "Input" dropdown is set to "Center/Width". Buttons at the bottom include "Apply", "OK", "Cancel", "Help", "Injection RF...", and "Activation...".

n	Parent Mass (m/z)	Isolation Width (m/z)	Normalized Collision Energy	Activation Q	Activation Time (ms)
2		1.0	20.0	0.250	10.000
3		1.0	20.0	0.250	10.000
4		1.0	20.0	0.250	10.000
5		1.0	20.0	0.250	10.000
6		1.0	20.0	0.250	10.000
7		1.0	20.0	0.250	10.000
8		1.0	20.0	0.250	10.000
9		1.0	20.0	0.250	10.000
10		1.0	20.0	0.250	10.000

#	Center Mass (m/z)	Width (m/z)
1	609.20	2.00
2		
3		
4		
5		
6		
7		
8		
9		
10		

2. Under Scan Description, do the following:
  - In the Mass Range list, select **Normal**.
  - In the Scan Rate list, select **Normal**.
  - In the Scan Type list, select **SIM**.
3. Under Scan Time, do the following:
  - In the Microscans box, enter **2**.
  - In the Max. Inject Time (ms) box, enter **200.000**.
4. Under Source Fragmentation, clear the **On** check box.

**Note** Selecting the Source Fragmentation check box allows collision-induced fragmentation to occur in the ion source. Because collision-induced fragmentation performed in the ion source is not very specific, this feature is rarely used.

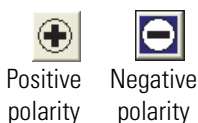
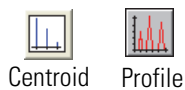
5. Under Scan Ranges, do the following:
  - a. In the Input list, select **Center/Width**.
  - b. In the Center Mass ( $m/z$ ) column, type the mass of the analyte to set the center mass for the scan range. If tuning on reserpine, type **609.20** for the center mass.
  - c. In the Width ( $m/z$ ) column, type **2.00**.
6. Verify the scan settings:
  - If tuning on reserpine, ensure that the settings in the Define Scan dialog box match those shown in [Figure 57](#) on [page 92](#).
  - If using another analyte, ensure that the settings in the Define Scan dialog box are appropriate for the analyte.
7. Click **OK**.

❖ **To set the data type and ion polarity mode**

1. In the Tune Plus window, click the **Centroid/Profile** button to select the **profile** data type.
2. Click the **Positive/Negative** button to select the **positive** ion polarity mode.

**Note** In the positive ion polarity mode, the mass spectrometer scans for positive ions. For basic compounds, such as amines, low pH solutions favor the formation of positive ions. Reserpine can form a positive ion  $[(M + H)^+ m/z = 609]$  in low pH solutions.

This completes the mass spectrometer setup with an analyte in ESI mode.



## Tuning the Mass Spectrometer Automatically

To maximize the transmission of ions for the analyte at a specified solvent flow rate, run the automatic tuning procedure and optimize on the target mass-to-charge ratio.

Before optimizing the tune of the LTQ Series mass spectrometer on a particular mass-to-charge ratio and creating a tune method for the analyte, for best results, first calibrate the mass accuracy of the mass spectrometer. The automated calibration procedure takes approximately 20 minutes. Or, for best results in a limited amount of time, infuse the ESI calibration mixture (or an analyte that you have mass spectral data for), and visually check the mass spectrum for mass accuracy. For information about calibrating the mass accuracy, see [“Calibrating Automatically in the Normal Mass Range” on page 79](#).

**Note** The most important parameters that affect the signal quality during ESI operation for the LXQ and LTQ XL mass spectrometers are the electrospray voltage, ion transfer tube temperature, heated tube voltage, tube lens voltage, gases, and solution flow rate. For the Velos Pro mass spectrometer, the important parameters are the electrospray voltage, transfer tube voltage, S-lens rf level, and solution flow rate.

If any of these parameters change, you must reoptimize the mass spectrometer parameters. Use the Semi-Automatic tune procedure to tune the mass spectrometer on individual parameters.

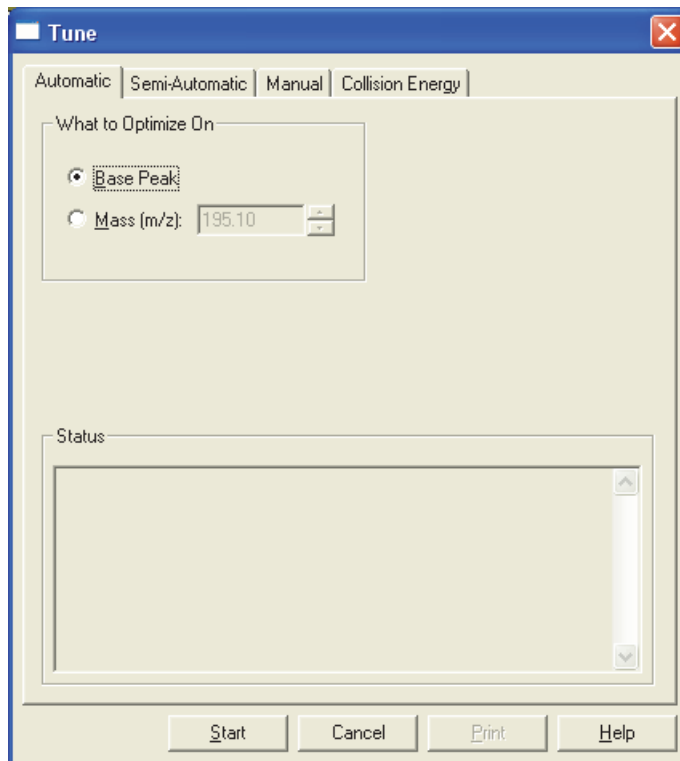
The following procedure uses a 100 pg/mL solution of reserpine to optimize the LTQ Series automatically on the reserpine peak at  $m/z$  609.2 at a flow rate of 0.4  $\mu\text{m}/\text{min}$ . You can substitute the reserpine solution with a sample solution that is suitable for analysis in ESI mode. For guidelines on setting an appropriate flow rate and temperatures, see [Table 3 on page 12](#).

❖ To automatically optimize the tune of the mass spectrometer for the analyte

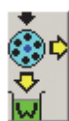


1. In the Tune Plus window, click the **Tune** button to open the Tune dialog box (Figure 58).

**Figure 58.** Tune dialog box showing the Automatic page



2. Under What to Optimize On, select the **Mass ( $m/z$ )** option.
3. In the Mass ( $m/z$ ) box, enter **609.2** (or the appropriate mass of the analyte).
4. Click the **Divert/Inject Valve** button to open the Divert/Inject Valve dialog box (Figure 59).



**Figure 59.** Divert/Inject Valve dialog box



5. Select the **Load Detector** option, and then click **Close**.

**Note** When the divert/inject valve is in the Detector position, the solvent flow from the LC pump enters and exits the divert/inject valve through ports 2 and 3, respectively (Figure 52 on page 88). Port 3 is connected to the ion source.

## 4 Tuning with an Analyte in ESI Mode

### Tuning the Mass Spectrometer Automatically

6. In the Tune dialog box, start the automatic tuning procedure as follows:

- a. Click **Start**.

A message appears:

Please ensure that the 500 microliter syringe is full.

- b. Ensure that the syringe pump contains at least 450  $\mu\text{L}$  of the 100  $\text{pg}/\mu\text{L}$  reserpine tuning solution or the analyte.
- c. Click **OK**.



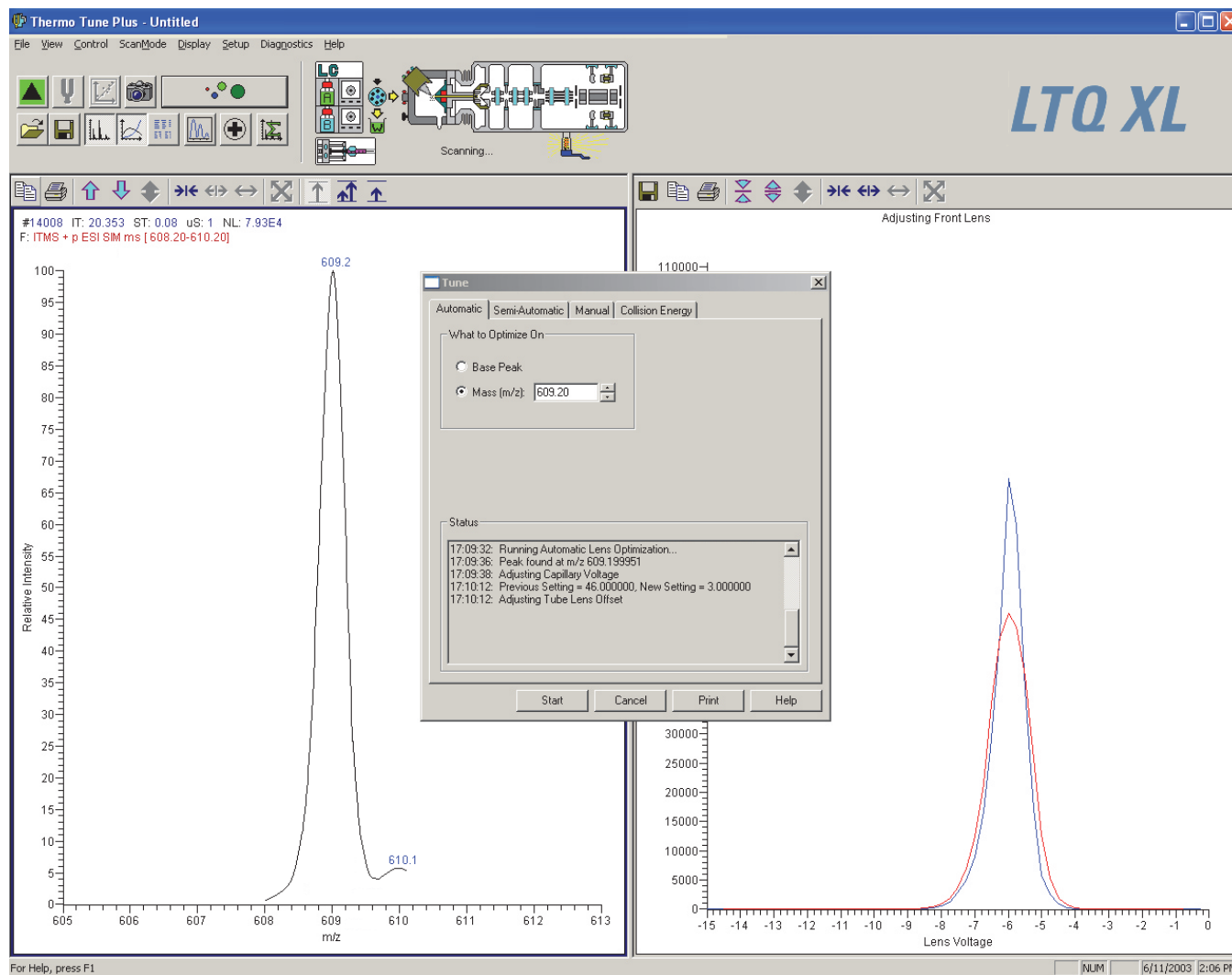
7. In the Tune Plus window, click the **Graph View** button.

8. Observe the Tune Plus window and the Tune dialog box.

During automatic tuning, the mass spectrometer displays the results of various tests in the Spectrum and Graph views in the Tune Plus window and displays various messages under Status in the Tune dialog box.

Check that the Tune Plus window looks similar to the one shown in [Figure 60](#).

Figure 60. Tune Plus window and Tune dialog box for automatic tuning in ESI mode of the LTQ XL



This completes the tuning of the mass spectrometer in ESI mode for the compound reserpine (or the analyte).

# Saving the ESI Tune Method

**Tip** You must save the tune method while the mass spectrometer is on.

#### ❖ To save the ESI tune method



1. In the Tune Plus window, click the **Save** button.
2. Browse to the *drive:\Thermo\Instruments\LTQ\methods* folder.
3. In the File Name box, type **reserpine** (or the name of the analyte).
4. Click **Save**.

If you tuned the mass spectrometer by using reserpine, clean the mass spectrometer as described in [Chapter 8, “Cleaning the Mass Spectrometer After Tuning and Calibrating.”](#) If you tuned with an analyte, as described in [Chapter 5, “Acquiring ESI Sample Data by Using Tune Plus,”](#) you are ready to acquire data on the analyte.



## Acquiring ESI Sample Data by Using Tune Plus

This chapter describes how to optimize the isolation width and collision energy for MS/MS experiments and how to acquire sample data in the selected ion monitoring (SIM) mode. In addition, this chapter describes how to set up the inlet for loop injections into the solvent flow from an LC pump.

For demonstration purposes only, the procedures in this chapter use a 1 µg/µL reserpine solution. You can use any analyte that is suitable for analysis in ESI mode.

**Note** Before beginning the analysis of the sample solution, verify that the mass spectrometer has been tuned and calibrated in ESI mode and that you have created and saved a tune method for the particular analyte by using a suitable flow rate.

### Contents

- [Setting Up to Acquire Full-Scan MS/MS Data](#)
- [Setting Up the Inlet for Flow Injection Analysis in ESI Mode](#)
- [Acquiring ESI Data in the SIM Scan Type](#)

## Setting Up to Acquire Full-Scan MS/MS Data

Before performing full-scan MS/MS experiments, optimize the isolation width to ensure the effective isolation of the ion, and then optimize the relative collision energy parameters to ensure efficient fragmentation of the parent ion. The relative collision energy for a particular analysis can depend on the type of sample being analyzed. Optimize the collision energy manually or use the automated process provided by the software.

The information in this section applies to both the ESI and APCI modes. The following procedures use reserpine for ESI mode. For APCI mode, use a suitable analyte.

- [Optimizing the Isolation Width for an MS/MS Experiment](#)
- [Optimizing the Collision Energy Manually for an MS/MS Experiment](#)
- [Optimizing the Collision Energy Automatically for an MS/MS Experiment](#)

## Optimizing the Isolation Width for an MS/MS Experiment

To optimize the isolation width for the analyte under the same or similar high-flow conditions intended for experiments, set up the inlet for high-flow infusion. Then, as you apply minor changes to the collision energy, observe the mass spectrum of the analyte. For this experiment, and for most applications, leave the **Activation Q** and **activation time** parameters set to their default values. For more information about these parameters, refer to the data system Help.

### ❖ To optimize the isolation width

1. Set up the inlet for high-flow infusion.

For ESI mode, see [page 85](#). For APCI mode, see [page 114](#).

2. Open the Tune Plus window (see [page 32](#)).

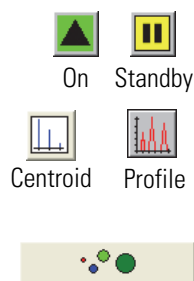
3. Click the **On/Standby** button to select the **On** mode.

4. Click the **Centroid/Profile** button to select the **centroid** data type.

5. Ensure that the scan parameters are defined to acquire full-scan MS/MS data for reserpine (or the analyte) as follows:
  - a. Click the **Define Scan** button to open the Define Scan dialog box ([Figure 61](#)).

The initial settings optimize the isolation width of an MS/MS experiment for reserpine.

**Figure 61.** Define Scan dialog box for acquiring reserpine full-scan data



The screenshot shows the 'Define Scan' dialog box. The 'Scan History' field contains 'ITMS + c Full ms [165.00-650.00]'. The 'Scan Description' section has 'Mass Range' set to 'Normal', 'Scan Rate' set to 'Normal', and 'Scan Type' set to 'Full'. The 'Scan Time' section has 'Microscans' set to '1' and 'Max. Inject Time (ms)' set to '200,000'. The 'Source Fragmentation' section has 'Qn' checked and 'Energy (V)' set to '20.0'. The 'Wideband Activation' checkbox is unchecked. The 'MSn Settings' table is as follows:

n	Parent Mass (m/z)	Isolation Width (m/z)	Normalized Collision Energy	Activation Q	Activation Time (ms)
2	609.20	3.0	20.0	0.250	10.000
3		1.0	20.0	0.250	10.000
4		1.0	20.0	0.250	10.000
5		1.0	20.0	0.250	10.000
6		1.0	20.0	0.250	10.000
7		1.0	20.0	0.250	10.000
8		1.0	20.0	0.250	10.000
9		1.0	20.0	0.250	10.000
10		1.0	20.0	0.250	10.000

The 'Scan Ranges' table is as follows:

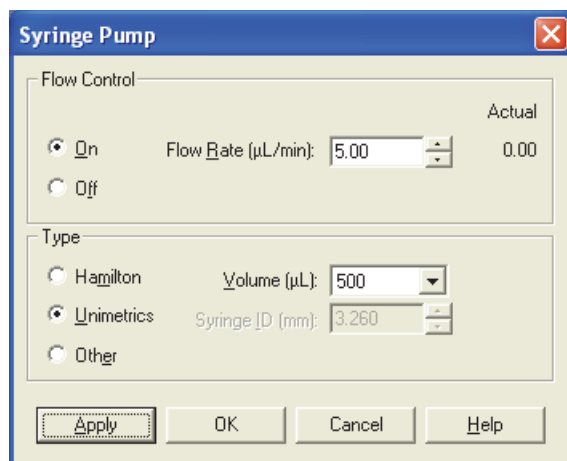
#	First Mass (m/z)	Last Mass (m/z)
1	165.00	650.00
2		
3		
4		
5		
6		
7		
8		
9		
10		

The 'Input' dropdown is set to 'From/To'. Buttons at the bottom include 'Apply', 'OK', 'Cancel', 'Help', 'Injection RE...', and 'Activation...'.

- b. If infusing reserpine, verify that the values in the dialog box match those shown in [Figure 61](#). If infusing a different analyte, ensure that the Parent Mass ( $m/z$ ) box contains the correct value and that the scan range is appropriate.

- c. Under MS<sup>n</sup> Settings, in the Isolation Width (*m/z*) column, start with a relatively wide isolation width of **2.0** Da.
  - d. After entering the values, click **Apply** and leave the Define Scan dialog box open.
6. Turn on the LC pump and specify a flow rate of **0.4 mL/min**.
  7. Verify that the inlet plumbing connections do not leak.
  8. Choose **Setup > Syringe Pump** to open the Syringe Pump dialog box (Figure 62).

Figure 62. Syringe Pump dialog box



9. Turn on the syringe pump and set an infusion flow rate of 5 µL/min as follows:
  - a. Under Flow Control, select the **On** option.
  - b. In the Flow Rate (µL/min) box, enter **5.00**.
  - c. Under Type, select the type of syringe, and do one of the following:
    - For a Hamilton or Unimetrics syringe, in the Volume (µL) list, select the volume of the syringe.
    - For another type of syringe, in the Syringe ID (mm) box, enter the ID of the syringe.
  - d. Click **Apply** to apply the syringe parameters and start the syringe pump.
10. Keep the Syringe Pump dialog box open and move it out of the way to the top of the screen.
11. Optimize the isolation width as follows:
  - a. In the Tune Plus window, observe the mass spectrum for the parent ion of reserpine, *m/z* 609.2 or the parent ion of the analyte. Ensure that the readback values for NL and IT are relatively stable.
  - b. In the Define Scan dialog box, under MS<sup>n</sup> Settings, type **2.0** in the Isolation Width (*m/z*) column.

- c. Click **Apply**.

**Note** The optimum value for the Isolation Width is the smallest  $m/z$  width (instrument minimum width =  $m/z$  0.1) that gives a mass spectrum of maximum intensity for only the target ions. When the optimum Isolation Width is obtained, the values for NL and IT are stable and the mass peak for the parent ion is at its maximum intensity and appears symmetrical. An Isolation Width value that is less than the optimum value causes a substantial drop in the NL reading. A significant drop in sensitivity indicates that the ions are not effectively isolated.

- d. Repeat [step 11a](#) and [step 11b](#), entering successively smaller values for the isolation width. Continue to observe the intensity of the mass spectrum of the parent ion, and ensure that the values for NL and IT are stable with each change made to the Isolation Width setting.

**Note** The Isolation Width setting is typically  $m/z$  1–3. After optimizing the isolation width, compensate for minor changes in tune stability by increasing the isolation width value a small amount. The adjustment should not exceed  $m/z$  = 1.

## Optimizing the Collision Energy Manually for an MS/MS Experiment

### ❖ To optimize the collision energy manually

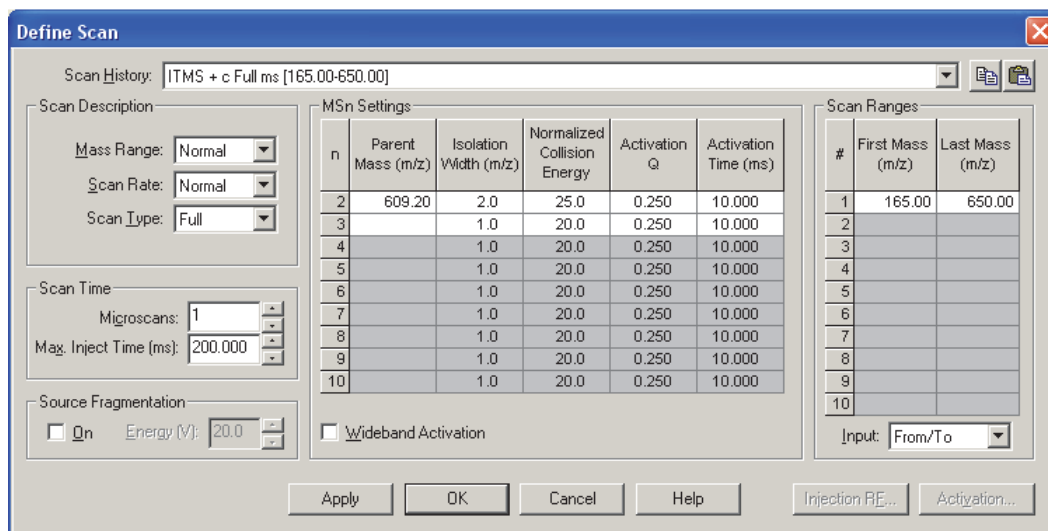


1. In the Tune Plus window, click the **Define Scan** button to open the Define Scan dialog box ([Figure 63](#)).
2. Under MS<sup>n</sup> Settings, in the Normalized Collision Energy column, type **20.0**.
3. Click **Apply**.
4. In the Tune Plus window, observe the mass spectrum of the product ions of reserpine (or the analyte).
5. If necessary, increase the value for the Normalized Collision Energy in increments of 5% until the intensity of the precursor ion is less than 5% of the intensity of the product ions.

