

LTQ Series

Getting Started Guide

97055-97073 Revision E September 2015



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Release history: Rev A, July 2009; Rev B, Aug 2010; Rev C, May 2011; Rev D, May 2012; Rev E, Sept 2015

Software version: Microsoft Windows 7 Professional (32-bit and 64-bit) SP1—Thermo Foundation 2.0 and later, and Thermo Xcalibur 2.2 and later; Windows XP Workstation SP3—Foundation 1.0.2 SP2 or earlier, and Xcalibur 2.1 SP1 or earlier; Thermo LTQ Tune Plus 2.7.0 and later

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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.

Changes that you make to your system may void compliance with one or more of these EMC and safety standards. Changes to your system include replacing a part or adding components, options, or peripherals not specifically authorized and qualified by Thermo Fisher Scientific. To ensure continued compliance with EMC and safety standards, replacement parts and additional components, options, and peripherals must be ordered from Thermo Fisher Scientific or one of its authorized representatives.

Regulatory compliance results for the following Thermo Scientific mass spectrometers (MSs):

- [Velos Pro MS](#)
- [LTQ XL MS](#)

Note Thermo Fisher Scientific no longer ships the LTQ Velos and LXQ mass spectrometers. Previously installed instruments were tested in accordance with applicable standards at that time (for example, EN 61326-0-1: 2006 (EMC) and EN/UL 61010-1 Second Edition (product safety)).

Velos Pro MS

EMC Directive 2004/108/EC

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

CFR 47, FCC Part 15, Subpart B, Class A: 2015	EN 61000-3-2: 2006 + A1+ A2
CISPR 11: 2009 + A1	EN 61000-3-3: 2013
CISPR 22: 2008	EN 61000-4-2: 2009
ICES-003: 2014	EN 61000-4-3: 2006 + A1+ A2
BSMI CNS 13438: 2006	EN 61000-4-4: 2004 + A1
EN 55011: 2009 + A1	EN 61000-4-5: 2006
EN 55022: 2010	EN 61000-4-6: 2009
EN 61326-1: 2013	EN 61000-4-11: 2004

Low Voltage Safety Compliance

This device complies with Low Voltage Directive 2006/95/EC and harmonized standard IEC/EN/CSA/UL 61010-1, Third Edition.

LTQ XL MS

EMC Directive 2004/108/EC

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

CFR 47, FCC Part 15, Subpart B, Class A: 2013

CISPR 11: 2009 + A1

CISPR 24: 2010

AS/NZS CISPR 22: 2009

ICES-003: 2012

EN 55011: 2009 + A1

EN 55024: 2010

EN 61326-1: 2013

EN 61000-3-2: 2006 + A1+ A2

EN 61000-3-3: 2008

EN 61000-4-2: 2009

EN 61000-4-3: 2006 + A1+ A2

EN 61000-4-4: 2004 + A1

EN 61000-4-5: 2006

EN 61000-4-6: 2009

EN 61000-4-11: 2004

Low Voltage Safety Compliance

This device complies with Low Voltage Directive 2006/95/EC and harmonized standard IEC/EN/CSA/UL 61010-1, Second Edition.

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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WEEE Directive

2012/19/EU



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- Appropriate pick-up time
- Declaration of decontamination, stating that all hazardous fluids or material have been removed from the product

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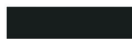
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Directive DEEE
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WEEE Direktive
2012/19/EU



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Preface

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

Contents

- [LTQ Series Models](#)
- [Related Documentation](#)
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❖ **To suggest changes to the documentation or to the Help**

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



LTQ Series Models

The LTQ Series documentation is intended for the following instrument models:

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

Note Unless otherwise noted:

- For the LTQ Velos MS, follow the Velos Pro MS information.
- For the LXQ MS, follow the LTQ XL MS information.

Related Documentation

An LTQ Series mass spectrometer includes complete documentation. In addition to this guide, you can also access the following documents as PDF files from the data system computer:

- *LTQ Series Preinstallation Requirements Guide*
- *LTQ Series Getting Connected Guide*
- *LTQ Series Hardware Manual*
- *Ion Max and Ion Max-S API Source Hardware Manual*
- *ETD Module Getting Started Guide* (with the MS/ETD system)
- *ETD Module Hardware Manual* (with the MS/ETD system)
- *MALDI Source Getting Started Guide* (with the MALDI LTQ XL system)
- *MALDI Source Hardware Manual* (with the MALDI LTQ XL system)
- *Achieving Peak Instrument Performance Reference Manual*

This document is for use with the following stand-alone mass spectrometers and hybrid Orbitrap™ systems: LTQ Velos, Velos Pro, LTQ Orbitrap Velos, Orbitrap Velos Pro™, and Orbitrap Elite™.

- *Safety and Regulatory Guide*

The LTQ Series also ships with a printed copy of the *Safety and Regulatory Guide*. This guide contains important safety information about Thermo Scientific liquid chromatography (LC) and mass spectrometry (MS) systems. Make sure that all lab personnel have read and have access to this document.

❖ To view the product manuals

From the Microsoft™ Windows™ taskbar, choose **Start > All Programs > Thermo Instruments > Manuals > *model***, where *model* is your specific model, and then open the PDF file to view it.

Note For Thermo Xcalibur™ version 2.0.7 or earlier (Microsoft Windows XP operating system), choose **Start > All Programs > Xcalibur > Manuals > LTQ > *model***.

The LTQ Series application also provides Help.

❖ To view the data system Help

- From the application window, choose **Help** from the menu bar.
- If information about setting parameters is available for a specific view, page, or dialog box, click **Help** or press the F1 key for information about setting parameters.

❖ To download user documentation from the Thermo Scientific website

1. Go to www.thermoscientific.com.
2. In the Search box, type the product name and press ENTER.
3. In the left pane, select **Documents & Videos**, and then under Refine By Category, click **Operations and Maintenance**.
4. (Optional) Narrow the search results or modify the display as applicable:
 - For all related user manuals and quick references, click **Operator Manuals**.
 - For installation and preinstallation requirements guides, click **Installation Instructions**.
 - For documents translated into a specific language, use the Refine By Language feature.
 - Use the Sort By options or the Refine Your Search box (above the search results display).
5. Download the document as follows:
 - a. Click the document title or click **Download** to open the file.
 - b. Save the file.

Getting a Trap-HCD License

Ion trap higher energy collision-induced dissociation (Trap-HCD) fragmentation is an optional feature for the Velos Pro MS. 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.

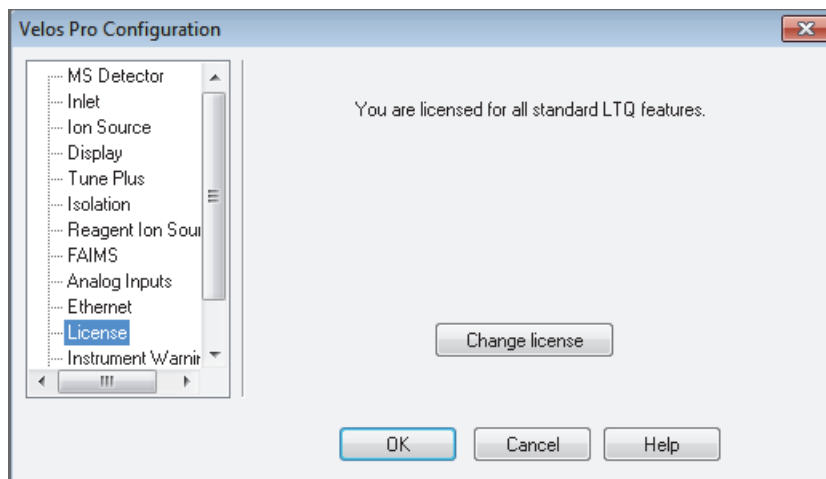
Note The Velos Pro MS includes a 90-day trial version of the Trap-HCD license. If you did not purchase this license, a reminder dialog box will appear notifying you that the trial period is close to expiring.

Getting a New License Code

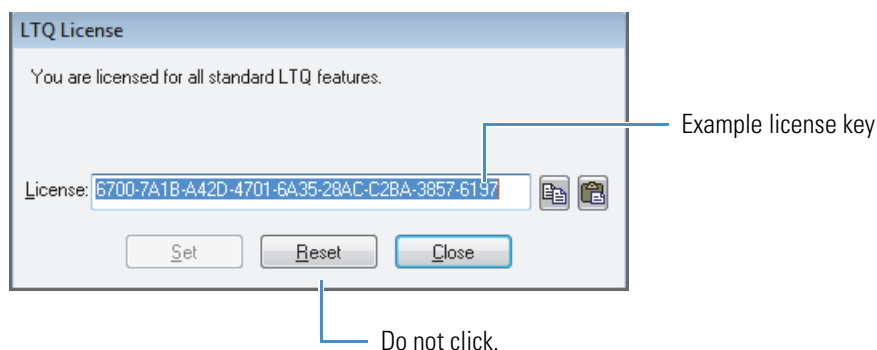
You can request a license code through email or fax (see [page xxiv](#)). These instructions cover email requests that you send to a specific Thermo Fisher Scientific email address.

❖ To get a Trap-HCD license code

1. Choose **Start > All Programs > Thermo Foundation x.x > 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. Click **Close**.

Note Do not click Reset in the LTQ License dialog box. If you do, you must reopen the LTQ License dialog box to copy a new license key for [step 8](#).

8. Send an email message to ThermoMSLicensing@thermofisher.com:

- In the Subject line, type **License Request**.
- In the body of the email 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 email 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.

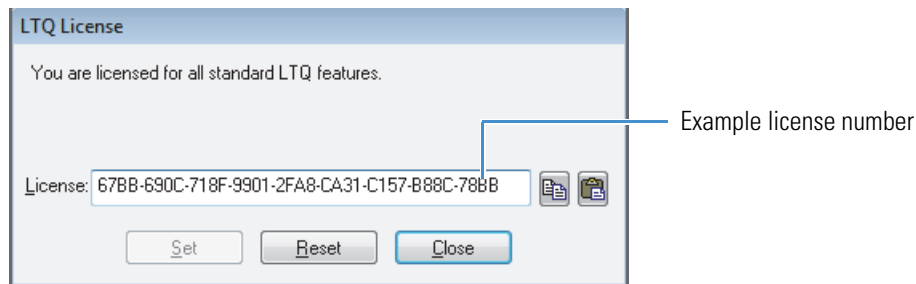
When Thermo Fisher Scientific Customer Support sends you a new license code, see [“Installing a New License Code.”](#)

Installing a New License Code

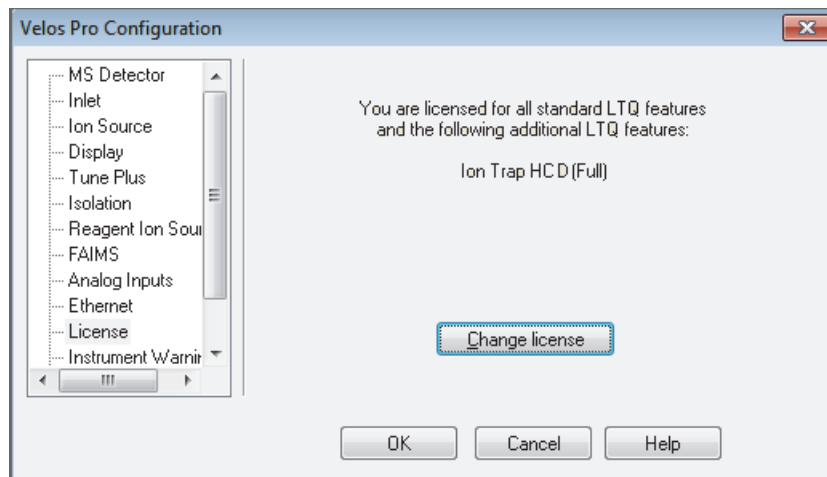
After you receive your new license code, follow this procedure.

❖ To install the HCD license number

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



3. Click **OK** when the following message appears:
The new license number has been set.
4. In the Velos Pro Configuration dialog box, verify the addition of the Ion Trap HCD (Full) feature and click **OK**.



5. Restart the data system and then the Velos Pro MS.

Cautions and Special Notices

Make sure you follow the cautions and special notices presented in this guide. Cautions and special notices appear in boxes; those concerning safety or possible system damage also have corresponding caution symbols.

This guide uses the following types of cautions and special notices.



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.

The *LTQ Series Getting Started Guide* contains the following caution-specific symbols (Table 1).

Table 1. Caution-specific symbols and their meanings (Sheet 1 of 2)











Symbol	Meaning
	Chemical hazard: Observe Good Laboratory Practices (GLP) when handling chemicals. Only work with volatile chemicals under a fume or exhaust hood. Wear gloves and other protective equipment, as appropriate, when handling toxic, carcinogenic, mutagenic, corrosive, or irritant chemicals. Use approved containers and proper procedures to dispose of waste oil and when handling wetted parts of the instrument.
	Hot surface: Allow heated components to cool before touching or servicing the instrument.

Table 1. Caution-specific symbols and their meanings (Sheet 2 of 2)

Symbol	Meaning
	Risk of electric shock: This instrument uses voltages that can cause electric shock and/or personal injury. Before servicing, shut down the instrument and disconnect it from line power. While operating the instrument, keep covers on.
	Risk of eye injury: Eye injury could occur from splattered chemicals or airborne particles. Wear safety glasses when handling chemicals or servicing the instrument.
	Sharp object: Avoid handling the tip of the syringe needle or the corona needle that is located inside the API source.

Contacting Us

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Introduction

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

Note Unless otherwise noted:

- For the LTQ Velos MS, follow the Velos Pro MS information.
- For the LXQ MS, follow the LTQ XL MS information.

The “[Glossary](#)” on [page 159](#) defines some of the terms used in this guide.

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- [Features of the LTQ Series Mass Spectrometer](#)
- [Sheath, Auxiliary, and Sweep Gases](#)
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Features of the LTQ Series Mass Spectrometer

The LTQ Series MS has the following features:

- [Linear Ion Trap Mass Analyzer](#)
- [ZoomScan and UltraZoom Analyses for Charge State Determination](#)
- [Wideband Activation](#)
- [Switchable API 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 high level of analytical specificity that the LTQ Series MS provides sets it 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 MS 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 MS 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, MSⁿ (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 [precursor 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 precursor 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 precursor 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 MS 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. MSⁿ techniques on the LTQ Series MS allow for stepwise fragmentation pathways, making interpretation of MSⁿ spectra relatively straightforward. The LTQ Series MS has several advanced features that make its MSⁿ capabilities extremely powerful for [qualitative analysis](#).

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

ZoomScan and UltraZoom Analyses for Charge State Determination

The [ZoomScan scan type](#) and UltraZoom scan type analyses provide 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. UltraZoom provides higher resolution than the ZoomScan type and can determine higher charge states than ZoomScan.

For additional information, see “Scan Rates” on [page 16](#).

Wideband Activation

Use the [WideBand Activation™](#) option for the LTQ Series MS 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 precursor 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 MS³ 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 101](#).

Switchable API Source

You can configure the LTQ Series MS with a standard [electrospray \(ESI\)](#) probe (see [page 42](#)), a [heated-electrospray \(H-ESI\)](#) probe (see [page 42](#)), or an [atmospheric pressure chemical ionization \(APCI\)](#) probe (see [page 56](#)). By using additional API source kits, you can operate the instrument in the [atmospheric pressure photoionization \(APPI\)](#) mode and the [nanoelectrospray \(nanoESI or NSI\)](#) 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 MS 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 nebulize and evaporate the sample solution by focusing the vapor plume and lowering the humidity in the API 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.

Refer to the Tune Plus Help for guidelines on the operating parameters.

Ionization Techniques

Typically, ESI or heated ESI (H-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 NH_4^+ ions in the solution and oxygen atoms in the polymer. With ESI, the range of molecular masses that the LTQ Series MS 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 instrument over a range of 0.1–1000 $\mu\text{L}/\text{min}$. (Refer to the Tune Plus Help for guidelines on the operating parameters.)

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.

IMPORTANT To obtain good ESI results, follow these guidelines:

- Keep nonvolatile salts and buffers out of the solvent system. For example, avoid the use of phosphates and salts that contain potassium or sodium. Use acetate or ammonium salts 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). The acidic pH tends to keep positive ions in solution.

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 MS in APCI mode is typically high (200–2000 $\mu\text{L}/\text{min}$). (Refer to the Tune Plus Help for guidelines on the operating parameters.)

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

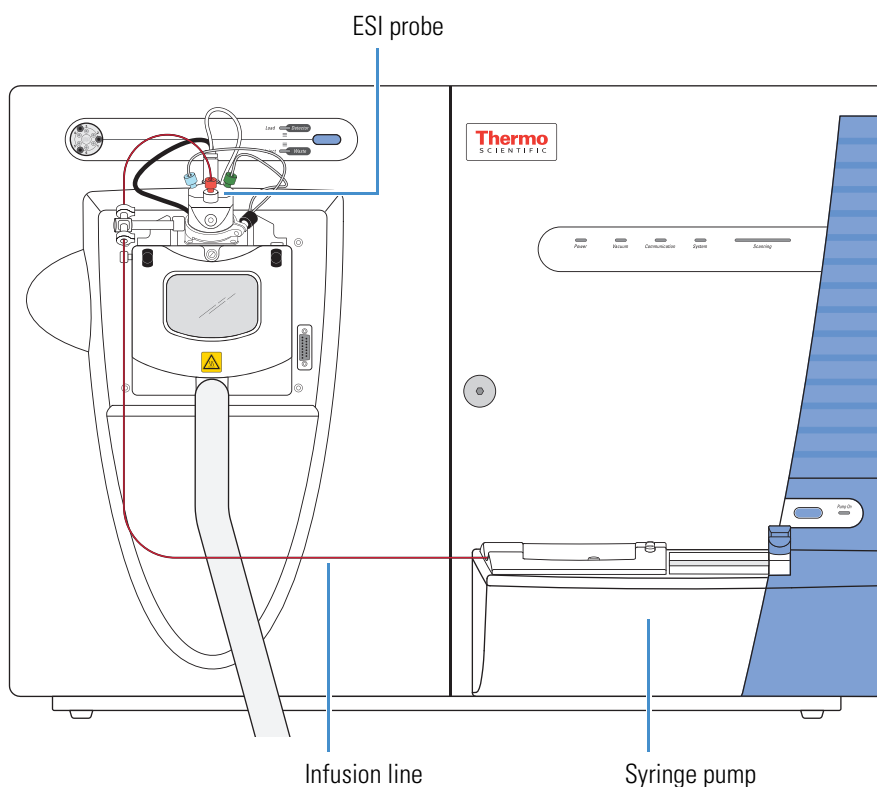
The LTQ Series MS has a [divert/inject valve](#) and a [syringe pump](#). The following techniques are available to introduce samples into the [API source](#):

- [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 API source, as shown in [Figure 1](#). Use this technique to introduce the 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



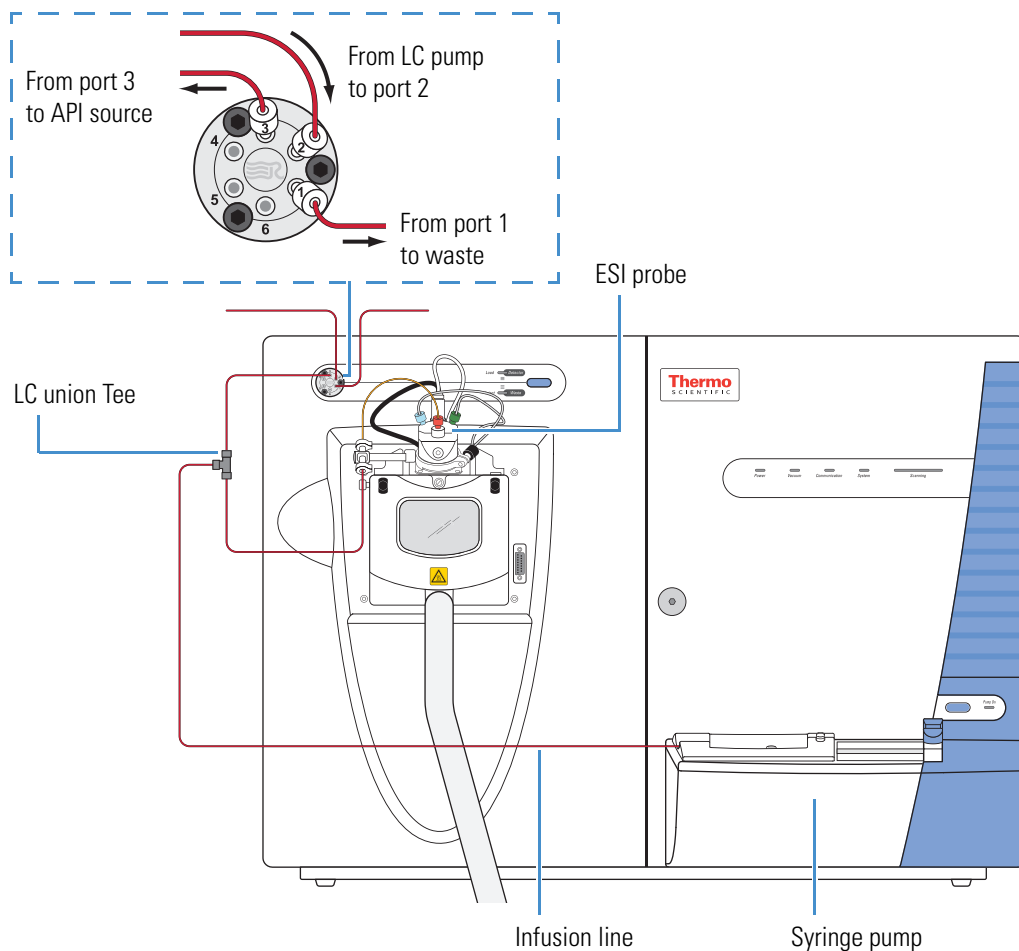
High-Flow Infusion

The high-flow infusion technique uses an LC union Tee 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 API 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 this infusion method to perform 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 ion 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 API 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. For plumbing instructions, see “Setting Up the Inlet for High-Flow Infusion in ESI Mode” on page 83.

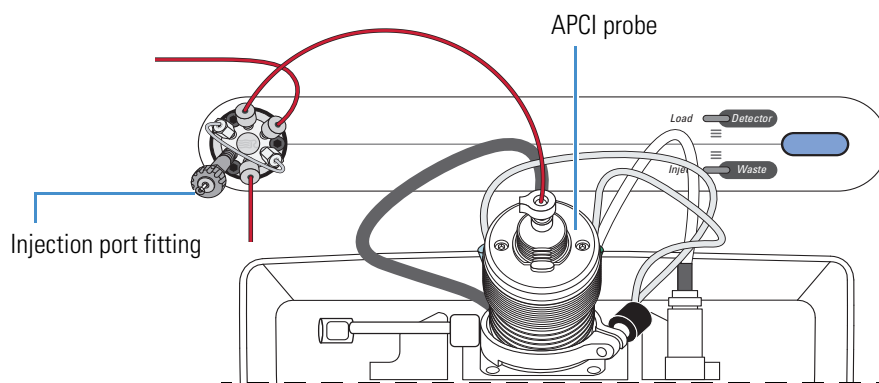
Figure 2. Sample introduction by using high-flow infusion in ESI mode



Loop Injection (Flow-Injection Analysis)

Use the loop injection technique in either ESI or APCI mode 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 API 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 API source.

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

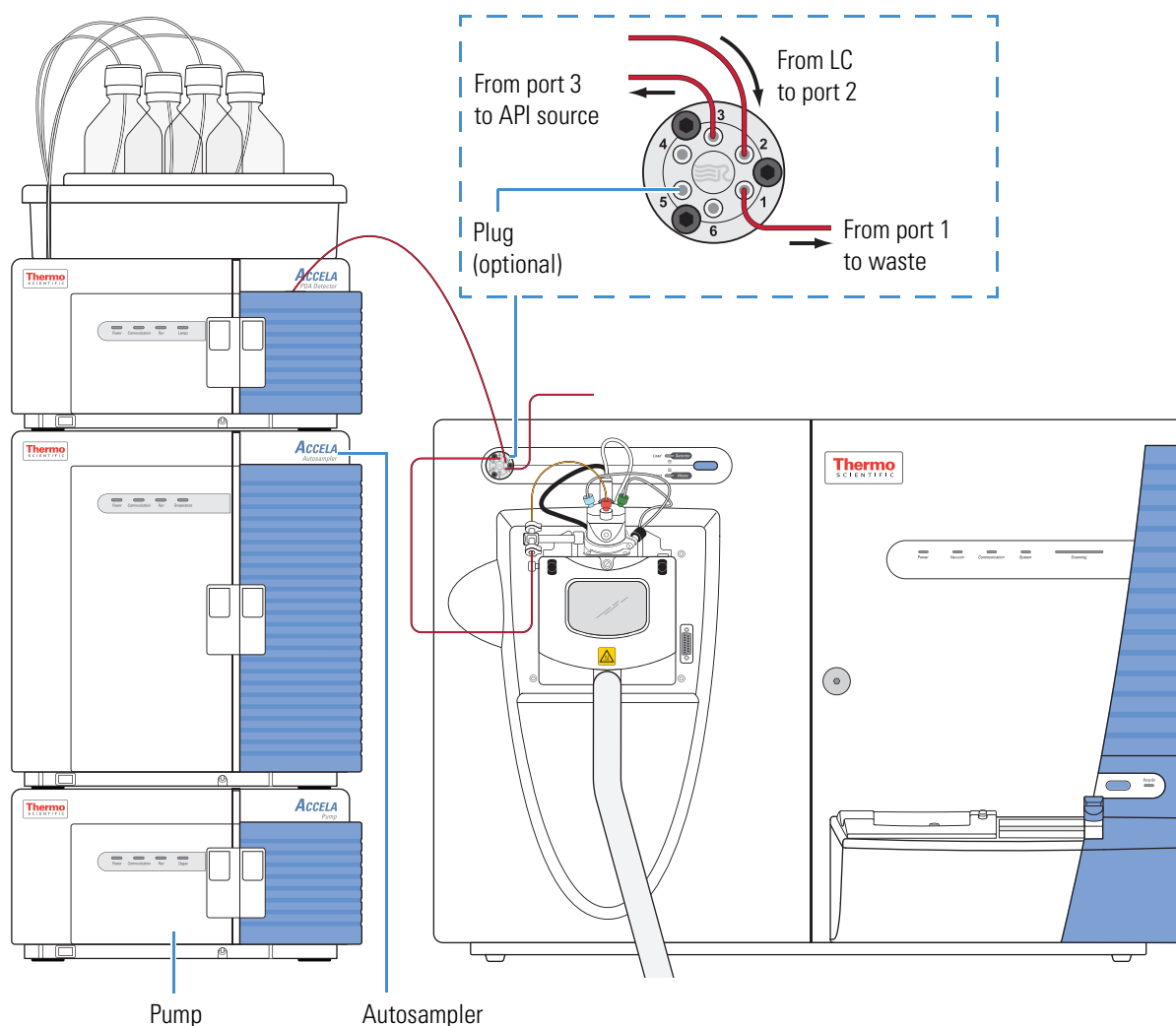


Liquid Chromatography

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

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 API 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 API source.

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



LC Flow Rate Ranges

The ESI probe can volatilize ions from liquid flows¹ 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 flows² 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.

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 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.

For guidelines for setting the operating parameters for an LC/ESI/MS, LC/H-ESI/MS, or LC/APCI/MS system, refer to the Tune Plus Help.

❖ To view the operating parameter guidelines

In the Tune Plus window, do any of the following:

- Choose **Setup** > **Type Source**, and then click **Help**.
- Open the Help and search for “API Source Settings for Various LC Flow Rates.”

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 API source, such as the ion transfer tube and nozzle of the ionization probe. Using nonvolatile buffers without also cleaning the API 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)

¹ 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.

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

For a list of recommended solvents, refer to the *LTQ Series Preinstallation Requirements Guide*.



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) or Safety Data Sheets (SDSs). The MSDSs and SDSs must be freely available to lab personnel to examine at any time. These data sheets describe the chemicals and summarize information on the hazard and toxicity of specific chemical compounds. They also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures to remedy spills or leaks.

Read the MSDS or SDS 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 or SDS.

Tuning and Calibrating the Mass Spectrometer

Calibrate the LTQ Series MS to ensure its mass accuracy, resolution, isolation efficiency, and dissociation efficiency. Before calibrating, tune the instrument with the ESI calibration solution to optimize the transmission of ions and ensure long-term performance.

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 directly from the syringe pump into the instrument in ESI/MS mode at a steady flow rate while the calibration is running.

To achieve the highest sensitivity or the lowest limits of detection for an analyte of interest, tune the instrument with the analyte. In addition, appropriate tuning can improve long-term performance by ensuring that the voltage gradient on the ion optics is set for a wide variety of conditions.

All LTQ Series instruments ship with default tune parameters that are specific to the instrument model and source type (for example, ESI, APCI, or NSI). In most cases, these tune parameters are sufficient for high sensitivity and long-term performance. You might be able to extend the performance for a particular analyte of interest by tuning with that 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.

IMPORTANT If you make changes to the tune parameters and do not save them before switching to another API source probe, the changes are lost. In addition, when you switch from one API source probe to another, the Tune Plus application automatically loads the last saved tune parameters for the newly installed API source probe and overwrites the tune parameters from the previously installed API source probe.

Automatic and semi-automatic tuning procedures (including optimizing the collision energy) require introducing the analyte into the instrument 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 manually tune several parameters.

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 Velos Pro and LTQ Velos MSs), gases, and solvent flow rate. For optimum sensitivity, tune the instrument in the operational mode that you plan to use for the experiments.

The Velos Pro and LTQ Velos MSs 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 2](#) lists the methods of sample introduction for each of the calibration and tuning procedures.

Table 2. Summary of methods of sample introduction for calibration and tuning

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

Ion Polarity Modes

The LTQ Series MS can operate in either positive or negative ion polarity modes. The instrument 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 MS 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 instrument so that you can easily see and measure mass resolution.

- Centroid data

With centroid data, you can see 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. Centroid data requires about one-tenth the computer disk space of what is required for profile data.

Scan Power and Scan Modes

Ions produced in the API source are often referred to as precursor 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 MS can first eject all ions, except for several selected precursor 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 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 instrument 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 LTQ Series MS support several scan powers.

- MS scan mode

The mass spectrometry (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 precursor ions, and no fragmentation of the precursor 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, precursor 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^n scan mode

An MS^n 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^n 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 MSs can operate in the following scan rates: Turbo, Rapid (for the Velos Pro only), Normal, Enhanced, ZoomScan, and UltraZoom. Table 3 lists the scan rates and their peak widths (resolutions) for the instruments. For additional information about the scan rates, refer to the data system Help.

Table 3. Scan rates and peak widths for the LTQ Series MSs

Scan rate	Parameter	Velos Pro	LTQ Velos	LTQ XL and LXQ
Turbo ^a	Scan rate (Da/sec):	125 000	125 000	125 000
	Peak width (FWHM):	≤ 3	≤ 3	≤ 3
Rapid	Scan rate (Da/sec):	66 666	N/A	N/A
	Peak width (FWHM):	≤ 0.6	N/A	N/A
Normal	Scan rate (Da/sec):	33 333	33 333	16 666
	Peak width (FWHM):	≤ 0.5	≤ 0.6	≤ 0.7
Enhanced	Scan rate (Da/sec):	10 000	10 000	5 000
	Peak width (FWHM):	≤ 0.35	≤ 0.35	≤ 0.45
ZoomScan	Scan rate (Da/sec):	2 222	2 222	1 111
	Peak width (FWHM):	≤ 0.25	≤ 0.25	≤ 0.3
UltraZoom	Scan rate (Da/sec):	27	27	27
	Peak width (FWHM):	≤ 0.075	≤ 0.1	≤ 0.15

^a The isotopes are partially unresolved. The actual observed peak widths are isotope-abundant dependent.

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 rates in which the instrument achieves a higher resolution. The resolution is sufficient to determine the ¹²C/¹³C isotopic separation that then allows the system to determine the charge state as follows:

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

With the UltraZoom scan rate, the preceding list can continue up to a charge state of ±10. You can then determine the molecular weight of the ion based on the charge state and mass-to-charge ratio of the ion.