**Note** After each change to the value in the Normalized Collision Energy column, you must click **Apply** to implement the change.

Figure 63 shows typical settings for acquiring full-scan MS/MS data on reserpine.

**Figure 63.** Define Scan dialog box (full-scan data)



## Optimizing the Collision Energy Automatically for an MS/MS Experiment

### ❖ To optimize the relative collision energy automatically

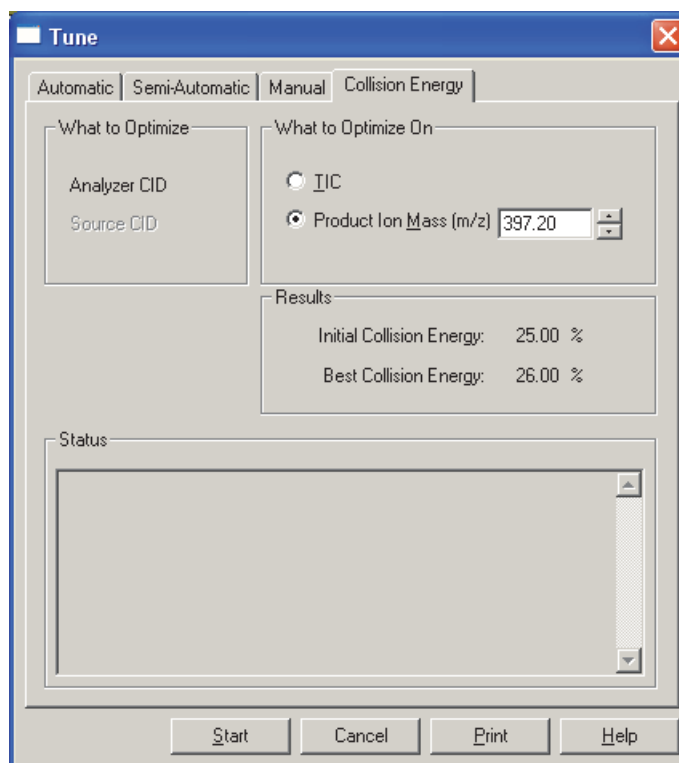
1. Identify the  $m/z$  of a product ion for the analyte.
2. In the Tune Plus window, click the **Tune** button to open the Tune dialog box.
3. Click the **Collision Energy** tab (Figure 64).



## 5 Acquiring ESI Sample Data by Using Tune Plus

Setting Up to Acquire Full-Scan MS/MS Data

**Figure 64.** Tune dialog box showing the Collision Energy page



4. Under What to Optimize On, select the **Product Ion Mass ( $m/z$ )** option and type **397.20** or a value for one of the product ions of the analyte.

The mass spectrometer can optimize the collision energy required to analyze the analyte automatically by using the  $m/z$  value of one of its product ions.

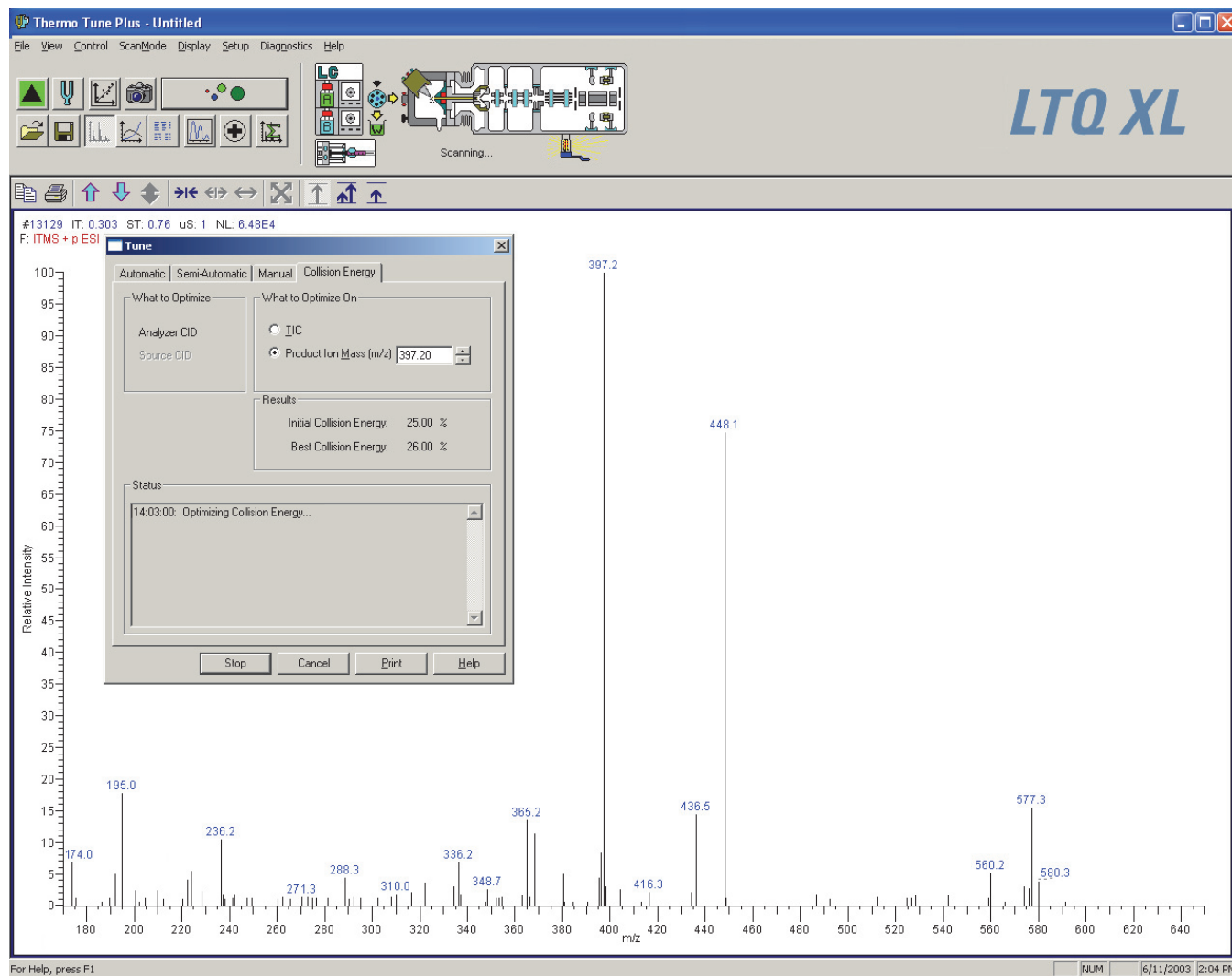
5. Click **Start**.

A message appears:

Ensure that the 500 microliter syringe is full.

6. Ensure that the syringe contains at least 450  $\mu\text{L}$  of the 100  $\text{pg}/\mu\text{L}$  reserpine tuning solution or the analyte.
7. Click **OK**.
8. Check that the Tune Plus window looks similar to the one shown in [Figure 65](#).

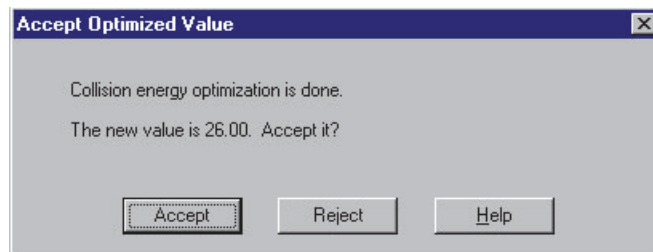
Figure 65. Tune Plus window and the Tune dialog box showing the Collision Energy page



9. In the Spectrum view of Tune Plus, observe the full-scan MS/MS spectrum of reserpine or the analyte.

After optimizing the collision energy, the Accept Optimized Value dialog box opens (Figure 66).

Figure 66. Accept Optimized Value dialog box



10. Click **Accept**.

The new value appears in the Define Scan dialog box.

11. In the Syringe Pump dialog box, select the **Off** option and click **Apply** to turn off the syringe pump. Click **OK** to close the dialog box.

12. Click **Cancel**.

After optimizing the relative collision energy, the mass spectrometer is ready to acquire MS/MS data on the analyte.

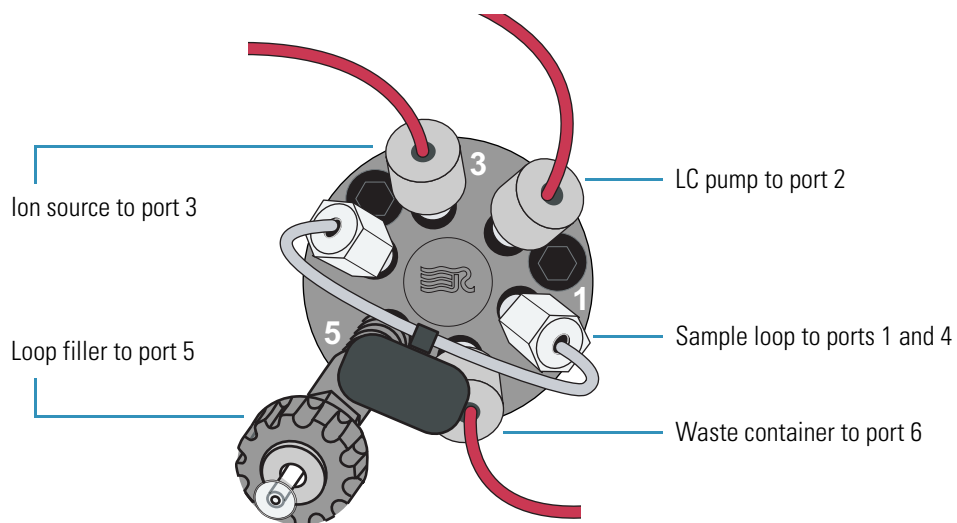
## Setting Up the Inlet for Flow Injection Analysis in ESI Mode

This section provides information about how to introduce sample by loop injection (flow injection analysis) into the solvent flow from an LC pump.

### ❖ To set up the inlet for loop injections

1. Connect a loop filler to port 5 of the divert/inject valve (Figure 67).

**Figure 67.** Divert/inject valve setup for loop injection

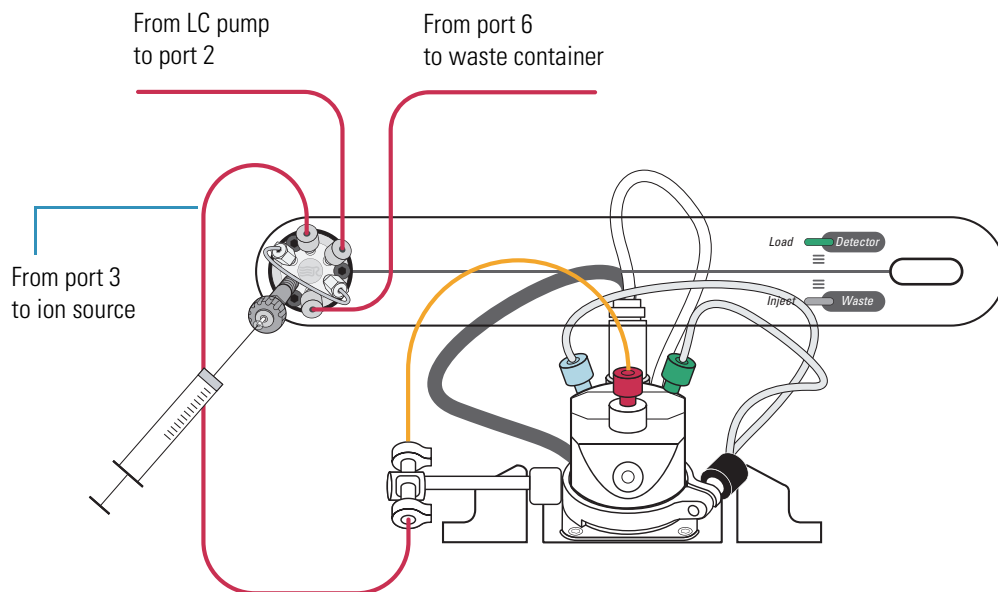


2. Connect a sample loop of the desired volume to ports 1 and 4.



3. Connect the LC pump to port 2 of the divert/inject valve as follows:
  - Use an appropriate fitting and a ferrule to connect one end of a length of red PEEK tubing to the outlet of the LC pump.  
To produce a stable solvent flow, the Accela 1250 Pump requires a minimum backpressure of 40 bar (580 psi). To connect the LC pump, use a length of 0.005 in. ID PEEK tubing sufficient to exert a backpressure of 40 bar (580 psi), or connect an inline backpressure regulator between the LC pump outlet and the divert/inject valve.
  - Use a fingertight fitting and a ferrule to connect the other end of the tubing to port 2.
4. For the ESI probe, use two fingertight fittings and two ferrules to connect a length of red PEEK tubing between port 3 of the divert/inject valve and the grounding union (Figure 68).
5. Connect the other end of the grounding union to the ESI probe sample inlet (see “Installing the ESI or HESI-II Probe” on page 40).

**Figure 68.** Plumbing for loop injection into the solvent flow from an LC into an ESI probe

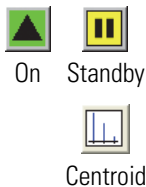


6. Connect the divert/inject valve to a waste container as follows:
  - Use a fingertight fitting and a ferrule to connect one end of a length of red PEEK tubing to port 6.
  - Connect the other end of the tubing to an appropriate waste container.

## Acquiring ESI Data in the SIM Scan Type

**Note** The data system computer automatically saves the acquired data to its hard disk drive.

### ❖ To acquire a data file containing SIM data



1. In the Tune Plus window, click the **On/Standby** button to select the **On** mode.

The mass spectrometer begins scanning and applies high voltage to the ESI probe. A real-time display appears in the Spectrum view.

2. Click the **Centroid/Profile** button to select the **centroid** data type.
3. Ensure that the scan parameters are defined to acquire SIM data for reserpine (or the analyte) as follows:
  - a. Click the **Define Scan** button.

Figure 69 shows the Define Scan dialog box with typical settings for acquiring SIM data for reserpine.

**Figure 69.** Define Scan dialog box (reserpine SIM-scan data)

n	Parent Mass (m/z)	Isolation Width (m/z)	Normalized Collision Energy	Activation Q	Activation Time (ms)
2		1.0	20.0	0.250	10.000
3		1.0	20.0	0.250	10.000
4		1.0	20.0	0.250	10.000
5		1.0	20.0	0.250	10.000
6		1.0	20.0	0.250	10.000
7		1.0	20.0	0.250	10.000
8		1.0	20.0	0.250	10.000
9		1.0	20.0	0.250	10.000
10		1.0	20.0	0.250	10.000

#	Center Mass (m/z)	Width (m/z)
1	609.20	2.00
2		
3		
4		
5		
6		
7		
8		
9		
10		

- b. Verify that the values in the dialog box match those in Figure 69, and then click **OK**.
4. Turn on the LC pump, and specify a flow rate of **0.4 mL/min**.

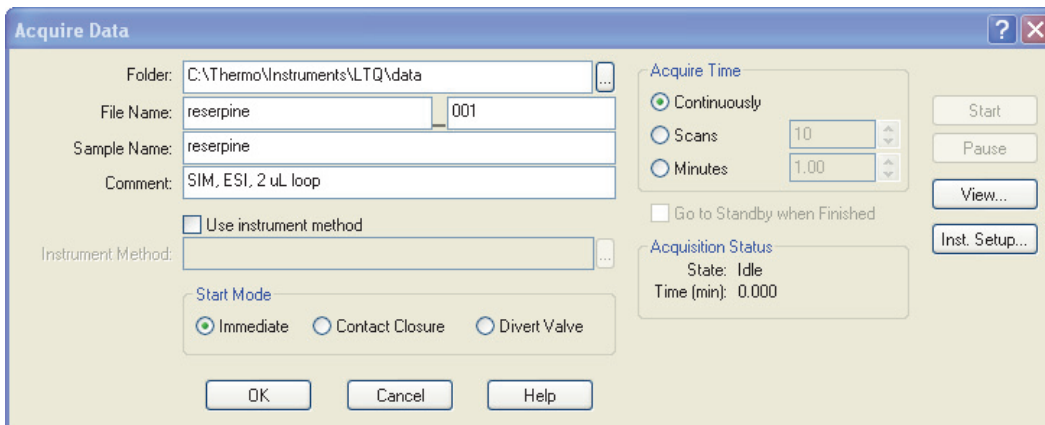
Ensure that the system is free of leaks.



5. In the Tune Plus window, click the **Acquire Data** button.

Figure 70 shows the acquisition status of the raw data file in the Acquire Data dialog box.

**Figure 70.** Acquire Data dialog box



6. Specify the acquisition parameters as follows:
  - a. In the File Name box, type **reserpine** (or the name of the analyte).
  - b. In the Sample Name box, type **reserpine** (or the name of the analyte).
  - c. In the Comment box, type a comment about the experiment.

For example, describe the scan mode, scan type, ionization mode, sample amount, or method of sample introduction. The Xcalibur data system includes the comment in the header information for the raw data file.

You can also add this information to reports created with the XReport reporting software. To open the XReport application, choose **Start > Programs > Thermo Xcalibur > XReport**.

- d. Under Acquire Time, select the **Continuously** option (acquires data until you stop the acquisition).
7. Click **Start**.
8. Leave the Acquire Data dialog box open during data acquisition and move it out of the way.
9. In the Tune Plus window, click the **Divert/Inject Valve** button to open the Divert/Inject Valve dialog box.



## 5 Acquiring ESI Sample Data by Using Tune Plus

Acquiring ESI Data in the SIM Scan Type

10. Inject the sample into the ESI source a total of four times as follows:

a. Select the **Load Detector** option.

When the valve is in the Load position, the solvent flow from the LC pump bypasses the sample loop.

b. Overfill the injector loop with a sample solution or a 1 pg/μL reserpine solution.

To prepare the reserpine tuning solution, follow one of these procedures:

- For the LXQ and LTQ XL, see “[Preparing the Reserpine Tuning and Sample Solutions for the ESI and APCI Modes](#)” on page 144.
- For the Velos Pro, see “[Preparing the Reserpine Tuning and Sample Solutions for ESI and APCI Modes](#)” on page 152.

c. Select the **Inject Waste** option.

When the valve is in the Inject position, the solvent flow from the LC pump backflushes the contents of the sample loop into the ion source.

d. In the Spectrum view, observe the reserpine peak ( $m/z$  609.2) or the peak of the analyte.

e. Repeat [step a](#) through [step d](#) three more times.

11. In the Divert/Inject Valve dialog box, click **Close**.

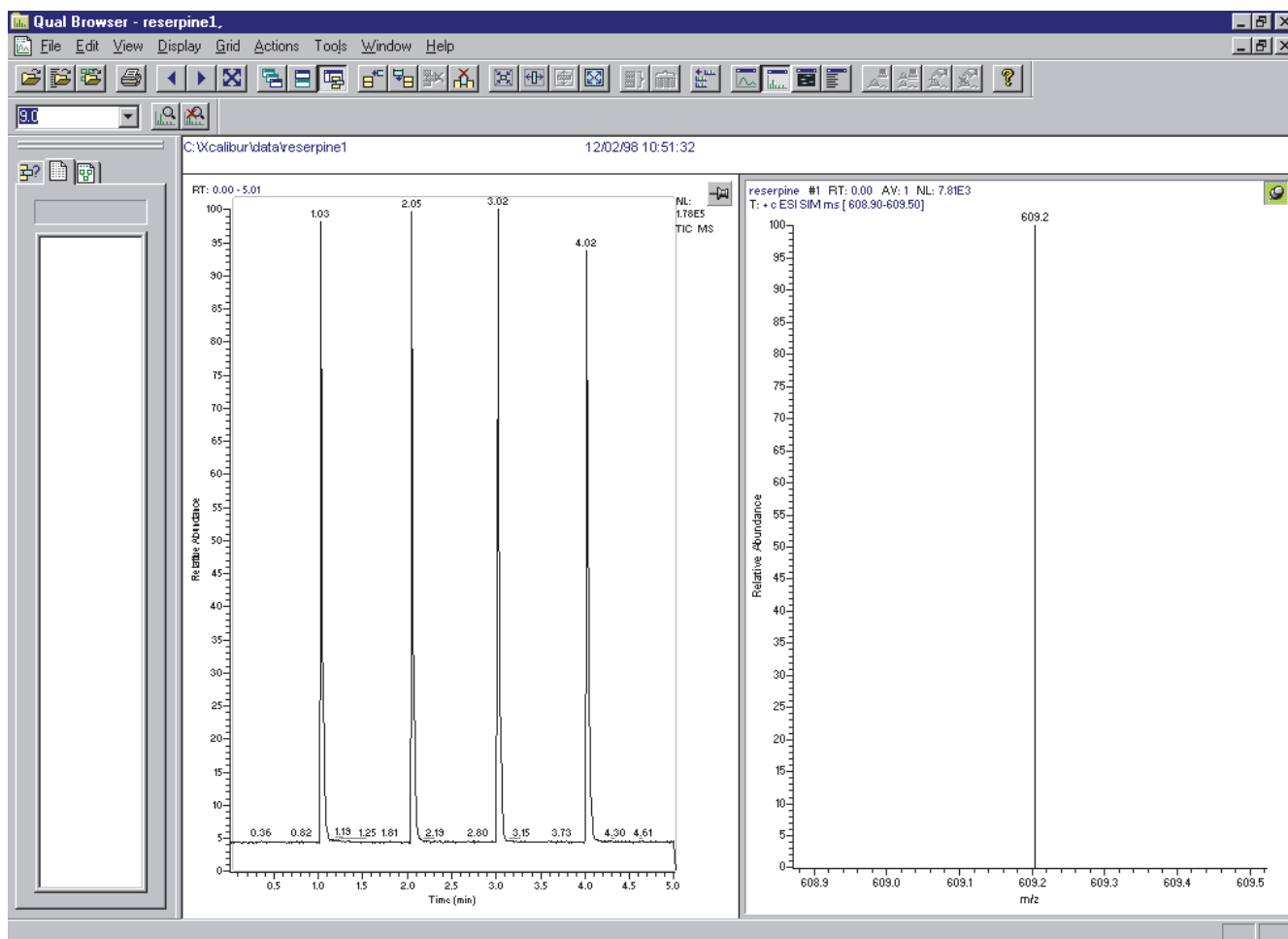
12. In the Acquire Data dialog box, click **Stop**, and then click **Cancel**.

Review the mass spectrum and chromatogram in the raw file just acquired by using the Xcalibur Qual Browser window. [Figure 71](#) shows a chromatogram (left side) containing the results from the four loop injections of reserpine and a mass spectrum of reserpine (right side).

For more information about reviewing the acquired data, refer to the *Thermo Xcalibur Qualitative Analysis User Guide* or the Qual Browser Help.

**Note** To acquire full-scan MS/MS data, see “[Setting Up to Acquire Full-Scan MS/MS Data](#)” on page 99.

Figure 71. Qual Browser window showing an ESI SIM chromatogram and mass spectrum





## Tuning with an Analyte in APCI Mode

This chapter describes how to create a tune method for an analyte in the APCI high-flow mode.

### Contents

- [Setting Up the Inlet for High-Flow Infusion in APCI Mode](#)
- [Setting Up the Mass Spectrometer to Tune with an Analyte in APCI Mode](#)
- [Tuning the Mass Spectrometer Automatically](#)
- [Saving the APCI Tune Method](#)

You do not have to recalibrate the LTQ Series mass spectrometer when switching to APCI operation. You can use the calibration settings obtained from the successful automatic calibration procedure performed in ESI mode.

The following mass spectrometer parameters have the most impact on signal quality in APCI mode:

- Electrospray voltage
- Tube lens voltage (LXQ and LTQ XL only)
- Heated ion transfer tube temperature (voltage)
- Ion transfer tube voltage (LXQ and LTQ XL only)
- Gas flow rates for the API gases
- S-lens rf level (Velos Pro only)
- Solution flow rate
- Vaporizer temperature

## 6 Tuning with an Analyte in APCI Mode

Setting Up the Inlet for High-Flow Infusion in APCI Mode

### Note

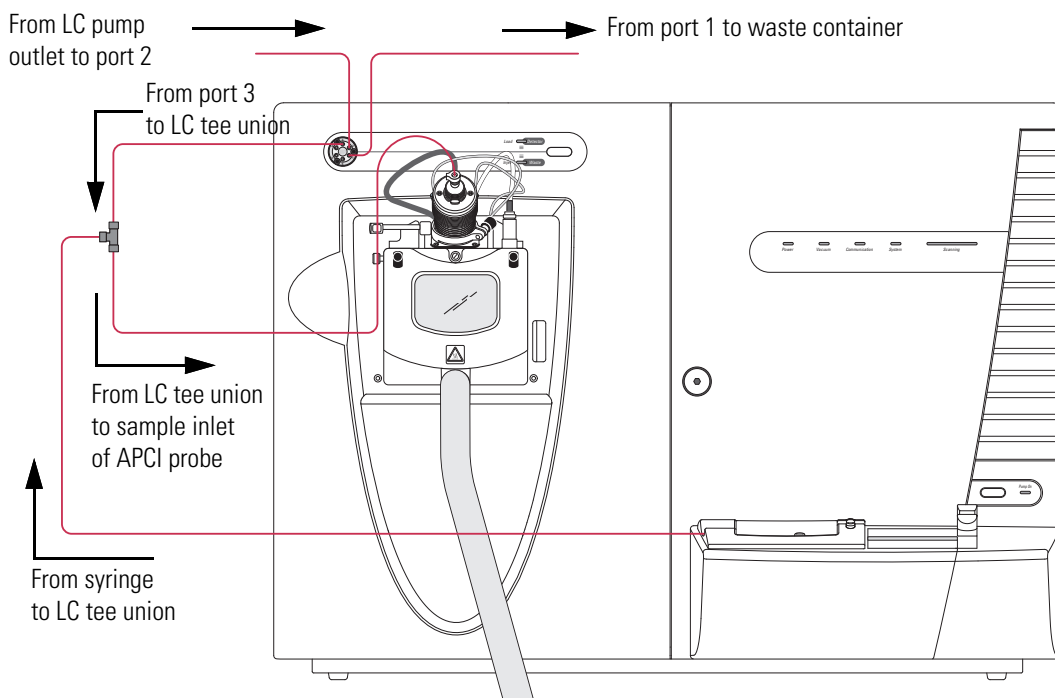
- These procedures assume that you are familiar with the LTQ Series mass spectrometers and the Tune Plus window. For more information, refer to the data system Help, *LTQ Series Getting Connected Guide*, and *LTQ Series Hardware Manual*, as needed.
- Before setting up the inlet plumbing, ensure that the ion source is set up for APCI mode and that you have tuned and calibrated the mass spectrometer in ESI mode.
- This chapter refers to a 100 pg/mL solution of reserpine as the sample analyte. You can substitute an analyte of your choice that is suitable for analysis in APCI mode.

## Setting Up the Inlet for High-Flow Infusion in APCI Mode

This section describes how to set up the LTQ Series mass spectrometer to infuse a sample solution with the syringe pump into the solvent flow from an LC pump. [Figure 72](#) shows the plumbing connections to introduce sample by high-flow infusion.

[Table 7](#) on [page 85](#) lists the inlet plumbing parts for high-flow infusion analysis.

**Figure 72.** Plumbing setup for APCI/MS sample introduction with high-flow infusion





❖ To set up the syringe pump for infusion



**CAUTION SHARP OBJECT** The syringe needle can puncture your skin. Handle it with care.

1. Load a clean, 500  $\mu\text{L}$  Unimetrics syringe with 450  $\mu\text{L}$  of a 100  $\text{pg}/\mu\text{L}$  solution of reserpine or the analyte.

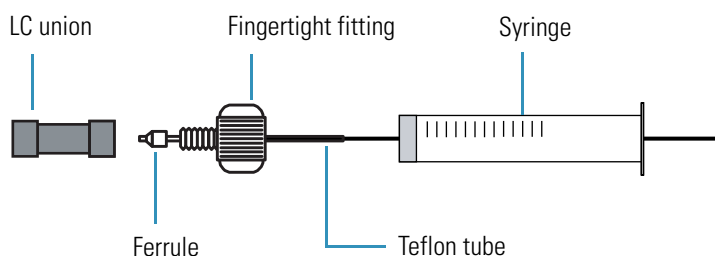
To prepare the reserpine tuning solution, follow one of these procedures:

- For the LXQ and LTQ XL, see “Preparing the Reserpine Tuning and Sample Solutions for the ESI and APCI Modes” on page 144.
- For the Velos Pro, see “Preparing the Reserpine Tuning and Sample Solutions for ESI and APCI Modes” on page 152.

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

2. Hold the plunger of the syringe in place and carefully insert the tip of the syringe needle into the end of a 4 cm (1.5 in.) Teflon tube with a fingertight fitting and a ferrule to the (black) LC union (Figure 73).

**Figure 73.** Plumbing connections for the syringe



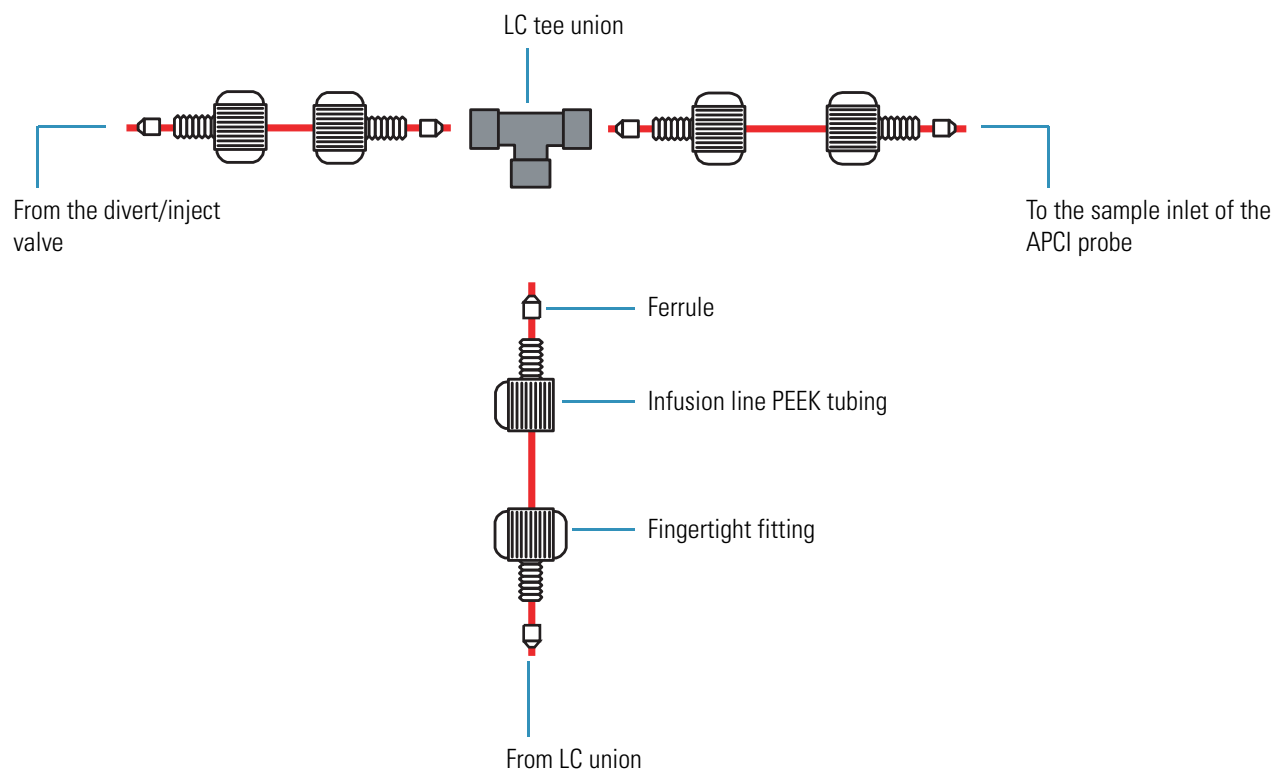
3. Place the syringe into the syringe holder of the syringe pump.
4. Connect a red PEEK infusion line from the LC union to the LC tee union as follows:
  - a. Use a fingertight fitting and ferrule to connect the infusion line to the free end of the LC union.
  - b. Use a fingertight fitting and ferrule to connect the other end of the infusion line to a leg of the LC tee union.

Figure 74 shows the connections to the LC tee union.

## 6 Tuning with an Analyte in APCI Mode

Setting Up the Inlet for High-Flow Infusion in APCI Mode

**Figure 74.** APCI/MS plumbing connections for the LC tee union



**Tip** Use a PEEK tubing cutter to cut the PEEK tubing. Use a PEEK cutter to ensure that the tubing is cut straight. To prevent adding dead volume to the inlet plumbing, ensure that the LC fittings, ferrules, and PEEK tubing are properly installed. Dead volume in the plumbing connections can broaden the chromatographic peaks and increase carryover.

5. Use a fingertight fitting and a ferrule to connect one end of a length of red PEEK tubing to the LC tee union (Figure 74).
6. Use a fingertight fitting and a ferrule to connect the other end of the Teflon tubing directly to the sample inlet of the APCI probe.

Figure 72 shows the connection between the LC tee union and the sample inlet of the APCI probe.

**Note** Do not use the grounding bar of the API source housing for the APCI probe. A knurled nut secures the grounding bar to the ion source. You do not need to remove the grounding bar to run the system in APCI mode.

7. Connect the LC pump to port 2 of the divert/inject valve as follows:
  - Use an appropriate fitting and a ferrule to connect one end of a length of tubing to the outlet of the LC pump.
  - Use a fingertight fitting and a ferrule to connect one end of the tubing to port 2 of the divert/inject valve.
8. Connect the divert/inject valve to a waste container as follows:
  - Use a fingertight fitting and a ferrule to connect one end of a length of tubing to port 1.
  - Connect the other end of the tubing to an appropriate waste container.

## Setting Up the Mass Spectrometer to Tune with an Analyte in APCI Mode

You can tune the LTQ Series mass spectrometer in APCI mode by using a high solvent flow: a solution of reserpine or a solution of the analyte. To prepare the reserpine tuning solution, follow one of these procedures:

- For the LXQ and LTQ XL, see [“Preparing the Reserpine Tuning and Sample Solutions for the ESI and APCI Modes”](#) on page 144.
- For the Velos Pro, see [“Preparing the Reserpine Tuning and Sample Solutions for ESI and APCI Modes”](#) on page 152.

To set up the mass spectrometer for tuning at the solvent flow rate for the experiments, follow these procedures:

- [To open a stored tune method with preset conditions for APCI mode](#)
- [To view the pre-tune APCI source settings](#)
- [To define the scan parameters for an analyte in APCI mode](#) on page 119
- [To set the scan type and ion polarity mode](#) on page 120

## 6 Tuning with an Analyte in APCI Mode

Setting Up the Mass Spectrometer to Tune with an Analyte in APCI Mode

### ❖ To open a stored tune method with preset conditions for APCI mode



On



Standby

1. Open the Tune Plus window (see [page 32](#)).
2. Click the **On/Standby** button to select the **On** mode.

The mass spectrometer begins scanning and applies high voltage to the corona needle. A real-time display appears in the Spectrum view.

3. Open the tune method file that stores the factory default tune settings for APCI mode as follows:



- a. In the Tune Plus window, click the **Open** button.
- b. Browse to the *drive:\Thermo\Instruments\LTQ\methods* folder, and then select **Default\_APCI.LTQTune**.

This file contains preset conditions for the APCI mode.

- c. Click **Open**.

Tune Plus downloads the tune method parameters to the mass spectrometer.

### ❖ To view the pre-tune APCI source settings

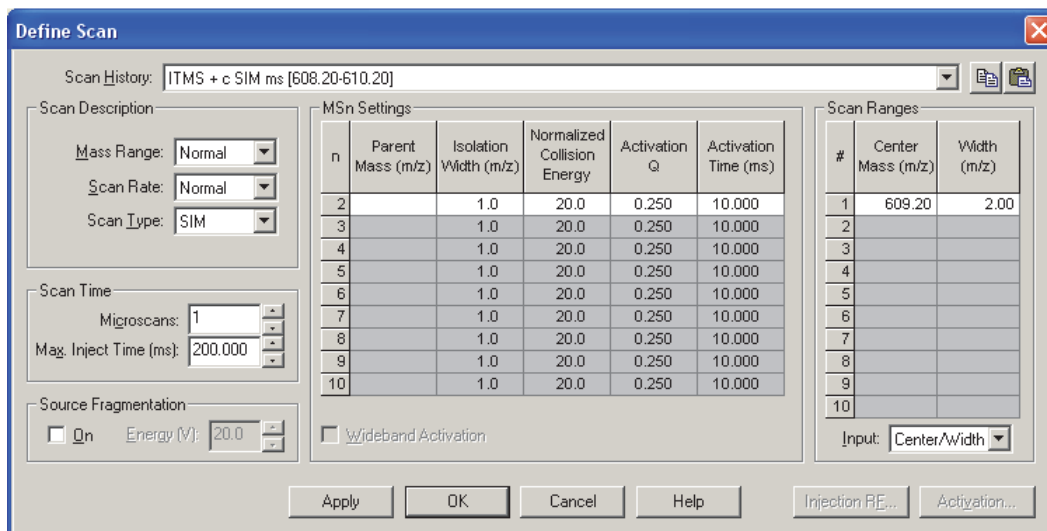
1. In the Tune Plus window, choose **Setup > APCI Source**.
2. Observe the settings.
3. Click **OK**.

❖ **To define the scan parameters for an analyte in APCI mode**

1. In the Tune Plus window, click the **Define Scan** button to open the Define Scan dialog box (Figure 75).

If the dialog box looks different from the one shown in Figure 75, the display is probably missing the advanced settings. If the advanced settings do not appear, in Tune Plus choose **ScanMode > Advanced Scan Features**.

**Figure 75.** Define Scan dialog box (reserpine SIM type data in APCI mode)



2. Under Scan Description, do the following:
  - In the Mass Range list, select **Normal**.
  - In the Scan Rate list, select **Normal**.
  - In the Scan Type list, select **SIM**.
3. Under Scan Time, do the following:
  - In the Microscans box, enter **2**.
  - In the Max. Inject Time (ms) box, enter **200.000**.
4. Under Source Fragmentation, clear the **On** check box.

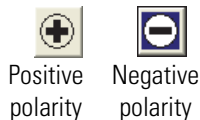
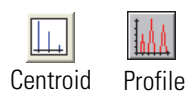
**Note** Selecting the Source Fragmentation check box allows collision-induced fragmentation to occur in the ion source. Because collision-induced fragmentation performed in the ion source is not very specific, this feature is rarely used.

## 6 Tuning with an Analyte in APCI Mode

Tuning the Mass Spectrometer Automatically

5. Under Scan Ranges, do the following:
  - a. In the Input list, select **Center/Width**.
  - b. In the Center Mass ( $m/z$ ) column, type the  $m/z$  value for the parent ion of the analyte, or type **609.20** for reserpine.
  - c. In the Width ( $m/z$ ) column, type **2.00**.
6. Verify that the settings in the Define Scan dialog box match those in [Figure 75](#) on [page 119](#).
7. Click **OK**.

### ❖ To set the scan type and ion polarity mode



1. In the Tune Plus window, click the **Centroid/Profile** button to select the **profile** data type.
2. Click the **Positive/Negative** button to select the **positive** ion polarity mode.

This completes the mass spectrometer set up to tune with an analyte in APCI mode.

## Tuning the Mass Spectrometer Automatically

Tune the LTQ Series mass spectrometer automatically in APCI mode to optimize the parameters listed on [page 113](#).

**Note** The most important parameters that affect the signal quality during APCI operation for the LXQ and LTQ XL mass spectrometers are the ion transfer tube temperature, vaporizer temperature, tube lens voltage, gases, and solution flow rate. For the Velos Pro mass spectrometer, the important parameters are the spray current, S-lens rf level, and solution flow rate.

If any of these parameters change, you must reoptimize the mass spectrometer parameters. Use the Semi-Automatic tune procedure to tune the mass spectrometer on individual parameters.

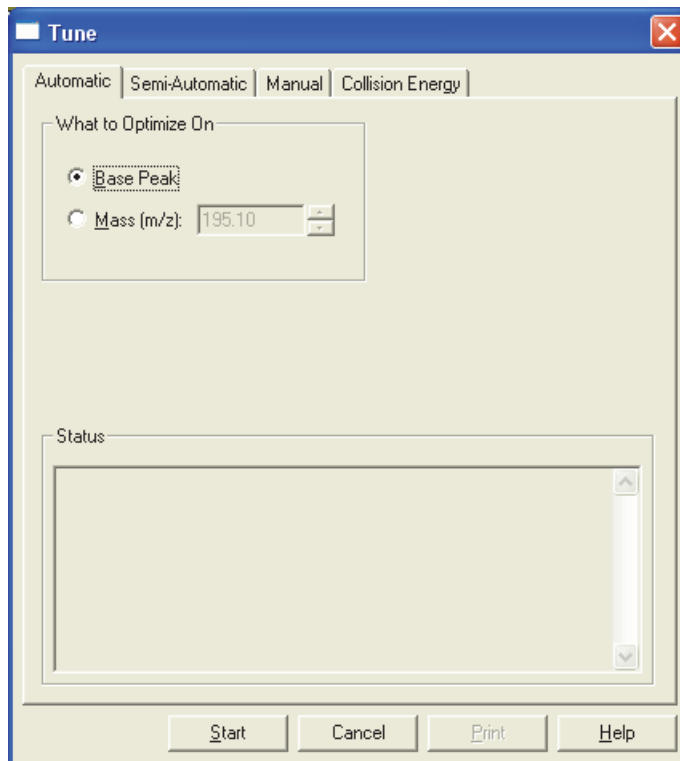
The following procedure uses a 100 pg/mL reserpine tuning solution to optimize the mass spectrometer automatically on the reserpine peak at  $m/z$  609.2 at a flow rate of 0.4  $\mu\text{m}/\text{min}$ . You can substitute the reserpine solution with a sample solution that is suitable for analysis in APCI mode. For guidelines on setting an appropriate flow rate, tube temperature, and vaporizer temperature for the application, see [Table 4](#) on [page 13](#).

❖ To automatically optimize the tune of the mass spectrometer for the analyte



1. In the Tune Plus window, click the **Tune** button to open the Tune dialog box (Figure 76).

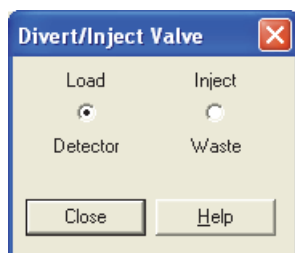
**Figure 76.** Tune dialog box showing the Automatic page



2. Under What to Optimize On, select the **Mass ( $m/z$ )** option.
3. In the Mass ( $m/z$ ) box, enter **609.20** (or the appropriate mass of the analyte).
4. Click the **Divert/Inject Valve** button to open the Divert/Inject dialog box (Figure 77).



**Figure 77.** Divert/Inject Valve dialog box



5. Select the **Load Detector** option, and then click **Close**.

**Note** When the divert/inject valve is in the Detector position, the solvent flow from the LC pump enters and exits the divert/inject valve through ports 2 and 3, respectively (Figure 52 on page 88). Port 3 is connected to the ion source.

6. In the Tune dialog box, start the automatic tuning procedure as follows:

a. Click **Start**.

A message appears:

Please ensure that the 500 microliter syringe is full.

b. Ensure that the syringe pump contains at least 450  $\mu\text{L}$  of a 100  $\text{pg}/\mu\text{L}$  reserpine tuning solution or the analyte.

To prepare the reserpine tuning solution, follow one of these instructions:

- For the LXQ and LTQ XL, see “[Preparing the Reserpine Tuning and Sample Solutions for the ESI and APCI Modes](#)” on [page 144](#).
- For the Velos Pro, see “[Preparing the Reserpine Tuning and Sample Solutions for ESI and APCI Modes](#)” on [page 152](#).

c. Click **OK**.