Scan Types

The LTQ Series MS can operate in the following scan types:

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

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 (MS¹)

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 API 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 API source. Then ions of one mass-to-charge ratio (the precursor ions) are selected, and all other ions are ejected from the mass analyzer. The precursor ions are excited and collide with background gas that is present in the mass analyzer. The collisions of the precursor 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 precursor and product ions to observe. To obtain this information you could use a one-stage full scan to determine the precursor mass spectrum and a two-stage full scan to determine the **product mass** spectrum for the precursor 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 API 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 precursor ion and product ion pairs.

In the first stage of mass analysis, the mass analyzer stores the ions formed in the API source. The mass spectrometer selects ions of one mass-to-charge ratio (the precursor ions) and ejects all other ions from the mass analyzer. The precursor ions are excited and collide with background gas that is present in the mass analyzer. The collisions of the precursor 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 precursor 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 precursor ion of the same mass-to-charge ratio as the selected precursor 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 API source. The mass spectrometer selects ions of one mass-to-charge ratio (the precursor ions) and ejects all other ions from the mass analyzer. The precursor ions are excited and collide with background gas that is present in the mass analyzer. The collisions of the precursor 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 precursor ions for the next stage of mass analysis. The new precursor ions are excited and collide with background gas. The collisions of the new precursor 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.

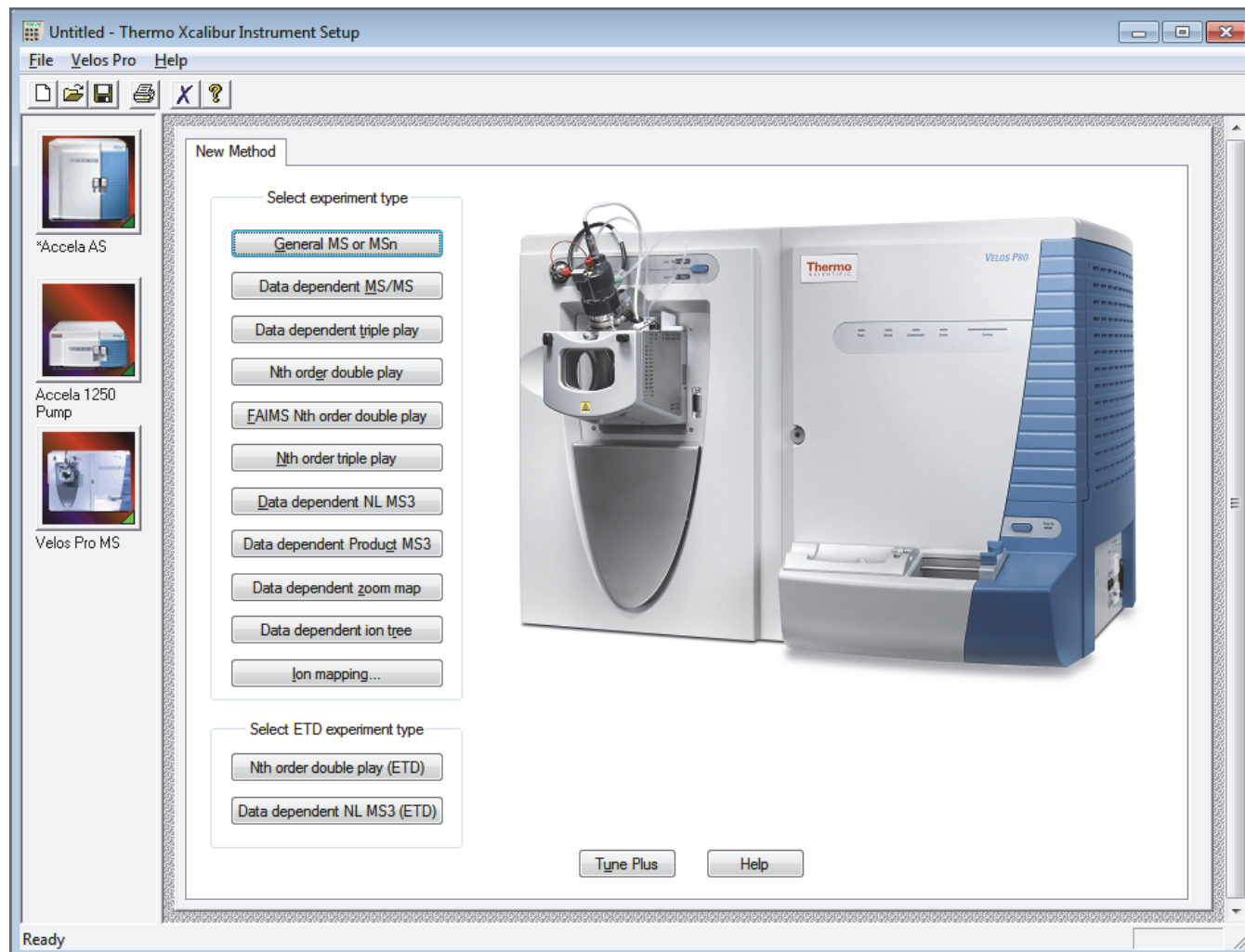
In the n^{th} stage of mass analysis, the mass analyzer stores the final product. The mass spectrometer then sequentially scans the m/z range of the selected ions out of the mass analyzer to produce a CRM final product ion mass spectrum.

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 precursor ion.

Types of Experiments

Use the New Method page of the Thermo Xcalibur Instrument Setup window to create the instrument methods for your experiments. To save time entering the parameters for an instrument method, open the system 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 extension).

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 MS. The experiments are grouped into the following categories:

- [General MS or MSⁿ Experiments](#)
- [Data Dependent Experiments](#)
- [Ion Mapping Experiments](#)
- [Ion Tree Experiments](#)

General MS or MSⁿ Experiments

A General MS or MSⁿ 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 MSⁿ experiment; click **General MS or MSⁿ** on the New Method page in the Xcalibur application (Figure 5 on page 20). Figure 6 shows an example of a General MS or MSⁿ experiment template.

In a General MS quantitation experiment, specify the following:

- Mass range of the analyte or analytes
- Precursor ion that fragments into distinctive product ions
- Mass-to-charge ratios of all the product ions

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

If using a General experiment to collect data for qualitative (structural) analysis, specify the scan mode (MS through MSⁿ) that you want data for on the MS Detector Setup page under Scan Event 1 Settings (Figure 6). If specifying MS/MS or MSⁿ, choose the precursor ion or ions that you want data for on the MS Detector Setup page under MSⁿ Settings. Under MSⁿ 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 xx). The LTQ Series MS then collects distinct qualitative information for structural analysis or for spectral reference.

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

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

MS Detector Setup

Run settings

Acquire time (min): 10.00 Segments: 1 Start delay (min): 0.00

To display a chromatogram here, use Velos Pro/Open raw file...

Segment 1

Retention time (min)

Segment 1 settings

Segment time (min): 10.00 Scan events: 1 Tune method: C:\Thermo\Instruments\LTQ\methods\Default_ESI.LT

Scan Event 1

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

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

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

Supplemental Activation
 Supplemental Activation

Wideband Activation SA Charge State: 2

Use MS/MS Mass List APCI Corona On APPI Lamp On

New method Tune Plus Help

Ready NOT SAVED

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 MS 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 precursor ions for fragmentation or let the instrument automatically select the ions for fragmentation. The instrument can automatically collect the structural information for every precursor 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 precursor ion. The instrument then collects full-scan MS data, picks the most intense precursor 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 precursor 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 precursor ion and calculate the molecular weight. The precursor ion is then fragmented into product ions (MS/MS). For example, if the instrument determines a charge state equal to two, and if the mass-to-charge ratio of the precursor 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 2×500). 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 MSⁿ data and arranging the data in formats that are easy to manipulate. For additional information, see “Ion Tree Experiments” on page 29.

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

- If you have some idea of the precursor ion, or if you expect a certain kind of precursor, set up a list of possible precursor ions. After detecting one of the specified precursor 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 instrument generates product spectra. (Decide later if the information is useful.) Parameters might include threshold values for the intensity of the MS or MSⁿ ion signal. Whatever threshold values you choose should isolate the precursor 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 MSⁿ experiment can identify process impurities. In the quality assurance process for aspirin, for example, the instrument 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 MSⁿ 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 MSⁿ spectra (Ion Tree).

A Data Dependent experiment can produce a composite spectrum of, for example, MS/MS, MS³, and MS⁴ data. The instrument can store the MSⁿ fingerprint data in a custom MSⁿ 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 the Thermo Xcalibur software. The window title is 'Untitled - Thermo Xcalibur Instrument Setup'. The interface includes a menu bar (File, Velos Pro, Help), a toolbar, and a sidebar with icons for 'Accela AS', 'Accela 1250 Pump', and 'Velos Pro MS'. The main area is divided into several sections:

- Run settings:** Acquire time (min): 10.00, Segments: 1, Start delay (min): 0.00. A message states: 'To display a chromatogram here, use Velos Pro/Open raw file...'. Below is a retention time scale from 0 to 10 minutes, with 'Segment 1' highlighted in yellow.
- Segment 1 settings:** Segment time (min): 10.00, Scan events: 3, Tune method: C:\Thermo\Instruments\LTQ\methods\Default_ESI.LT. Below is a scan event timeline with 'Scan Event 1' highlighted in yellow.
- 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:**

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

 - Supplemental Activation:
 - Wideband Activation: SA Charge State: 2
 - Use MS/MS Mass List: APCI Corona On: APPI Lamp On:
- Scan Ranges:**

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

 - Input: From/To

Buttons at the bottom include 'New method', 'Tune Plus', and 'Help'. The status bar shows 'Ready' and 'NOT SAVED'.

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 precursor ion over a specified mass range. An Ion Mapping experiment can help to identify automatically which precursor ions were fragmented to yield a specified product ion. The experiment “maps” one or more precursor ions by using the information from product ion scans.

These experiments require that sample solution enter the instrument 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 MS 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 precursor ion in a specified mass range to determine which precursor 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 precursor ions are related to specific product ions (precursor 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 precursor 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 precursor (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 precursor 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

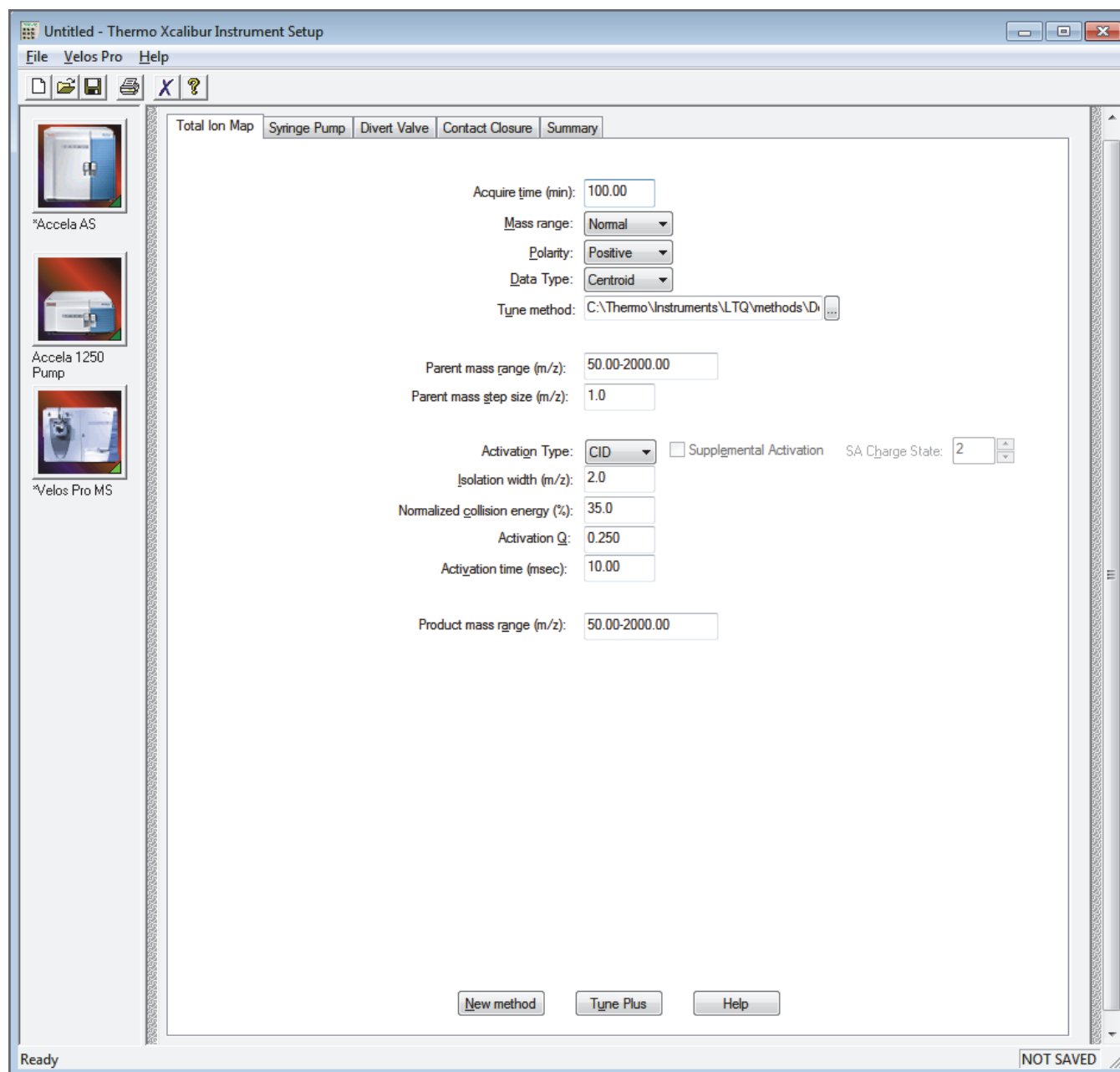
A Data Dependent Zoom Map is an Ion Map experiment that collects ZoomScan data on every scan interval in a specified mass range and Data Dependent MS/MS product spectra on the most intense mass peak above an intensity threshold with each ZoomScan.

- FAIMS CV Ion Map

A FAIMS CV Ion Map experiment performs full-scan MS scans while stepping the FAIMS compensation voltage (CV). The resulting FAIMS CV ion map, which you can view in the Xcalibur Qual Browser window, is a 3D plot of the ion intensity versus m/z versus CV. From the 3D plot, you can visualize the selected or rejected ion at a particular CV.

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

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



Ion Tree Experiments

In an Ion Tree experiment, the LTQ Series MS can collect MSⁿ data automatically. To maximize the structural information acquired for a particular sample, perform an Ion Tree experiment. Specify a particular precursor ion for MSⁿ fragmentation or let the instrument find the precursor ions automatically and fragment them to any level between MS/MS and MS¹⁰. The instrument 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 instrument gathers information in the allowed time, choose one of two options:

- Depth focus
Characterizes an ion by performing a series of MSⁿ-level fragmentations (for example, MS/MS, MS³, MS⁴, and so on) before characterizing the next most intense ion in the MSⁿ series.
- Breadth focus
Characterizes all ions to the same MSⁿ level before advancing to the next MSⁿ 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 instrument first scans for precursor ions (MS) over the specified mass range. Next, it selects the first most intense ion of the MS spectrum for fragmentation (MS/MS).

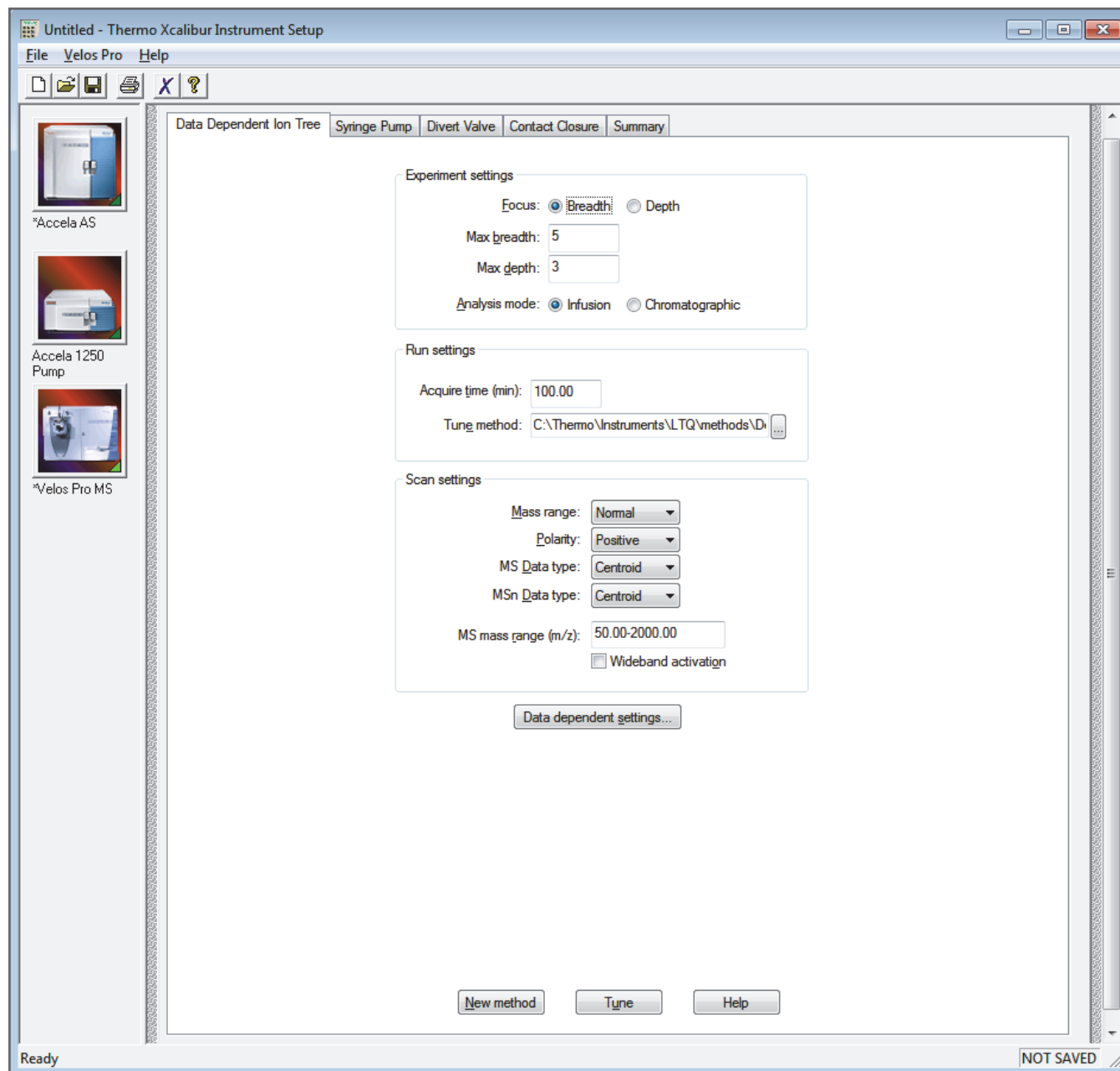
- For depth focus, after fragmenting the most intense ion of the MS spectrum—producing an MS/MS spectrum—the instrument selects and fragments the most intense ion of the MS/MS spectrum. This results in an MS³ spectrum, the level specified as the maximum depth for this example. The instrument then backs up one level and fragments the second most intense ion of the MS/MS spectrum, creating more product ions on the level of MS³ from this precursor 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 instrument selects and fragments the second-most intense ion in the same MS spectrum. The fragmentation of precursor ions continues to the Max Breadth level (2, for this example). After the two most intense peaks on the MS level are fragmented, the instrument selects and fragments the two most intense ions in the first MS/MS spectrum. This results in product ions on the level of MS³, 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 precursor ion.

1 Introduction

Scan Mass-to-Charge Range

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



Scan Mass-to-Charge Range

The LTQ Series MS has three mass-to-charge (m/z) range modes:

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

Setting Up the API Source

This chapter provides information about setting up the API source. Use the Ion Max™ or Ion Max-S™ source for ESI, H-ESI, or APCI experiments. The API source ships with the LTQ Series MS and consists of the source housing for use with an ESI, a HESI-II, or an APCI probe.

Note Tune and calibrate the LTQ Series mass spectrometer in ESI or H-ESI mode before you acquire data in ESI, H-ESI, 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 ways to open the Tune Plus window (Figure 10).

❖ To open the Tune Plus window

Do one of the following:

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

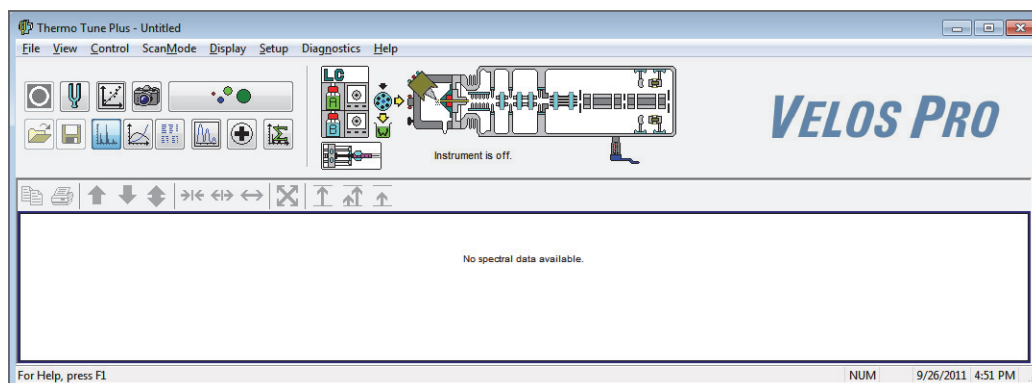
Note For LTQ Series version 2.5.0 or earlier, choose **Start > All Programs > Xcalibur > model Tune**.

- In the Xcalibur application, click the **Roadmap View** icon (under the menu bar), the **Instrument Setup** icon, the *model* MS icon, and then **Tune Plus**.

—or—

- In the Xcalibur application, click the **Roadmap View** icon (under the menu bar), the **Instrument Setup** icon, and the *model* MS icon. Then, from the main toolbar choose *model* > **Start Tune Plus**.

Figure 10. Tune Plus window for the Velos Pro



Placing the Mass Spectrometer in Standby Mode

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

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

❖ To place the instrument in Standby mode

1. Complete all data acquisition, if any.
2. Open the Tune Plus window (see [page 32](#)).
3. If your LC/MS system includes an LC pump, turn off the liquid flow to the API source.
For instructions, refer to the LC pump's manual.
4. In the Tune Plus window, do one of the following:
 - If the instrument is off, choose **Control > Standby**.

–or–

- If the instrument 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 API source probe or API source housing after it has cooled to room temperature.



CAUTION If you are using APPI and the mass spectrometer is in standby mode, turn off the LC or other liquid delivery device. Otherwise, the absence of sheath and auxiliary gases while in standby mode can cause the hot VUV vacuum lamp to break upon contact with liquids.

The instrument 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 instrument 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 instrument components when it 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 MS 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](#)
- [Removing the API Source Housing](#)

Installing the API Source Housing

❖ To install the API source housing

1. Place the instrument 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 instrument ([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)

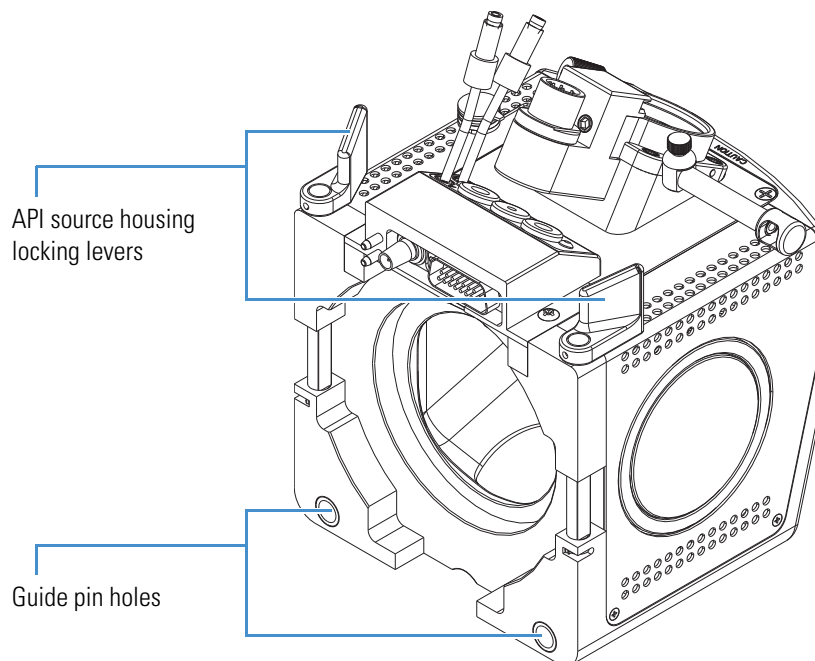
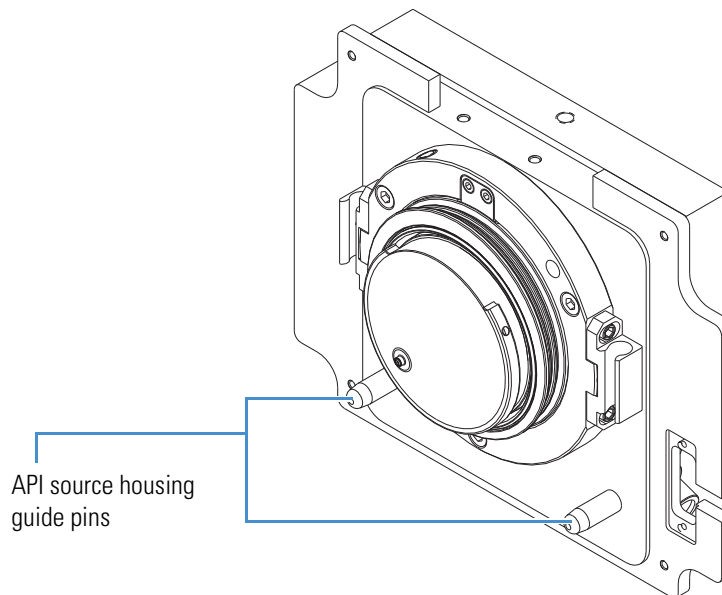


Figure 12. MS API source mount assembly showing the guide pins



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 API source and the instrument. Always make sure 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 API source interface, you must remove the API source housing from the instrument.



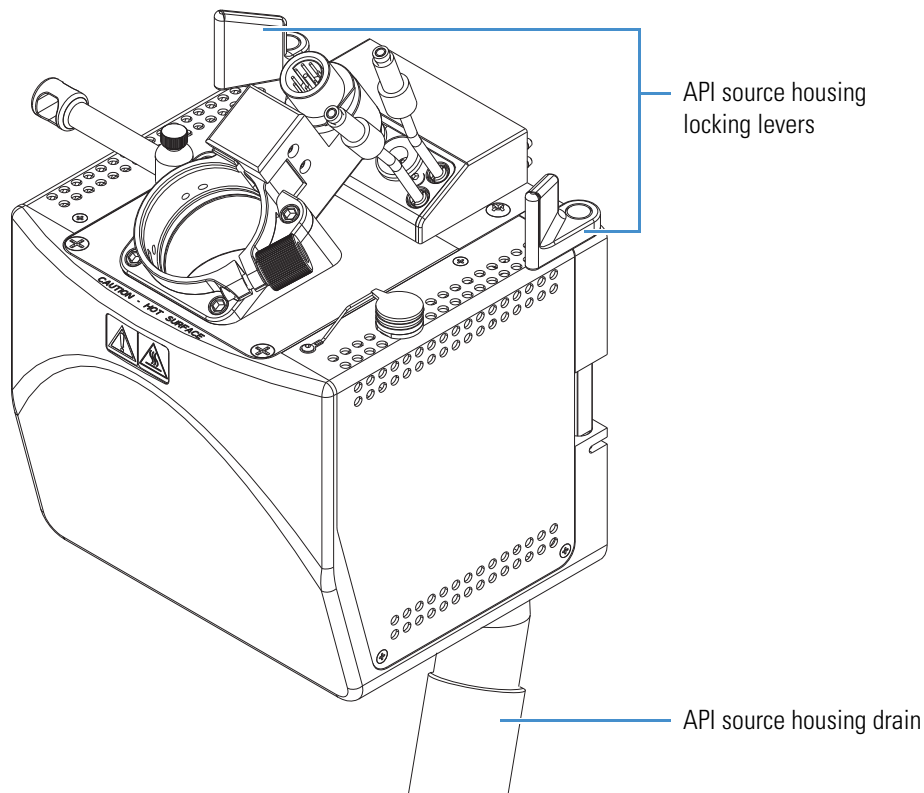
CAUTION Hot surface. Accidental contact with the part of the API probe located inside the housing can severely burn you, as this area of the probe can reach 350 °C (662 °F). In addition, although the exterior surface of the housing and the exposed part of the probe fall below the limit of 70 °C (158 °F) for accessible metal surfaces, they too pose a burn hazard. Therefore, handle ALL parts of the probe and housing with extreme caution.

- Before removing the API probe or housing, allow the part to cool to room temperature (approximately 20 minutes) before you touch 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 instrument 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 locking levers on the API source housing 90 degrees away from the housing to unlock it.
5. Pull the API source housing straight off of the MS 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

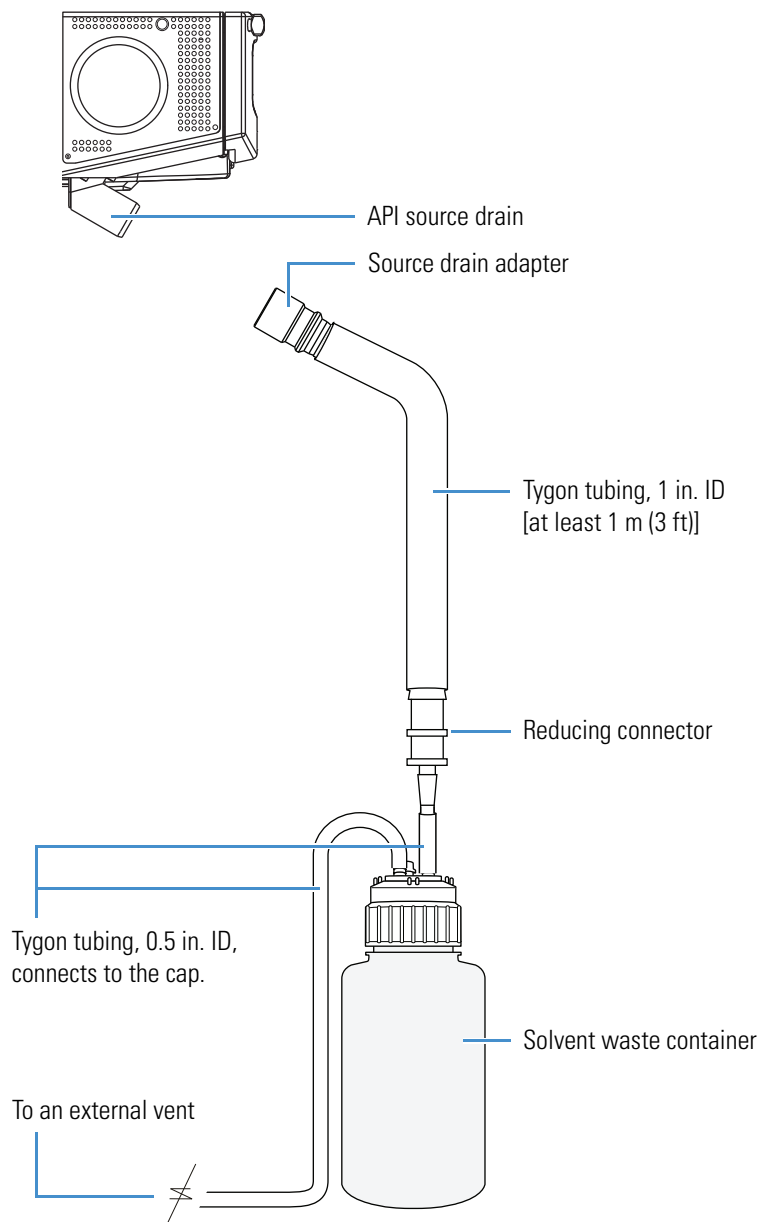


Table 4 lists the components of the solvent waste system. During the initial installation of the instrument, a Thermo Fisher Scientific field service engineer installs the solvent waste system.