7. In the Tune Plus window, click the **Graph View** button.

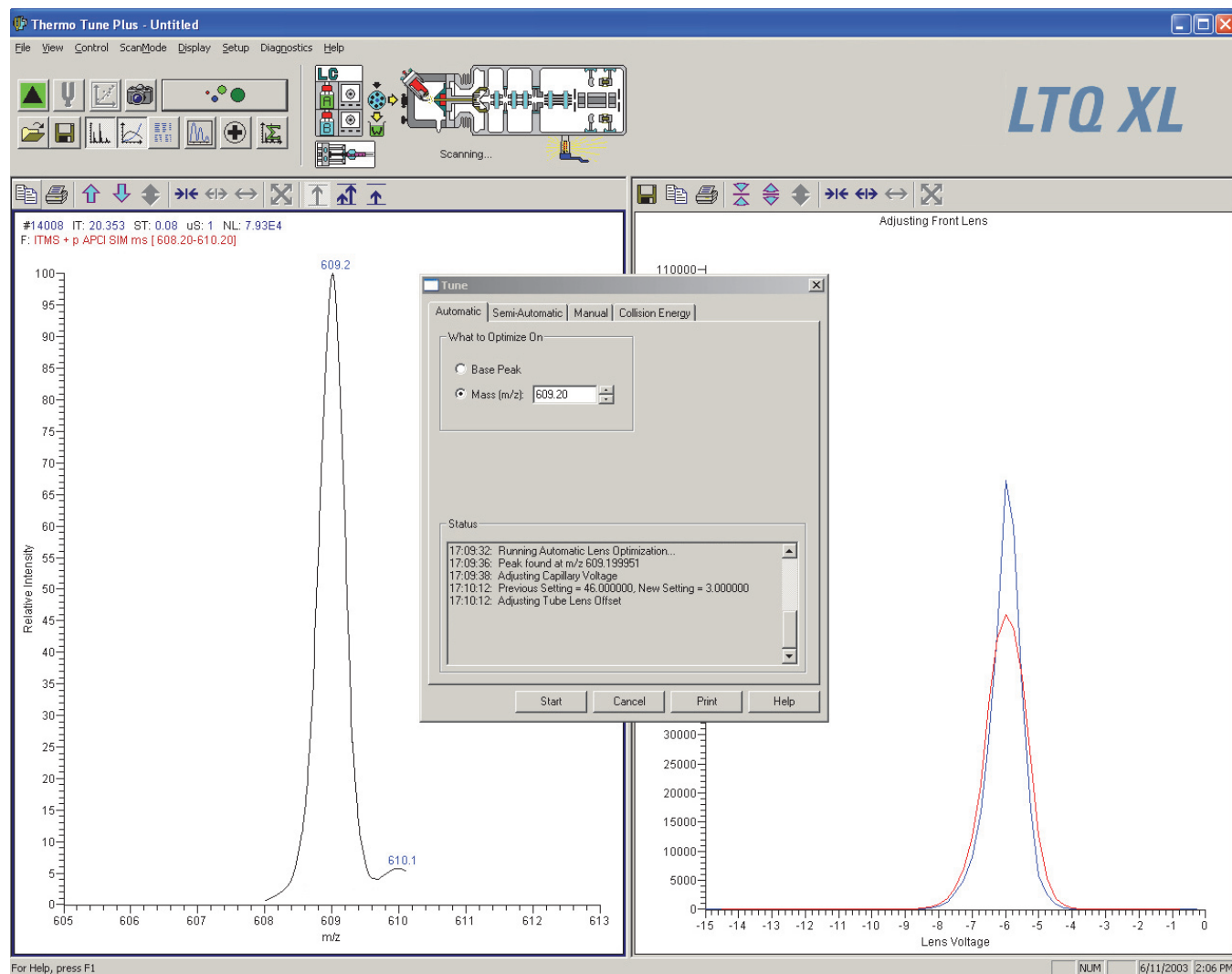
8. Observe the Tune Plus window and the Tune dialog box.

During automatic tuning, the mass spectrometer displays the results of various tests in the Spectrum and Graph views in the Tune Plus window and displays various messages under Status in the Tune dialog box.

Check that the Tune Plus window looks similar to the one shown in [Figure 78](#).



**Figure 78.** Tune Plus window and Tune dialog box for automatic tuning in APCI mode for the LTQ XL



9. After the automatic tuning process ends, choose **Setup > APCI Source** to observe the post-tune settings for the APCI source.

10. Choose **Setup > Ion Optics** to observe the post-tune settings for the ion optics.

This completes the tuning of the mass spectrometer in APCI mode for the compound reserpine (or the analyte).

# Saving the APCI Tune Method

You can save the parameters that you just set in a tune method specific to the particular analyte and solvent flow rate. Recall the tune method and use it as a starting point for optimizing the mass spectrometer on a different analyte or at a different flow rate.

**Tip** You must save the tune method while the mass spectrometer is on.

#### ❖ To save the APCI tune method



1. In the Tune Plus window, click the **Save** button.
2. Browse to the *drive:\Thermo\Instruments\LTQ\methods* folder.
3. In the File Name box, type a name, such as **APCImyTune**, to identify the tune method.
4. Click **Save**.

If you tuned the mass spectrometer by using reserpine, clean the mass spectrometer as described in [Chapter 8, “Cleaning the Mass Spectrometer After Tuning and Calibrating.”](#) If you tuned with an analyte, as described in [Chapter 7, “Acquiring APCI Sample Data by Using Tune Plus,”](#) you are ready to acquire data on the analyte.

## Acquiring APCI Sample Data by Using Tune Plus

This chapter describes how to set up the inlet to make loop injections in APCI mode and how to acquire sample data in the selected ion monitoring (SIM) mode.

For demonstration purposes only, the procedures in this chapter use a 1 pg/mL reserpine solution. You can use any analyte that is suitable for analysis in APCI mode. If you do not have a sample suitable for analysis in APCI mode, use the reserpine provided in the instrument's chemicals kit to prepare a 1 pg/mL reserpine solution as described in one of the following:

- For the LXQ and LTQ XL, see [“Preparing the Reserpine Tuning and Sample Solutions for the ESI and APCI Modes”](#) on page 144.
- For the Velos Pro see [“Preparing the Reserpine Tuning and Sample Solutions for ESI and APCI Modes”](#) on page 152.

### Note

- Before beginning the analysis of the sample solution, verify that the mass spectrometer has been tuned and calibrated in ESI mode and that you have created and saved a tune method for the particular analyte by using a suitable flow rate.
- Before performing full-scan MS/MS experiments, follow the procedures in [“Setting Up to Acquire Full-Scan MS/MS Data”](#) on page 99.

### Contents

- [Setting Up the Inlet for Flow Injection Analysis in APCI Mode](#)
- [Acquiring APCI Data in the SIM Scan Mode](#)

## Setting Up the Inlet for Flow Injection Analysis in APCI Mode

This section provides information about how to introduce sample by loop injection (flow injection analysis) into the solvent flow from an LC pump.

### ❖ To set up the inlet for loop injection

1. Connect a 2  $\mu$ L sample loop to ports 1 and 4 of the divert/inject valve.
2. Connect the LC pump to port 2 of the divert/inject valve as follows:
  - Use an appropriate fingertight fitting and a ferrule to connect one end of a length of red PEEK tubing to the outlet of the LC pump.  
To produce a stable solvent flow, the Accela 1250 Pump requires a minimum backpressure of 40 bar (580 psi). To connect the LC pump, use a length of 0.005 in. ID PEEK tubing sufficient to exert a backpressure of 40 bar (580 psi), or connect an inline backpressure regulator between the LC pump outlet and the divert/inject valve.
  - Use a fitting and a ferrule to connect the other end of the tubing to port 2 of the divert/inject valve.
3. For the APCI probe, use fingertight fittings to connect an appropriate length of red PEEK tubing between port 3 of the divert/inject valve to the sample inlet fitting on the APCI probe (Figure 79 and Figure 80).

**Figure 79.** Divert/inject valve setup for loop injection

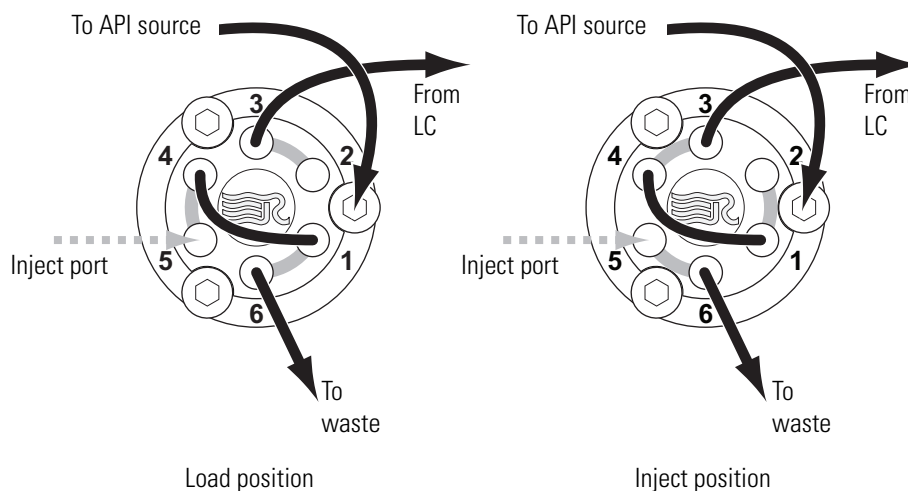
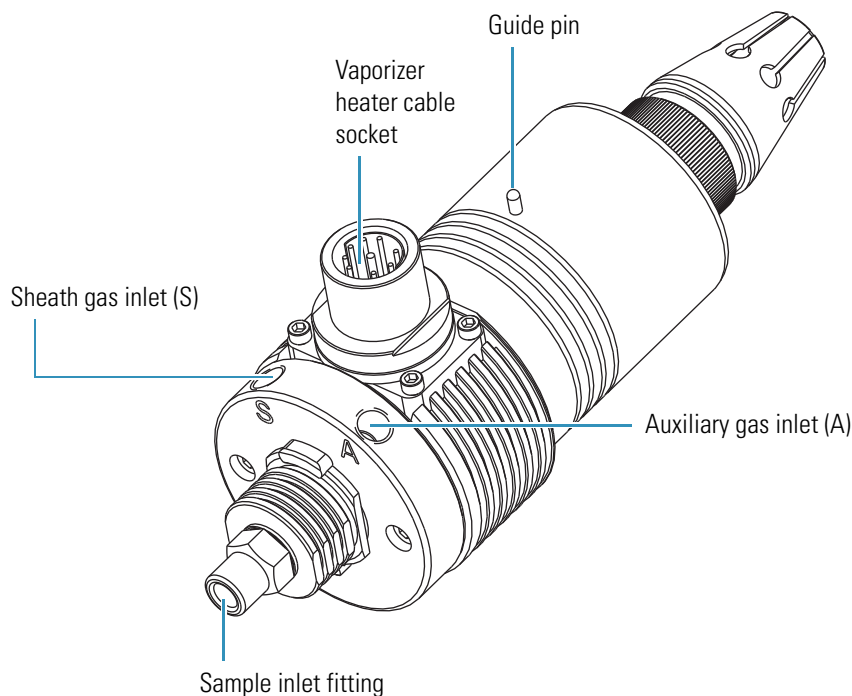


Figure 80. APCI probe



4. Connect the divert/inject valve to a waste container as follows:

- Use a fingertight fitting and a ferrule to connect one end of a length of red PEEK tubing to port 6.
- Connect the other end of the tubing to an appropriate waste container.

The mass spectrometer is now set up to introduce sample by loop injection into the solvent flow from an LC pump.

## Acquiring APCI Data in the SIM Scan Mode

**Note** The data system automatically saves the acquired data to its hard disk drive.

### ❖ To acquire a data file containing SIM data

1. Open the Tune Plus window (see [page 32](#)).
2. Click the **On/Standby** button to select the **On** mode.

The mass spectrometer begins scanning and applies high voltage to the APCI corona needle. A real-time display appears in the Spectrum view.

3. Click the **Centroid/Profile** button to select the **centroid** data type.



On



Standby



Centroid



Profile

## 7 Acquiring APCI Sample Data by Using Tune Plus

Acquiring APCI Data in the SIM Scan Mode

4. Ensure that the scan parameters are defined to acquire SIM data for reserpine (or the analyte) as follows:



- a. Click the **Define Scan** button.

Figure 81 shows the Define Scan dialog box with typical settings for acquiring SIM data for reserpine.

**Figure 81.** Define Scan dialog box (reserpine SIM-scan data)

n	Parent Mass (m/z)	Isolation Width (m/z)	Normalized Collision Energy	Activation Q	Activation Time (ms)
2		1.0	20.0	0.250	10.000
3		1.0	20.0	0.250	10.000
4		1.0	20.0	0.250	10.000
5		1.0	20.0	0.250	10.000
6		1.0	20.0	0.250	10.000
7		1.0	20.0	0.250	10.000
8		1.0	20.0	0.250	10.000
9		1.0	20.0	0.250	10.000
10		1.0	20.0	0.250	10.000

#	Center Mass (m/z)	Width (m/z)
1	609.20	2.00
2		
3		
4		
5		
6		
7		
8		
9		
10		

- b. Verify that the values in the dialog box are the same as those in Figure 81, and then click **OK**.

5. Turn on the LC pump and specify a flow rate of **0.4 mL/min**.

Ensure that the system is free of leaks.



6. In the Tune Plus window, click the **Acquire Data** button.

Figure 82 shows the acquisition status of the raw data file in the Acquire Data dialog box.

**Figure 82.** Acquire Data dialog box

Folder: C:\Thermo\Instruments\LTQ\data

File Name: reserpine\_001

Sample Name: reserpine

Comment: SIM, ESI, 2 uL loop

Use instrument method

Instrument Method:

Start Mode

Immediate  Contact Closure  Divert Valve

Acquire Time

Continuously  Scans  Minutes

10

1.00

Go to Standby when Finished

Acquisition Status

State: Idle

Time (min): 0.000

Start

Pause

View...

Inst. Setup...

OK

Cancel

Help

7. Specify the acquisition parameters as follows:

- In the File Name box, type **reserpine** (or the name of the analyte).
- In the Sample Name box, type **reserpine** (or the name of the analyte).
- In the Comment box, type a comment about the experiment.

For example, describe the scan mode, scan type, ionization mode, sample amount, or method of sample introduction. The Xcalibur data system includes the comment in the header information for the raw data file.

You can also add this information to reports created with the XReport reporting software. To open the XReport application, choose **Start > Programs > Thermo Xcalibur > XReport**.

- Under Acquire Time, select the **Continuously** option (acquires data until you stop the acquisition).

8. Click **Start**.

9. Leave the Acquire Data dialog box open during data acquisition and move it out of the way.



10. Click the **Divert/Inject Valve** button.

11. Inject the sample into the APCI source a total of four times as follows:

- a. Select the **Load Detector** option.

When the valve is in the Load position, the solvent flow from the LC pump bypasses the sample loop.

- b. Overfill the 2  $\mu$ L injector loop with a sample solution or a 1 pg/ $\mu$ L reserpine solution.

To prepare the reserpine tuning solution, follow these procedures:

- For the LXQ and LTQ XL, see [“Preparing the Reserpine Tuning and Sample Solutions for the ESI and APCI Modes”](#) on page 144.
- For the Velos Pro, see [“Preparing the Reserpine Tuning and Sample Solutions for ESI and APCI Modes”](#) on page 152.

- c. Select the **Inject Waste** option.

When the valve is in the Inject position, the solvent flow from the LC pump backflushes the contents of the sample loop into the ion source.

- d. In the Spectrum view, observe the reserpine peak ( $m/z$  609.2) or the peak of the analyte.
- e. Wait approximately one minute before the next injection.
- f. Repeat [step 11a](#) to [step 11e](#) three more times.

## 7 Acquiring APCI Sample Data by Using Tune Plus

Acquiring APCI Data in the SIM Scan Mode

12. In the Divert/Inject Valve dialog box, click **Close**.

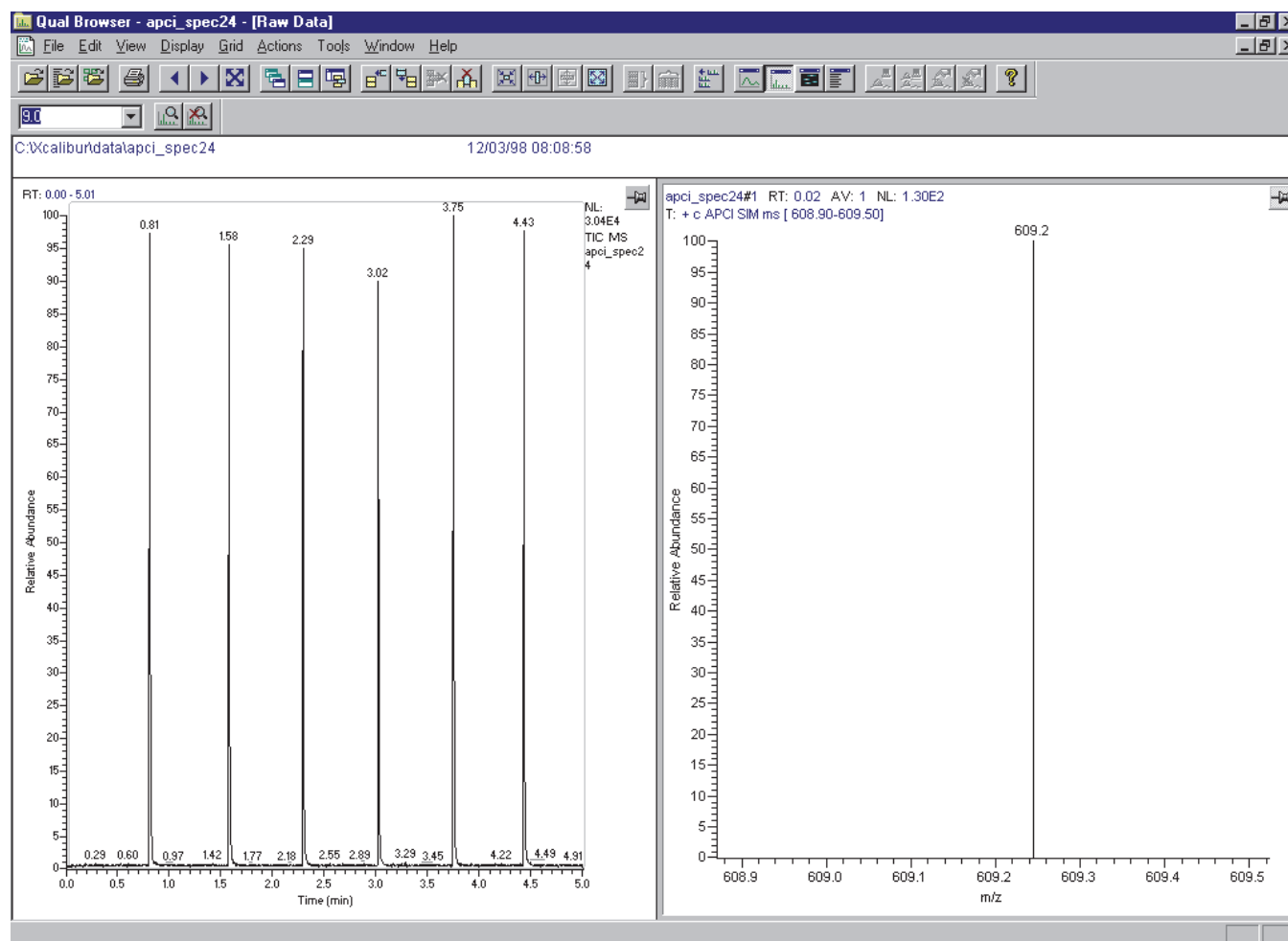
13. In the Acquire Data dialog box, click **Stop**, and then click **Cancel**.

Review the mass spectrum and chromatogram in the raw file just acquired by using the Xcalibur Qual Browser window. [Figure 83](#) shows a chromatogram (left side) containing the results from the four loop injections of reserpine and a mass spectrum of reserpine (right side).

For more information about reviewing the acquired data, refer to the *Thermo Xcalibur Qualitative Analysis User Guide* or the Qual Browser Help.

**Note** To acquire full-scan MS/MS data, see “[Setting Up to Acquire Full-Scan MS/MS Data](#)” on page 99.

**Figure 83.** Qual Browser window showing an APCI SIM chromatogram and mass spectrum





# Cleaning the Mass Spectrometer After Tuning and Calibrating

After infusing the calibration solution, you must clean the system before tuning with an analyte. Complete all data acquisition before proceeding.

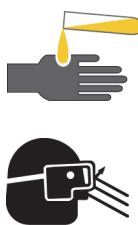
## Contents

- Cleaning Supplies and Chemicals
- Flushing the Sample Transfer Line, Sample Tube, and API Probe
- Removing and Cleaning the Syringe
- Cleaning the Ion Sweep Cone, Spray Cone, and Ion Transfer Tube

## Cleaning Supplies and Chemicals

You need the following supplies and chemicals.

Supplies	Chemicals
Gloves, lint-free and powder-free	Acetone
Graduated cylinder or beaker (for use with methanol)	Formic acid
Kimwipes or lint-free industrial tissues	Methanol, LCMS-grade
Sonicator	Water, LCMS-grade



### CAUTION AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS

By law, producers and suppliers of chemical compounds are required to provide their customers with the most current health and safety information in the form of Material Safety Data Sheets (MSDSs). The MSDSs describe the chemicals and must be freely available to lab personnel to examine at any time. MSDSs provide summarized information on the hazard 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.

Read the MSDS for each chemical you use. Store and handle all chemicals in accordance with standard safety procedures. Always wear protective gloves and safety glasses when you use solvents or corrosives. Also, contain waste streams, use proper ventilation, and dispose of all laboratory reagents according to the directions in the MSDS.

## Flushing the Sample Transfer Line, Sample Tube, and API Probe

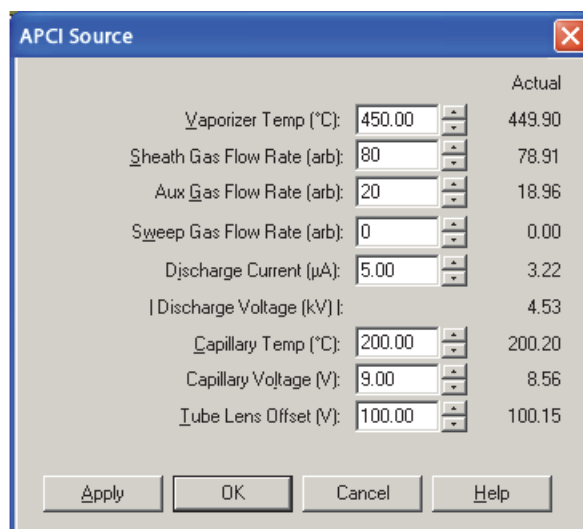
Flush the sample transfer line, sample tube, and API probe at the end of each work day (or more often if you suspect they are contaminated) with a 50:50 methanol/water solution from the LC system through the API source at a flow rate of 200–400  $\mu\text{L}/\text{min}$  for approximately 15 minutes to remove contamination.