Table 4. Solvent waste system parts

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

Use these guidelines for the API source drain:

- Use the PVC tubing provided with the solvent waste container to connect the solvent waste container to a fume exhaust system. 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.



CAUTION Do not vent the PVC drain tube (or any vent tubing connected to the waste container) to the same fume exhaust system that connects to the forepump. Vent the waste container to a dedicated fume exhaust system. The exhaust system for the API source must accommodate a flow rate of up to 30 L/min (64 ft³/h).

- Use the Teflon source drain adapter (see [Table 4](#)). Do not connect Tygon tubing directly to the API source drain. At high temperatures, Tygon releases volatile contaminants.



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

- To prevent solvent waste from backing up into the mass spectrometer, make sure that all tubing is above the level of liquid in the waste container as follows:
 - Tygon tubing from the mass spectrometer to the solvent waste container
 - PVC tubing from the waste container to the exhaust system

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. Accidental contact with the part of the API probe located inside the housing can severely burn you, as this area of the probe can reach 350 °C (662 °F). In addition, although the exterior surface of the housing and the exposed part of the probe fall below the limit of 70 °C (158 °F) for accessible metal surfaces, they too pose a burn hazard. Therefore, handle ALL parts of the probe and housing with extreme caution.

- Before removing the API probe or housing, allow the part to cool to room temperature (approximately 20 minutes) before you touch 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](#)
- [Removing the ESI or HESI-II Probe](#)

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.

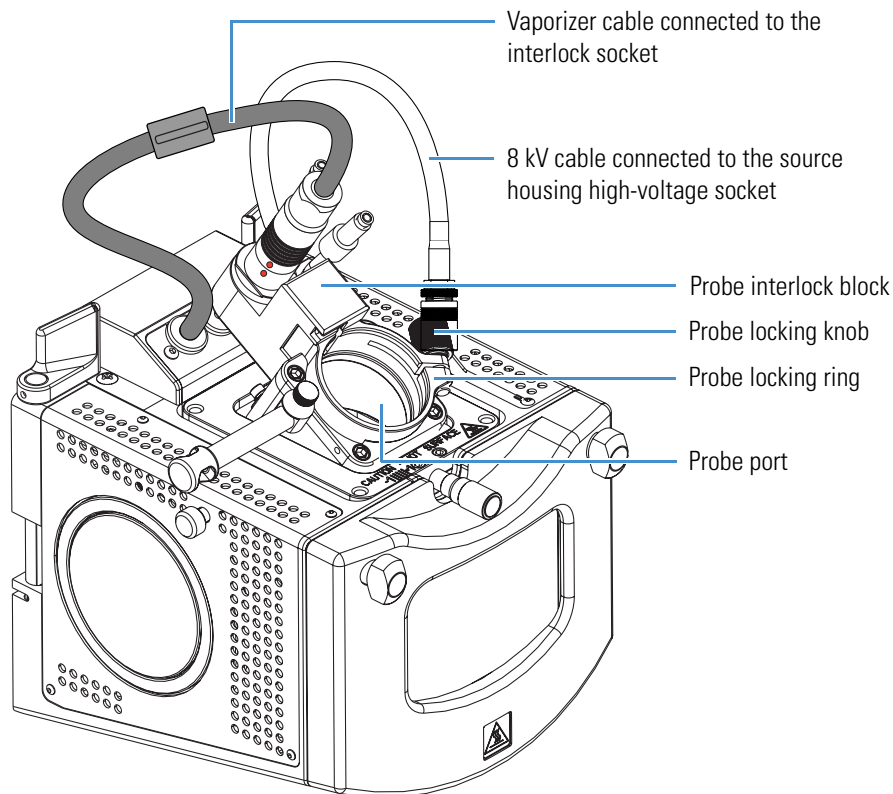
❖ To install the ESI or HESI-II probe

1. Place the instrument in **Standby** mode (see [page 33](#)) and let it cool to room temperature. See the preceding CAUTION statement.
2. If the instrument 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, make sure 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

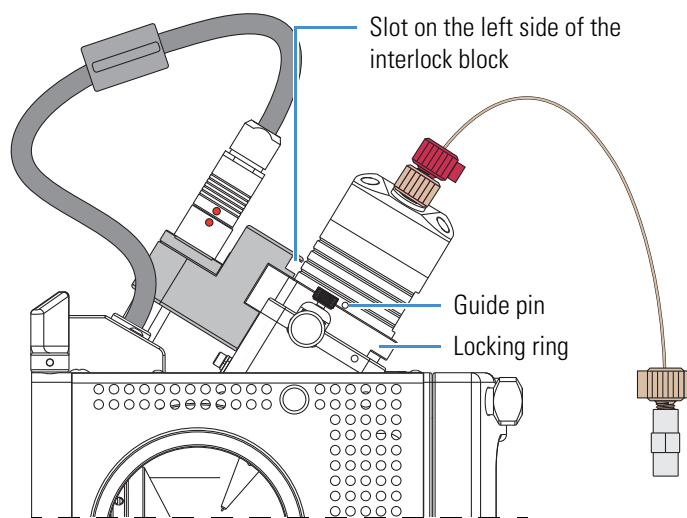
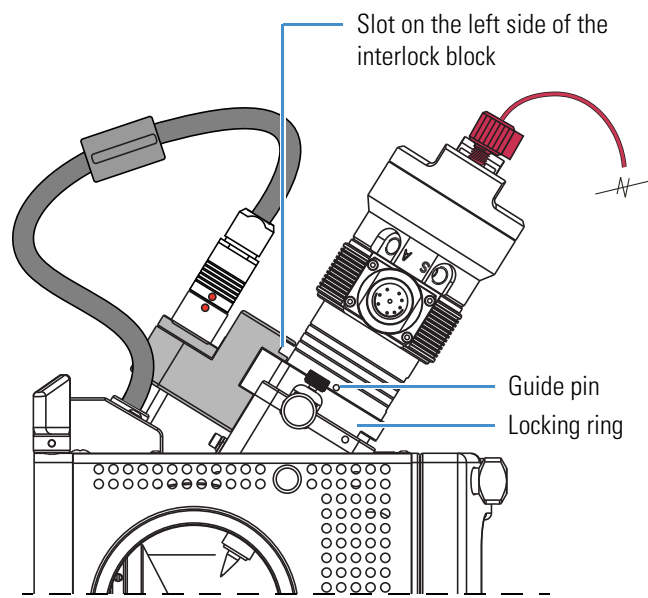


Figure 17. HESI-II probe showing the guide pin and locking ring



- 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

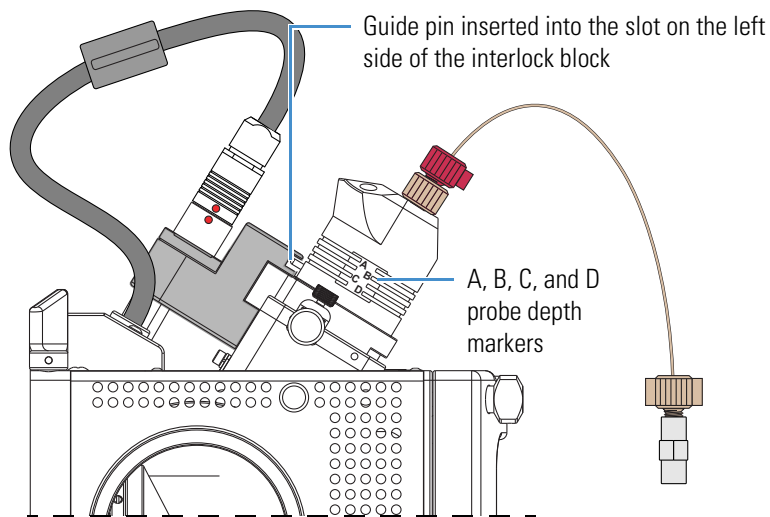
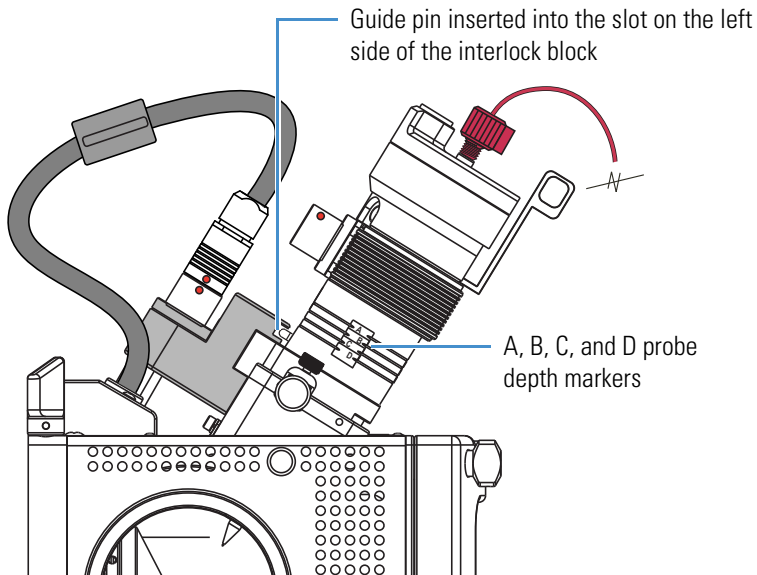


Figure 19. HESI-II probe showing the guide pin inserted into the interlock block slot



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.



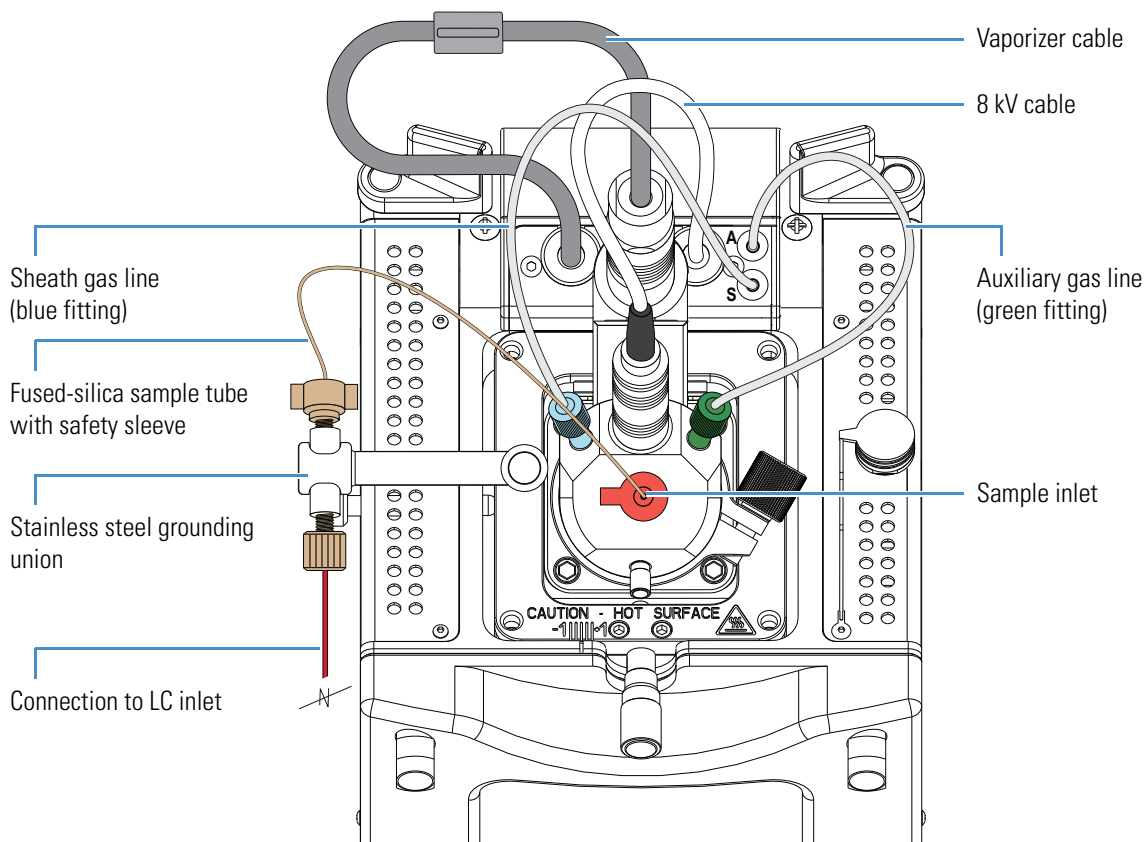
CAUTION High voltage. To prevent electric shock, make sure that the mass spectrometer is in standby mode before you disconnect or connect the 8 kV cable.

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. Make sure 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 MS auto-senses the installed probe type.

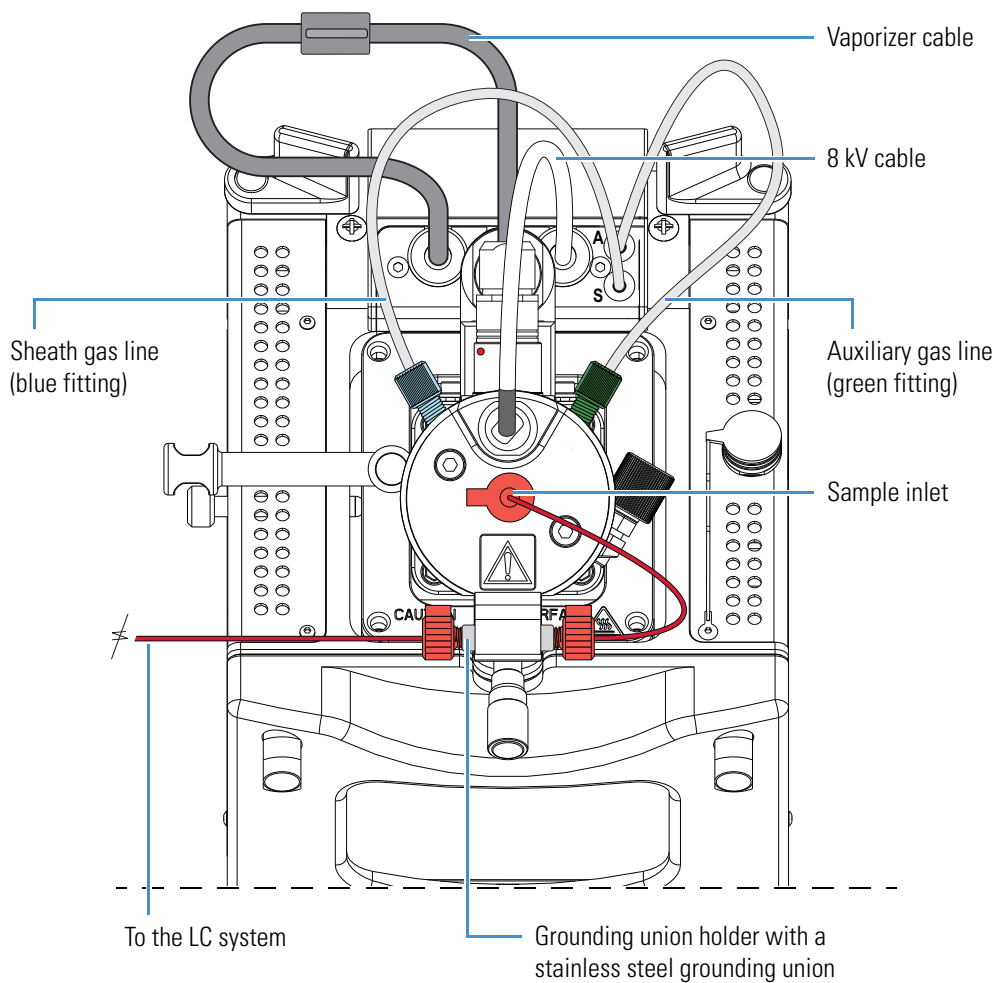
Figure 20. ESI probe installed in the Ion Max API source housing



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



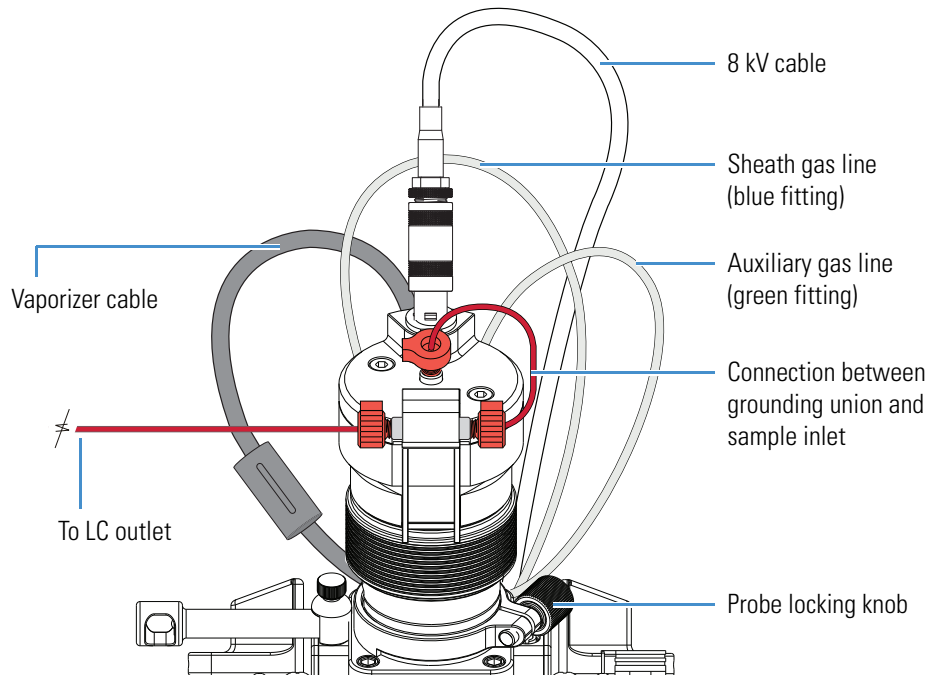
Removing the ESI or HESI-II Probe

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 instrument 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



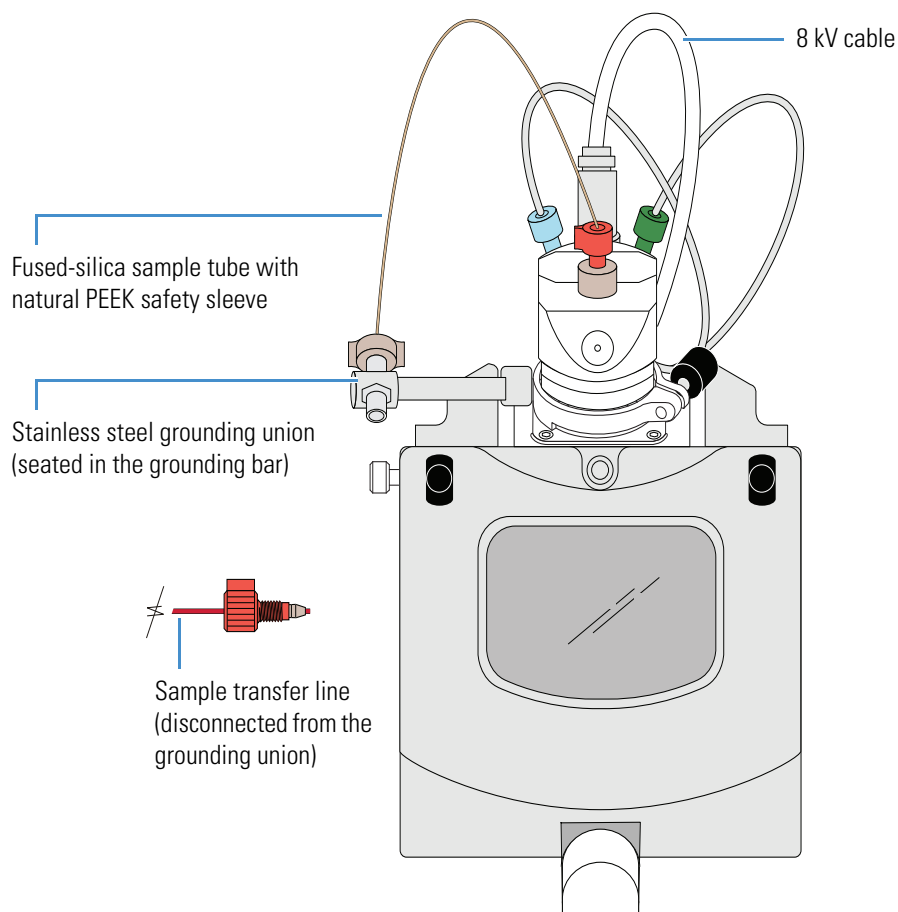
2 Setting Up the API Source

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. To prevent electric shock, make sure that the mass spectrometer is 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

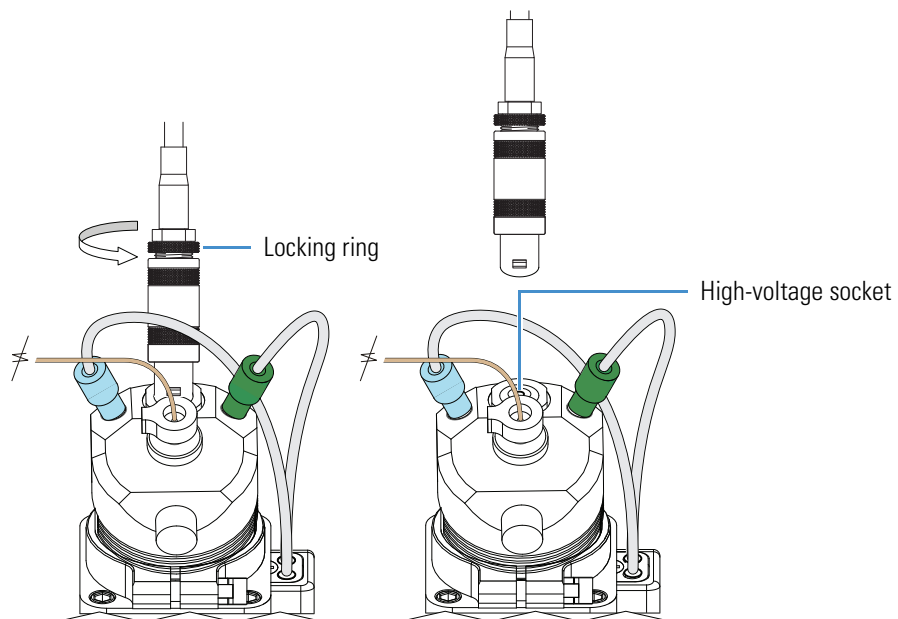
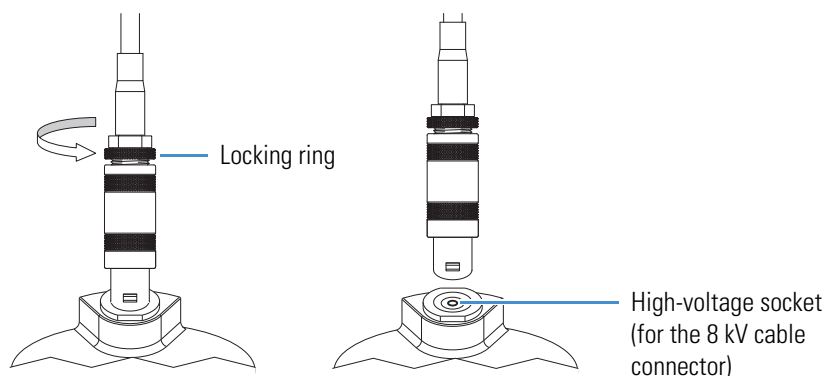


Figure 25. 8 kV cable connector removed from the HESI-II probe (enlarged view)

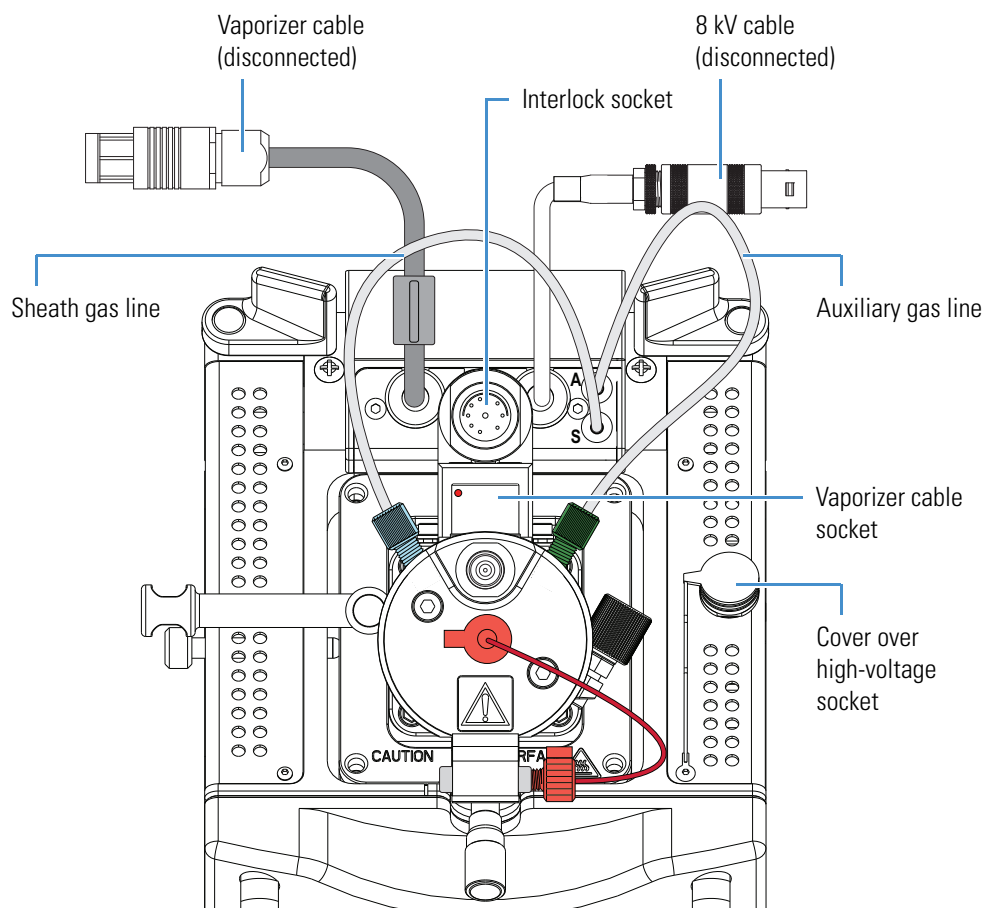


2 Setting Up the API Source

ESI or HESI-II Probe Installation and Removal

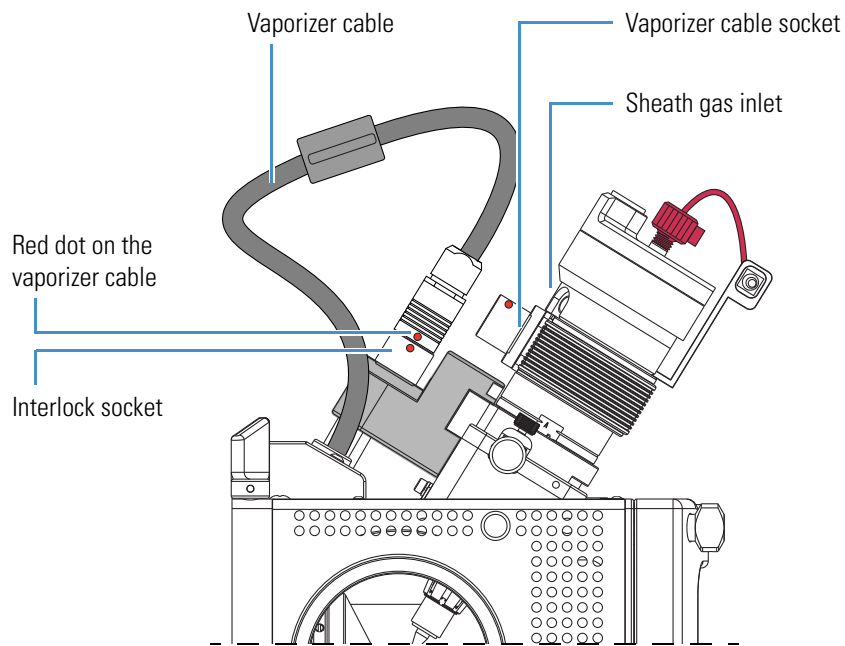
6. Disconnect the vaporizer cable from the probe vaporizer cable socket (Figure 26).

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).

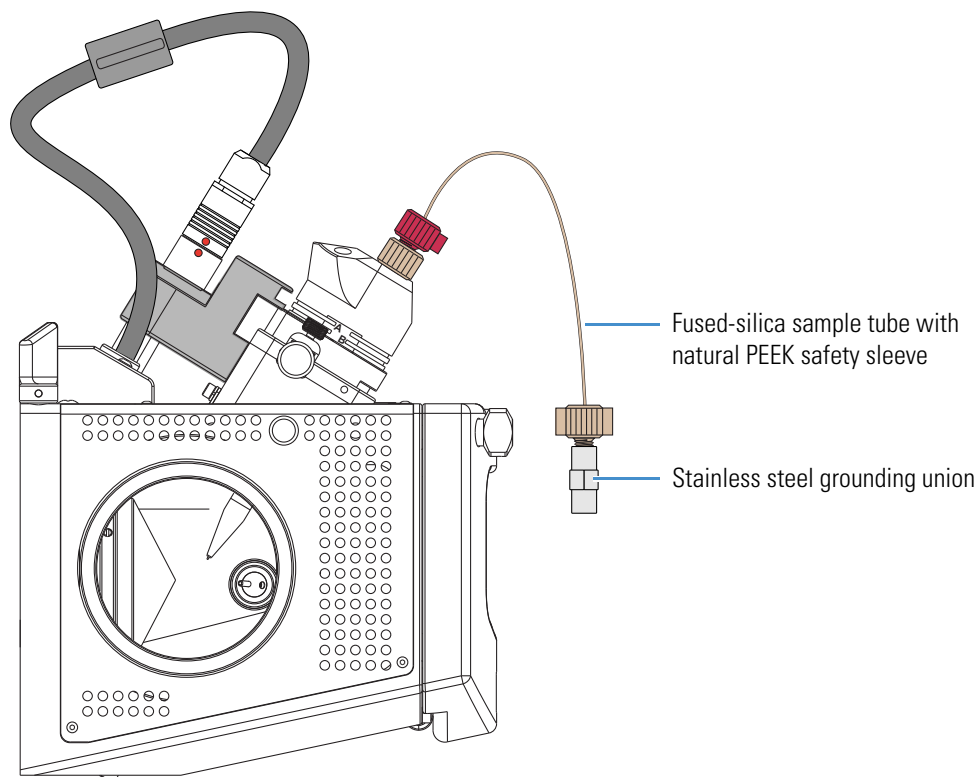
2 Setting Up the API Source

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](#)
- [Removing the APCI Probe and the Corona Needle](#)



CAUTION Hot surface. Accidental contact with the part of the API probe located inside the housing can severely burn you, as this area of the probe can reach 350 °C (662 °F). In addition, although the exterior surface of the housing and the exposed part of the probe fall below the limit of 70 °C (158 °F) for accessible metal surfaces, they too pose a burn hazard. Therefore, handle ALL parts of the probe and housing with extreme caution.

- Before removing the API probe or housing, allow the part to cool to room temperature (approximately 20 minutes) before you touch 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.