### ❖ To flush the sample transfer line, sample tube, and API probe

1. Open the Tune Plus window (see [page 32](#)).
2. In the Tune Plus window, ensure that the **On/Standby** button indicates the **On** mode, and then do one of the following:
  - If operating in APCI or APPI mode, go to [step 3](#).
  - If operating in ESI mode, go to [step 4](#).
3. To flush the APCI source:
  - a. Choose **Setup > APCI Source** to open the APCI Source dialog box ([Figure 84](#)).



**Figure 84.** APCI Source dialog box



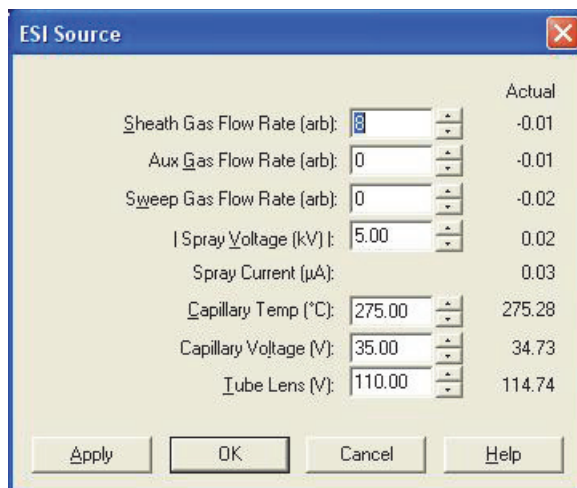
- b. In the Vaporizer Temp (°C) box, enter **500.00**.
- c. In the Sheath Gas Flow Rate (arb) box, enter **30**.
- d. In the Aux Gas Flow Rate (arb) box, enter **5**.
- e. In the Sweep Gas Flow Rate (arb) box, enter **0**.
- f. In the Discharge Current box, enter **0**.
- g. Click **OK**.
- h. Go to [step 5](#).

## 8 Cleaning the Mass Spectrometer After Tuning and Calibrating

Flushing the Sample Transfer Line, Sample Tube, and API Probe

4. To flush the ESI source:
  - a. Choose **Setup > ESI Source** to open the ESI Source dialog box (Figure 85).

**Figure 85.** ESI Source dialog box



- b. In the Sheath Gas Flow Rate (arb) box, enter **30**.
    - c. In the Aux Gas Flow Rate (arb) box, enter **5**.
    - d. In the Sweep Gas Flow Rate (arb) box, enter **0**.
    - e. In the Spray Voltage (kV) box, enter **0**.
    - f. Click **OK**.
5. To set up and start a flow of 50:50 methanol/water solution from the LC system to the API source:
  - a. Choose **Setup > Inlet Direct Control** to open the Inlet Direct Control dialog box.

**Note** The Xcalibur data system controls the LC pumps from several manufacturers including Thermo Fisher Scientific Inc., Agilent™ Technologies, and Waters™ Corporation. Contact your Thermo Fisher Scientific sales representative for information about the liquid chromatography systems compatible with the LTQ Series mass spectrometer.

- b. Click the **LC Pump** tab.
      - c. Set the solvent proportions to 50% methanol and 50% water.
      - d. Start the solvent flow.
6. Let the solution flow through the sample transfer line, sample tube, and API probe for 15 minutes.

7. After 15 minutes, turn off the flow of liquid from the LC to the API source as follows:
  - a. Leave the API source (including the APCI vaporizer, sheath gas, and auxiliary gas) on for an additional 5 minutes.
  - b. Click the **Pump Off** or **Stop Pump** button.
8. After another 5 minutes, place the mass spectrometer in Standby mode (see [page 33](#)).



## Removing and Cleaning the Syringe

### ❖ To remove and clean the syringe

1. Squeeze the blue pusher blocks and pull back on the syringe pump handle to free the syringe.
2. Remove the syringe from the holder.
3. Disconnect the tip of the syringe needle from the Teflon tubing.
4. Clean the syringe with a solution of 5% formic acid in water.
5. Rinse the syringe with a solution of 50:50 methanol/water.
6. Rinse the syringe with acetone several times.

## Cleaning the Ion Sweep Cone, Spray Cone, and Ion Transfer Tube

### IMPORTANT

- Prepare a clean work surface by covering the area with lint-free paper or a sheet of aluminum foil.
- Put on a new pair of lint- and powder-free gloves before starting each cleaning and component installation procedure.



**CAUTION HOT SURFACE** At operating temperatures above 350 °C (662 °F), the probe and API source housing can severely burn you.

- Before removing the probe or API source housing, allow the part to cool to room temperature (approximately 20 minutes) before touching it.
- If the mass spectrometer connects to an LC system, leave the solvent flow from the LC pump on while the probe cools to room temperature.

## 8 Cleaning the Mass Spectrometer After Tuning and Calibrating

Cleaning the Ion Sweep Cone, Spray Cone, and Ion Transfer Tube

### ❖ To clean the ion sweep cone, spray cone, and ion transfer tube

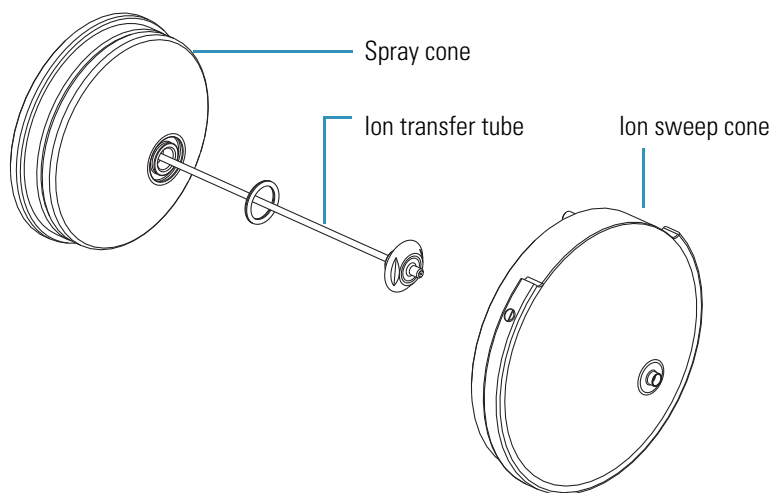
1. Remove the API source housing (see [page 36](#)).
2. Remove the ion sweep cone by grasping the outer ridges of the ion sweep cone and pulling it off of the API cone seal.

You might need to loosen the ball plungers on the ion sweep cone. There are two versions of the ion sweep cone. [Figure 86](#) shows the version with a nipple. [Figure 88](#) on [page 138](#) shows the version with an offset orifice.

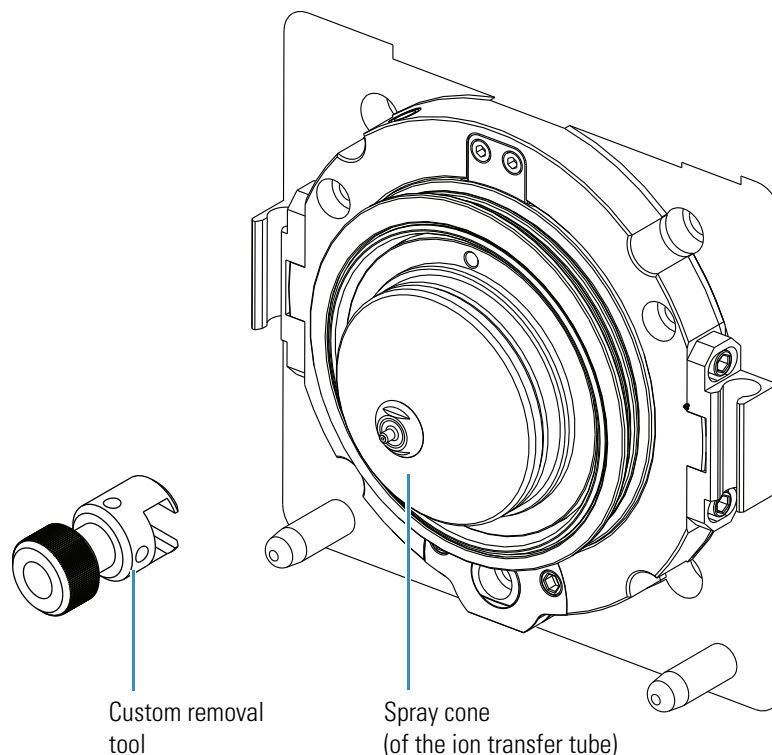


**CAUTION** To avoid contaminating the ion transfer tube, do not touch its exposed entrance.

**Figure 86.** Ion source interface components for the LXQ and LTQ XL (exploded view)



3. Remove the ion transfer tube by turning its exposed spray cone counterclockwise with the custom removal tool provided in the MS Accessory Kit ([Figure 87](#)). When the tube is free of the spray cone, pull it straight out of the ion source interface.

**Figure 87.** Ion transfer tube removal tool

4. Clean the ion sweep cone and optional spray cone by wiping the insides and outsides with Kimwipe tissues soaked in methanol.
5. Clean the ion transfer tube as follows:
  - a. Place it into a graduated cylinder containing 50:50 methanol/water.
  - b. Sonicate the component for 15 minutes.



**CAUTION** Take these precautions when reinstalling the ion transfer tube:

- Ensure that everything is properly aligned to prevent stripping the threads on the ion transfer tube.
- Do not bend the ion transfer tube. Rotate it as you insert it.

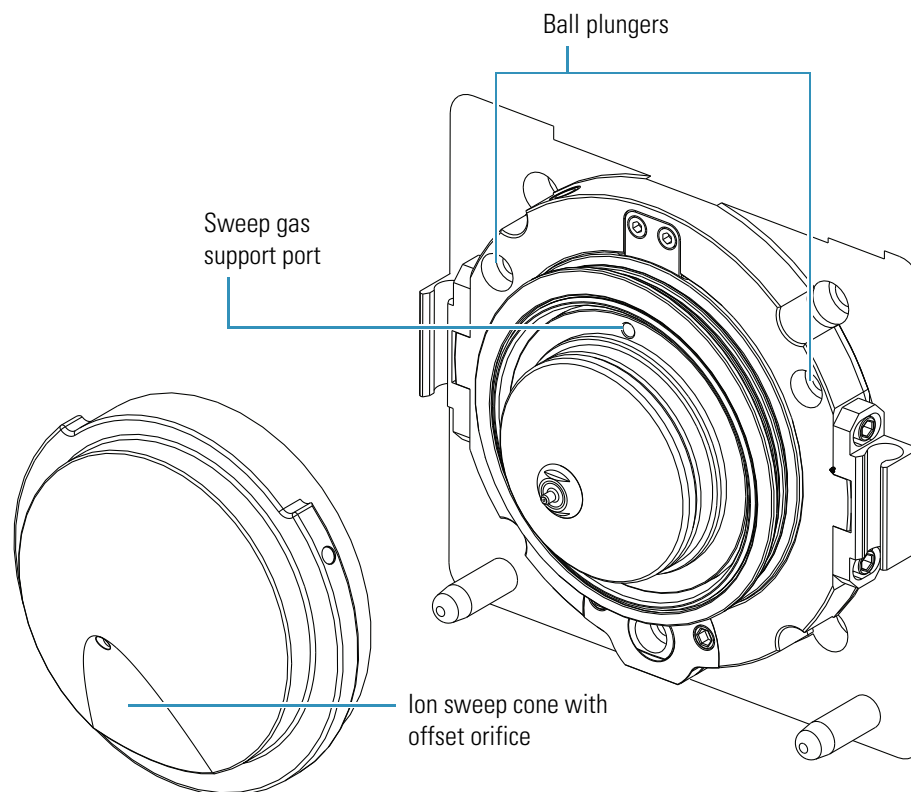
6. Reinstall the ion transfer tube as follows:
  - a. Rotate the ion transfer tube while reinserting it into the heater block.
  - b. Use the custom tool to turn the tube clockwise until tight.
7. Reinstall the ion sweep cone as follows:
  - a. Put on a new pair of lint- and powder-free gloves.
  - b. Carefully align the gas inlet on the ion sweep cone with the sweep gas supply port (Figure 88). Firmly press the ion sweep cone into position.

## 8 Cleaning the Mass Spectrometer After Tuning and Calibrating

Cleaning the Ion Sweep Cone, Spray Cone, and Ion Transfer Tube

- c. If necessary to achieve a proper ion sweep cone installation, adjust the ball plungers around the perimeter of the ion sweep cone (Figure 88).

**Figure 88.** Sweep gas supply port in the API cone seal



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



# Sample Formulations for the LXQ and LTQ XL Mass Spectrometers

This appendix describes how to prepare the tuning and calibration solutions for the LXQ and LTQ XL mass spectrometers.



**CAUTION** NEVER use the solutions described in this appendix to calibrate the Velos Pro mass spectrometer. Instead, use the solutions described in [Appendix B](#), “Sample Formulations for the Velos Pro Mass Spectrometer.”

## Contents

- [Preparing the Normal Mass Range Calibration Solution for ESI Mode](#)
- [Preparing the Reserpine Tuning and Sample Solutions for the ESI and APCI Modes](#)
- [Preparing the High Mass Range Calibration Solution](#)

The Chemical Accessory Kit provides the caffeine, Met-Arg-Phe-Ala acetate salt (MRFA), Ultramark 1621, and reserpine needed to make the solutions. You can order replacement Chemical Accessory Kits (P/N 97455-62045) from Thermo Fisher Scientific.

You can also order specific chemicals from Thermo Fisher Scientific, which are sold under its Fisher Chemical brand. As specified in [Table 8](#), use only LCMS-grade chemicals for calibrating the mass spectrometers.

**Table 8.** Recommended chemicals

Solvent/reagent	Specifications
Acetic acid (modifier)	LCMS-grade
Acetonitrile	LCMS-grade
Formic acid (modifier)	99–100% (Must be supplied in a glass bottle.)
Isopropyl alcohol	LCMS-grade
Methanol	LCMS-grade
Water	LCMS-grade

## A Sample Formulations for the LXQ and LTQ XL Mass Spectrometers

Preparing the Normal Mass Range Calibration Solution for ESI Mode

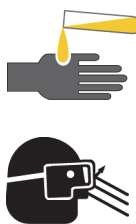
For a complete selection of LCMS-grade consumables from Thermo Fisher Scientific, visit [www.FisherLCMS.com](http://www.FisherLCMS.com).

**Note** Do not filter solvents. Filtering solvents can introduce contamination.

Potentially hazardous chemicals used in procedures throughout this appendix include the following:

- Acetonitrile
- Formic acid
- Glacial acetic acid
- Methanol
- Reserpine

**IMPORTANT** Do not use plastic pipettes to prepare the tuning and calibration standards. Plastic products can release phthalates that can interfere with the analyses.



### CAUTION AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS

By law, producers and suppliers of chemical compounds are required to provide their customers with the most current health and safety information in the form of Material Safety Data Sheets (MSDSs). The MSDSs describe the chemicals and must be freely available to lab personnel to examine at any time. MSDSs provide summarized information on the hazard 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.

Read the MSDS for each chemical you use. Store and handle all chemicals in accordance with standard safety procedures. Always wear protective gloves and safety glasses when you use solvents or corrosives. Also, contain waste streams, use proper ventilation, and dispose of all laboratory reagents according to the directions in the MSDS.

## Preparing the Normal Mass Range Calibration Solution for ESI Mode

For tuning and calibrating the LXQ and LTQ XL in ESI mode, use a calibration solution of caffeine, MRFA, and Ultramark 1621 in an acetonitrile/methanol/water solution containing 1% acetic acid. To prepare the ESI calibration solution, follow these procedures:

- “Caffeine Stock Solution” on page 142
- “Preparing the MRFA Stock Solution” on page 142
- “Preparing the Ultramark 1621 Stock Solution” on page 143
- “Preparing the Normal Mass Range Calibration Solution for ESI Mode” on page 143

**Note** The Chemical Accessory Kit contains vials of caffeine and MRFA. The following compound part numbers are available from Sigma Chemical Co.:

- P/N C6035 is for caffeine at a concentration of 1 mg/mL in methanol.
- P/N M1170 is for MRFA.

To order these compounds from Sigma, write or call:

Sigma Chemical Company

P. O. Box 14508

St. Louis, Missouri U.S. 63178-9916

(800) 325-3010 (U.S.)

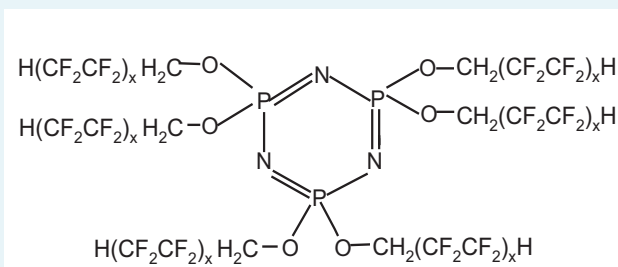
(905) 829-9500 (Canada)

(314) 771-3750 (outside the U.S. or Canada)

[www.sigmaaldrich.com](http://www.sigmaaldrich.com)

**Note** The Chemical Accessory Kit contains a vial of Ultramark 1621 (neat liquid). This compound is available from Lancaster Synthesis.

The structure of Ultramark 1621 follows ( $x$  is 1, 2, or 3):



The Lancaster product number for Ultramark 1621 is L16698 (Ultramark 1621, Mass Spec Std.). To order this compound from Lancaster Synthesis, write or call:

Lancaster Synthesis, Inc.

P.O. Box 1000

Windham, NH U.S. 03087-9977

(603) 889-3306, (800) 238-2324 (in the U.S. and Canada)

+44 (0)1524 36101 (U.K. and International)

[www.lancastersynthesis.com](http://www.lancastersynthesis.com)

## A Sample Formulations for the LXQ and LTQ XL Mass Spectrometers

Preparing the Normal Mass Range Calibration Solution for ESI Mode



**CAUTION** Always wear protective gloves and safety glasses when you use solvents or corrosives.

### Caffeine Stock Solution

The LXQ and LTQ XL mass spectrometers ships with a 1 mg/mL stock solution of caffeine in 100% methanol. You can also order this solution through Sigma Chemical (P/N C6035).

### Preparing the MRFA Stock Solution

#### ❖ To prepare the MRFA stock solution

1. Weigh out 6 mg of the MRFA compound provided in the accessory kit, and then transfer the sample to a clean, minimum 20 mL glass vial.

The MRFA provided in the accessory kit has an average molecular weight of 523.7 Da.

2. Add 2.0 mL of 50:50 methanol/water to the vial.
3. Mix the solution thoroughly.
4. Label the vial **MRFA Stock Solution 3 mg/mL**.

#### ❖ To prepare the MRFA diluted stock solution

1. Transfer 100  $\mu$ L of the 3 mg/mL MRFA stock solution into a clean, minimum 20 mL glass vial.
2. Add 2.9 mL of 50:50 methanol/water to the vial.
3. Mix the solution thoroughly.
4. Label the vial **Diluted MRFA Stock Solution 0.1 mg/mL**.

## Preparing the Ultramark 1621 Stock Solution

### ❖ To prepare the Ultramark 1621 stock solution

1. Use a syringe to transfer 25  $\mu\text{L}$  of Ultramark 1621 to a clean 25 mL volumetric glass flask.
2. Fill the flask to volume with 100% acetonitrile.
3. Mix the solution thoroughly.
4. Transfer the solution to a vial and label the vial  
**Ultramark 1621 stock solution (1/1000 dilution).**

## Preparing the Normal Mass Range Calibration Solution for ESI Mode

The following ready-to-use normal mass range calibration solution is available from Thermo Fisher Scientific at [www.thermo.com/pierce](http://www.thermo.com/pierce):

P/N 88322, Pierce LTQ ESI Positive Ion Calibration Solution, 10 mL

**IMPORTANT** Use only glass pipets or stainless steel syringes when measuring glacial acetic acid. Using plastic pipet tips causes contamination of acid stock solutions that can introduce contaminants in the calibration solution.

### ❖ To prepare the normal mass range calibration solution

1. Pipet 200  $\mu\text{L}$  of the caffeine stock solution into a clean 10 mL volumetric glass flask.
2. Pipet 100  $\mu\text{L}$  of the diluted MRFA stock solution (0.1 mg/mL) into the flask.
3. Pipet 100  $\mu\text{L}$  of the Ultramark 1621 stock solution into the flask.
4. Pipet 100  $\mu\text{L}$  of the LCMS-grade glacial acetic acid into the flask.
5. Pipet 5 mL of LCMS-grade acetonitrile into the flask.
6. Bring the flask to volume with 50:50 methanol/water.
7. Mix the solution thoroughly.
8. Transfer the solution to a clean, dry vial.
9. Label the vial **LXQ/LTQ XL ESI Calibration Solution** and store it in a refrigerator at 2–8  $^{\circ}\text{C}$  (36– 46  $^{\circ}\text{F}$ ) until needed.

## Preparing the Reserpine Tuning and Sample Solutions for the ESI and APCI Modes

Ideally, you should prepare the reserpine solutions just before using them. If you must store the solutions, keep them in a light-resistant container in the refrigerator until needed.

### ❖ To prepare the reserpine stock solution

1. Weigh out 10 mg of reserpine, and then transfer the sample into a clean 10 mL volumetric glass flask.

The average molecular weight of reserpine is 608.7 Da.

2. Fill the flask to volume with a solution of 1% acetic acid in methanol.
3. Mix the solution thoroughly.
4. Transfer the solution to a clean, dry, light-resistant vial.
5. Label the vial **Reserpine Stock Solution (1 µg/µL)**.

### ❖ To prepare the reserpine tuning solution

1. Pipet 100 µL of the reserpine stock solution (1 µg/µL) into a clean, minimum 1.5 mL polypropylene microcentrifuge tube.
2. Add 900 µL of 1% acetic acid in 50:50 methanol/water to the tube.
3. Mix the solution (100 ng/µL) thoroughly.
4. Transfer 10 µL of the 100 ng/µL solution into a clean polypropylene tube.
5. Add 990 µL of 1% acetic acid in 50:50 methanol/water to the tube.
6. Mix the solution (1 ng/µL) thoroughly.
7. Transfer 100 µL of the 1 ng/µL solution into a clean, minimum 1.5 mL polypropylene tube.
8. Add 900 µL of 1% acetic acid in 50:50 methanol/water to the tube.
9. Mix the solution thoroughly.
10. Label the tube **Reserpine Tuning Solution (100 pg/µL)**.

❖ **To prepare the reserpine sample solution**

1. Transfer 100  $\mu\text{L}$  of the 100  $\text{pg}/\mu\text{L}$  reserpine tuning solution into a clean, minimum 1.5 mL polypropylene tube.
2. Add 900  $\mu\text{L}$  of 1% acetic acid in 50:50 methanol/water to the tube.
3. Mix the solution (10  $\text{pg}/\mu\text{L}$ ) thoroughly.
4. Transfer 100  $\mu\text{L}$  of the 10  $\text{pg}/\mu\text{L}$  solution into a clean, minimum 1.5 mL polypropylene tube.
5. Add 900  $\mu\text{L}$  of 1% acetic acid in 50:50 methanol/water to the tube.
6. Mix the solution (1  $\text{pg}/\mu\text{L}$ ) thoroughly.
7. Transfer 100  $\mu\text{L}$  of the 1  $\text{pg}/\mu\text{L}$  solution into a clean, minimum 1.5 mL polypropylene tube.
8. Add 700  $\mu\text{L}$  of 1% acetic acid in 50:50 methanol/water to the tube.
9. Mix the solution thoroughly.
10. Label the tube **Reserpine Sample Solution (125  $\text{fg}/\mu\text{L}$ )**.

## Preparing the High Mass Range Calibration Solution

The high mass range calibrant is a solution of 3.5  $\text{mg}/\mu\text{L}$  polypropylene glycol (PPG) in a solvent of 70:30 methanol/23 mM sodium acetate.

The high mass range calibration procedure is designed to work with a PPG that has an average molecular weight of approximately 2700 ( $M_n \sim 2700$ ), which is Aldrich product number 202347. PPG 2700 is a viscous liquid. To order this compound from Sigma-Aldrich, write or call:

Sigma Chemical Company  
P. O. Box 14508  
St. Louis, Missouri U.S. 63178-9916  
(800) 325-3010 (U.S.)  
(905) 829-9500 (Canada)  
(314) 771-3750 (outside the U.S. or Canada)  
[www.sigmaldrich.com](http://www.sigmaldrich.com)

## A Sample Formulations for the LXQ and LTQ XL Mass Spectrometers

Preparing the High Mass Range Calibration Solution

### ❖ To prepare the sodium acetate stock solution

Dissolve 0.082 gm of sodium acetate in 10 mL of water in a clean 20 mL glass vial and label the container **Sodium Acetate Stock Solution**.

### ❖ To prepare the PPG stock solution

1. Dissolve 0.7 gm of PPG 2700 in 7 mL of methanol in a clean 20 mL glass vial.

**Tip** Because PPG 2700 is a viscous liquid, use a glass pipette to transfer 0.7 gm of the liquid into a weigh boat, or weigh the liquid directly into a minimum 20 mL glass vial.

2. Add 2.3 mL of water to the vial.
3. Add 0.7 mL of the sodium acetate stock solution and label the container **PPG 2700 Stock Solution (70 mg/mL)**.

### ❖ To prepare the calibration solution

1. Pipette 7 mL of methanol into a clean 20 mL glass vial.
2. Add 2.3 mL of water to the vial.
3. Add 700  $\mu$ L of the sodium acetate stock solution to the vial.
4. Add 10  $\mu$ L of the PPG 2700 stock solution and label the container **LXQ/LTQ XL PPG 2700 Calibration Solution (70 ng/ $\mu$ L)**.



# Sample Formulations for the Velos Pro Mass Spectrometer

This appendix describes how to prepare the tuning and calibration solutions for the Velos Pro mass spectrometer.



**CAUTION** NEVER use the solutions described in this appendix to calibrate the LXQ and LTQ XL mass spectrometers. Instead, use the solutions described in [Appendix A, “Sample Formulations for the LXQ and LTQ XL Mass Spectrometers.”](#)

## Contents

- [Preparing the Normal Mass Range Calibration Solution for ESI Mode](#)
- [Preparing the Reserpine Tuning and Sample Solutions for ESI and APCI Modes](#)
- [Preparing the High Mass Range Calibration Solution](#)

The Chemical Accessory Kit provides the caffeine, MRFA, Ultramark 1621, and reserpine needed to make the solutions. You can order replacement Chemical Accessory Kits (P/N 97455-62045) from Thermo Fisher Scientific.

You can also order specific chemicals from Thermo Fisher Scientific, which are sold under its Fisher Chemical brand. As specified in [Table 9](#), use only LCMS-grade chemicals for calibrating the mass spectrometers.

**Table 9.** Recommended chemicals

Solvent/reagent	Specifications
Acetic acid (modifier)	LCMS-grade
Acetonitrile	LCMS-grade
Formic acid (modifier)	99–100% (Must be supplied in a glass bottle.)
Isopropyl alcohol	LCMS-grade
Methanol	LCMS-grade
Water	LCMS-grade

## B Sample Formulations for the Velos Pro Mass Spectrometer

Preparing the Normal Mass Range Calibration Solution for ESI Mode

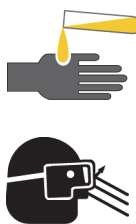
For a complete selection of LCMS-grade consumables from Thermo Fisher Scientific, visit [www.FisherLCMS.com](http://www.FisherLCMS.com).

**Note** Do not filter solvents. Filtering solvents can introduce contamination.

Potentially hazardous chemicals used in the procedures throughout this appendix include the following:

- Acetonitrile
- Formic acid
- Glacial acetic acid
- Methanol
- Reserpine

**IMPORTANT** Do not use plastic pipettes to prepare the tuning and calibration standards. Plastic products can release phthalates that can interfere with the analyses.



### CAUTION AVOID EXPOSURE TO POTENTIALLY HARMFUL MATERIALS

By law, producers and suppliers of chemical compounds are required to provide their customers with the most current health and safety information in the form of Material Safety Data Sheets (MSDSs). The MSDSs describe the chemicals and must be freely available to lab personnel to examine at any time. MSDSs provide summarized information on the hazard 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.

Read the MSDS for each chemical you use. Store and handle all chemicals in accordance with standard safety procedures. Always wear protective gloves and safety glasses when you use solvents or corrosives. Also, contain waste streams, use proper ventilation, and dispose of all laboratory reagents according to the directions in the MSDS.

## Preparing the Normal Mass Range Calibration Solution for ESI Mode

For tuning and calibrating the Velos Pro in ESI mode, use a calibration solution of caffeine, MRFA, Ultramark 1621, and N-butylamine in an acetonitrile/methanol/water solution containing 1% acetic acid. To prepare the ESI calibration solution, follow these procedures:

- “Caffeine Stock Solution” on page 150
- “Preparing the MRFA Stock Solution” on page 150
- “Preparing the Ultramark 1621 Stock Solution” on page 151
- “Preparing the N-butylamine Stock Solution” on page 151
- “Preparing the Normal Mass Range Calibration Solution for ESI Mode” on page 151

**Note** The Chemical Accessory Kit contains vials of caffeine and MRFA. The following compound part numbers are available from Sigma Chemical Co.:

- P/N C6035 is for caffeine at a concentration of 1 mg/mL in methanol.
- P/N M1170 is for MRFA.

To order these compounds from Sigma, write or call:

Sigma Chemical Company

P. O. Box 14508

St. Louis, Missouri U.S. 63178-9916

(800) 325-3010 (U.S.)

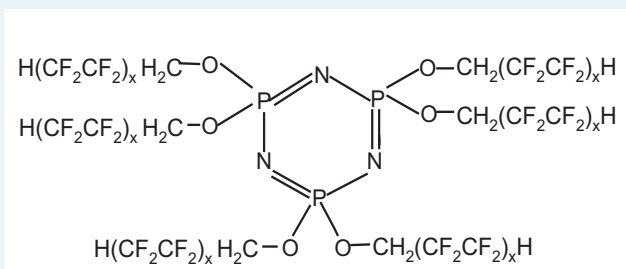
(905) 829-9500 (Canada)

(314) 771-3750 (outside the U.S. or Canada)

[www.sigmaaldrich.com](http://www.sigmaaldrich.com)

**Note** The Chemical Accessory Kit contains a vial of Ultramark 1621 (neat liquid). This compound is available from Lancaster Synthesis.

The structure of Ultramark 1621 follows ( $x$  is 1, 2, or 3):



The Lancaster product number for Ultramark 1621 is L16698 (Ultramark 1621, Mass Spec Std.). To order this compound from Lancaster Synthesis, write or call:

Lancaster Synthesis, Inc.

P.O. Box 1000

Windham, NH U.S. 03087-9977

(603) 889-3306, (800) 238-2324 (in the U.S. and Canada)

+44 (0)1524 36101 (U.K. and International)

[www.lancastersynthesis.com](http://www.lancastersynthesis.com)

## B Sample Formulations for the Velos Pro Mass Spectrometer

Preparing the Normal Mass Range Calibration Solution for ESI Mode



**CAUTION** Always wear protective gloves and safety glasses when you use solvents or corrosives.

### Caffeine Stock Solution

The Velos Pro mass spectrometer ships with a 1 mg/mL stock solution of caffeine in 100% methanol. You can also order this solution through Sigma Chemical (P/N C6035).

### Preparing the MRFA Stock Solution

#### ❖ To prepare the MRFA stock solution

1. Weigh out 6 mg of the MRFA compound provided in the accessory kit, and then transfer the sample to a clean, minimum 20 mL glass vial.

The MRFA provided in the accessory kit has an average molecular weight of 523.7 Da.

2. Add 2.0 mL of 50:50 methanol/water to the vial.
3. Mix the solution thoroughly.
4. Label the vial **MRFA Stock Solution 3 mg/mL**.

#### ❖ To prepare the MRFA diluted stock solution

1. Transfer 100  $\mu$ L of the 3 mg/mL MRFA stock solution into a clean, minimum 20 mL glass vial.
2. Add 2.9 mL of 50:50 methanol/water to the vial.
3. Mix the solution thoroughly.
4. Label the vial **Diluted MRFA Stock Solution 0.1 mg/mL**.

## Preparing the Ultramark 1621 Stock Solution

### ❖ To prepare the Ultramark 1621 stock solution

1. Use a syringe to transfer 25  $\mu\text{L}$  of Ultramark 1621 to a clean 25 mL volumetric glass flask.
2. Fill the flask to volume with 100% acetonitrile.
3. Mix the solution thoroughly.
4. Transfer the solution to a vial and label the vial  
**Ultramark 1621 stock solution (1/1000 dilution).**

## Preparing the N-butylamine Stock Solution

### ❖ To prepare the N-butylamine stock solution

1. Use a syringe to transfer 5  $\mu\text{L}$  of N-butylamine to a clean, 25 mL volumetric glass flask.
2. Add 9.995 mL of 50:50 methanol/water to the vial.
3. Mix the solution thoroughly.
4. Transfer the solution to a vial and label the vial  
**N-butylamine (5/10 000 dilution) stock solution.**

## Preparing the Normal Mass Range Calibration Solution for ESI Mode

The following ready-to-use normal mass range calibration solution is available from Thermo Fisher Scientific at [www.thermo.com/pierce](http://www.thermo.com/pierce):

P/N 88323, Pierce LTQ Velos Positive Ion Calibration Solution, 10 mL

**IMPORTANT** Use only glass pipets or stainless steel syringes when measuring glacial acetic acid. Using plastic pipet tips causes contamination of acid stock solutions that can introduce contaminants in the calibration solution.

### ❖ To prepare the normal mass range calibration solution

1. Pipet 20  $\mu\text{L}$  of the caffeine stock solution into a clean 10 mL volumetric glass flask.
2. Pipet 100  $\mu\text{L}$  of the diluted MRFA stock solution (0.1 mg/mL) into the flask.
3. Pipet 100  $\mu\text{L}$  of the Ultramark 1621 stock solution into the flask.
4. Pipet 100  $\mu\text{L}$  of the N-butylamine stock solution into the flask.
5. Pipet 100  $\mu\text{L}$  of LCMS-grade glacial acetic acid into the flask.
6. Pipet 5 mL of LCMS-grade acetonitrile into the flask.
7. Bring the flask to volume with 50:50 methanol/water.

## B Sample Formulations for the Velos Pro Mass Spectrometer

Preparing the Reserpine Tuning and Sample Solutions for ESI and APCI Modes

- Mix the solution thoroughly.
- Transfer the solution to a clean, dry vial.
- Label the vial **Velos Pro ESI Calibration Solution** and store it in a refrigerator at 2–8 °C (36–46 °F) until needed.

## Preparing the Reserpine Tuning and Sample Solutions for ESI and APCI Modes

Ideally, you should prepare the reserpine solutions just before using them. If you must store the solutions, keep them in a light-resistant container in the refrigerator until needed.

### ❖ To prepare the reserpine stock solution

1. Weigh out 10 mg of reserpine, and then transfer the sample into a clean 10 mL volumetric glass flask.

The average molecular weight of reserpine is 608.7 Da.

2. Fill the flask to volume with a solution of 1% acetic acid in methanol.
3. Mix the solution thoroughly.
4. Transfer the solution to a clean, dry, light-resistant vial.
5. Label the vial **Reserpine Stock Solution (1 µg/µL)**.

### ❖ To prepare the reserpine tuning solution

1. Pipet 100 µL of the reserpine stock solution (1 µg/µL) into a clean, minimum 1.5 mL polypropylene microcentrifuge tube.
2. Add 900 µL of 1% acetic acid in 50:50 methanol/water to the tube.
3. Mix the solution (100 ng/µL) thoroughly.
4. Transfer 10 µL of the 100 ng/µL solution into a clean polypropylene tube.
5. Add 990 µL of 1% acetic acid in 50:50 methanol/water to the tube.
6. Mix the solution (1 ng/µL) thoroughly.
7. Transfer 100 µL of the 1 ng/µL solution into a clean, minimum 1.5 mL polypropylene tube.
8. Add 900 µL of 1% acetic acid in 50:50 methanol/water to the tube.
9. Mix the solution thoroughly.
10. Label the tube **Reserpine Tuning Solution (100 pg/µL)**.

❖ **To prepare the reserpine sample solution**

1. Transfer 100  $\mu\text{L}$  of the 100  $\text{pg}/\mu\text{L}$  reserpine tuning solution into a clean, minimum 1.5 mL polypropylene tube.
2. Add 900  $\mu\text{L}$  of 1% acetic acid in 50:50 methanol/water to the tube.
3. Mix the solution (10  $\text{pg}/\mu\text{L}$ ) thoroughly.
4. Transfer 100  $\mu\text{L}$  of the 10  $\text{pg}/\mu\text{L}$  solution into a clean, minimum 1.5 mL polypropylene tube.
5. Add 900  $\mu\text{L}$  of 1% acetic acid in 50:50 methanol/water to the tube.
6. Mix the solution (1  $\text{pg}/\mu\text{L}$ ,) thoroughly.
7. Transfer 50  $\mu\text{L}$  of the 1  $\text{pg}/\mu\text{L}$  solution into a clean, minimum 1.5 mL polypropylene tube.
8. Add 950  $\mu\text{L}$  of 1% acetic acid in 50:50 methanol/water to the tube.
9. Mix the solution thoroughly.
10. Label the tube **Reserpine Sample Solution (50  $\text{fg}/\mu\text{L}$ )**.

## Preparing the High Mass Range Calibration Solution

The high mass range calibrant is a solution of 3.5  $\text{mg}/\mu\text{L}$  polypropylene glycol (PPG) in a solvent of 65:35 methanol/10 mM sodium acetate.

The high mass range calibration procedure is designed to work with a PPG that has an average molecular weight of approximately 2700 ( $M_n$ -2700), which is Aldrich product number 202347. PPG 2700 is a viscous liquid. To order this compound from Sigma-Aldrich, write or call:

Sigma Chemical Company  
P. O. Box 14508  
St. Louis, Missouri U.S. 63178-9916  
(800) 325-3010 (U.S.)  
(905) 829-9500 (Canada)  
(314) 771-3750 (outside the U.S. or Canada)  
[www.sigmaaldrich.com](http://www.sigmaaldrich.com)

## B Sample Formulations for the Velos Pro Mass Spectrometer

Preparing the High Mass Range Calibration Solution

### ❖ To prepare the sodium acetate stock solution

Dissolve 0.082 gm of sodium acetate in 10 mL of water in a clean 20 mL glass vial and label the container **Sodium Acetate Stock Solution**.

### ❖ To prepare the PPG stock solution

1. Dissolve 0.7 gm of PPG 2700 in 7 mL of methanol in a clean 20 mL glass vial.

**Tip** Because PPG 2700 is a viscous liquid, use a glass pipette to transfer 0.7 gm of the liquid into a weigh boat, or weigh the liquid directly into a minimum 20 mL glass vial.

2. Add 2.3 mL of water to the vial.
3. Add 0.7 mL of the sodium acetate stock solution and label the container **PPG 2700 Stock Solution (70 µg/µL)**.

### ❖ To prepare the calibration solution

1. Pipette 6.65 mL of methanol into a clean 20 mL glass vial.
2. Add 2.15 mL of water to the vial.
3. Add 700 µL of the sodium acetate stock solution to the vial.
4. Add 500 µL of the PPG 2700 stock solution and label the container **Velos Pro PPG 2700 Calibration Solution (3.5 µg/µL)**.



## High Mass Range Calibration

This appendix applies to all LTQ Series mass spectrometers, unless otherwise noted.

Before calibrating an LTQ Series mass spectrometer in the high mass range, you must calibrate it in the normal mass range as described in [Chapter 3, “Automatic Tuning and Calibration in ESI Mode.”](#)

### Note

Prepare the appropriate Normal mass range calibration solution as follows:

- For the LXQ and LTQ XL, see [“Preparing the Normal Mass Range Calibration Solution for ESI Mode”](#) on [page 140](#) in Appendix A.
- For the Velos Pro, see [“Preparing the Normal Mass Range Calibration Solution for ESI Mode”](#) on [page 148](#) in Appendix B.

Prepare the appropriate High mass range calibration solution as follows:

- For the LXQ and LTQ XL, see [“Preparing the High Mass Range Calibration Solution”](#) on [page 145](#) in Appendix A.
- For the Velos Pro, see [“Preparing the High Mass Range Calibration Solution”](#) on [page 153](#) in Appendix B.

Follow these procedures:

1. [“Verifying Coarse Calibration for the High Mass Range by Using the Normal Mass Range Calibration Solution”](#) on [page 156](#)
2. [“Calibrating the High Mass Range by Using the Normal Mass Range Calibration Solution”](#) on [page 162](#)
3. [“Performing Two-Point Manual Coarse Calibration—High Mass Range Mode by Using Normal Mass Range Calibration Solution”](#) on [page 164](#)
4. [“Calibrating the High Mass Range by Using the High Mass Range Calibration Solution”](#) on [page 168](#)
5. [“Cleaning the System After Calibration”](#) on [page 171](#)

## Verifying Coarse Calibration for the High Mass Range by Using the Normal Mass Range Calibration Solution

To verify that the high mass range is coarsely calibrated, use the normal mass range calibration solution to view the peaks in the normal and high mass ranges. When you are sure that the system performs properly in the normal and high mass ranges with the normal mass range calibration solution, proceed to “[Calibrating the High Mass Range by Using the Normal Mass Range Calibration Solution](#)” on page 162.

To verify coarse calibration, follow these procedures:

1. [Checking the Mass Calibration in the Normal Mass Range](#)
2. [Checking the Mass Calibration in the High Mass Range](#) on page 160

### Checking the Mass Calibration in the Normal Mass Range

View the peaks in the spectrum in the normal mass range to determine if the system is calibrated correctly in this mass range.

#### ❖ To check the mass calibration in the normal mass range

1. Load the syringe with the normal mass range calibration solution that is appropriate for your mass spectrometer and turn on the syringe pump.
2. Open the Tune Plus window (see [page 32](#)).



3. Click the **On/Standby** button to select the **On** mode.

The mass spectrometer begins scanning, nitrogen flows into the ESI probe, and high voltage is applied to the ESI nozzle.



4. Click the **Display Spectrum View** button.

A real-time mass spectrum view appears in the Spectrum view of the Tune window.



- Click the **Define Scan** button to open the Define Scan dialog box (Figure 89).

**Figure 89.** Define Scan dialog box

Define Scan

Scan History: ITMS + p cv=0.0 Full ms [150.00-2000.00]

Scan Description

Mass Range: Normal

Scan Rate: Normal

Scan Type: Full

Scan Time

Microscans: 1

Max. Inject Time (ms): 10.000

Source Fragmentation

On Energy (V): 35.0

MSn Settings

n	Parent Mass (m/z)	Act. Type	Iso. Width (m/z)	Normalized Collision Energy	Act. Q	Act. Time (ms)
2		CID	1.0	0.0	0.250	30.000

Scan Ranges

#	First Mass (m/z)	Last Mass (m/z)
1	150.00	2000.00

Supplemental Activation

SA Energy: 15.0

SA Charge State: 2

Wideband Activation

Input: From/To

Apply OK Cancel Help Injection RE... Activation...

- Under Scan Description, do the following:
  - In the Mass Range list, select **Normal**.
  - In the Scan Rate list, select **Normal**.
  - In the Scan Type list, select **Full**.
- Under Scan Time, do the following:
  - In the Microscans box, enter **1**.
  - In the Max. Inject Time (ms) box, enter **10.000**.
- Under Source Fragmentation, clear the **On** check box.