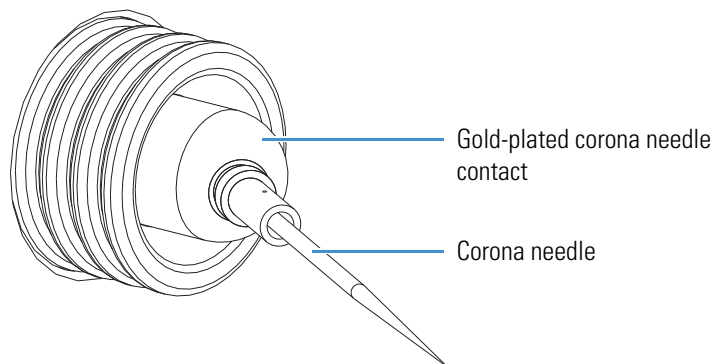
❖ To install the corona needle

1. Place the instrument in **Standby** mode (see [page 33](#)) and let it cool to room temperature. See the preceding CAUTION statement.
2. If the instrument 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

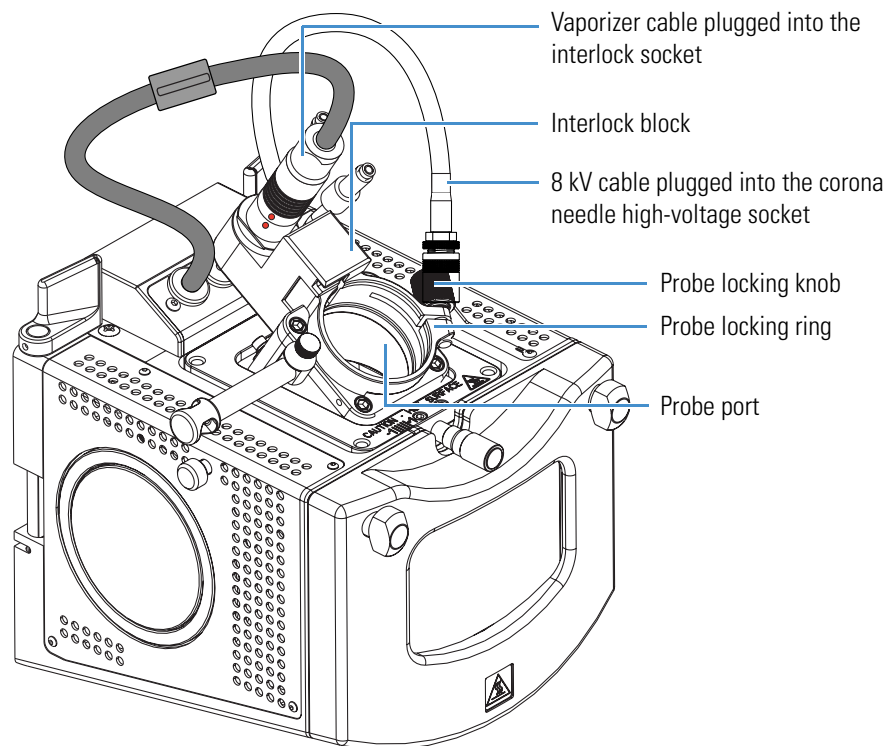


5. Make sure that the needle tip aligns with the travel path between the APCI probe and the API source interface.
6. Reinstall the API source housing (see [page 34](#)).

❖ **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.

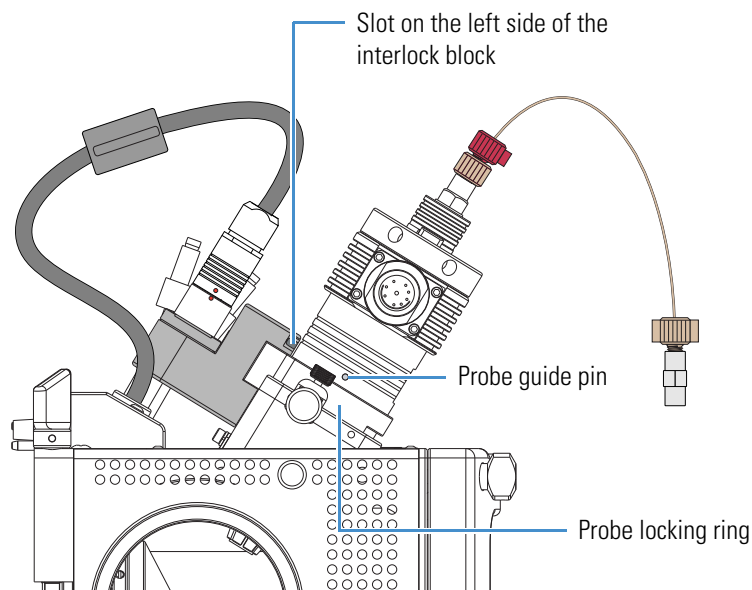
Figure 30. Ion Max API source housing



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

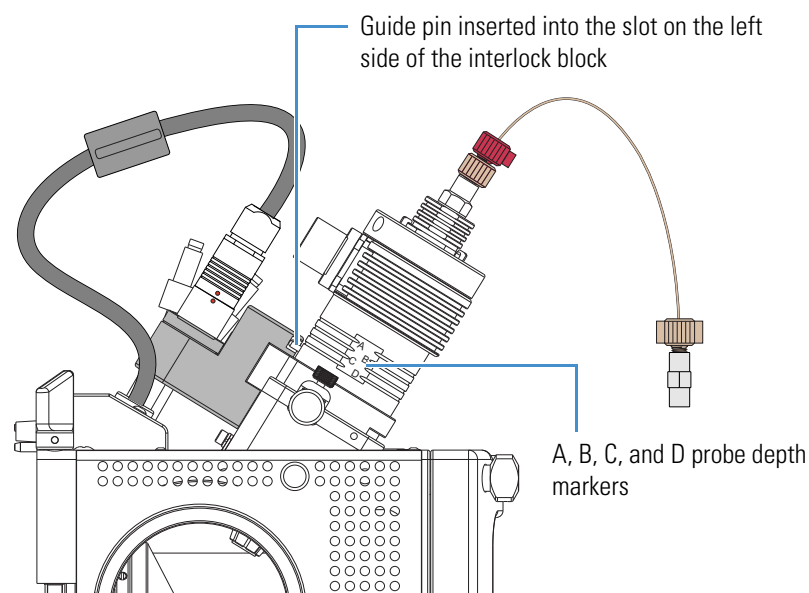
3. Position the probe in the API source housing probe port as follows:
 - a. Hold the probe with the nozzle facing down and the guide pin facing toward the left, and then slowly insert the probe into the port until the guide pin meets the locking ring on the API source housing (Figure 31).

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.

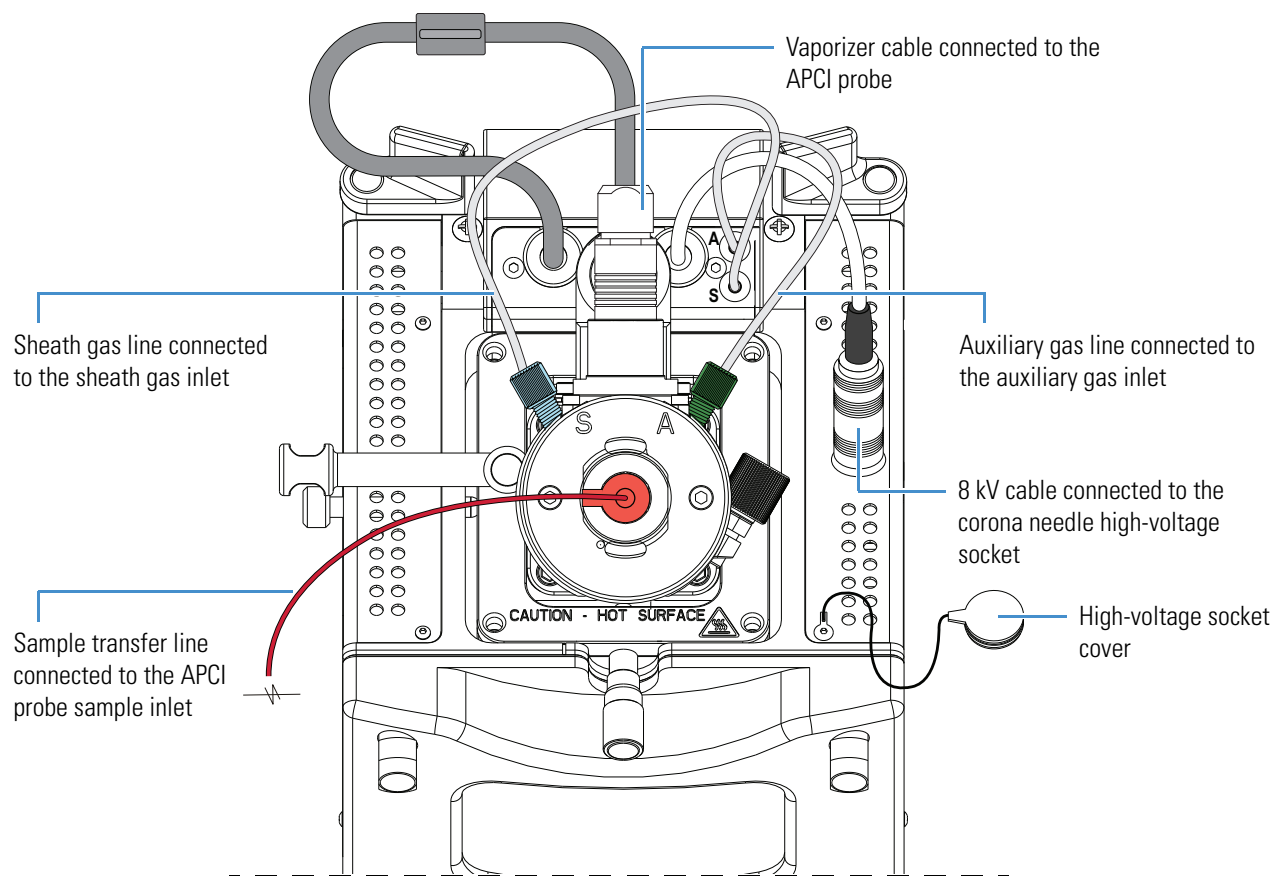
Insert the probe to a depth of B, C, or D. For high solvent flow rates, adjust the probe depth so that the nozzle is farther away from the ion interface (depth C or D). Conversely, for low solvent flow rates, adjust the probe depth so that the nozzle is closer to the ion interface (depth B or C).

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.

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



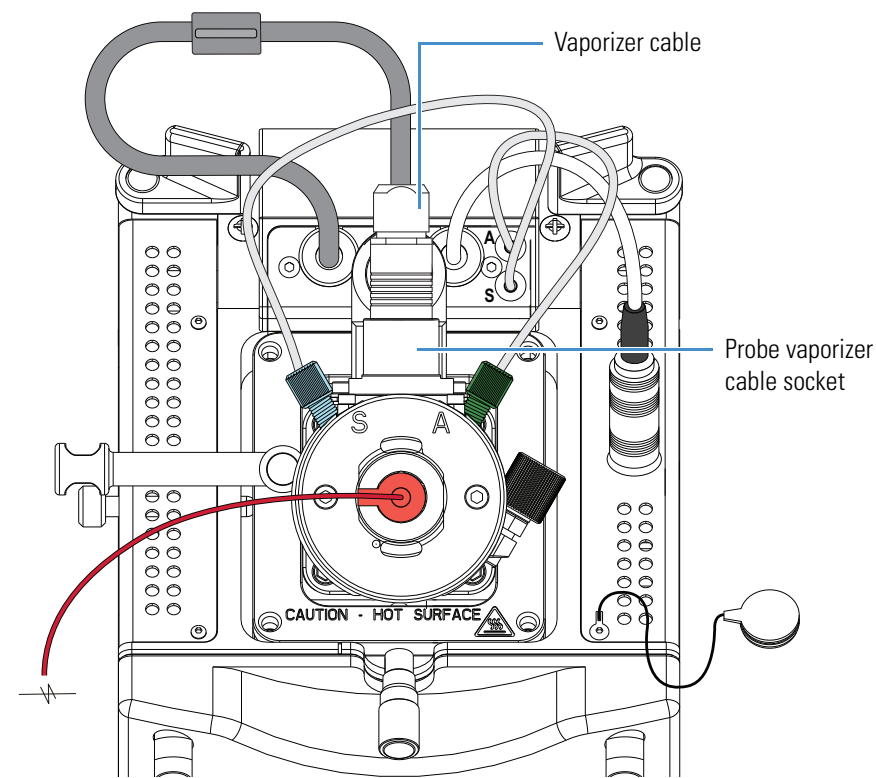
Removing the APCI Probe and the Corona Needle

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

❖ To remove the APCI probe

1. Place the instrument in **Standby** mode (see [page 33](#)) and let it cool to room temperature. See the CAUTION statement on [page 40](#).
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**

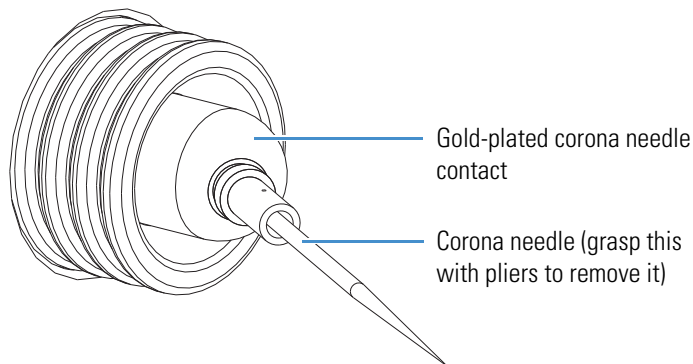


CAUTION Sharp object. The corona needle is very sharp and can puncture your skin. Handle it with care.

1. Place the instrument in **Standby** mode (see [page 33](#)) and let it cool to room temperature. See the CAUTION statement on [page 40](#).
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 MS 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).

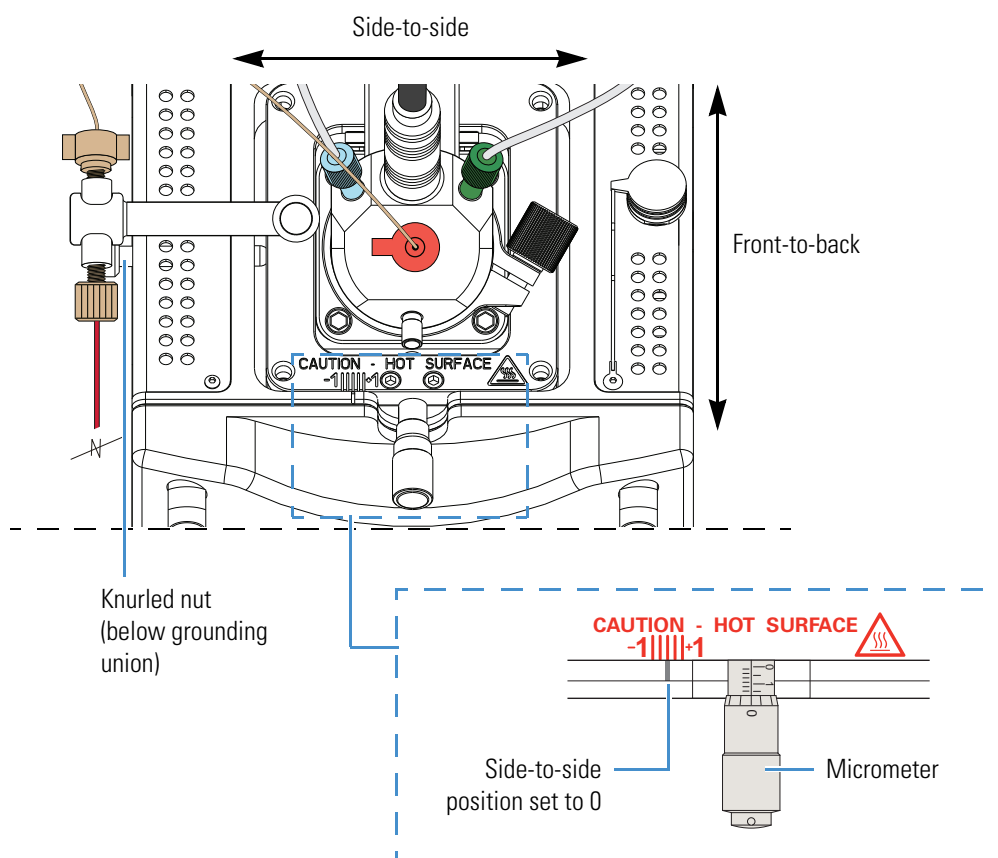
Note The probe position on the Ion Max-S API source housing is not adjustable.

❖ To adjust the probe position

Make any of the following adjustments:

- 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



2 Setting Up the API Source

Adjusting the Probe Position on the Ion Max API Source Housing

Automatic Tuning and Calibration in ESI Mode

This chapter describes how to tune and calibrate the LTQ Series MS. First, tune and calibrate the instrument in ESI mode—that is, set up the instrument 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 API source while running the automatic tune and automatic calibration options provided within the data system.

Generally, you must calibrate the instrument every 1 to 3 months of operation for optimum performance over the entire mass range of the detector.

Contents

- [Plumbing Connections](#)
- [Setting Up the Syringe Pump](#)
- [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](#)

Plumbing Connections

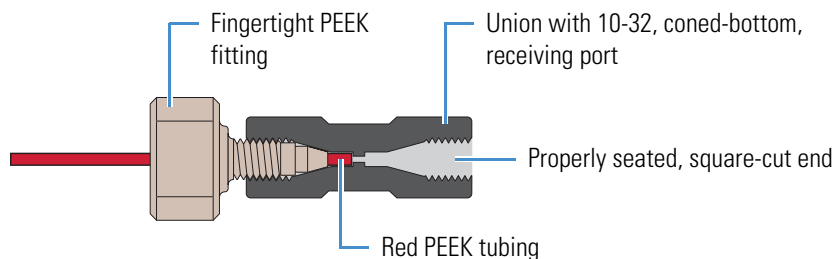
The divert/inject valve is a six-port, two-position, Rheodyne™ injection valve. The ports use standard 10-32 fittings for high-pressure and 1/16 in. OD tubing. To connect the high-pressure tubing to the valve, use the one-piece fingertight fittings provided in the MS Accessory Kit (P/N 97055-62055).

IMPORTANT To help ensure spray stability, make sure that all PEEK tubing is not crimped, kinked, or otherwise damaged.

Ensure the following when you make the plumbing connections:

- The ends of the PEEK tubing are squarely cut (Figure 37). For best results, use a polymeric tubing cutter to ensure square cuts. Poorly cut tubing can cause flow restrictions.
- The PEEK tubing makes contact with the bottom of the receiving port (Figure 37). Tubing that is not properly seated can add dead volume to a chromatographic system.
- The fittings are not overtightened. Tighten the PEEK fittings by using your fingers only, not a wrench. Overtightening the PEEK fittings can cause leaks.

Figure 37. Proper connection for the PEEK tubing and fitting (syringe adapter assembly)



Setting Up the Syringe Pump

Use the syringe pump to directly infuse solution for tuning and calibration.

IMPORTANT To minimize the possibility of cross-contamination, do the following:

- Use a different syringe and length of PEEK tubing for each type of solution.
- Wipe off the needle tip with a clean, lint-free tissue before reinserting the syringe into the syringe adapter assembly.

❖ To set up the syringe pump for direct infusion



CAUTION Sharp object. The syringe needle can puncture your skin. Handle it with care.

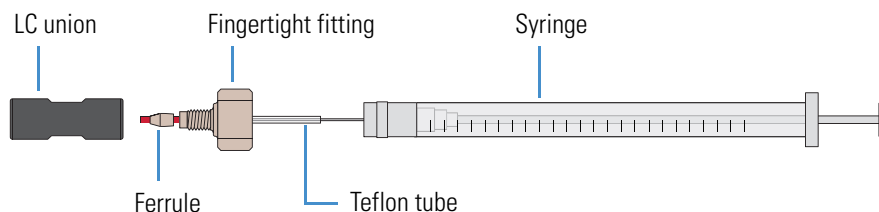
1. Load a clean, 500 μL Unimetrics™ syringe with 450 μL of the ESI calibration solution. The Chemical Kit contains the ESI calibration solution.

IMPORTANT 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 38).

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

Figure 38. Plumbing connection for the syringe



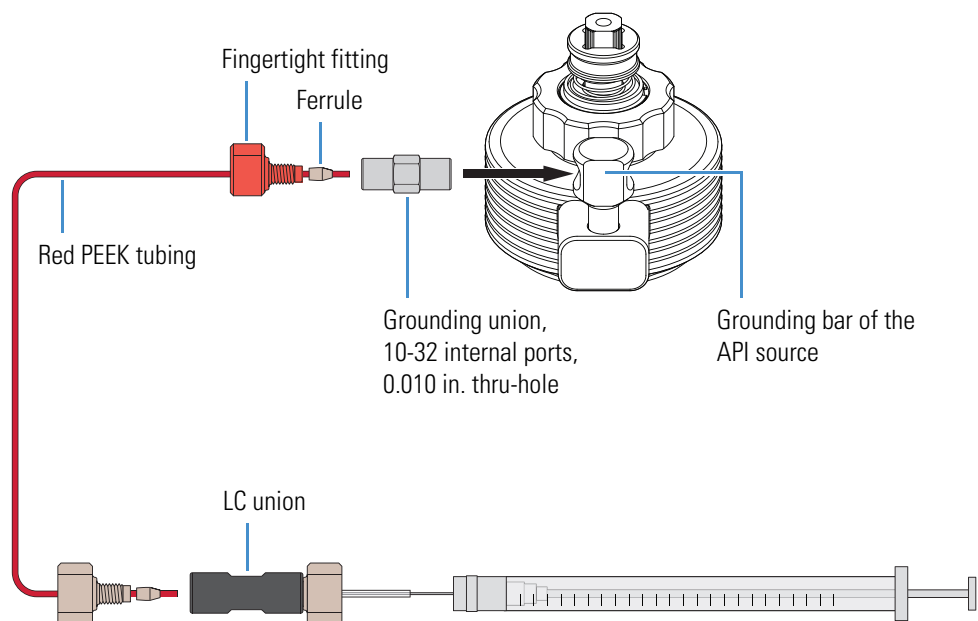
3. Place the syringe into the syringe holder of the syringe pump.
4. Use a fingertight fitting and ferrule to connect an infusion line of red PEEK tubing (0.005 in. ID, 1/16 in. OD) between the LC union and the grounding union held by the grounding bar of the API source.

Figure 39 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.

3 Automatic Tuning and Calibration in ESI Mode

Setting Up the Mass Spectrometer for Tuning and Calibration

Figure 39. 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 MS, 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 instrument automatically to optimize the parameters that affect ion detection.



CAUTION Before beginning normal operation of the mass spectrometer each day, verify 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 source. The presence of oxygen in the source when the mass spectrometer is on can be unsafe. In addition, if the mass spectrometer turns off during an analytical run, you might lose data.

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](#)

❖ **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 **C:\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 instrument.

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

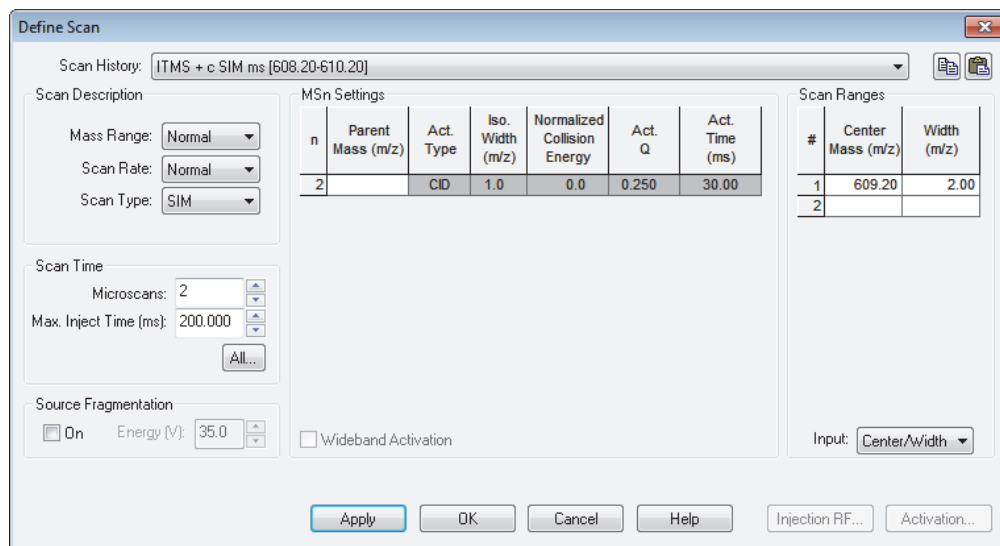
1. In the Tune Plus window, choose **Setup > ESI Source**.
2. Observe the settings.
3. Click **OK** when finished.

❖ **To define the scan parameters**



1. In the Tune Plus window, click the **Define Scan** button to open the Define Scan dialog box ([Figure 40](#)).

Figure 40. Define Scan dialog box



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 Automatic Tuning and Calibration in ESI Mode

Setting Up the Mass Spectrometer for Tuning and Calibration

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 Velos Pro MS, 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.

- For the LTQ XL and LXQ MSs, clear the **On** check box.


Note Selecting the Source Fragmentation check box allows collision-induced fragmentation to occur in the API source. Because collision-induced fragmentation performed in the API 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, type **150.00**.
 - c. In the Last Mass (m/z) column, type **2000.00**.
6. Make sure the settings in the Define Scan dialog box are the same as those shown in [Figure 40](#) on [page 67](#).
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.

Centroid 

Profile 

Positive polarity 

Negative polarity 

Testing the Mass Spectrometer in ESI Mode

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

IMPORTANT Do not infuse the calibration solution at syringe pump flow rates above 10 $\mu\text{L}/\text{min}$. If you use high concentrations of the Ultramark 1621 component, you can contaminate the sample.

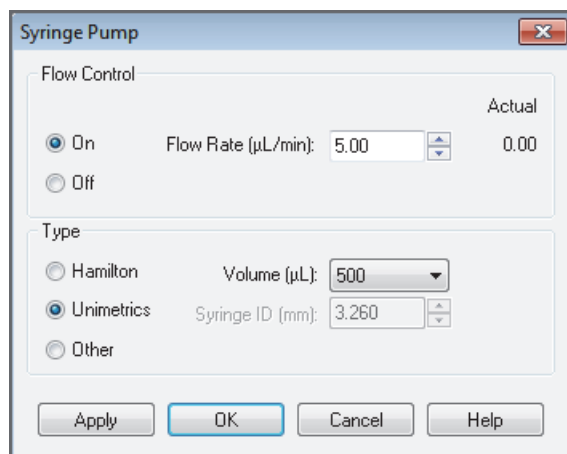
Follow these procedures:

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

❖ To infuse the calibration solution into the API source

1. In the Tune Plus window, choose **Setup > Syringe Pump** to open the Syringe Pump dialog box (Figure 41).

Figure 41. 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 Automatic Tuning and Calibration in ESI Mode

Testing the Mass Spectrometer in ESI Mode

3. Under Type, do the following:

a. Select the type of syringe.

A 500 μL Unimetrics syringe is supplied with the LTQ Series MS. 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 (μL) list becomes unavailable.

b. 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.

4. Click **OK**.

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

Before starting the next procedure, make sure that you have set up the syringe pump to infuse the ESI calibration solution as described in “[To infuse the calibration solution into the API source](#)” on [page 69](#).

❖ 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 instrument, it begins scanning, nitrogen flows into the ESI API source, high voltage is applied to the ESI API 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.



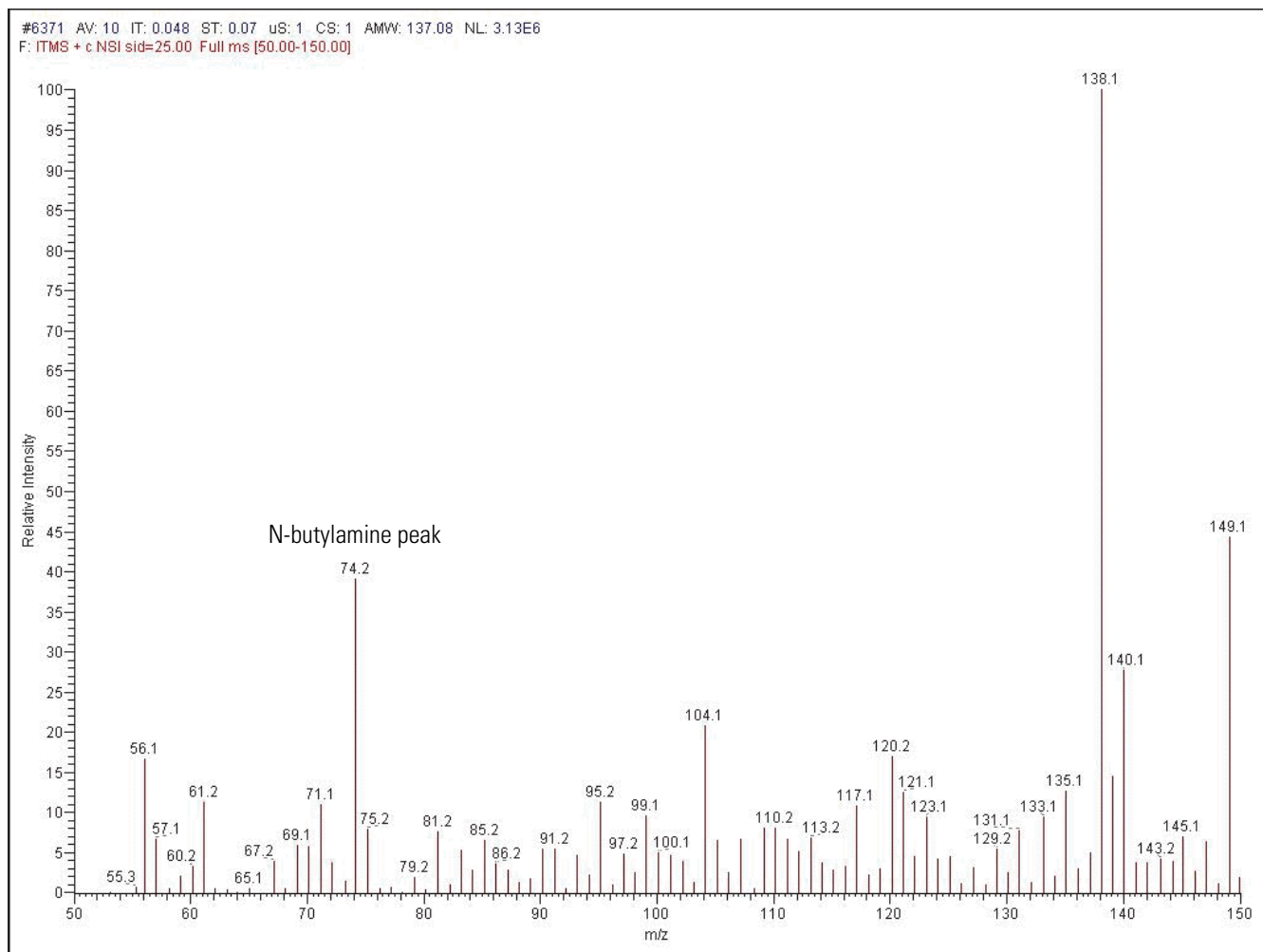
2. Click the **Display Spectrum View** button.

3. Observe the mass spectra of the singly-charged ions in the calibration solution ([Figure 42](#), [Figure 43](#), and [Figure 44](#) on pages [71](#) to [73](#)).