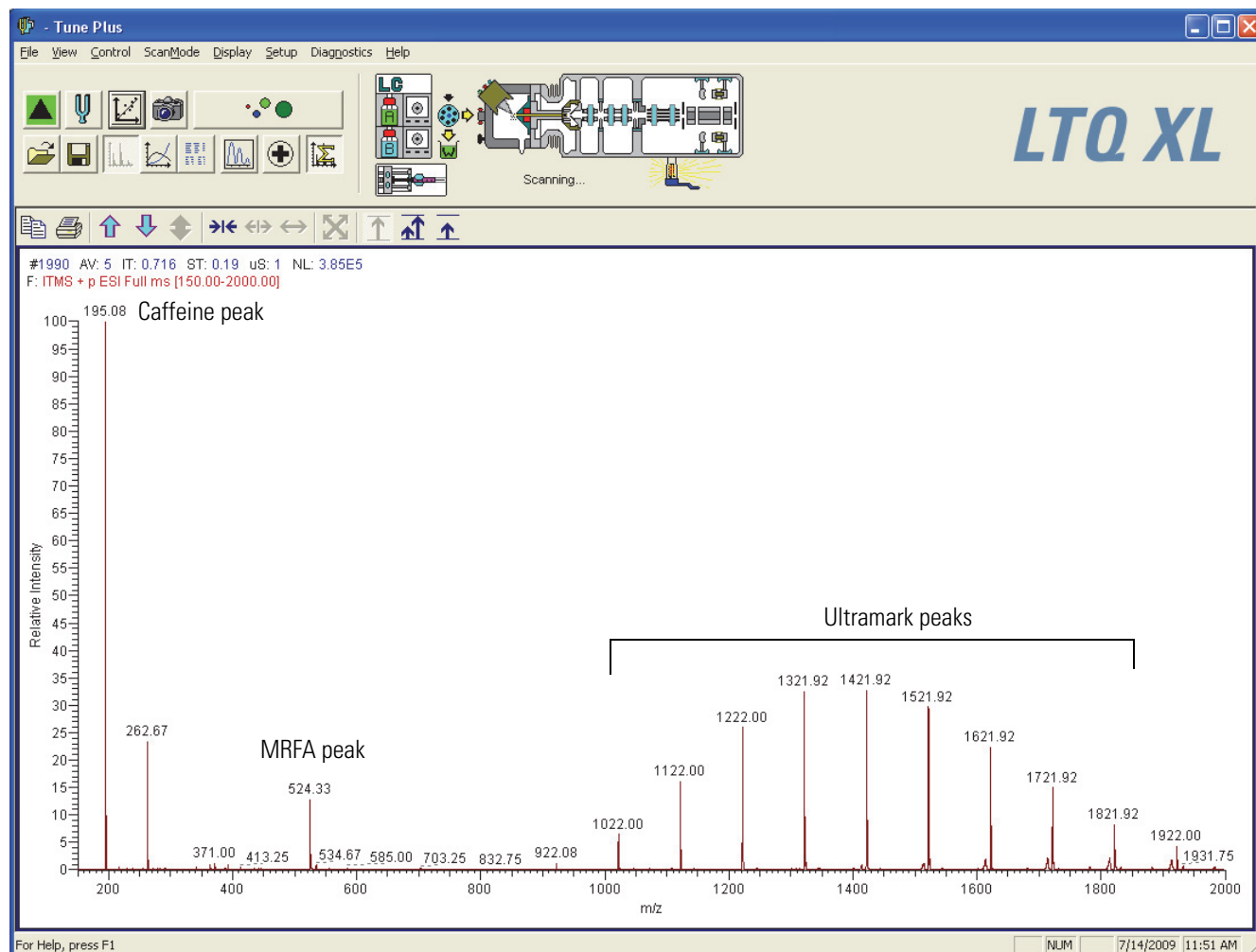
**Note** Selecting the Source Fragmentation check box allows collision-induced fragmentation to occur in the ion source. Because collision-induced fragmentation performed in the ion source is not very specific, this feature is rarely used.

- Under Scan Ranges, do the following:
  - In the Input list, select **From/To**.
  - In the First Mass ( $m/z$ ) column, type **150.00**.
  - In the Last Mass ( $m/z$ ) column, type **2000.00**.
- Click **Apply**.

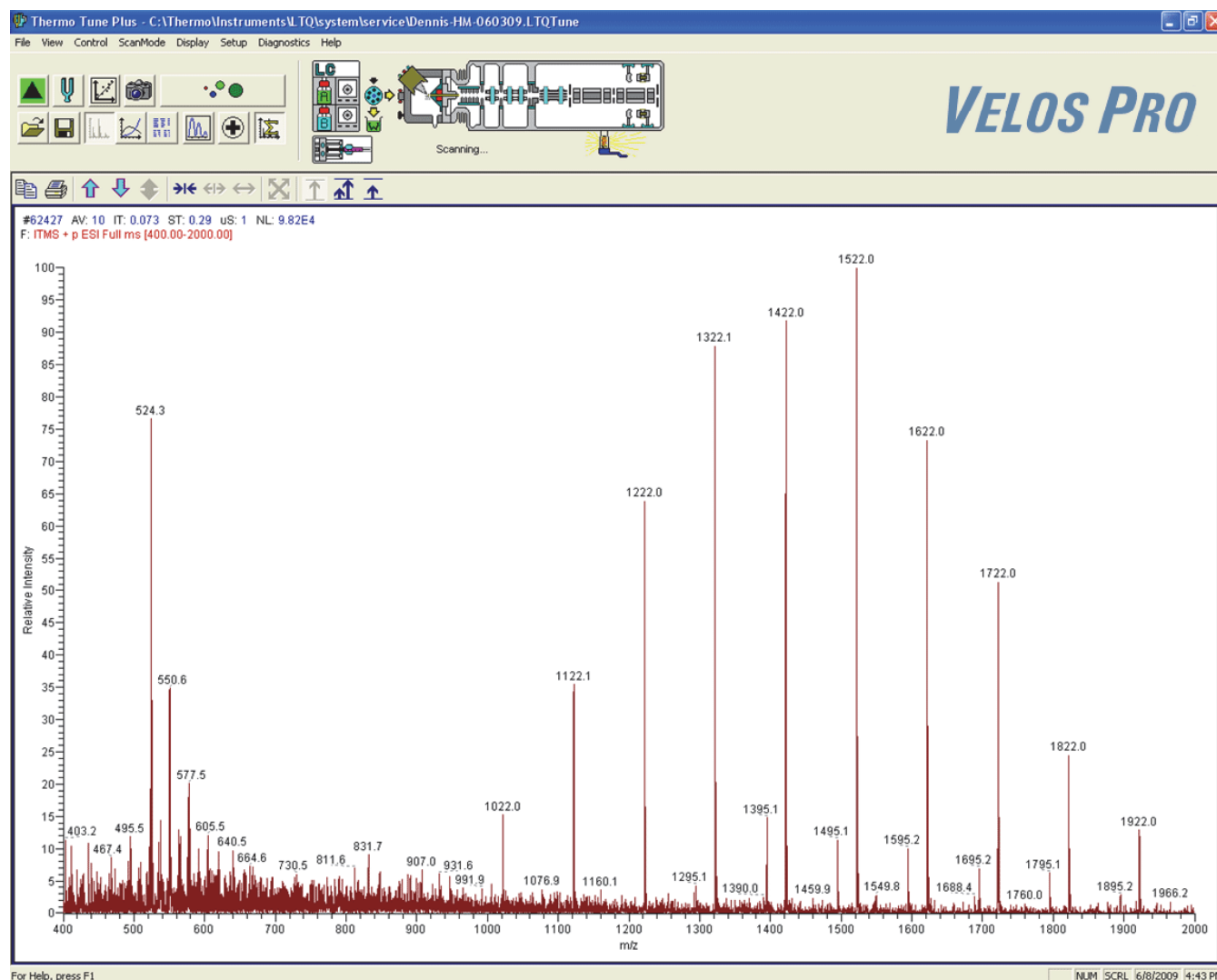
11. Observe the mass spectra of the singly-charged ions in the calibration solution (Figure 90 for the LXQ and LTQ XL, or Figure 91 for the Velos Pro). The integer  $m/z$  values of the calibration ions are as follows:

- Caffeine:  $m/z$  195 (only for the LXQ and LTQ XL)
- MRFA:  $m/z$  524
- Ultramark 1621:  $m/z$  1022, 1122, 1222, 1322, 1422, 1522, 1622, 1722, and 1822

Figure 90. Calibration solution in the normal mass range spectrum (LTQ XL, coarse calibration)



**Figure 91.** Calibration solution in the normal mass range spectrum (Velos Pro, coarse calibration)



12. Do one of the following:

- If the  $m/z$  of the observed ions are  $\pm 1$  Da for the LXQ and LTQ XL or  $\pm 3$  Da for the Velos Pro of the integer mass-to-charge ratios stated in [step 11](#) (see the example in [Figure 90](#)), proceed to section “[Checking the Mass Calibration in the High Mass Range.](#)”
- If the ions are not  $\pm 1$  Da for the LXQ and LTQ XL or  $\pm 3$  Da for the Velos Pro of the integer mass-to-charge ratios stated in [step 11](#), perform the normal mass range calibration before proceeding (see “[Calibrating Automatically in the Normal Mass Range](#)” on [page 79](#)).

## Checking the Mass Calibration in the High Mass Range

The next step in the process is to view the peaks of the normal mass range calibration solution when the scan is set to the high mass range.

### ❖ To check the mass calibration in the high mass range



1. In the Tune Plus window, click the **Define Scan** button to open the Define Scan dialog box.
2. Under Scan Description, do the following:
  - In the Mass Range list, select **High**.
  - In the Scan Rate list, select **Normal**.
  - In the Scan Type list, select **Full**.
3. Under Scan Time, do the following:
  - In the Microscans box, enter **1**.
  - In the Max. Inject Time (ms) box, enter **10.000**.
4. Under Source Fragmentation, clear the **On** check box.

**Note** Selecting the Source Fragmentation check box allows collision-induced fragmentation to occur in the ion source. Because collision-induced fragmentation performed in the ion source is not very specific, this feature is rarely used.

5. Under Scan Ranges, do the following:
  - a. In the Input list, select **From/To**.
  - b. In the First Mass ( $m/z$ ) column, do one of the following:
    - For the LXQ and LTQ XL, type **150.00**.
    - For the Velos Pro, type **400.00**.
  - c. In the Last Mass ( $m/z$ ) column, type **2000.00**.
6. Click **Apply**.
7. Observe the mass spectra of the singly-charged ions in the calibration solution (Figure 90 on page 158 for the LXQ and LTQ XL, or Figure 91 on page 159 for the Velos Pro). The ions are as follows:
  - Caffeine:  $m/z$  195 (only for the LXQ and LTQ XL)
  - MRFA:  $m/z$  524
  - Ultramark 1621:  $m/z$  1022, 1122, 1222, 1322, 1422, 1522, 1622, 1722, and 1822

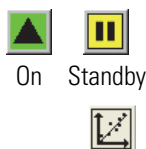
8. Do one of the following:

- If the high mass range spectrum shows that the observed ions are no more than  $\pm 10$  Da for the LXQ and LTQ XL or  $\pm 5$  Da for the Velos Pro at  $m/z$  1822, calibrate in the high mass range with the normal mass range calibration solution (see “[Calibrating the High Mass Range by Using the Normal Mass Range Calibration Solution](#)” on page 162).
- If the ions are more than  $\pm 10$  Da for the LXQ and LTQ XL or  $\pm 5$  Da for the Velos Pro at  $m/z$  1822, note the observed low mass (195) and observed high mass (1822), and then perform the two-point calibration (see “[Performing Two-Point Manual Coarse Calibration—High Mass Range Mode by Using Normal Mass Range Calibration Solution](#)” on page 164).

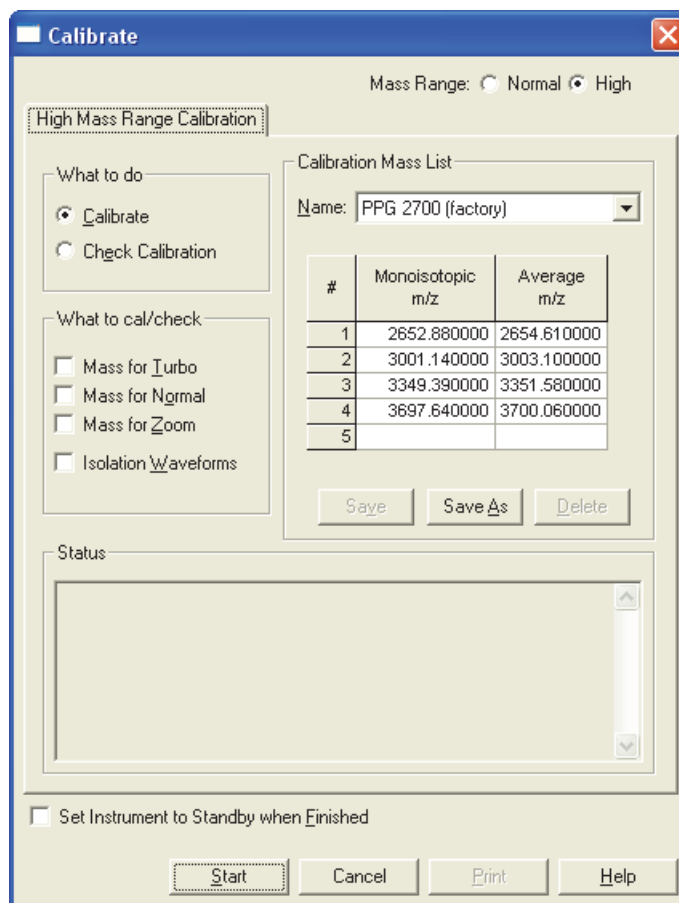
## Calibrating the High Mass Range by Using the Normal Mass Range Calibration Solution

❖ **To calibrate in the high mass range by using the normal mass calibration solution**

1. Load the syringe pump with the normal mass range calibration solution that is appropriate for your mass spectrometer and turn on the syringe pump.
2. Click the **On/Standby** button to select the **On** mode.
3. In the Tune Plus window, click the **Calibrate** button to open the Calibrate dialog box.
4. For Mass Range, select the **High** option (Figure 92).



**Figure 92.** Calibrate dialog box showing the High Mass Range Calibration page

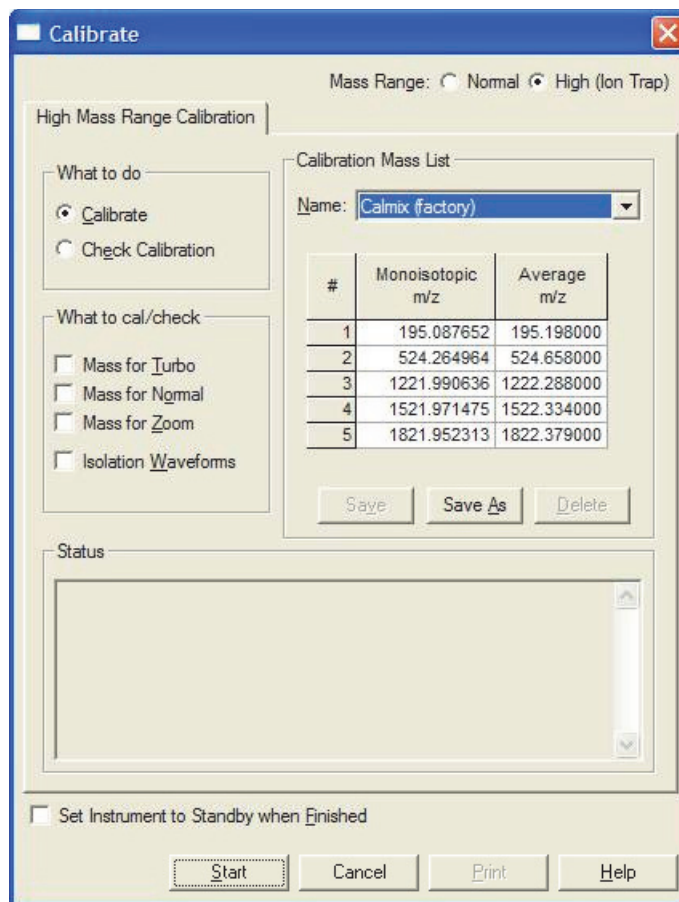


5. Under What To Do, select the **Calibrate** option.
6. Under What to Cal/Check, select all four check boxes: **Mass for Turbo**, **Mass for Normal**, **Mass for Zoom**, and **Isolation Waveforms**.



7. (For the LXQ and LTQ XL only) Under Calibration Mass List, in the Name list, select **Calmix (factory)** (Figure 93).

**Figure 93.** Calibrate dialog box showing the Calmix calibration mix (LXQ and LTQ XL only)



8. Specify the instrument state after completing the calibration:
- To switch to Standby mode after the calibration, select the **Set Instrument to Standby when Finished** check box.
  - To remain in the On mode after the calibration, clear the **Set Instrument to Standby when Finished** check box.
9. Click **Start**.

A message appears:

Please ensure that the 500 microliter syringe is full.

10. Ensure that the syringe contains at least 450  $\mu\text{L}$  of the normal mass range calibration solution and that the syringe pump is turned on.
11. Click **OK**.

12. Observe the Tune Plus window and the Calibrate dialog box.

While the automatic calibration is in progress, test results appear in the Spectrum and Graph views and messages appear in the Status box in the Calibrate dialog box. The system automatically uses the average mass values for the Turbo Scan calibration. It automatically uses monoisotopic masses for the normal, Zoom Scan, and isolation waveform calibrations. The results appear when the calibration process finishes.

13. Check the calibration results.

14. Do one of the following:

- If the calibration passes and the masses are correct, proceed to “[Calibrating the High Mass Range by Using the High Mass Range Calibration Solution](#)” on page 168.
- If the calibration does not pass or if the masses are off by more than  $\pm 10$  Da, note the observed low mass (195) and observed high mass (1822) before “[Performing Two-Point Manual Coarse Calibration—High Mass Range Mode by Using Normal Mass Range Calibration Solution.](#)”

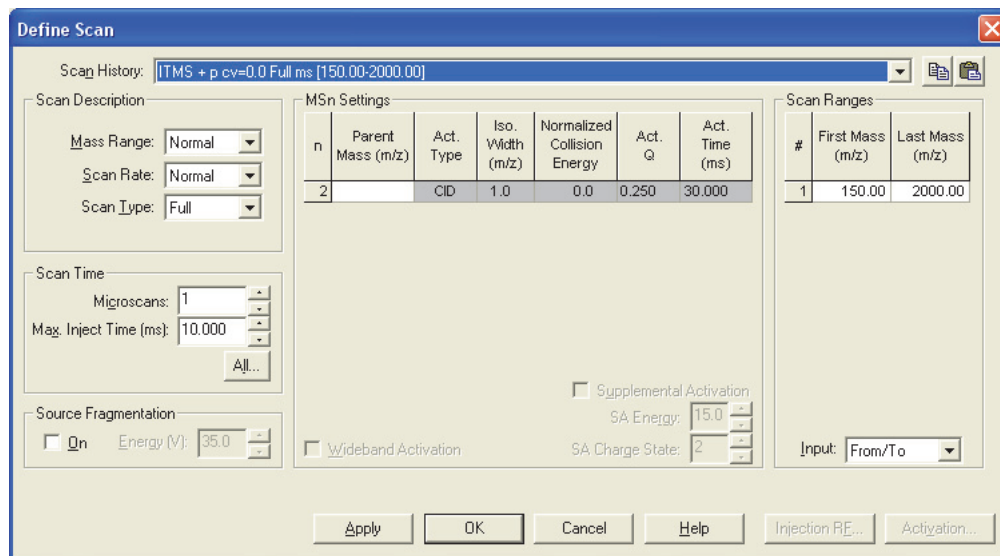
## Performing Two-Point Manual Coarse Calibration—High Mass Range Mode by Using Normal Mass Range Calibration Solution

- ❖ **To perform a two-point calibration in the high mass range mode by using the normal mass range calibration solution**



1. In the Tune Plus window, click the **Define Scan** button to open the Define Scan dialog box (Figure 94).

**Figure 94.** Define Scan dialog box (default ESI settings)



2. Under Scan Description, do the following:
  - In the Mass Range list, select **High**.
  - In the Scan Rate list, select **Normal**.
  - In the Scan Type list, select **Full**.
3. Under Scan Time, do the following:
  - In the Microscans box, enter 1.
  - In the Max. Inject Time (ms) box, enter **10.000**.
4. Under Source Fragmentation, clear the **On** check box.

**Note** Selecting the Source Fragmentation check box allows collision-induced fragmentation to occur in the ion source. Because collision-induced fragmentation performed in the ion source is not very specific, this feature is rarely used.

5. Under Scan Ranges, do the following:
  - a. In the Input list, select **From/To**.
  - b. In the First Mass ( $m/z$ ) column, do one of the following:
    - For the LXQ and LTQ XL, type **150.00**.
    - For the Velos Pro, type **400.00**.
  - c. In the Last Mass ( $m/z$ ) column, type **2000.00**.
6. Click **Apply**.
7. Observe the mass spectra of the singly-charged ions in the calibration solution.

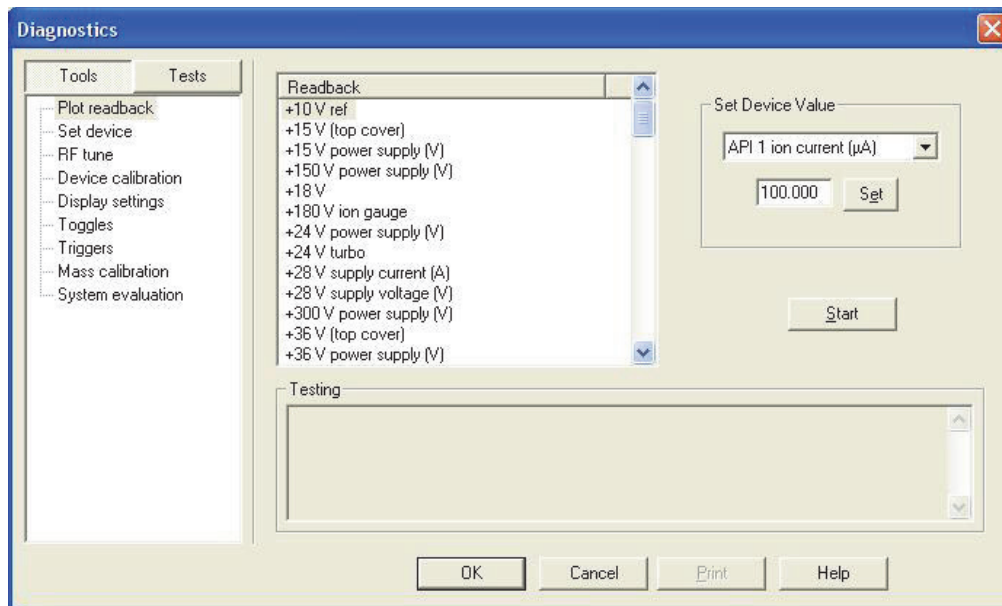
The  $m/z$  of the expected ions are as follows:

- Caffeine:  $m/z$  195 (only for the LXQ and LTQ XL)
- MRFA:  $m/z$  524
- Ultramark 1621:  $m/z$  1022, 1122, 1222, 1322, 1422, 1522, 1622, 1722, and 1822

See [Figure 90](#) for the LXQ and LTQ XL, or [Figure 91](#) for the Velos Pro.

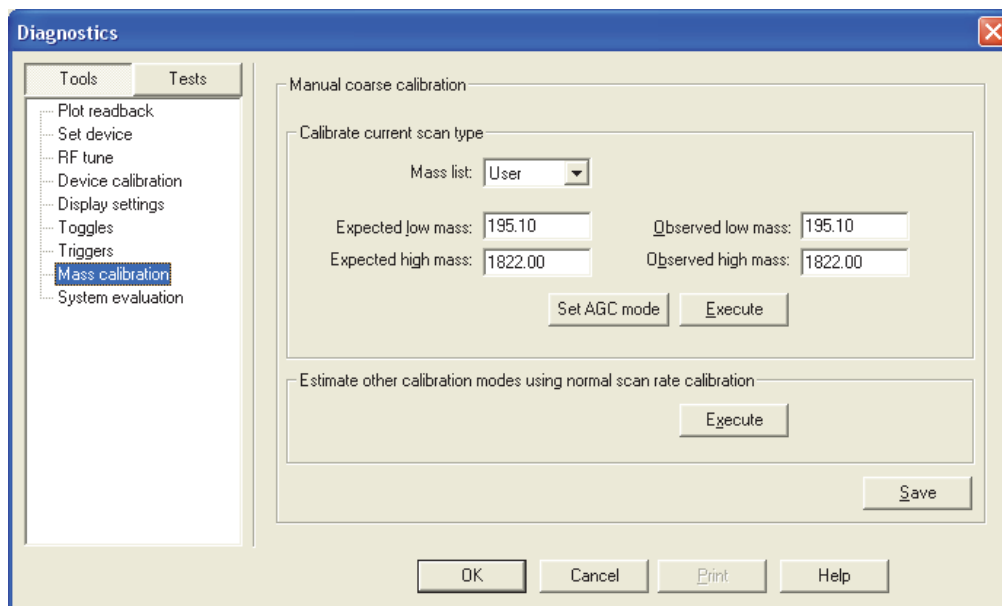
- In the Tune Plus window, choose **Diagnostics > Diagnostics** to open the Diagnostics dialog box (Figure 95).

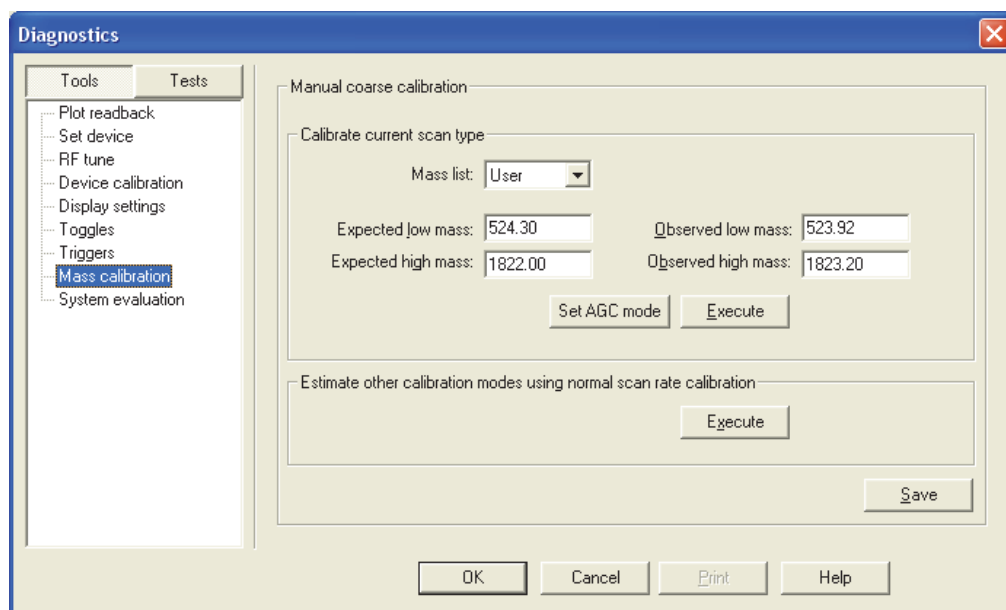
**Figure 95.** Diagnostics dialog box



- In the Tools list, click **Mass Calibration** to open the Manual Coarse Calibration page (Figure 96).

**Figure 96.** Diagnostics dialog box showing the mass calibration page (LXQ and LTQ XL)



**Figure 97.** Diagnostics dialog box showing the mass calibration page (Velos Pro)

10. Under Calibrate Current Scan Type, do the following:
  - In the Expected Low Mass box, do one of the following:
    - For LXQ and LTQ XL, type **195.10**.
    - For Velos Pro, type **524.3**.
  - In the Expected High Mass box, type **1822.00**.
  - In the Observed Low Mass box, do one of the following:
    - For LXQ and LTQ XL, type the mass closest to caffeine (**195.10**).
    - For Velos Pro, type the observed mass closest to MRFA (**524.3**).
  - In the Observed High Mass box, type whatever mass was the closest to 1822, observed in “[Checking the Mass Calibration in the High Mass Range](#)” on page 160.
11. Under Calibrate Current Scan Type, click **Execute**.
12. Click **OK**.
13. Again, observe the masses in the spectrum, which should be very close to the ones shown in [Figure 90](#) on page 158.

14. Do one of the following:

- If any of the midrange Ultramark peaks (1122, 1222, 1322, 1422, 1522, or 1622) are more than  $\pm 10$  Da off, pick a different peak for 1822. If the Ultramark peaks are less than 100 Da apart, pick the next lower mass peak ( $\sim 1725$ ), or if they are greater than 100 Da apart, pick the next higher peak ( $\sim 1925$ ). Enter this peak as the observed mass and repeat [step 11](#) through [step 13](#) before proceeding.
- If the masses are now  $\pm 3$  Da of those specified above, click **Execute** under Estimate Other Calibration Modes Using Normal Scan Rate Calibration, and then click **Save**.

**IMPORTANT** Ensure that the mass range in the Define Scan dialog box is set to High so that the normal mass range calibration values are not affected.

15. Do one of the following:

- If the calibration passed, proceed to the next procedure, “[Calibrating the High Mass Range by Using the High Mass Range Calibration Solution](#).”
- If the calibration failed, repeat this entire procedure.

If the calibrations become severely off, or if error messages are reported in the process of doing this procedure, you can restore the factory defaults to return to the valid calibration default values.

## Calibrating the High Mass Range by Using the High Mass Range Calibration Solution

The final step is to calibrate in the high mass range with the high mass range calibration solution (PPG 2700). Follow these procedures:

1. [To prepare the mass spectrometer](#)
2. [To calibrate in the high mass range by using the high mass range calibration solution](#)

**IMPORTANT** To minimize the possibility of cross-contamination, use a clean syringe and a new length of PEEK tubing for the high mass range calibration solution.

### ❖ To prepare the mass spectrometer

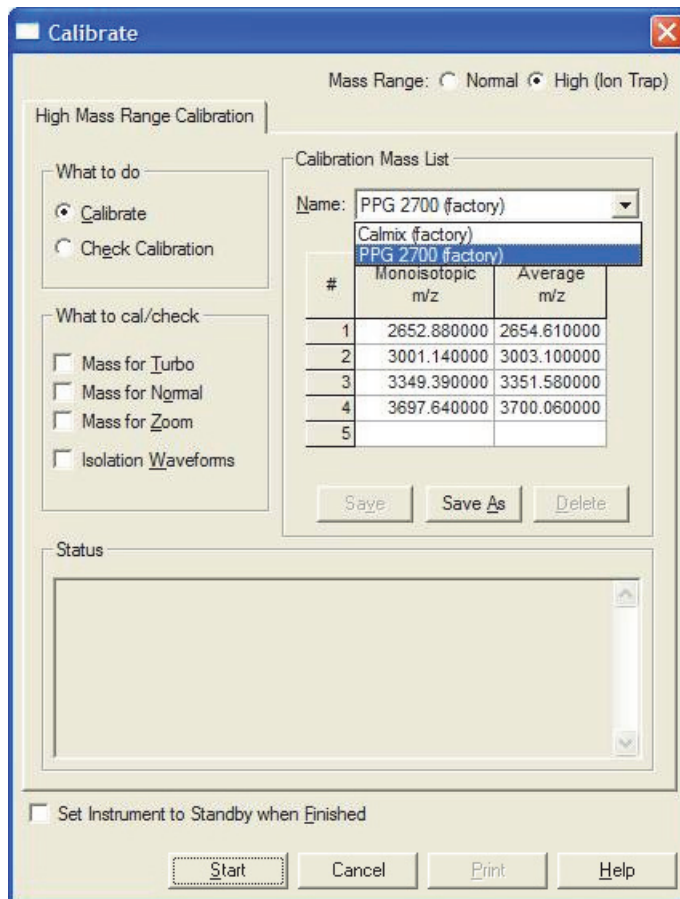
1. Install new red PEEK tubing.
2. Load a clean, 500  $\mu\text{L}$  Unimetrics syringe with 450  $\mu\text{L}$  of the PPG 2700 calibration solution.
3. Turn on the mass spectrometer.
4. Turn the syringe pump on at 5  $\mu\text{L}/\text{min}$  and purge the system until high mass peaks appear.

❖ **To calibrate in the high mass range by using the high mass range calibration solution**

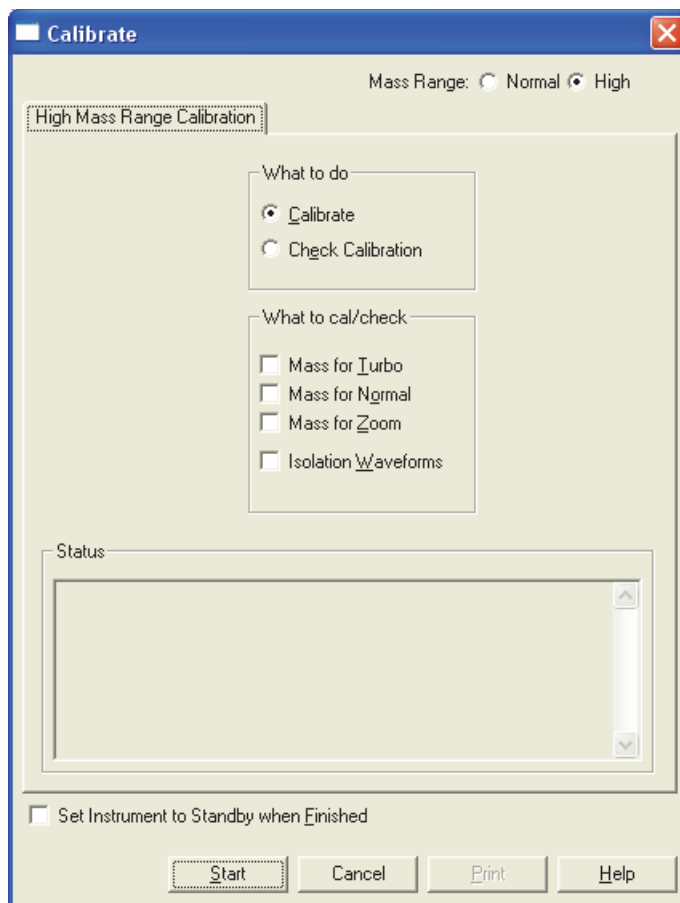


1. In the Tune Plus window, click the **Calibrate** button to open the Calibrate dialog box.
2. For the Mass Range, select the **High** option.

**Figure 98.** Calibrate dialog box showing the high mass range calibration page with PPG 2700 (factory) (LXQ and LTQ XL)



**Figure 99.** Calibrate dialog box showing the high mass range calibration page (Velos Pro)



3. Under What To Do, select the **Calibrate** option.
4. Under What to Cal/Check, select all four check boxes: **Mass for Turbo**, **Mass for Normal**, **Mass for Zoom**, and **Isolation Waveforms**.
5. (For the LXQ and LTQ XL only) Under Calibration Mass List, in the Name list, select **PPG 2700 (factory)** (Figure 98).
6. Specify the instrument state after completing the calibration:
  - To switch to Standby mode after the calibration, select the **Set Instrument to Standby when Finished** check box.
  - To remain in the On mode after the calibration, clear the **Set Instrument to Standby when Finished** check box.
7. Click **Start**.

A message appears:

Please ensure that the 500 microliter syringe is full.



8. Ensure that the syringe contains at least 450  $\mu\text{L}$  of the high mass range calibration solution, that the syringe pump is on, and that it is set to 5  $\mu\text{L}/\text{min}$ .
9. Click **OK**.
10. Observe the Tune Plus window and the Calibrate dialog box.

While the automatic calibration is in progress, test results appear in the Spectrum and Graph views and messages appear in the Status box in the Calibrate dialog box. The system automatically uses the average mass values for the Turbo Scan calibration. It automatically uses monoisotopic masses for the normal, Zoom Scan, and isolation waveform calibrations. The results appear when the calibration process finishes.

## Cleaning the System After Calibration

After infusing the high mass calibration solution, you must clean the system. For instructions, see [Chapter 8, “Cleaning the Mass Spectrometer After Tuning and Calibrating.”](#)



# Glossary

A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

## A

**Activation Q** Directly relates to the rf frequency used to fragment ions in an ion trap. If the Advanced Features option is turned off, the default value for Activation Q is 0.25. Activation Q can be set from the Instrument Setup window or the Tune Plus window by using the Advanced Features option.

**activation time** The time in milliseconds that the rf used for fragmentation is applied in an ion trap. The activation time default value is 10 ms for the Velos Pro mass spectrometer and 30 ms for the LXQ and LTQ XL mass spectrometers. In general, shorter activation time results in less fragmentation and a longer activation time results in more fragmentation.

**API ion transfer tube** A tube assembly that assists in desolvating ions that are produced by the ESI, NSI, or APCI probe.

**API ion transfer tube offset voltage** A dc voltage applied to the ion transfer tube. The voltage is positive for positive ions and negative for negative ions.

**API source** The sample interface between the LC and the mass spectrometer. It consists of the API probe (ESI, HESI-II, or APCI) and API stack.

**API tube lens** A lens in the API source that separates ions from neutral particles as they leave the ion transfer tube. A potential applied to the tube lens focuses the ions toward the opening of the skimmer and helps to dissociate adduct ions.

**API tube lens offset voltage** A DC voltage applied to the tube lens. The value is normally tuned for a specific compound.

**API tube-skimmer region** The area between the tube and the skimmer, which is surrounded by the tube lens. It is also the area of first-stage evacuation in the API source.

**atmospheric pressure chemical ionization (APCI)** A soft ionization technique done in an ion source operating at atmospheric pressure. Electrons from a corona discharge initiate the process by ionizing the mobile phase vapor molecules. A reagent gas forms, which efficiently produces positive and negative ions of the analyte through a complex series of chemical reactions.

**atmospheric pressure ionization (API)** Ionization performed at atmospheric pressure by using atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI), or nanospray ionization (NSI).

**atmospheric pressure photoionization (APPI)** A soft ionization technique that shows an ion generated from a molecule when it interacts with a photon from a light source.

**Automatic Gain Control™ (AGC)** Sets the ion injection time to maintain the optimum quantity of ions for each scan. With AGC on, the scan function consists of a prescan and an analytical scan.

## Glossary

**autosampler** The device used to inject samples automatically into the inlet of a chromatograph.

## C

**collision energy** The energy used when ions collide with the collision gas.

**collision-induced dissociation (CID)** A method of fragmentation where molecular ions are accelerated to high-kinetic energy and then allowed to collide with neutral gas molecules such as helium for the LTQ Series mass spectrometer. The collisions break the bonds and fragment the ions into smaller pieces.

**consecutive reaction monitoring (CRM) scan type**

A scan type with three or more stages of mass analysis and where a particular multi-step reaction path is monitored.

**conversion dynode** A highly polished metal surface that converts ions from the mass analyzer into secondary particles, which enter the electron multiplier.

## D

**divert/inject valve** A valve on the mass spectrometer that can be plumbed as a divert valve or as a loop injector.

## E

**electron multiplier** A device used for current amplification through the secondary emission of electrons. Electron multipliers can have a discrete dynode or a continuous dynode.

**electrospray ionization (ESI)** A type of atmospheric pressure ionization that is currently the softest ionization technique available to transform ions in solution into ions in the gas phase.

## F

**full-scan type** Provides a full mass spectrum of each analyte or parent ion. With the full-scan type, the mass analyzer is scanned from the first mass to the last mass without interruption. Also known as singlestage full-scan type.

## H

**heated-electrospray ionization (H-ESI)** Converts ions in solution into ions in the gas phase by using electrospray ionization (ESI) in combination with heated auxiliary gas.

**high performance liquid chromatography (HPLC)**

Liquid chromatography where the liquid is driven through the column at high pressure. Also known as high pressure liquid chromatography.

**higher energy collision-induced dissociation (HCD)** Collision-induced dissociation that occurs in an HCD collision cell. A voltage offset between the mass analyzer and HCD collision cell accelerates parent ions into the collision gas inside the HCD cell, which causes the ions to fragment into product ions. The product ions are then returned to the mass analyzer for mass analysis. HCD produces triple quadrupole-like product ion mass spectra.

## I

**ion detection system** The ion detection system is a high sensitivity, off-axis system for detecting ions. It produces a high signal-to-noise ratio and allows for switching of the voltage polarity between positive ion and negative ion modes of operation. The ion detection system includes a  $\pm 15$  kV conversion dynode and a channel electron multiplier.

**ion optics** Focuses and transmits ions from the API source to the mass analyzer.

**ion sweep cone** A removable cone-shaped metal cover that fits on top of the API ion transfer tube and acts as a physical barrier to protect the entrance of the tube.

**L**

**LC pump** A high pressure solvent pump in the liquid chromatograph (LC) that provides the pressure on the input side of a column to drive the eluent and sample through the column.

**lens** A metal disk with a circular hole in the center that allows the ion beam to pass.

**M**

**mass analysis** A process that produces a mixture of ionic species that is then separated according to the mass-to-charge ratios ( $m/z$ ) of the ions to produce a mass spectrum.

**mass-to-charge ratio ( $m/z$ )** An abbreviation used to denote the quantity formed by dividing the mass of an ion (in u) by the number of charges carried by the ion. For example, for the ion  $C_7H_7^+$ ,  $m/z = 45.5$ .

**mass analyzer** A device that determines the mass-to-charge ratios of ions by one of a variety of techniques.

**MS scan modes** Scan modes where only one stage of mass analysis is performed. The scan types used with the MS scan modes are full-scan type and selected ion monitoring (SIM) scan type.

**N**

**nanospray ionization (NSI)** A type of electrospray ionization (ESI) that accommodates very low flow rates of sample and solvent on the order of 1 to 20 nL/min (for static nanospray) or 100 to 1000 nL/min (for dynamic nanospray).

**P**

**parent ion** An electrically charged molecular species that can dissociate to form fragments. The fragments can be electrically charged or neutral species. A parent ion can be a molecular ion or an electrically charged fragment of a molecular ion. Also called a precursor ion.

**product ion** An electrically charged product of reaction of a selected parent ion. In general, product ions have a direct relationship to a particular parent ion and can correlate to a unique state of the parent ion.

**product mass** The mass-to-charge ratio of a product ion. The location of the center of a target production peak in mass-to-charge ratio ( $m/z$ ) units.

**pulsed Q collision-induced dissociation (PQD)** A method of fragmentation where precursor ions are activated at high Q, a time delay occurs to allow the precursor to fragment, and then a rapid pulse is applied to low Q where all fragment ions are trapped. The product ions can then be scanned out of the ion trap and detected. PQD fragmentation produces precise, reproducible fragmentation and has been used for iTRAQ peptide quantitation on the LTQ by using both electrospray and MALDI source ionization. PQD eliminates the "1/3 Rule" low mass cut-off for MS/MS data.

**Q**

**qualitative analysis** Chemical analysis designed to determine the identity of the components of a substance.

**quantitative analysis** Chemical analysis designed to determine the quantity or concentration of a specific substance in a sample.

**S**

**sample loop** A loop of calibrated volume that is used to perform flow injection analysis.

**scan** Comprised of one or more microscans. Each microscan is one mass analysis (ion injection and storage/scan-out of ions) followed by ion detection. After the microscans are summed, the scan data is sent to the data system for display and/or storage. The process of ramping the amplitude of the rf and dc voltages on the quadrupole rods in the mass analyzer to transmit ions from the lowest mass to the highest mass of a specified scan range.

## Glossary

**scan power** The power  $n$  in the expression  $MS^n$ . The number of stages of mass analysis, expressed as  $MS^n$ , where  $n$  is the scan power. For example, a scan power of  $n = 1$  corresponds to an  $MS1$  (or  $MS$ ) scan with one stage of mass analysis. A scan power of  $n = 2$  corresponds to an  $MS2$  (or  $MS/MS$ ) scan with two stages of mass analysis. A scan power of  $n = 3$  corresponds to an  $MS3$  scan with three stages of mass analysis, and so on.

**selected ion monitoring (SIM) scan type** A scan type where the mass spectrometer acquires and records ion current at only one or a few selected mass-to-charge ratio values.

**selected reaction monitoring (SRM) scan type** A scan type with two stages of mass analysis and where a particular reaction or set of reactions, such as the fragmentation of an ion or the loss of a neutral moiety, is monitored. In SRM a limited number of product ions is monitored.

**signal-to-noise ratio (S/N)** The ratio of the signal height ( $S$ ) to the noise height ( $N$ ). The signal height is the baseline corrected peak height. The noise height is the peak-to-peak height of the baseline noise.

**syringe pump** A device that delivers a solution from a syringe at a specified rate.

## W

**WideBand Activation** A type of resonance excitation in ion traps with a wide range of excitation frequencies, used during mass analyzer, collision-induced dissociation. Wideband activation causes multiple fragmentations of the parent ion, providing more structural information but less sensitivity.

## Z

**ZoomScan scan type** A scan type that provides information about the charge state of one or more ions of interest. ZoomScans are slower scans with higher resolution than normal scans.

**ZoomScan scan type, multi-stage** A scan type with three or more stages of mass analysis and where a particular multi-step reaction path is monitored to determine the charge-state of one final product ion.

**ZoomScan scan type, single-stage** A ZoomScan scan type with one stage of mass analysis and where ions in one to ten  $10\text{-u}$  scan-range windows are monitored to determine the charge-state of up to ten ions.

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