The ions are as follows:

- Caffeine: m/z 138.1 and 195 for the Velos Pro MS; m/z 195 for the LTQ XL and LXQ MSs
- MRFA (met-arg-phe-ala acetate salt): m/z 524
- Ultramark 1621: m/z 1022, 1122, 1222, 1322, 1422, 1522, 1622, 1722, 1822, and 1922

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



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 MS (upper range)

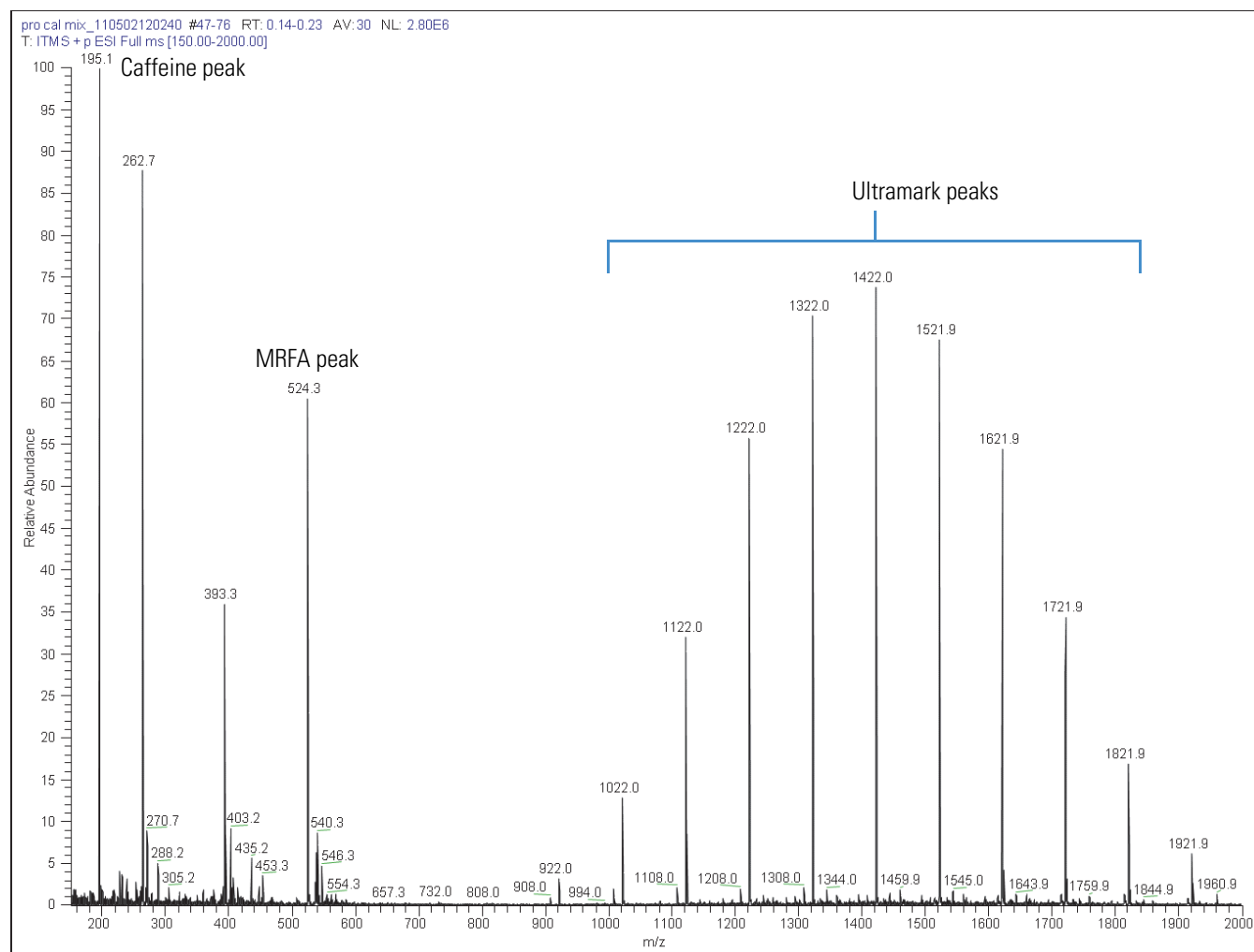
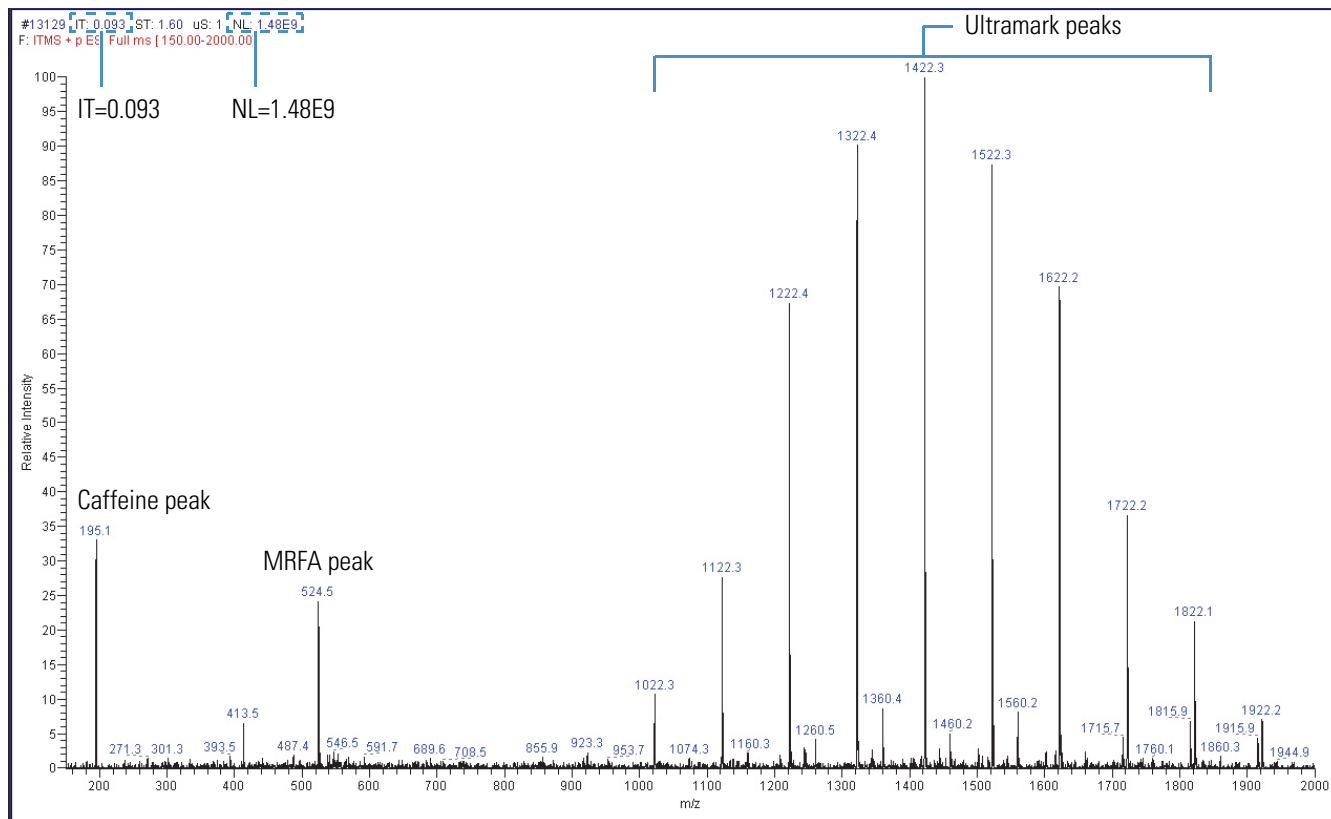


Figure 44. Spectrum of the calibration solution for the LTQ XL MS



- At the top of the Spectrum view, check the values for the ionization time (IT) and normalization level (NL) (Figure 44).
- Choose **Setup > ESI Source**.
- Check the Spray Current readback.
- 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 these questions, then the instrument is operating properly.

If you answered “no” to either of these questions, go to [step 8](#).

8. Try the following troubleshooting measures:
 - Make sure that the fused-silica sample tube on an 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*.
 - Make sure that the entrance to the ion transfer tube is clean and not blocked.
 - Make sure that the solution entering the probe is free of air bubbles and that the tubing and connectors are free of leaks.
 - Make sure that the flow rate of the sheath gas is 5–8 units.

If you have demonstrated that the instrument is operating properly in ESI mode, you are now ready to tune and calibrate the instrument. Leave the instrument 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

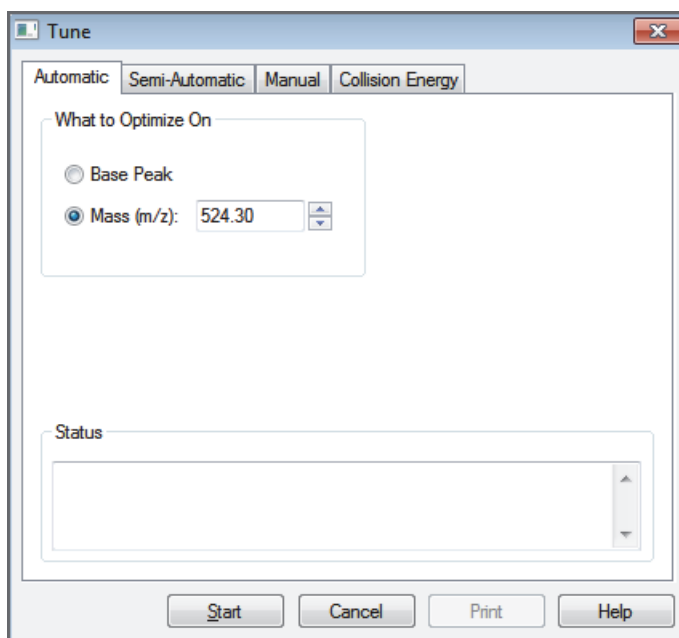
To optimize important parameters, tune the LTQ Series MS automatically in ESI mode.

❖ To automatically tune the instrument in ESI mode



1. In the Tune Plus window, click the **Tune** button to open the Tune dialog box ([Figure 45](#)).

Figure 45. 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, **524.3** in the Mass (*m/z*) box.

The instrument optimizes the tune on the peak at *m/z* 524.3. 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. Make sure that the syringe contains at least 450 μL of calibration solution.

The Chemical Kit contains the ESI calibration solution

6. Click **OK**.



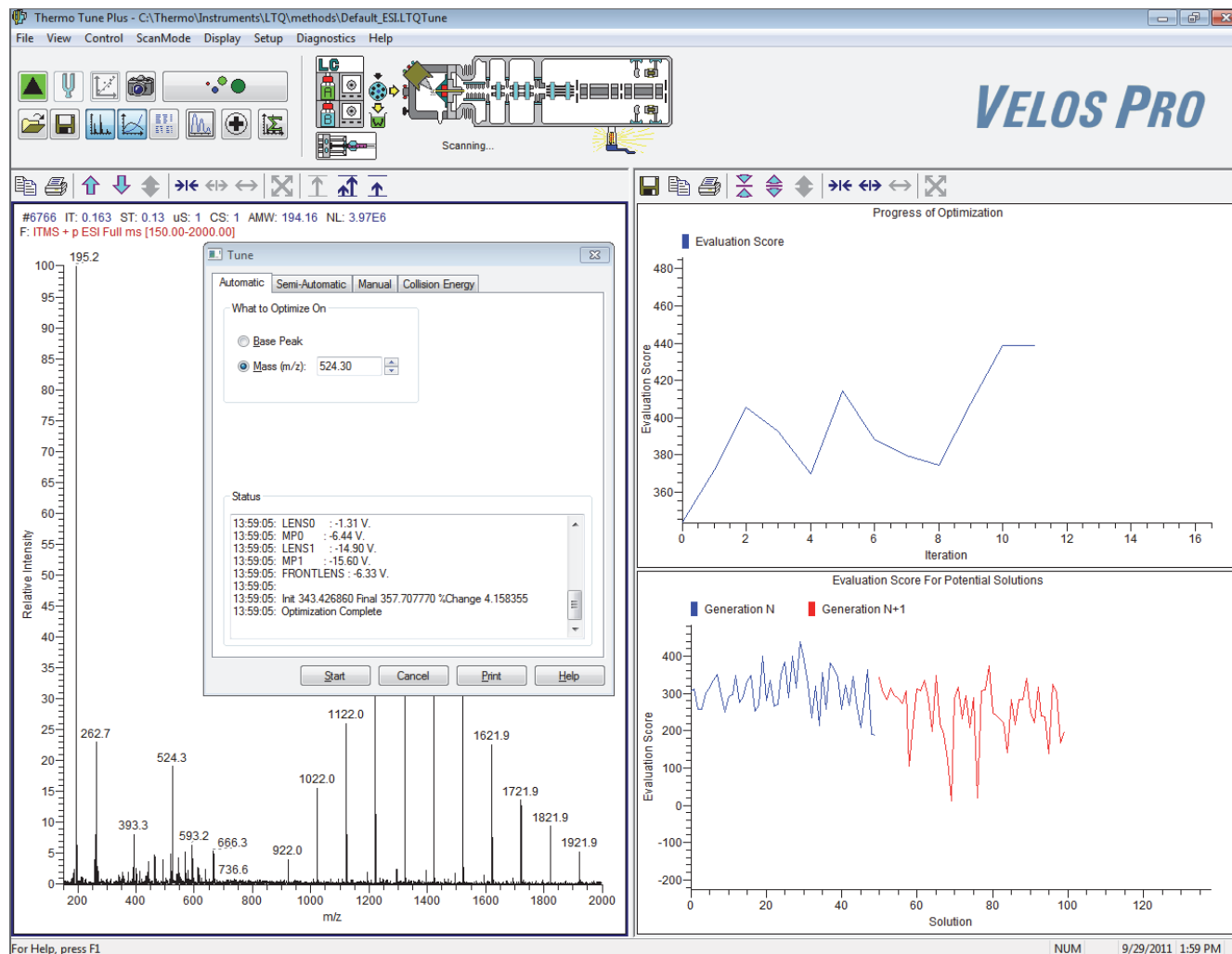
7. In the Tune Plus window, click the **Display Graph View** button.
8. Observe the Tune Plus window and the Tune dialog box.

While automatic tuning is in progress, the instrument 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. [Figure 46](#) shows the Velos Pro spectrum.

3 Automatic Tuning and Calibration in ESI Mode

Tuning the Mass Spectrometer Automatically in ESI Mode

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



When the Optimization Complete message appears in the Status box of the Tune dialog box, it 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 instrument on a different analyte or at a different flow rate.

Note You must save the tune method while the instrument is on.

❖ To save the ESI tune method



1. In the Tune Plus window, click the **Save** button.
2. Browse to the **C:\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

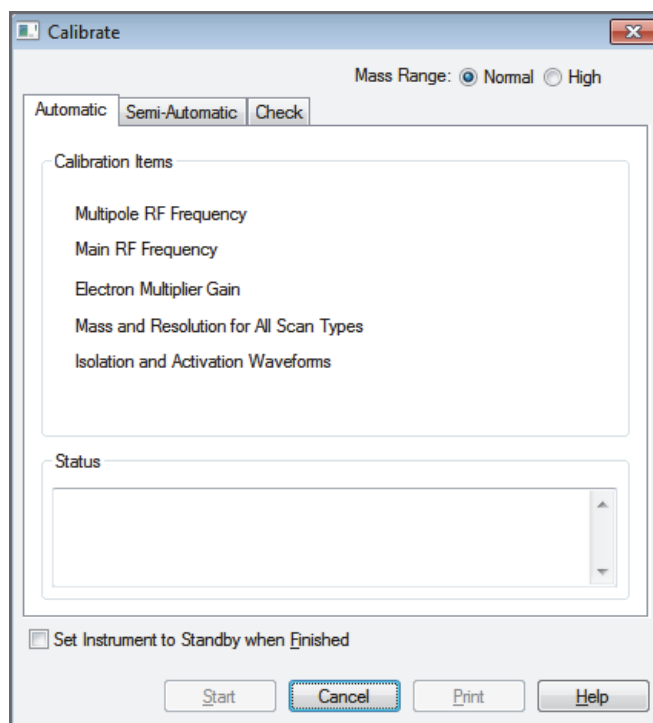
Note To calibrate the instrument in the high mass range, see [Appendix C, “High Mass Range Calibration.”](#)

❖ To automatically calibrate the instrument 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 instrument switch to Standby mode after the calibration, click the **Set Instrument to Standby when Finished** check box.
 - To have the instrument remain in the On mode after the calibration, clear the **Set Instrument to Standby when Finished** check box.
3. Click **Start**.

A message appears:

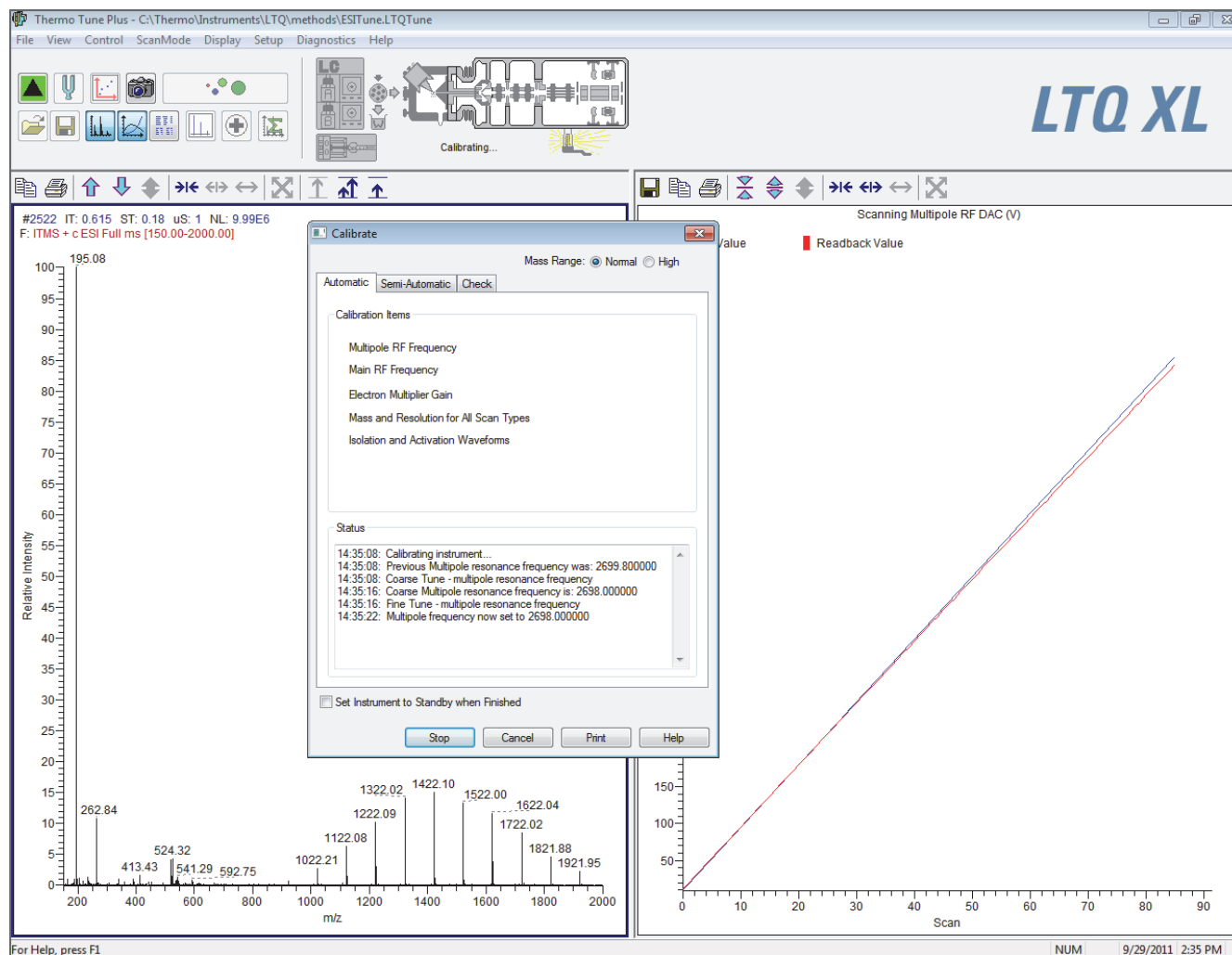
Please ensure that the 500 microliter syringe is full.

4. Make sure that the syringe contains at least 450 μL of the calibration solution.
The Chemical Kit contains the ESI calibration solution.

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 MS



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 79).

- If a calibration item is successful, the instrument 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 the automatic calibration, green checks ✓, red X marks ✗, or both appear in the Result column of the Semi-Automatic page of the Calibrate dialog box. A green check indicates a successful calibration and a red X indicates a failed calibration for the corresponding item. The Status box at the bottom of the Calibrate dialog box provides additional information.

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

After all the calibration items are successful, the instrument 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 Velos Pro MS: m/z 74, 138, 195, 524, 1222, 1522, and 1822
- For the LTQ XL and LXQ MSs: m/z 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 instrument in the high mass range, see Appendix C, “High Mass Range Calibration.”

Before tuning with an analyte, you must clean the instrument 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 81.

Tuning with an Analyte in ESI Mode

This chapter describes how to tune the LTQ Series MS 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 instrument, 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 instrument parameters significantly impact signal quality in ESI mode:

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

The settings for these parameters depend on the solvent flow rate and target analyte composition. In general, fine-tune the instrument 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 instrument's sheath, auxiliary, and sweep gas pressures; ESI needle (or "spray") voltage; ion transfer tube temperature; 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 instrument. For ESI only, set the ion transfer tube temperature proportional to the flow rate of the solution. Refer to the Tune Plus Help for guidelines on the operating parameters.

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 instrument on the particular analyte, see [Chapter 5, "Acquiring ESI Sample Data by Using Tune Plus,"](#) for instructions about acquiring sample data.

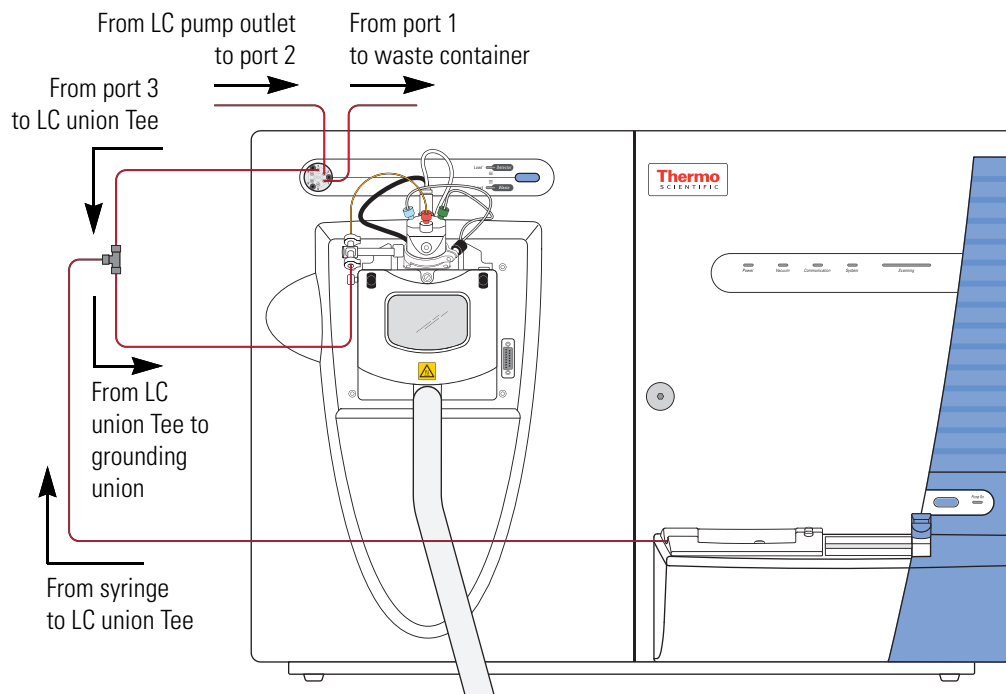
Before optimizing the tune for the analyte, make sure that the instrument has been calibrated within the last 3 months. If the system needs to be calibrated, follow the procedures in [Chapter 3, "Automatic Tuning and Calibration in ESI Mode."](#)

IMPORTANT If you make changes to the tune parameters and do not save them before switching to another API source probe, the changes are lost. In addition, when you switch from one API source probe to another, the Tune Plus application automatically loads the last saved tune parameters for the newly installed API source probe and overwrites the tune parameters from the previously installed API source probe.

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

This section describes how to set up the LTQ Series MS 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 5](#), which are in the instrument accessory kit.

Table 5. Inlet plumbing parts for high-flow infusion analysis (Sheet 1 of 2)

Description	Part number
Grounding union (ESI mode)	00101-18182
LC union Tee	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 5. 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

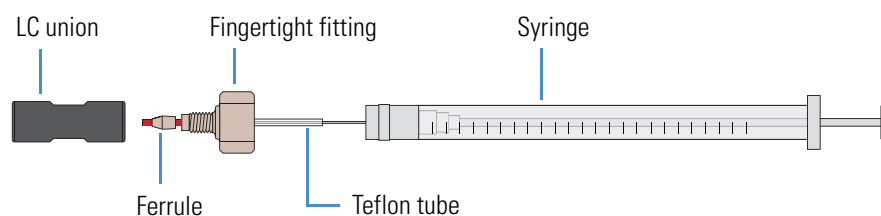
❖ To set up the syringe pump



CAUTION Sharp object. The syringe needle can puncture your skin. Handle it with care.

1. Load a clean, 500 μL Unimetrics syringe with the 100 $\text{pg}/\mu\text{L}$ reserpine stock tuning solution or the analyte.
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 API source ([Figure 39](#) on [page 66](#)).
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

Follow these procedures to connect solvent flow from the syringe pump and the LC pump to the API source:

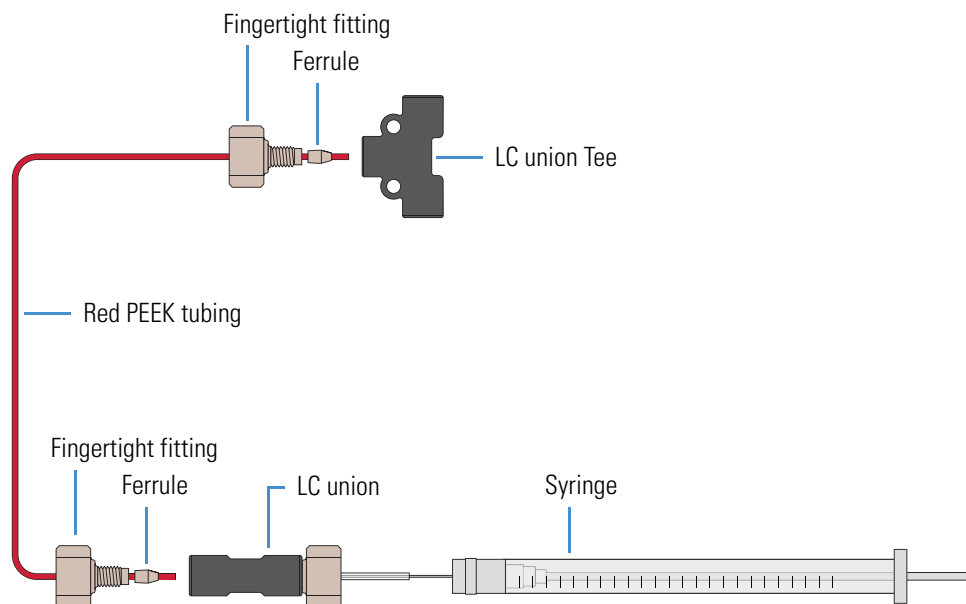
1. To connect the syringe to the LC union Tee
2. To connect the LC union Tee to the divert/Inject valve
3. To connect the LC pump to the divert/inject valve
4. To connect the divert/ inject valve to a waste container
5. To connect the LC union Tee to the grounding union
6. To connect the grounding union to the ESI probe sample inlet

❖ To connect the syringe to the LC union Tee

1. Set up the syringe pump (see [page 84](#)).
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 union Tee.

[Figure 51](#) shows the fittings required to connect the LC union to the union Tee.

Figure 51. Plumbing connections between the LC union Tee and the LC union



❖ **To connect the LC union Tee 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 union Tee (Figure 52 and Figure 53).

Figure 52. Six-port divert/inject valve connections

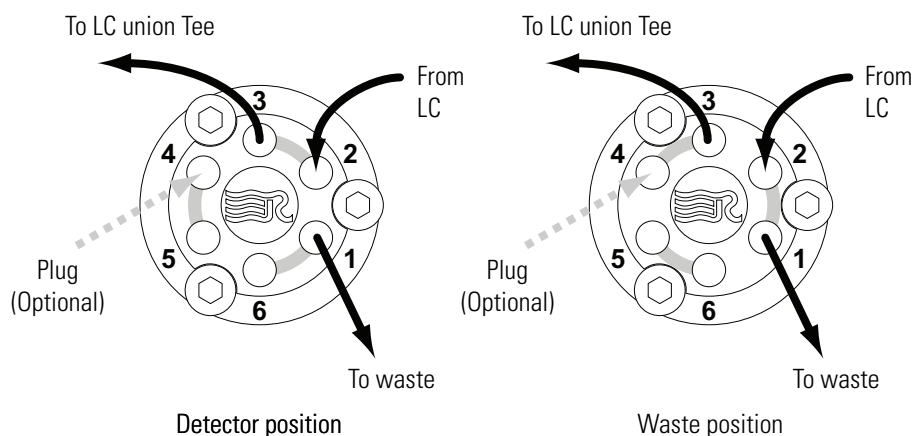
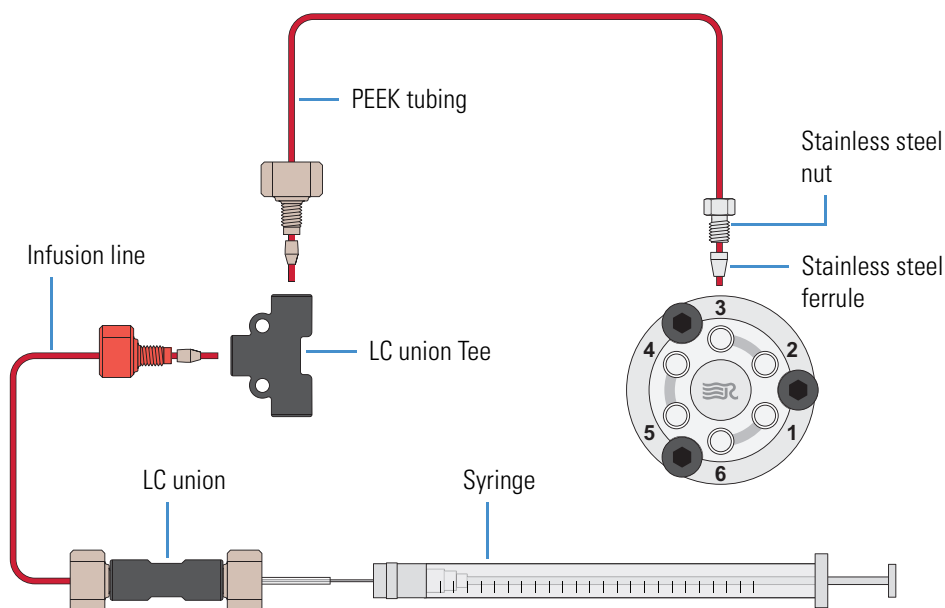


Figure 53. Connections between the LC union Tee and the divert/inject valve



❖ **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 86).
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**

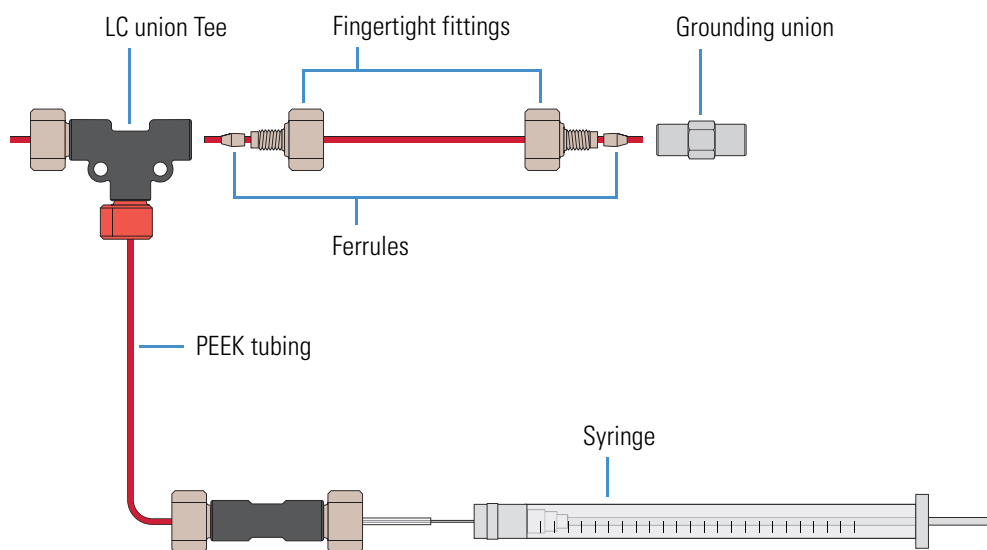
1. Use a fingertight fitting and a ferrule to connect a length of red PEEK tubing to port 1 of the divert/inject valve (Figure 52 on page 86).
2. Insert the other end of the tubing into a suitable waste container.

❖ **To connect the LC union Tee 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 union Tee (Figure 55).

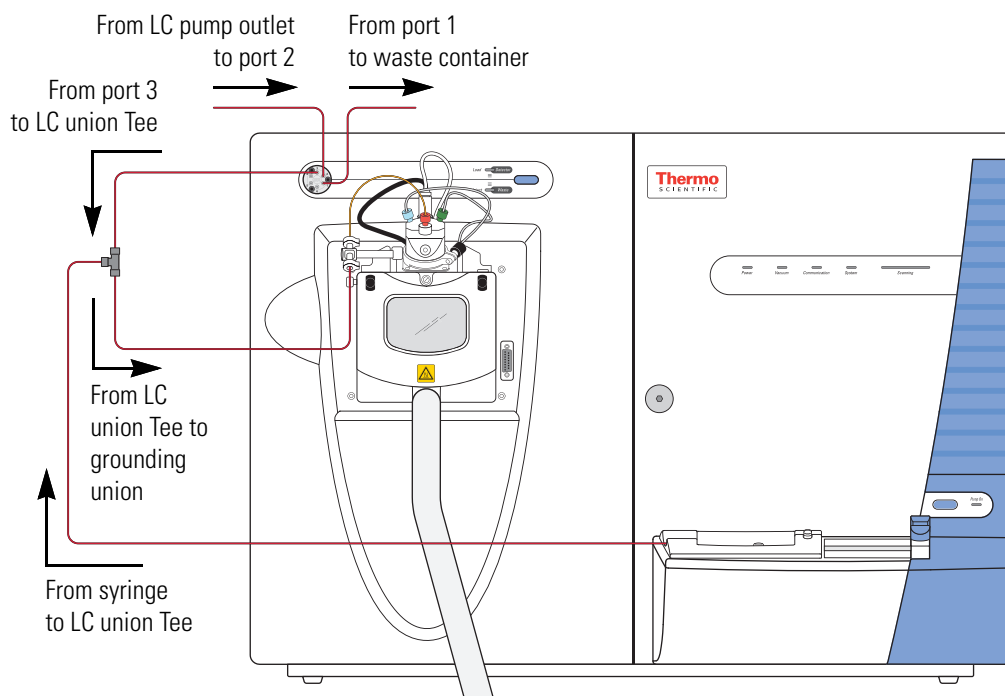
The grounding union slides into the grounding bar on the API source as shown in Figure 54. For instructions about connecting the grounding union to the ESI probe sample inlet, refer to the *LTQ Series Hardware Manual*. Figure 56 shows the complete connection.

Figure 54. Connections between the LC union Tee 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 87).

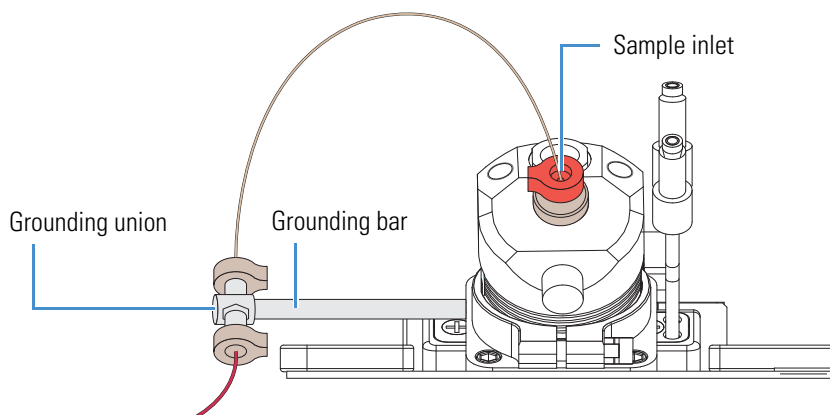
Figure 55. Plumbing setup for ESI/MS sample introduction with high-flow infusion



❖ **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 MS in ESI mode by using a high solvent flow of the 100 pg/ μ L reserpine tuning solution or a solution of the analyte. 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

❖ 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 instrument 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 **C:\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 instrument, and the title bar in the Tune Plus window lists the name of the current tune method, for example, **C:\Thermo\Instruments\LTQ\ESImyTune**.

4 Tuning with an Analyte in ESI Mode

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

❖ 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

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	10.00

#	Center Mass (m/z)	Width (m/z)
1	609.20	2.00
2		

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 API source. Because collision-induced fragmentation performed in the API 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, make sure that the settings in the Define Scan dialog box match those shown in [Figure 57](#) on [page 90](#).
 - If using another analyte, make sure 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 instrument 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 instrument 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 MS 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 instrument. The automated calibration procedure takes approximately 20 minutes. Or, for best results in a limited amount of time, infuse the ESI calibration solution (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 78](#).

Centroid 

Profile 

Positive polarity 

Negative polarity 

Note The following are the most important parameters that affect signal quality during ESI operation:

- (For the Velos Pro MS) Electrospray voltage, ion transfer tube temperature and voltage, S-lens rf level, gases, and solution flow rate
- (For the LTQ XL and LXQ MSs) Electrospray voltage, ion transfer tube temperature and voltage, tube lens voltage, gases, and solution flow rate

The most important parameters for long-term performance are the voltage gradients between MP00, MP0, and the front lens voltage. Automatic tuning ensures a sufficient voltage gradient so that the instrument continues to perform well under extended usage.

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

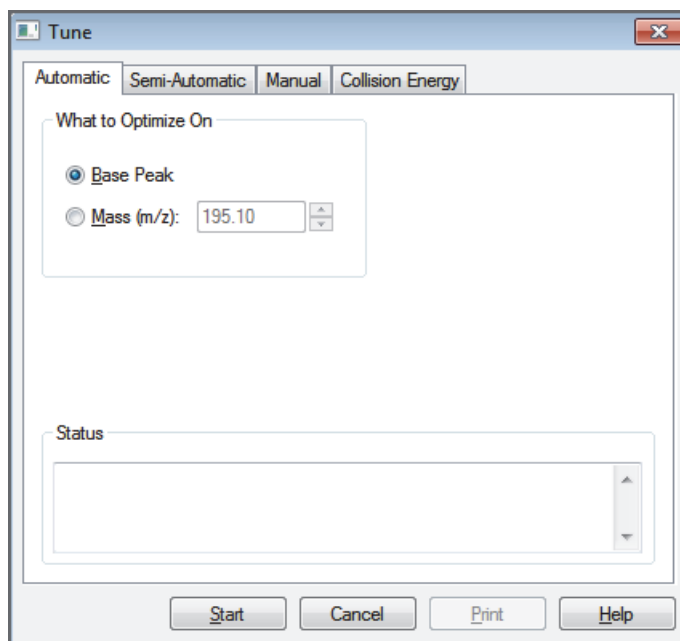
The following procedure uses a 100 pg/mL solution of reserpine to optimize the LTQ Series automatically on the reserpine peak at m/z 609.2 at a flow rate of 0.4 $\mu\text{m}/\text{min}$. You can substitute the reserpine solution with a sample solution that is suitable for analysis in ESI mode.

❖ **To automatically optimize the tune of the instrument 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

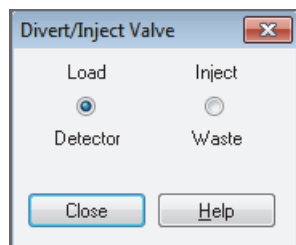


2. Under What to Optimize On, select the **Mass (m/z)** option.
3. In the Mass (m/z) box, enter **609.2** (or the appropriate mass of the analyte).



- Click the **Divert/Inject Valve** button to open the Divert/Inject Valve dialog box (Figure 59).

Figure 59. Divert/Inject Valve dialog box



- 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 86). Port 3 is connected to the API source.

- In the Tune dialog box, start the automatic tuning procedure as follows:
 - Click **Start**.
A message appears:
Please ensure that the 500 microliter syringe is full.
 - Make sure that the syringe pump contains at least 450 μL of the 100 $\text{pg}/\mu\text{L}$ reserpine tuning solution or the analyte.
 - Click **OK**.



- In the Tune Plus window, click the **Display Graph View** button.
- Observe the Tune Plus window and the Tune dialog box.

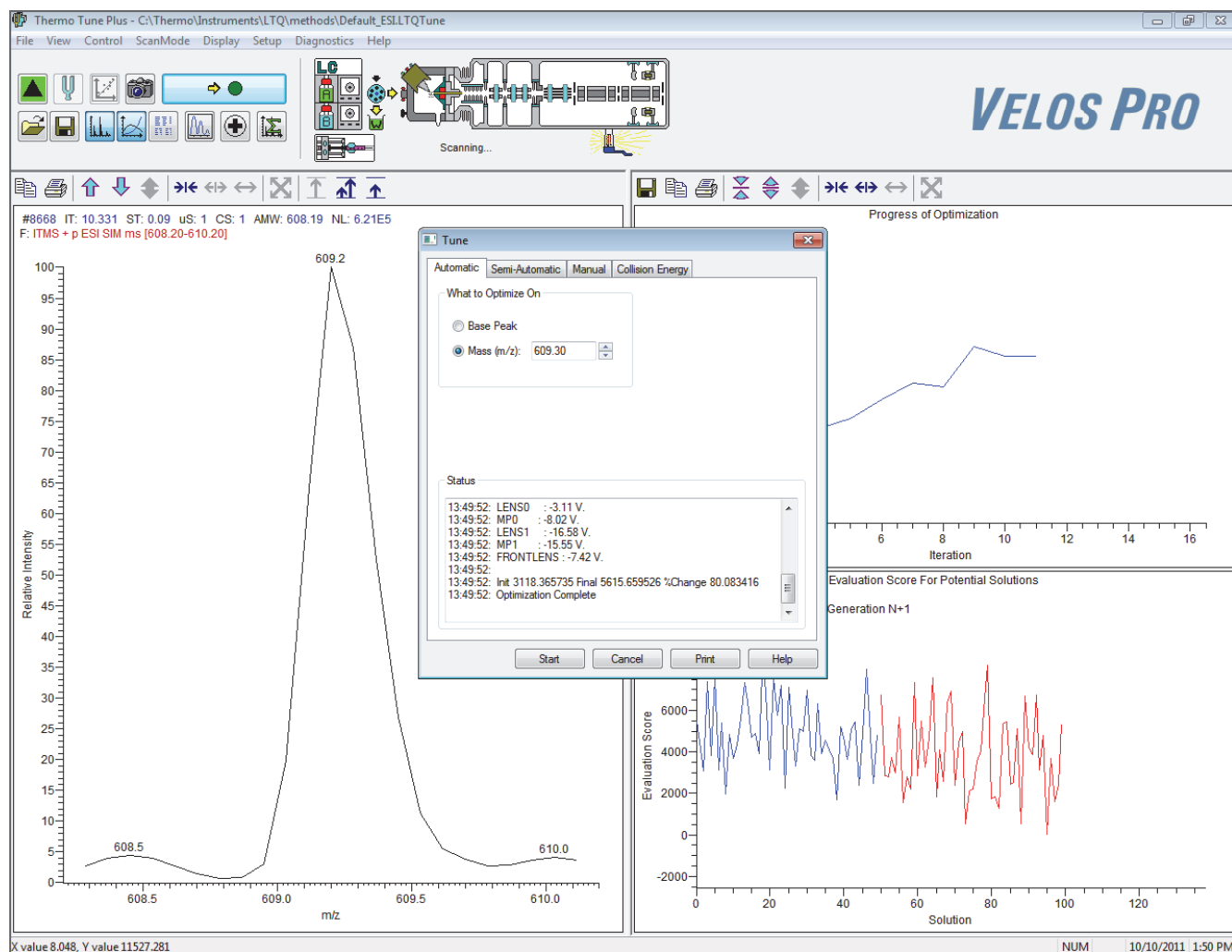
During automatic tuning, the instrument displays the results of various tests in the Spectrum and Graph views in the Tune Plus window and displays various messages under Status in the Tune dialog box.

Check that the Tune Plus window looks similar to the one shown in Figure 60.

4 Tuning with an Analyte in ESI Mode

Tuning the Mass Spectrometer Automatically

Figure 60. Tune Plus window and Tune dialog box for Velos Pro MS automatic tuning in ESI mode



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

Saving the ESI Tune Method

IMPORTANT Save the tune method before you change the type of probe.

❖ To save the ESI tune method



1. In the Tune Plus window, click the **Save** button.
2. Browse to the **C:\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 instrument by using reserpine, clean it 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.

4 Tuning with an Analyte in ESI Mode

Saving the ESI Tune Method

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 from the LTQ Series MS 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 instrument 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 precursor 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 application.

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 83](#). For APCI mode, see [page 110](#).

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. Make sure 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.

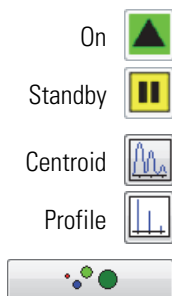
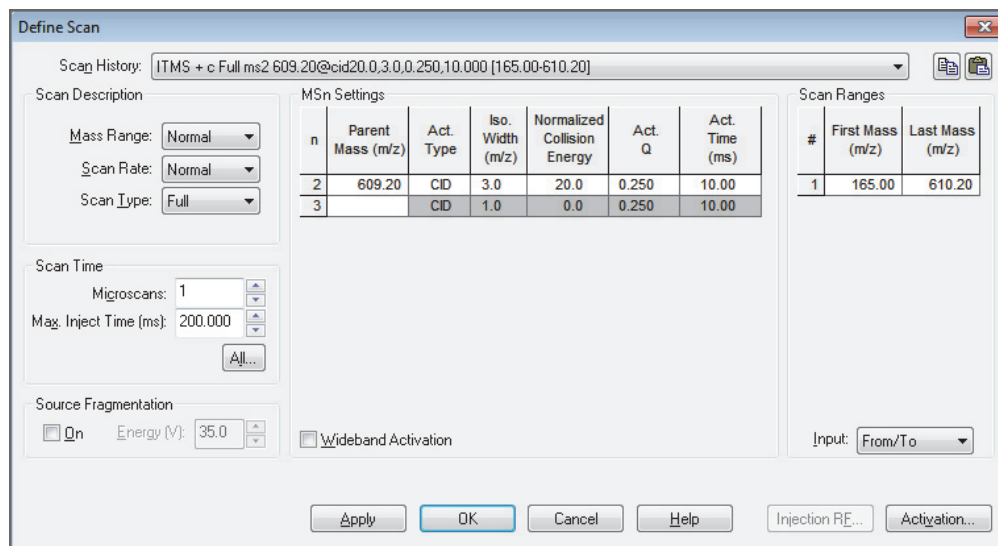


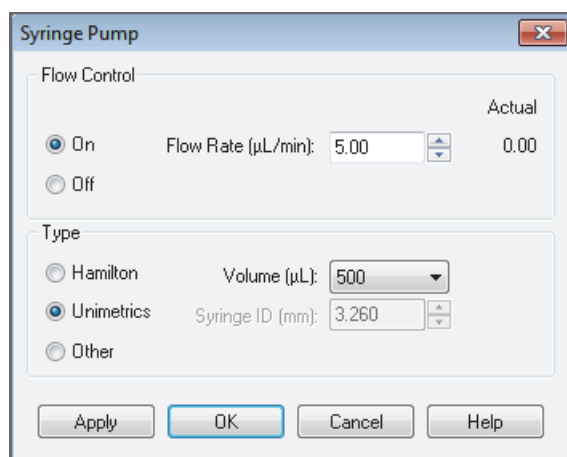
Figure 61. Define Scan dialog box for acquiring reserpine full-scan data



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

- c. Under MSⁿ 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 precursor ion of reserpine, *m/z* 609.2, or the precursor ion of the analyte. Make sure that the readback values for NL and IT are relatively stable.
 - b. In the Define Scan dialog box, under MSⁿ 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 precursor 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 precursor ion, and make sure 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

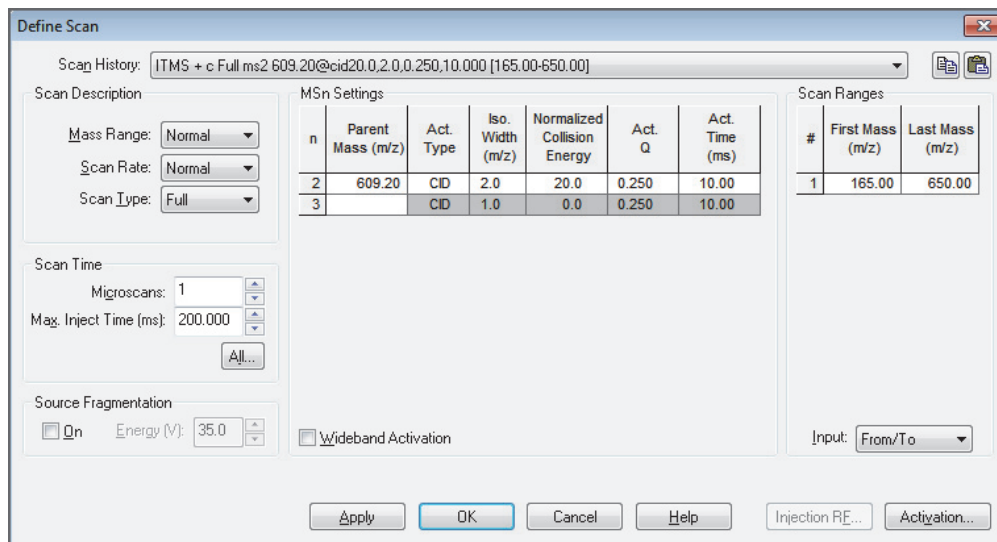


1. In the Tune Plus window, click the **Define Scan** button to open the Define Scan dialog box ([Figure 63](#)).
2. Under MSⁿ Settings, in the Normalized Collision Energy column, type **20.0**.
3. Click **Apply**.
4. In the Tune Plus window, observe the mass spectrum of the product ions of reserpine (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)



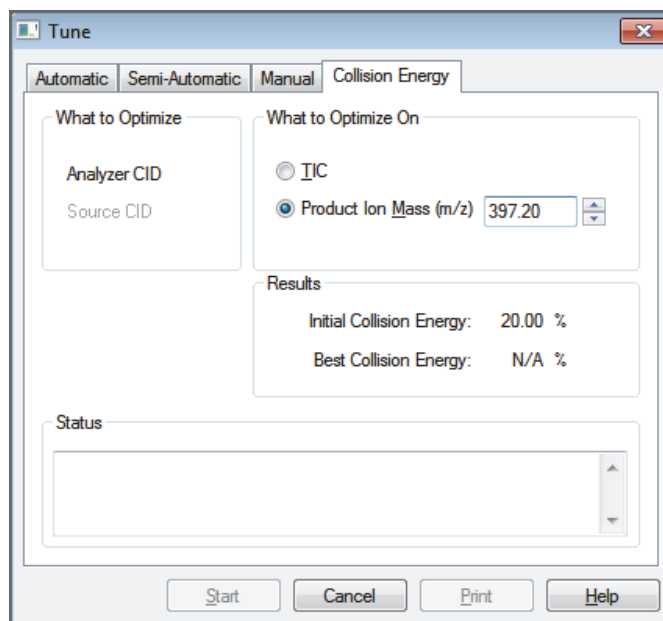
Optimizing the Collision Energy Automatically for an MS/MS Experiment

❖ 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).



Figure 64. Tune dialog box showing the Collision Energy page



5 Acquiring ESI Sample Data by Using Tune Plus

Setting Up to Acquire Full-Scan MS/MS Data

- 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 instrument can optimize the collision energy required to analyze the analyte automatically by using the m/z value of one of its product ions.

- Click **Start**.

A message appears:

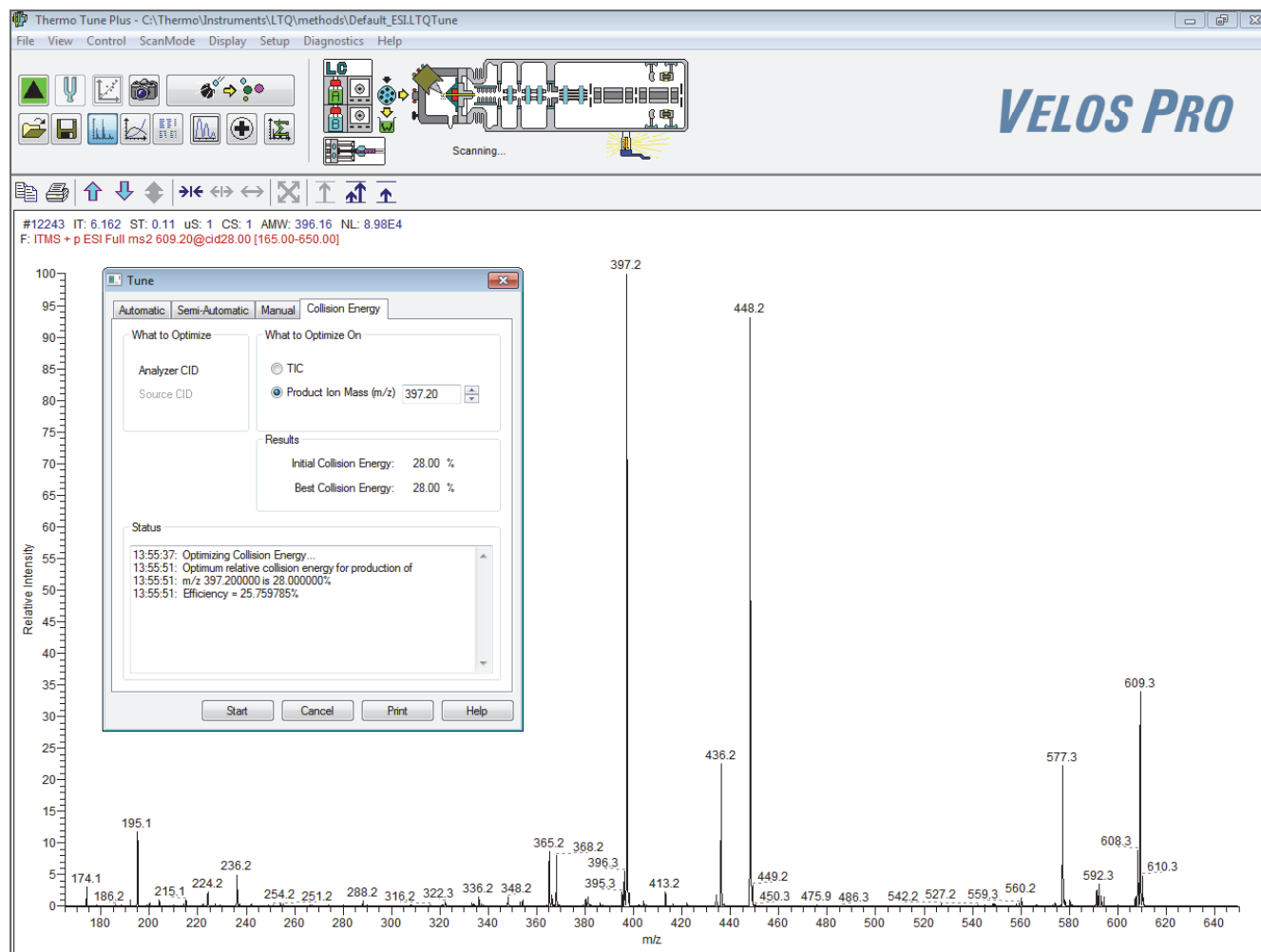
Ensure that the 500 microliter syringe is full.

- Make sure that the syringe contains at least 450 μL of the 100 $\text{pg}/\mu\text{L}$ reserpine tuning solution or the analyte.

- Click **OK**.

- Check that the Tune Plus window looks similar to the one shown in [Figure 65](#).

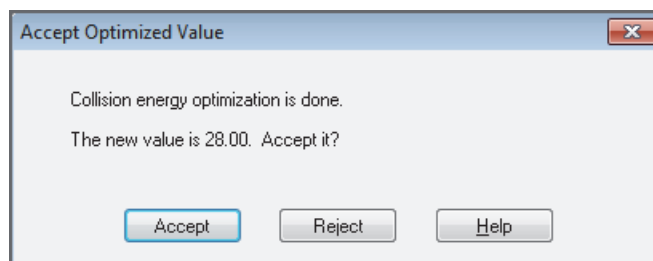
Figure 65. Tune Plus window and the Tune dialog box showing the Collision Energy page



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 instrument is ready to acquire MS/MS data on the analyte.

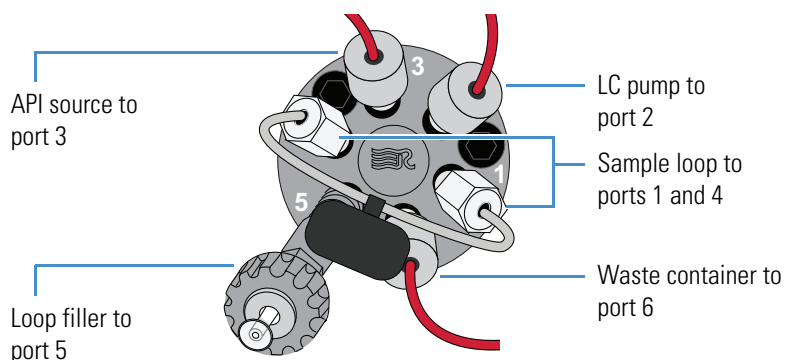
Setting Up the Inlet for Flow Injection Analysis in ESI Mode

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

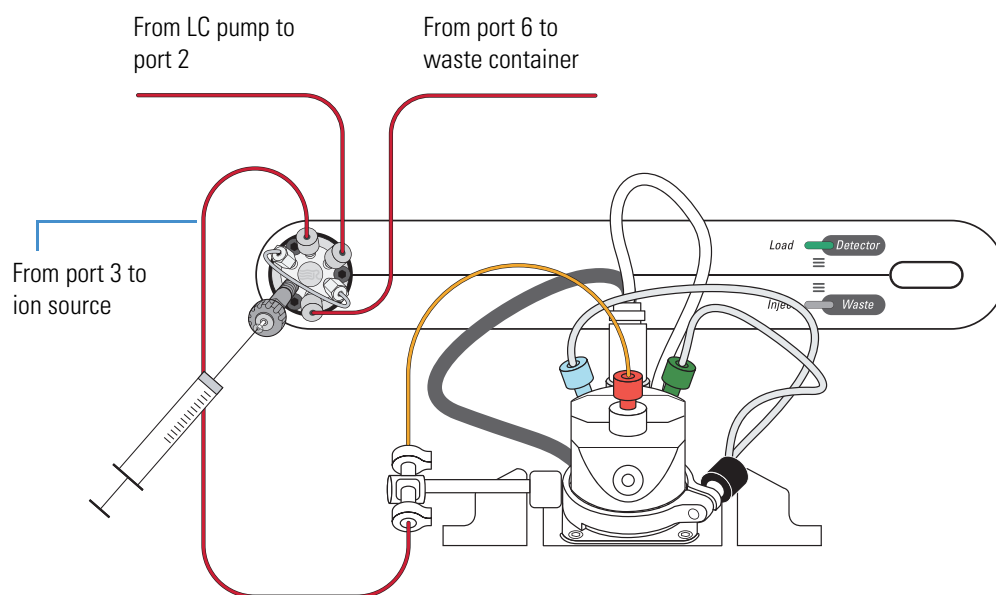


2. Connect a sample loop of the desired volume to ports 1 and 4.
3. Connect the LC pump to port 2 of the divert/inject valve as follows:
 - Use an appropriate fitting and a ferrule to connect one end of a length of red PEEK tubing to the outlet of the LC pump.

To 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

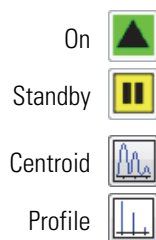


6. Connect the divert/inject valve to a waste container as follows:
 - Use a fingertight fitting and a ferrule to connect one end of a length of red PEEK tubing to port 6.
 - Connect the other end of the tubing to an appropriate waste container.

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

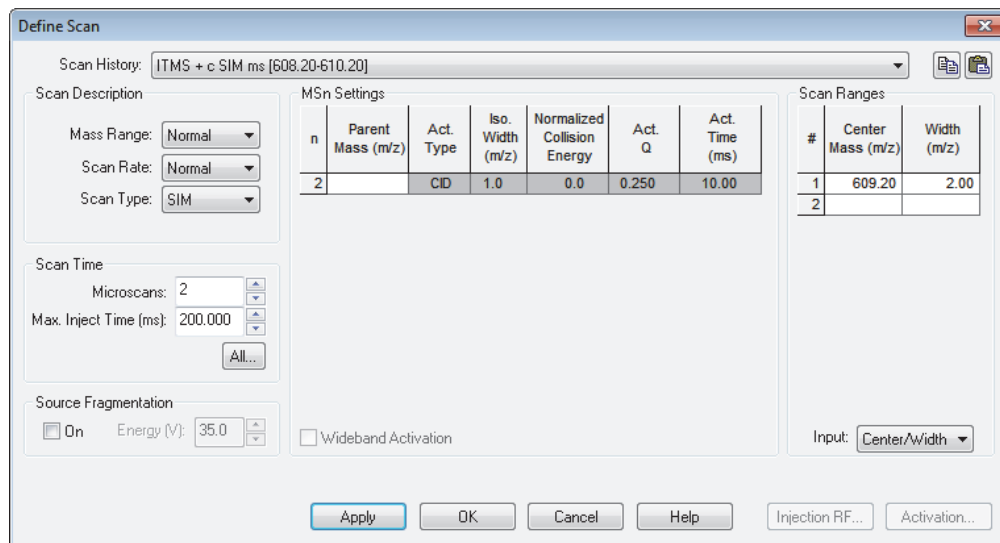
2. Click the **Centroid/Profile** button to select the **centroid** data type.

3. Make sure 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)



- 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**.

Make sure that the system is free of leaks.

5 Acquiring ESI Sample Data by Using Tune Plus

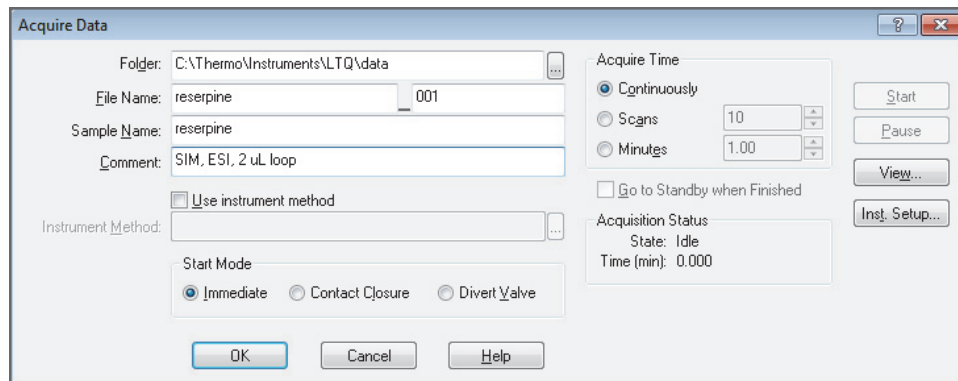
Acquiring ESI Data in the SIM Scan Type



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 application. To open the XReport application, choose **Start > All 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.



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 solution, follow one of these procedures:

- For the Velos Pro MS, see “Preparing the Reserpine Sample Solution for ESI and APCI Modes” on page 136.
- For the LTQ XL and LXQ MSs, see “Preparing the Reserpine Sample Solution for the ESI and APCI Modes” on page 140.

- 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 API 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 data file just acquired by using the 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).

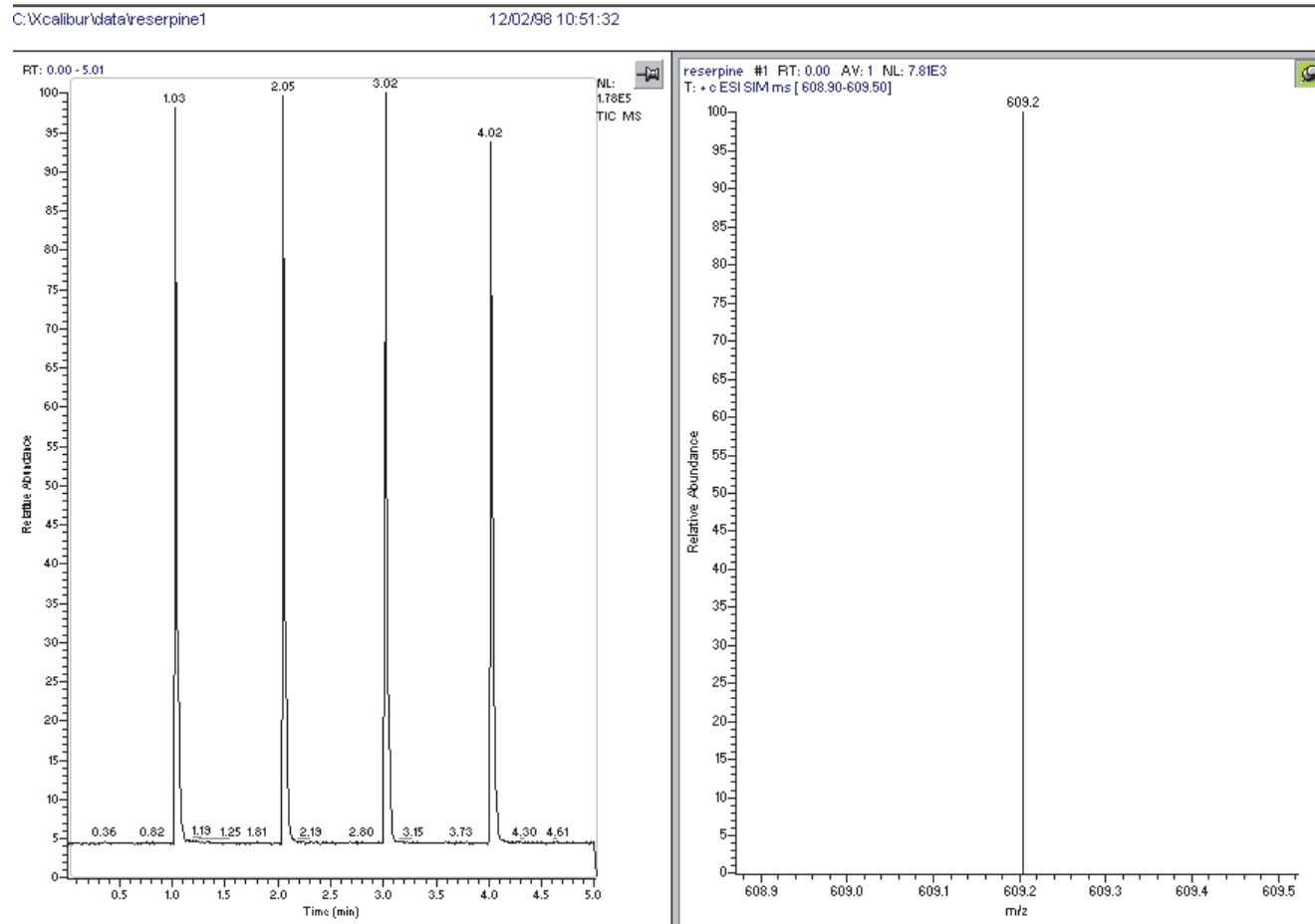
For more information about reviewing the acquired data, refer to the *Thermo Xcalibur Qualitative Analysis User Guide* or the Qual Browser Help.

Note To acquire full-scan MS/MS data, see “Setting Up to Acquire Full-Scan MS/MS Data” on page 97.

5 Acquiring ESI Sample Data by Using Tune Plus

Acquiring ESI Data in the SIM Scan Type

Figure 71. ESI SIM chromatogram and mass spectrum from the Qual Browser window



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 MS when switching to APCI operation. You can use the calibration settings obtained from the successful automatic calibration procedure performed in ESI mode.

IMPORTANT If you make changes to the tune parameters and do not save them before switching to another API source probe, the changes are lost. In addition, when switching from one API source probe to another, the Tune Plus application automatically loads the last saved tune parameters for the newly installed API source probe and overwrites the tune parameters from the previously installed API source probe.

The following instrument parameters significantly impact signal quality in APCI mode:

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

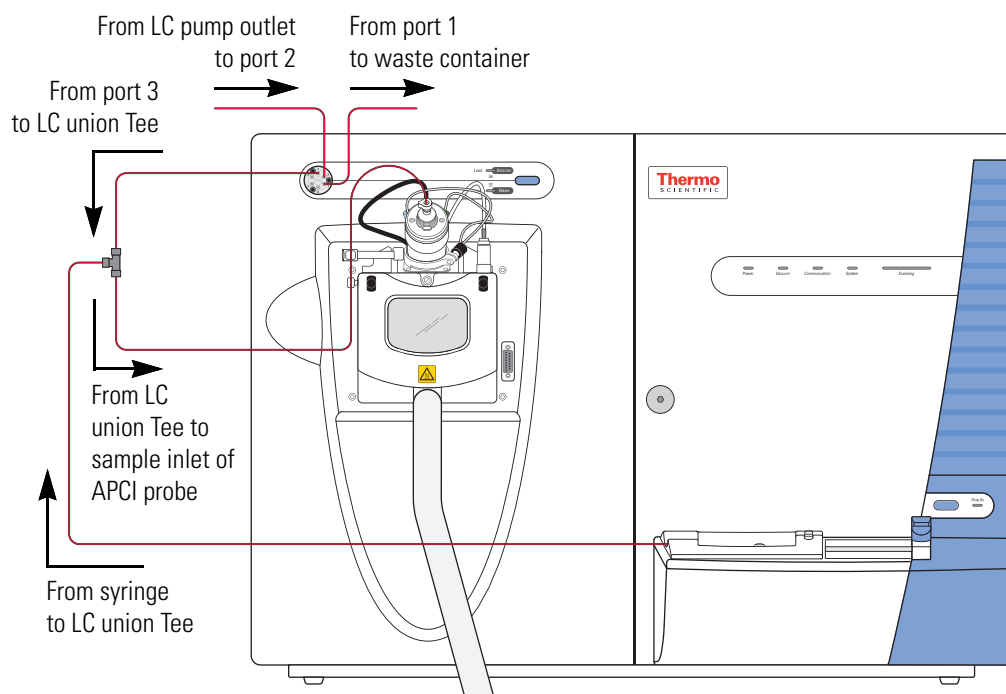
- These procedures assume that you are familiar with the LTQ Series MS and the Tune Plus window. For more information, refer to the data system Help, *LTQ Series Getting Connected Guide*, and *LTQ Series Hardware Manual*, as needed.
- Before setting up the inlet plumbing, make sure that the API source is set up for APCI mode and that you have tuned and calibrated the instrument in ESI mode.
- This chapter refers to a 100 pg/ μ L reserpine tuning solution 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 MS 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 5](#) on [page 83](#) 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



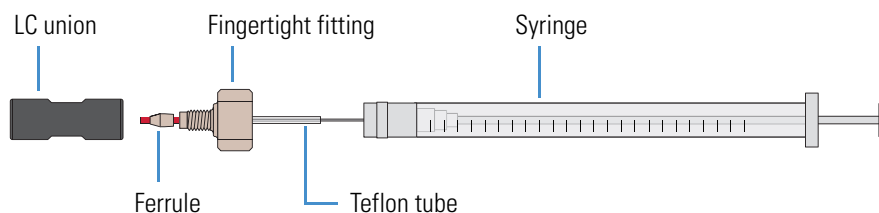
CAUTION Sharp object. The syringe needle can puncture your skin. Handle it with care.

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

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



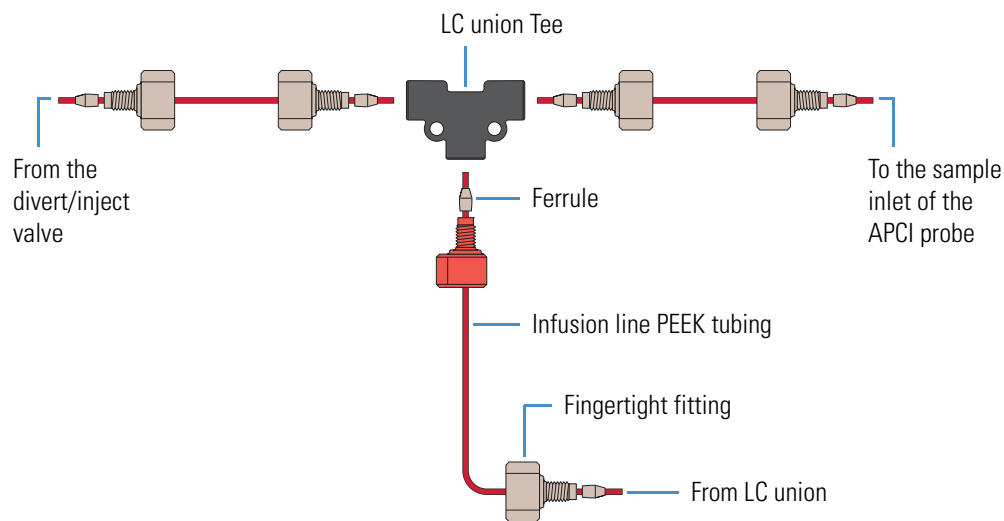
3. Place the syringe into the syringe holder of the syringe pump.
4. Connect a red PEEK infusion line from the LC union to the LC union Tee as follows:
 - a. Use a fingertight fitting and ferrule to connect the infusion line to the free end of the LC union.
 - b. Use a fingertight fitting and ferrule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

Figure 74 shows the connections to the LC union Tee.

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 union Tee



Tip Use a PEEK tubing cutter to ensure that the tubing is cut straight. To prevent adding dead volume to the inlet plumbing, make sure 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 union Tee (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 on page 110 shows the connection between the LC union Tee 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 API 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 MS in APCI mode by using a high solvent flow of the 100 pg/ μ L reserpine tuning solution or a solution of the analyte. 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
- To set the scan type and ion polarity mode

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

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



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

The instrument 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 **C:\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 instrument.

❖ 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



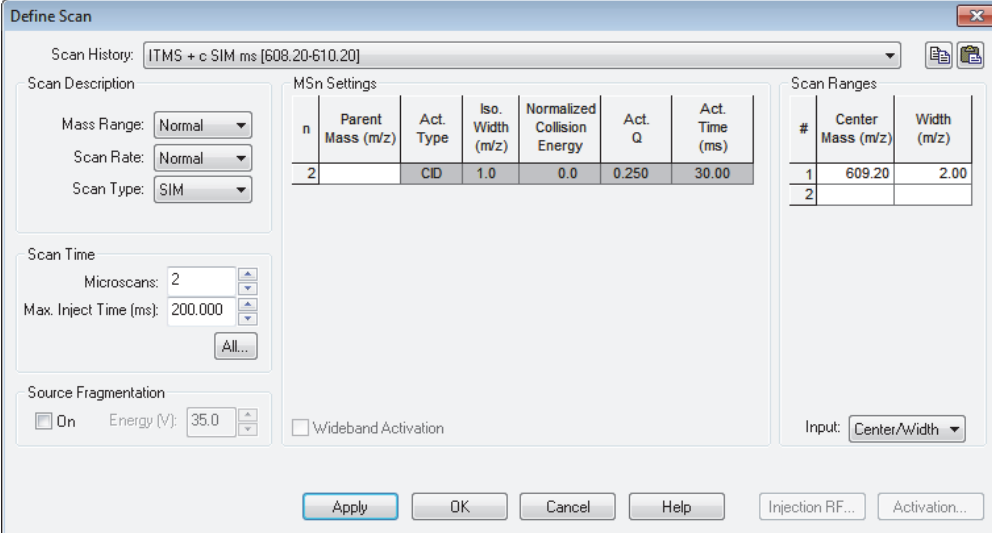
1. In the Tune Plus window, click the **Define Scan** button to open the Define Scan dialog box ([Figure 75](#)).

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**.

6 Tuning with an Analyte in APCI Mode

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

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



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	30.00

#	Center Mass (m/z)	Width (m/z)
1	609.20	2.00
2		

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 API source. Because collision-induced fragmentation performed in the API 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 m/z value for the precursor 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 114](#).
7. Click **OK**.

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

Centroid 

Profile 

Positive polarity 

Negative polarity 

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 instrument set up to tune with an analyte in APCI mode.

Tuning the Mass Spectrometer Automatically

Tune the LTQ Series MS automatically in APCI mode to optimize the parameters listed on [page 109](#).

Note The following are the most important parameters that affect signal quality during APCI operation:

- (For the Velos Pro MS) Discharge current, ion transfer tube temperature and voltage, vaporizer temperature, S-lens rf level, gases, and solution flow rate
- (For the LTQ XL and LXQ MSs) Discharge current, ion transfer tube temperature and voltage, vaporizer temperature, tube lens voltage, gases, and solution flow rate

The most important parameters for long-term performance are the voltage gradients between MP00, MP0, and the front lens voltage. Automatic tuning ensures a sufficient voltage gradient so that the instrument continues to perform well under extended usage.

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

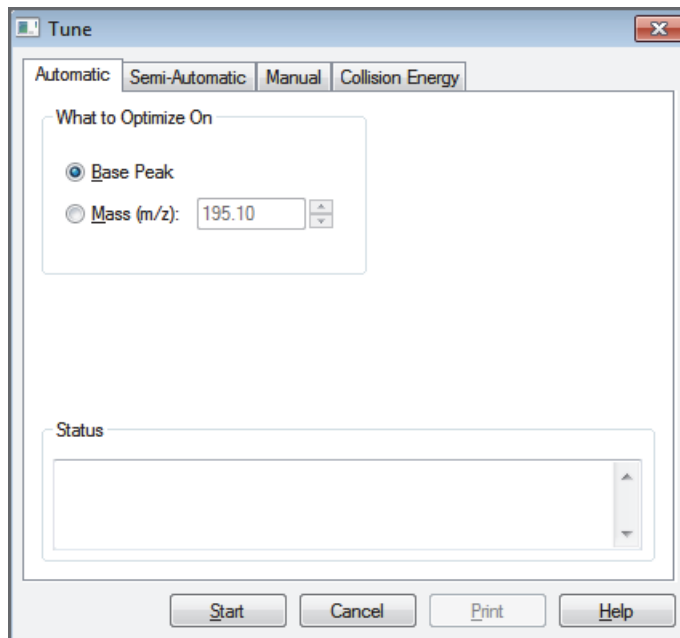
The following procedure uses the 100 pg/μL reserpine tuning solution to optimize the instrument automatically on the reserpine peak at m/z 609.2 at a flow rate of 0.4 μm/min. You can substitute the reserpine solution with a sample solution that is suitable for analysis in APCI mode.

❖ To automatically optimize the tune of the instrument for the analyte



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

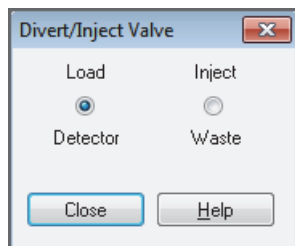
Figure 76. Tune dialog box showing the Automatic page



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 86). Port 3 is connected to the API 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. Make sure that the syringe pump contains at least 450 μL 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 **Display Graph View** button.

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

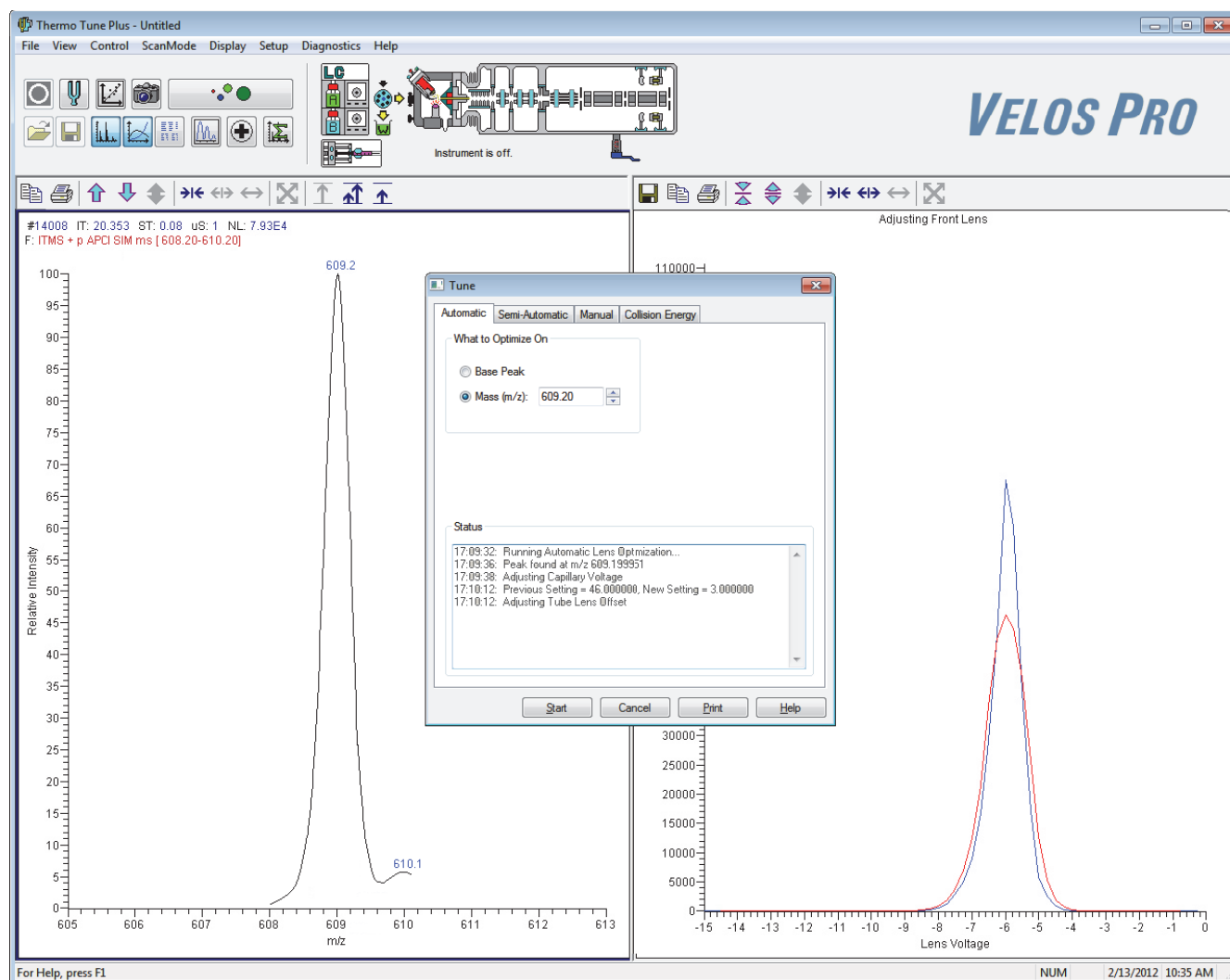
During automatic tuning, the instrument displays the results of various tests in the Spectrum and Graph views in the Tune Plus window and displays various messages under Status in the Tune dialog box.

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

6 Tuning with an Analyte in APCI Mode

Tuning the Mass Spectrometer Automatically

Figure 78. Tune Plus window and Tune dialog box for Velos Pro MS automatic tuning in APCI mode



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 instrument 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 instrument on a different analyte or at a different flow rate.

IMPORTANT Save the tune method before you change the type of probe.

❖ To save the APCI tune method



1. In the Tune Plus window, click the **Save** button.
2. Browse to the **C:\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 instrument by using reserpine, clean it 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.

6 Tuning with an Analyte in APCI Mode

Saving the APCI Tune Method

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 Velos Pro MS, see [“Preparing the Reserpine Sample Solution for ESI and APCI Modes”](#) on page 136.
- For the LTQ XL and LXQ MSs, see [“Preparing the Reserpine Sample Solution for the ESI and APCI Modes”](#) on page 140.

Note

- Before beginning the analysis of the sample solution, verify that the instrument 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 97.

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 μ 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 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

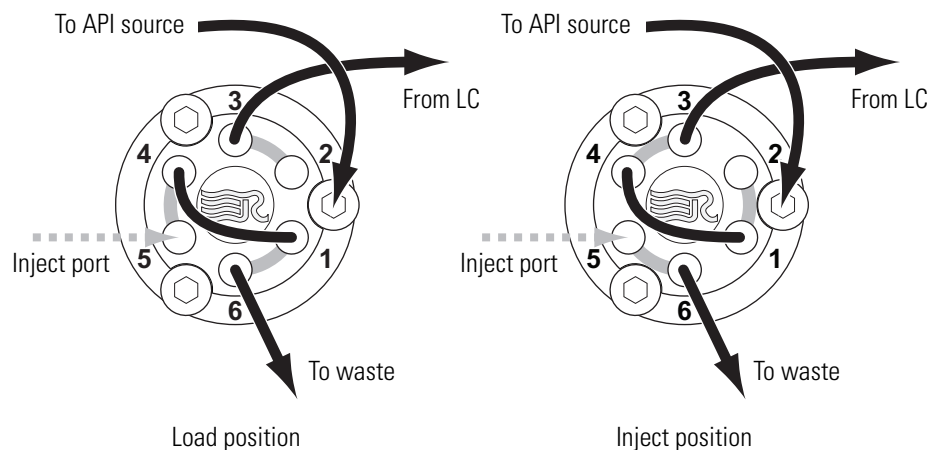
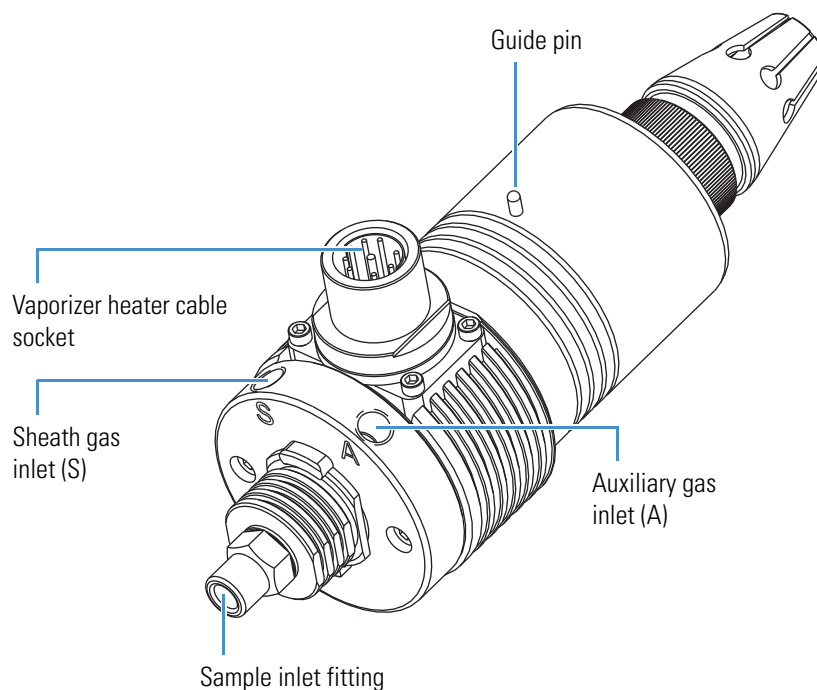


Figure 80. APCI probe



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 instrument 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.

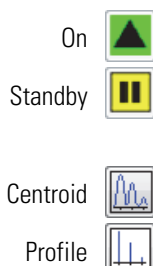
❖ To acquire a data file containing SIM data

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

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

The instrument 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.



7 Acquiring APCI Sample Data by Using Tune Plus

Acquiring APCI Data in the SIM Scan Mode

4. Make sure 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)	Act. Type	Iso. Width (m/z)	Normalized Collision Energy	Act. Q	Act. Time (ms)
2		CID	1.0	0.0	0.250	10.00

#	Center Mass (m/z)	Width (m/z)
1	609.20	2.00
2		

- 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**.

Make sure 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

Instrument Method: [Empty]

Start Mode: Immediate Contact Closure Divert Valve

Acquire Time: Continuously Scans (10) Minutes (1.00)

Acquisition Status: State: Idle, Time (min): 0.000

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 application. To open the XReport application, choose **Start > All 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 μ L injector loop with a sample solution or a 1 pg/ μ L reserpine solution.

To prepare the reserpine solution, follow these procedures:

- For the Velos Pro MS, see [“Preparing the Reserpine Sample Solution for ESI and APCI Modes”](#) on page 136.
- For the LTQ XL and LXQ MSs, see [“Preparing the Reserpine Sample Solution for the ESI and APCI Modes”](#) on page 140.

- 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 API 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

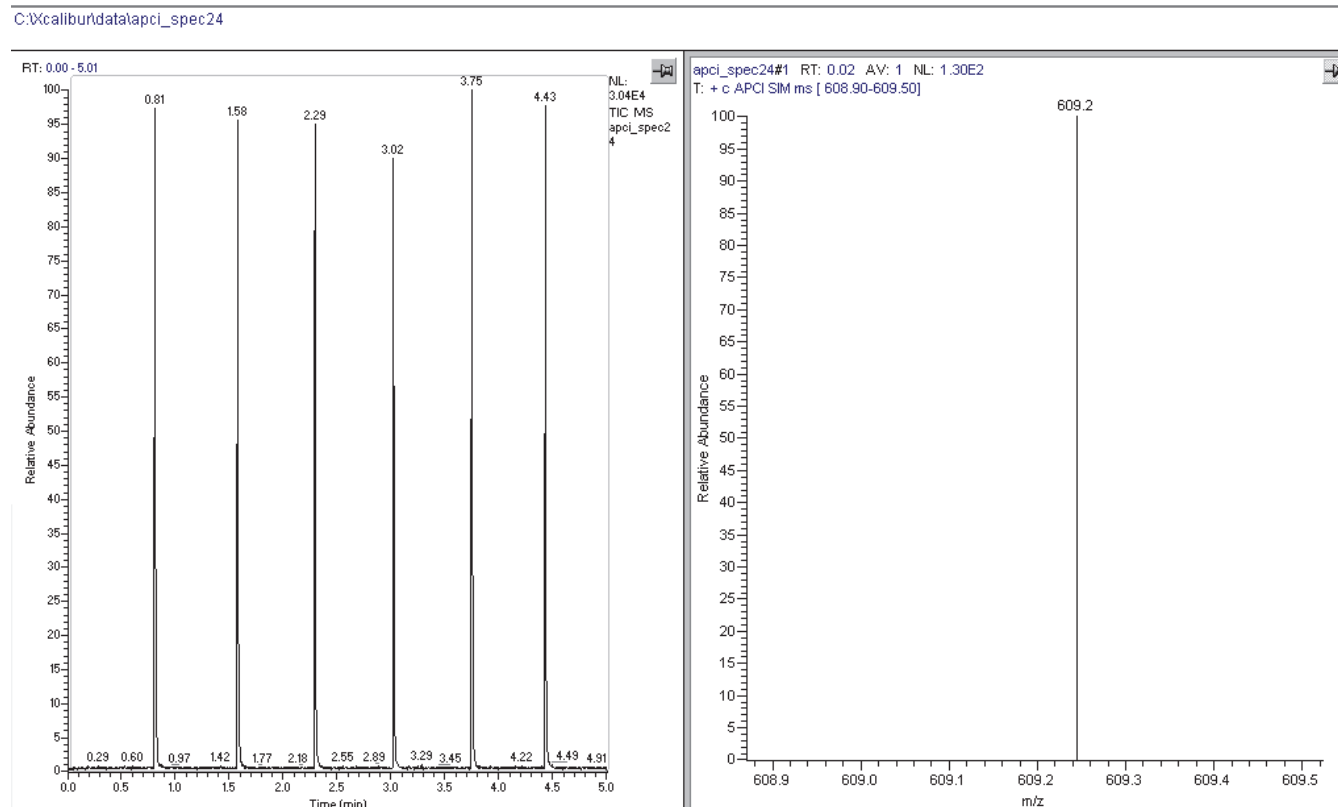
12. In the Divert/Inject Valve dialog box, click **Close**.
13. In the Acquire Data dialog box, click **Stop**, and then click **Cancel**.

Review the mass spectrum and chromatogram in the raw data file just acquired by using the 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 97.

Figure 83. APCI SIM chromatogram and mass spectrum from the Qual Browser window



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
Industrial tissues, lint-free	Methanol, LC/MS grade
Sonicator	Water, LC/MS grade

8 Cleaning the Mass Spectrometer After Tuning and Calibrating

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



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) or Safety Data Sheets (SDSs). The MSDSs and SDSs must be freely available to lab personnel to examine at any time. These data sheets describe the chemicals and summarize information on the hazard and toxicity of specific chemical compounds. They also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures to remedy spills or leaks.

Read the MSDS or SDS 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 or SDS.

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, make sure 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

Parameter	Value	Actual
Vaporizer Temp (°C)	500.00	0.00
Sheath Gas Flow Rate (arb)	30	0.00
Aux Gas Flow Rate (arb)	5	0.00
Sweep Gas Flow Rate (arb)	0	0.00
Discharge Current (µA)	0.00	0.00
Discharge Voltage (kV)	0.00	0.00
Capillary Temp (°C)	275.00	0.00
Capillary Voltage (V)	35.00	0.00
Tube Lens (V)	110.00	0.00

- 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](#).
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

Parameter	Value	Actual
Sheath Gas Flow Rate (arb)	30	0.00
Aux Gas Flow Rate (arb)	5	0.00
Sweep Gas Flow Rate (arb)	0	0.00
Spray Voltage (kV)	0.00	0.00
Spray Current (µA)	0.00	0.00
Capillary Temp (°C)	275.00	0.00
Capillary Voltage (V)	35.00	0.00
Tube Lens (V)	110.00	0.00

8 Cleaning the Mass Spectrometer After Tuning and Calibrating

Removing and Cleaning the Syringe

- 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, Agilent™ Technologies, and Waters™ Corporation. Contact your Thermo Fisher Scientific sales representative for information about the liquid chromatography systems compatible with the LTQ Series MS.

- 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 liquid flow to the API source.
- For instructions, refer to the LC pump's manual.
8. After another 5 minutes, place the instrument 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. Accidental contact with the part of the API probe located inside the housing can severely burn you, as this area of the probe can reach 350 °C (662 °F). In addition, although the exterior surface of the housing and the exposed part of the probe fall below the limit of 70 °C (158 °F) for accessible metal surfaces, they too pose a burn hazard. Therefore, handle ALL parts of the probe and housing with extreme caution.

- Before removing the API probe or housing, allow the part to cool to room temperature (approximately 20 minutes) before you touch 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 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.

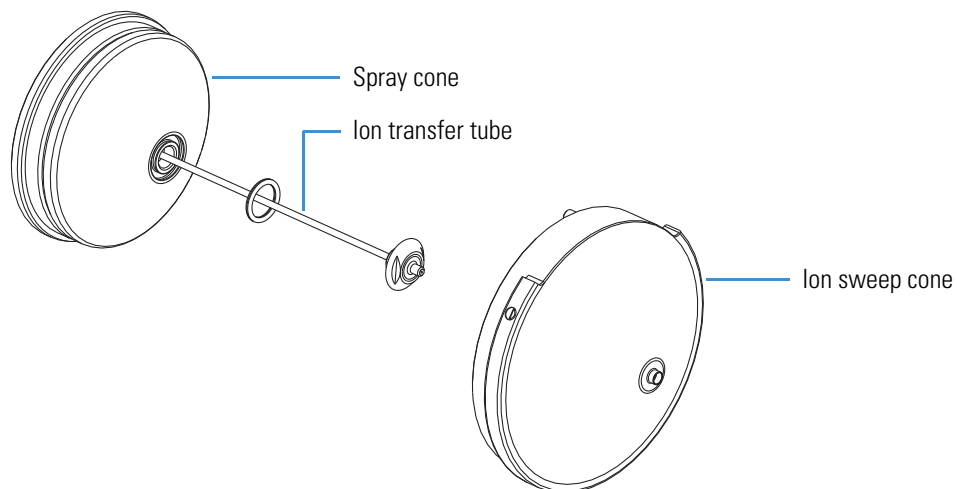
You might need to loosen the ball plungers on the ion sweep cone. There are two versions of the ion sweep cone: [Figure 86](#) shows the version with a nipple, and [Figure 88](#) on [page 133](#) shows the version with an offset orifice.

CAUTION To avoid contaminating the ion transfer tube, do not touch its exposed entrance.

8 Cleaning the Mass Spectrometer After Tuning and Calibrating

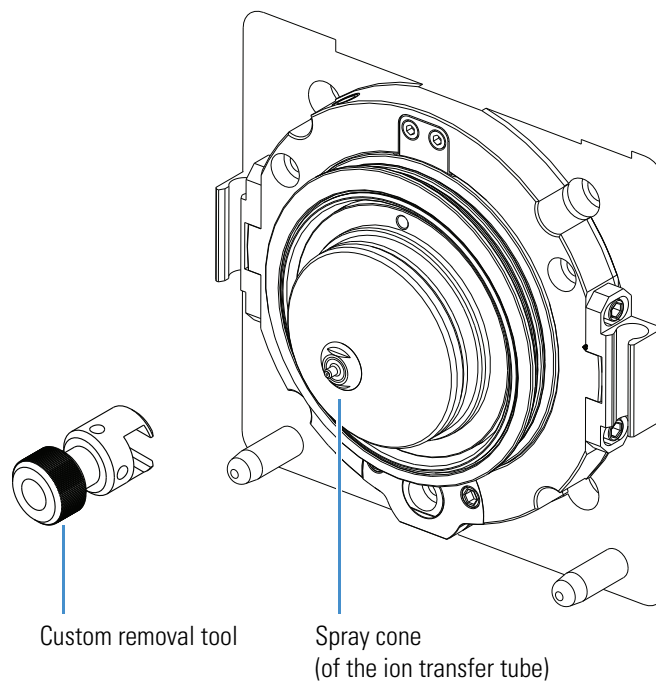
Cleaning the Ion Sweep Cone, Spray Cone, and Ion Transfer Tube

Figure 86. API source interface components for the LTQ XL and LXQ MSs (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 API source interface.

Figure 87. Ion transfer tube removal tool



4. Clean the ion sweep cone and optional spray cone by wiping them, both inside and outside, with the lint-free tissues soaked in methanol.

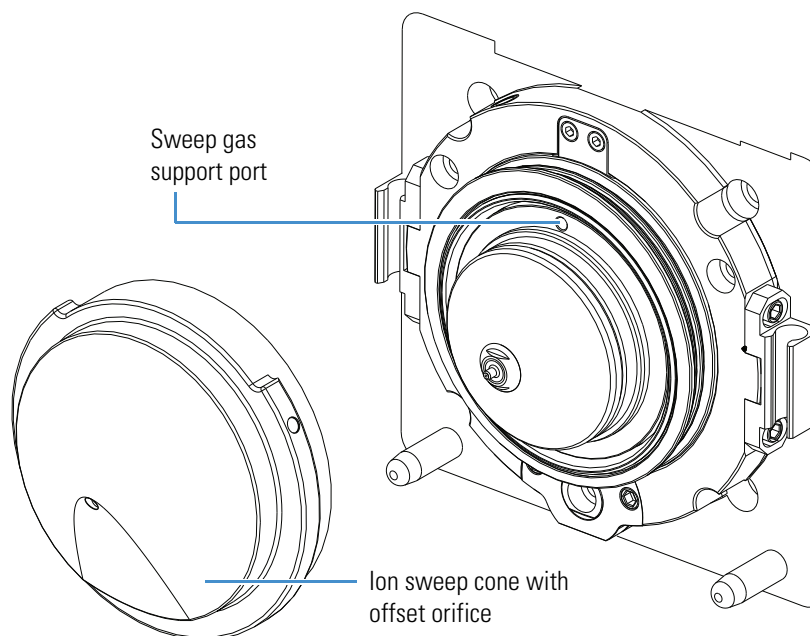
5. Clean the ion transfer tube as follows:
 - a. Place it into a graduated cylinder containing 50:50 methanol/water.
 - b. Sonicate the component for 15 minutes.

CAUTION Take these precautions when reinstalling the ion transfer tube:

- Make sure 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.
 - 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. Sweep gas supply port in the API cone seal



The ion sweep cone is now properly installed on the instrument.

8 Cleaning the Mass Spectrometer After Tuning and Calibrating

Cleaning the Ion Sweep Cone, Spray Cone, and Ion Transfer Tube

Sample Formulations for the Velos Pro Mass Spectrometer

This appendix describes how to prepare the sample and calibration solutions for the Velos Pro MS. You can order a replacement Chemical Kit, OPTON-20042 (P/N 97655-62070), from Thermo Fisher Scientific. You can also order specific chemicals from Thermo Fisher Scientific, which are sold under its Fisher Chemical™ brand. For a complete selection of LC/MS-grade consumables, visit www.fishersci.com.

IMPORTANT NEVER use the solutions described in this appendix to calibrate the LTQ XL and LXQ MSs. Instead, use the solutions described in [Appendix B, “Sample Formulations for the LTQ XL and LXQ Mass Spectrometers.”](#)

Contents

- [Normal Mass Range Calibration Solution for ESI Mode](#)
- [Preparing the Reserpine Sample Solution for ESI and APCI Modes](#)
- [Preparing the High Mass Range Calibration Solution](#)



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) or Safety Data Sheets (SDSs). The MSDSs and SDSs must be freely available to lab personnel to examine at any time. These data sheets describe the chemicals and summarize information on the hazard and toxicity of specific chemical compounds. They also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures to remedy spills or leaks.

Read the MSDS or SDS 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 or SDS.

A Sample Formulations for the Velos Pro Mass Spectrometer

Preparing the Reserpine Sample Solution for ESI and APCI Modes

Use only LC/MS-grade chemicals for calibrating the instrument. For suggested consumable part numbers, refer to Chapter 5, “Gases and Solvents,” in the *LTQ Series Preinstallation Requirements Guide*.

Potentially hazardous chemicals used in the procedures throughout this appendix include the following:

- Glacial acetic acid
- Methanol
- Reserpine

IMPORTANT

- Do not filter solvents. Filtering solvents can introduce contamination.
- Do not use plastic pipettes to prepare the tuning and calibration solutions. Plastic products can release phthalates that can interfere with the analyses.

Preparing the Reserpine Sample Solution for ESI and APCI Modes

Ideally, you should prepare the reserpine solution just before using it. If you must store the solution, keep it in a light-resistant container in the refrigerator until needed.

❖ To prepare the reserpine sample solution

1. Transfer 100 μL of the 100 $\text{pg}/\mu\text{L}$ reserpine stock solution into a clean, minimum 1.5 mL polypropylene tube.
2. Add 900 μL of 1% glacial acetic acid in 50:50 methanol/water to the tube.
3. Mix the solution (10 $\text{pg}/\mu\text{L}$) thoroughly.
4. Transfer 100 μL of the 10 $\text{pg}/\mu\text{L}$ solution into a clean, minimum 1.5 mL polypropylene tube.
5. Add 900 μL of 1% glacial acetic acid in 50:50 methanol/water to the tube.
6. Mix the solution (1 $\text{pg}/\mu\text{L}$,) thoroughly.
7. Transfer 50 μL of the 1 $\text{pg}/\mu\text{L}$ solution into a clean, minimum 1.5 mL polypropylene tube.
8. Add 950 μL of 1% glacial acetic acid in 50:50 methanol/water to the tube.
9. Mix the solution thoroughly.
10. Label the tube **Reserpine Sample Solution (50 $\text{fg}/\mu\text{L}$)**.

Normal Mass Range Calibration Solution for ESI Mode

For tuning and calibrating the Velos Pro MS 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.

Tip You can order the following ready-to-use normal mass range calibration solution from Thermo Fisher Scientific at www.thermoscientific.com/pierce:

Pierce LTQ Velos ESI Positive Ion Calibration Solution, 10 mL (Pierce P/N 88323)

Preparing the High Mass Range Calibration Solution

The high mass range calibrant is a solution of 3.5 mg/μL 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 ($M_n \sim 2700$), which is Sigma-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
(U.S.) 1 (800) 325-3010; (Canada) 1 (905) 829-9500
(Outside the U.S. or Canada) (314) 771-3750
www.sigmaaldrich.com

To prepare the high mass range calibration solution, follow these procedures:

1. [To prepare the sodium acetate stock solution](#)
2. [To prepare the PPG stock solution](#)
3. [To prepare the calibration solution](#)

❖ **To prepare the sodium acetate stock solution**

Dissolve 0.082 g of sodium acetate in 10 mL of water in a clean 20 mL glass vial and label the container **Sodium Acetate Stock Solution**.

A Sample Formulations for the Velos Pro Mass Spectrometer

Preparing the High Mass Range Calibration Solution

❖ To prepare the PPG stock solution

1. Dissolve 0.7 g of the 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 g 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)**.

Sample Formulations for the LTQ XL and LXQ Mass Spectrometers

This appendix describes how to prepare the sample and calibration solutions for the LTQ XL and LXQ MSs. You can order a replacement Chemical Kit, OPTON-20041 (P/N 97355-62070), from Thermo Fisher Scientific. You can also order specific chemicals from Thermo Fisher Scientific, which are sold under its Fisher Chemical brand. For a complete selection of LC/MS-grade consumables, visit www.fishersci.com.

IMPORTANT NEVER use the solutions described in this appendix to calibrate the Velos Pro MS. Instead, use the solutions described in [Appendix A, “Sample Formulations for the Velos Pro Mass Spectrometer.”](#)

Contents

- [Preparing the Reserpine Sample Solution for the ESI and APCI Modes](#)
- [Normal Mass Range Calibration Solution for ESI Mode](#)
- [Preparing the High Mass Range Calibration Solution](#)



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) or Safety Data Sheets (SDSs). The MSDSs and SDSs must be freely available to lab personnel to examine at any time. These data sheets describe the chemicals and summarize information on the hazard and toxicity of specific chemical compounds. They also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures to remedy spills or leaks.

Read the MSDS or SDS 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 or SDS.

B Sample Formulations for the LTQ XL and LXQ Mass Spectrometers

Preparing the Reserpine Sample Solution for the ESI and APCI Modes

Use only LC/MS-grade chemicals for calibrating the instruments. For suggested consumable part numbers, refer to Chapter 5, “Gases and Solvents,” in the *LTQ Series Preinstallation Requirements Guide*.

Potentially hazardous chemicals used in the procedures throughout this appendix include the following:

- Glacial acetic acid
- Methanol
- Reserpine

IMPORTANT

- Do not filter solvents. Filtering solvents can introduce contamination
- Do not use plastic pipettes to prepare the tuning and calibration solutions. Plastic products can release phthalates that can interfere with the analyses.

Preparing the Reserpine Sample Solution for the ESI and APCI Modes

Ideally, you should prepare the reserpine solution just before using it. If you must store the solution, keep it in a light-resistant container in the refrigerator until needed.

❖ To prepare the reserpine sample solution

1. Transfer 100 μL of the 100 $\text{pg}/\mu\text{L}$ reserpine stock solution into a clean, minimum 1.5 mL polypropylene tube.
2. Add 900 μL 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 μL of the 10 $\text{pg}/\mu\text{L}$ solution into a clean, minimum 1.5 mL polypropylene tube.
5. Add 900 μL 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 μL of the 1 $\text{pg}/\mu\text{L}$ solution into a clean, minimum 1.5 mL polypropylene tube.
8. Add 700 μL 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 $\text{fg}/\mu\text{L}$)**.

Normal Mass Range Calibration Solution for ESI Mode

For tuning and calibrating the LTQ XL and LXQ in ESI mode, use a calibration solution of caffeine, MRFA, and Ultramark 1621 in an acetonitrile/methanol/water solution containing 1% acetic acid.

Tip You can order the following ready-to-use normal mass range calibration solution from Thermo Fisher Scientific at www.thermoscientific.com/pierce:

Pierce LTQ ESI Positive Ion Calibration Solution, 10 mL (Pierce P/N 88322)

Preparing the High Mass Range Calibration Solution

The high mass range calibrant is a solution of 3.5 mg/μL 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 Sigma-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
(U.S.) 1 (800) 325-3010; (Canada) 1 (905) 829-9500
(Outside the U.S. or Canada) (314) 771-3750
www.sigmaaldrich.com

To prepare the high mass range calibration solution, follow these procedures:

1. [To prepare the sodium acetate stock solution](#)
2. [To prepare the PPG stock solution](#)
3. [To prepare the calibration solution](#)

❖ **To prepare the sodium acetate stock solution**

Dissolve 0.082 g of sodium acetate in 10 mL of water in a clean 20 mL glass vial and label the container **Sodium Acetate Stock Solution**.

B Sample Formulations for the LTQ XL and LXQ Mass Spectrometers

Preparing the High Mass Range Calibration Solution

❖ To prepare the PPG stock solution

1. Dissolve 0.7 g of the 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 g 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 μL of the sodium acetate stock solution to the vial.
4. Add 10 μL of the PPG 2700 stock solution and label the container **LTQ XL/LXQ PPG 2700 Calibration Solution (70 ng/ μL)**.

High Mass Range Calibration

This appendix applies to all LTQ Series mass spectrometers, unless otherwise noted. Before calibrating an LTQ Series MS 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

- The Chemical Kits contain the applicable ESI calibration solution for Normal mass range calibration. To order additional solution, see the following appendices:
 - For the Velos Pro MS, see [“Normal Mass Range Calibration Solution for ESI Mode”](#) on [page 137](#) in Appendix A.
 - For the LTQ XL and LXQ MSs, see [“Normal Mass Range Calibration Solution for ESI Mode”](#) on [page 141](#) in Appendix B.
- Prepare the appropriate High mass range calibration solution as follows:
 - For the Velos Pro MS, see [“Preparing the High Mass Range Calibration Solution”](#) on [page 137](#) in Appendix A.
 - For the LTQ XL and LXQ MSs, see [“Preparing the High Mass Range Calibration Solution”](#) on [page 141](#) in Appendix B.

Follow the procedures in this appendix in order.

Contents

- [Verifying Coarse Calibration for the High Mass Range by Using the Normal Mass Range Calibration Solution](#)
- [Calibrating the High Mass Range by Using the Normal Mass Range Calibration Solution](#)
- [Performing Two-Point Manual Coarse Calibration—High Mass Range Mode by Using Normal Mass Range Calibration Solution](#)
- [Calibrating the High Mass Range by Using the High Mass Range Calibration Solution](#)
- [Cleaning the System After Calibration](#)

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 149.

Follow these procedures:

1. [Checking the Mass Calibration in the Normal Mass Range](#)
2. [Checking the Mass Calibration in the High Mass Range](#)

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 instrument and turn on the syringe pump.
2. Open the Tune Plus window (see [page 32](#)).

On 

Standby 

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

The instrument 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 + c SIM ms [608.20-610.20]

Scan Description

Mass Range: Normal

Scan Rate: Normal

Scan Type: SIM

Scan Time

Microscans: 2

Max. Inject Time (ms): 200.000

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	30.00	

Wideband Activation

Scan Ranges

#	Center Mass (m/z)	Width (m/z)
1	609.20	2.00
2		

Input: Center/Width

Apply OK Cancel Help Injection RF... 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 API source. Because collision-induced fragmentation performed in the API 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 Velos Pro MS, or Figure 91 for the LTQ XL and LXQ MSs). The integer m/z values of the calibration ions are as follows:

- Caffeine: m/z 195 (only for the LTQ XL and LXQ MSs)
- 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 (Velos Pro MS, coarse calibration)

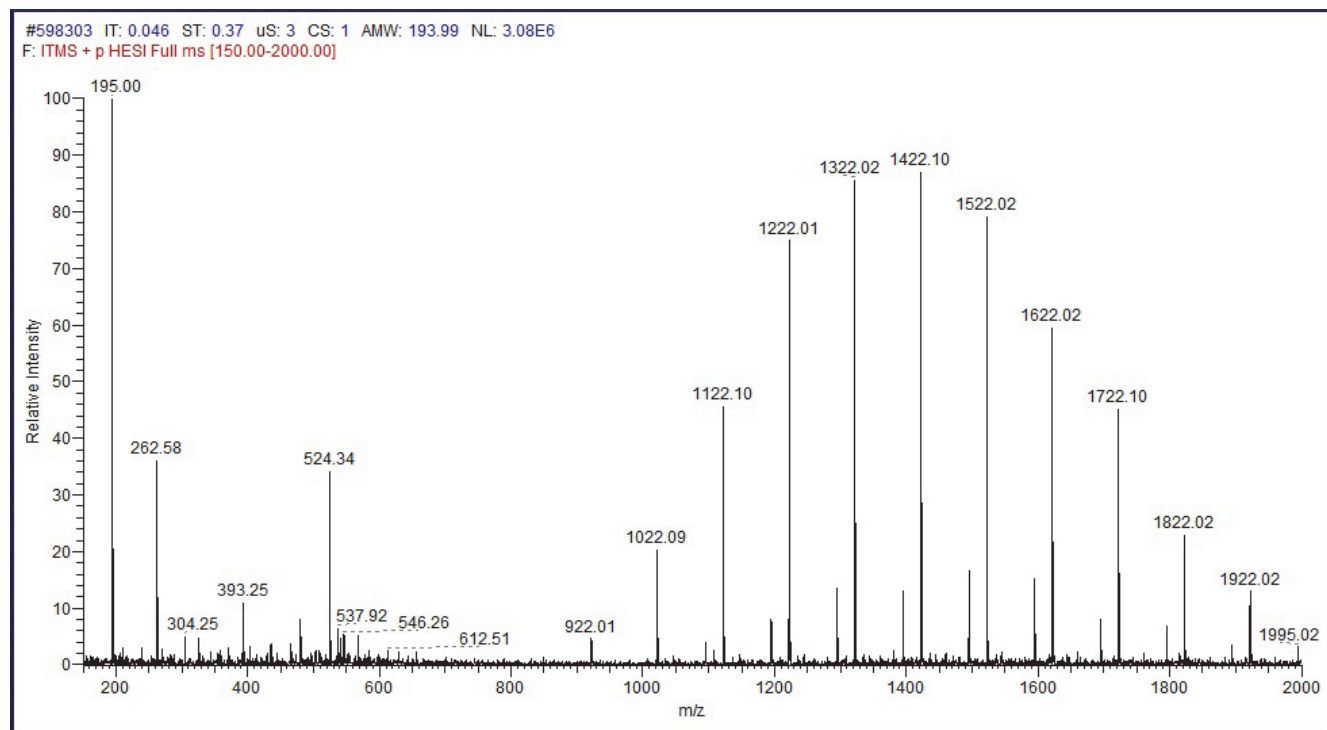
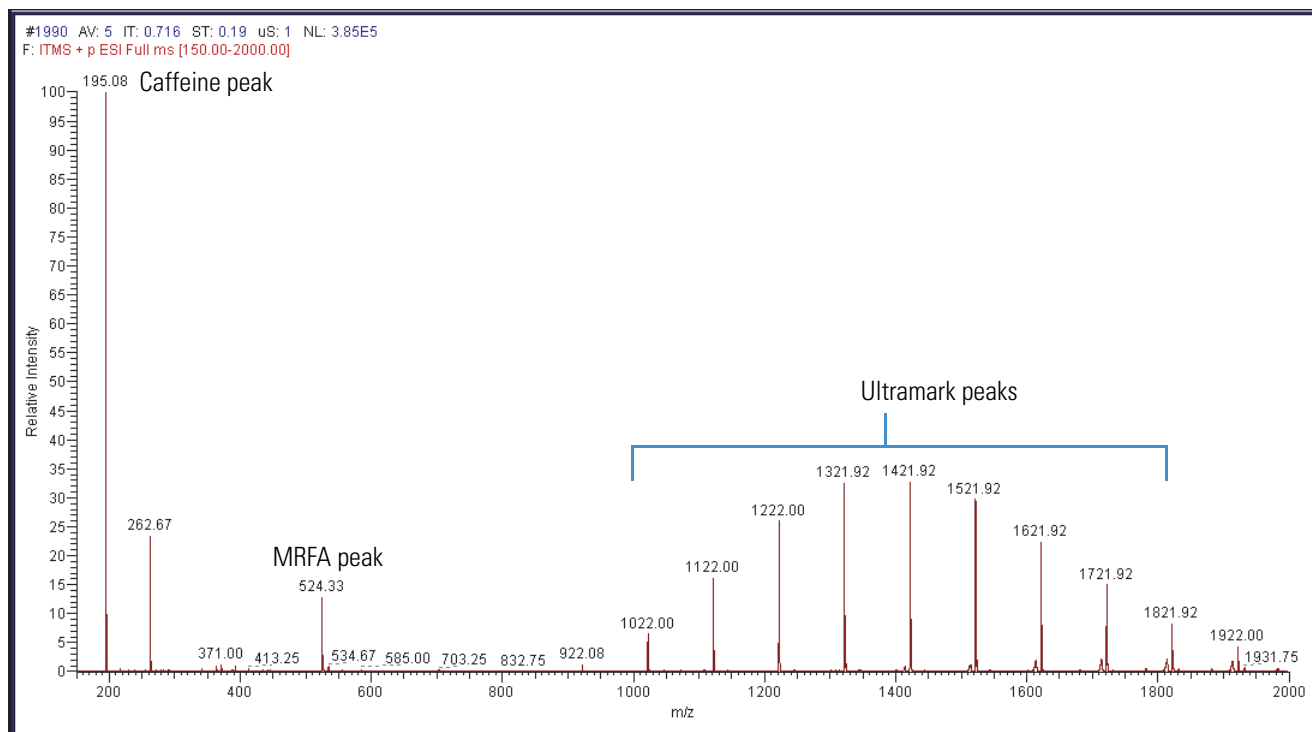


Figure 91. Calibration solution in the normal mass range spectrum (LTQ XL MS, coarse calibration)

12. Do one of the following:

- If the m/z of the observed ions are ± 3 Da for the Velos Pro MS or ± 1 Da for the LTQ XL and LXQ MSs of the integer mass-to-charge ratios stated in [step 11](#) (see the example in [Figure 91](#)), proceed to section “[Checking the Mass Calibration in the High Mass Range.](#)”
- If the ions are not ± 3 Da for the Velos Pro MS or ± 1 Da for the LTQ XL and LXQ MSs 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 78](#)).

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 API source. Because collision-induced fragmentation performed in the API 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 Velos Pro MS, type **400.00**.
 - For the LTQ XL and LXQ MSs, type **150.00**.
 - 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 146 for the Velos Pro MS, or Figure 91 on page 147 for the LTQ XL and LXQ MSs). The ions are as follows:
 - Caffeine: m/z 195 (only for the LTQ XL and LXQ MSs)
 - 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 ± 5 Da for the Velos Pro MS or ± 10 Da for the LTQ XL and LXQ MSs 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 149).
- If the ions are more than ± 5 Da for the Velos Pro MS or ± 10 Da for the LTQ XL and LXQ MSs 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 152).

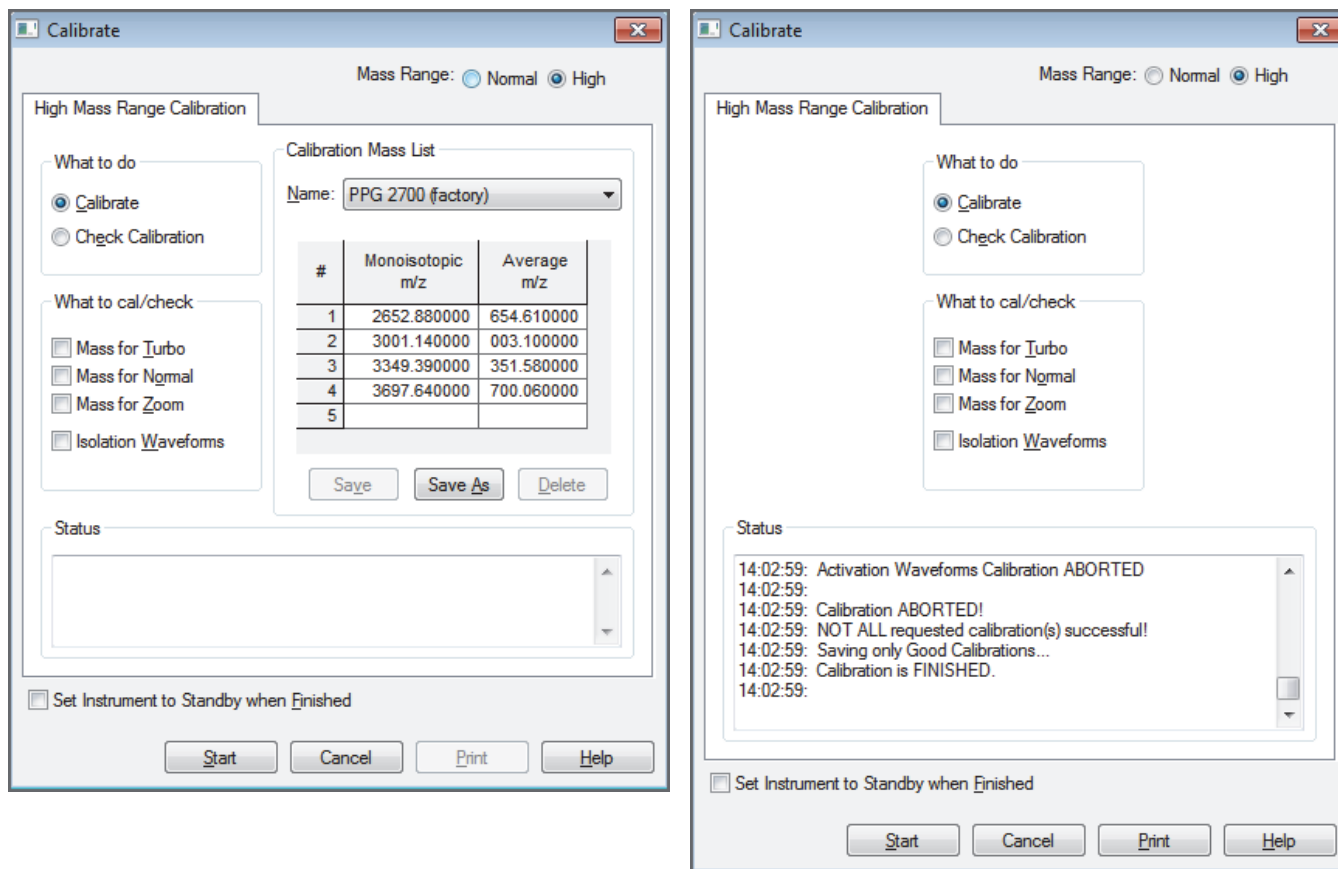
Calibrating the High Mass Range by Using the Normal Mass Range Calibration Solution

❖ 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 instrument 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](#)).



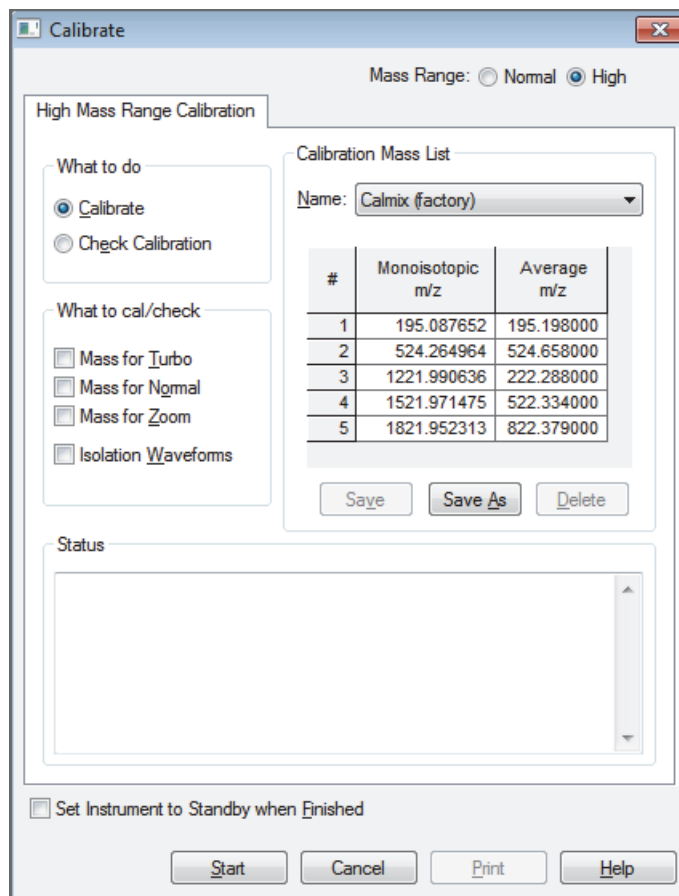
Figure 92. Calibrate dialog boxes showing the High Mass Range Calibration page
 LTQ XL and LXQ MSs Velos Pro MS



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**
 - **Isolation Waveforms**

7. (For the LTQ XL and LXQ MSs only) Under Calibration Mass List, in the Name list, select **Calmix (factory)** (Figure 93).

Figure 93. Calibrate dialog box showing the Calmix calibration mix (LTQ XL and LXQ 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. Make sure that the syringe contains at least 450 μL 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, ZoomScan, 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 156.
- If the calibration does not pass or if the masses are off by more than ± 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)

Define Scan

Scan History: ITMS + c SIM ms [608.20-610.20]

Scan Description

Mass Range: Normal

Scan Rate: Normal

Scan Type: SIM

Scan Time

Microscans: 2

Max. Inject Time (ms): 200.000

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	30.00

Wideband Activation

Scan Ranges

#	Center Mass (m/z)	Width (m/z)
1	609.20	2.00
2		

Input: Center/Width

Apply OK Cancel Help Injection RF... Activation...

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 API source. Because collision-induced fragmentation performed in the API 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 Velos Pro MS, type **400.00**.
 - For the LTQ XL and LXQ MSs, type **150.00**.
 - 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.

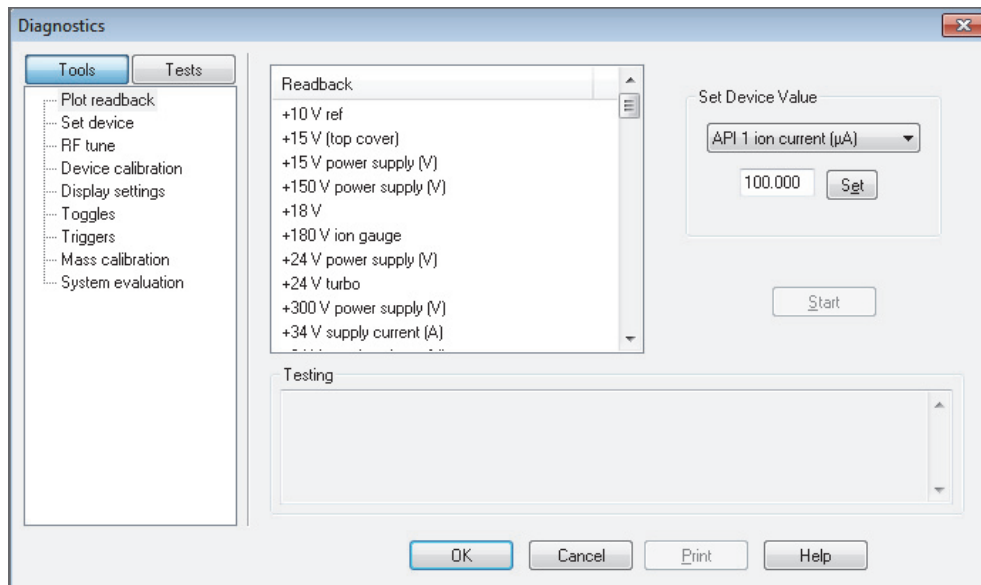
The m/z of the expected ions are as follows:

- Caffeine: m/z 195 (only for the LTQ XL and LXQ MSs)
- MRFA: m/z 524
- Ultramark 1621: m/z 1022, 1122, 1222, 1322, 1422, 1522, 1622, 1722, and 1822

See [Figure 90](#) on [page 146](#) for the Velos Pro MS, or [Figure 91](#) on [page 147](#) for the LTQ XL and LXQ MSs.

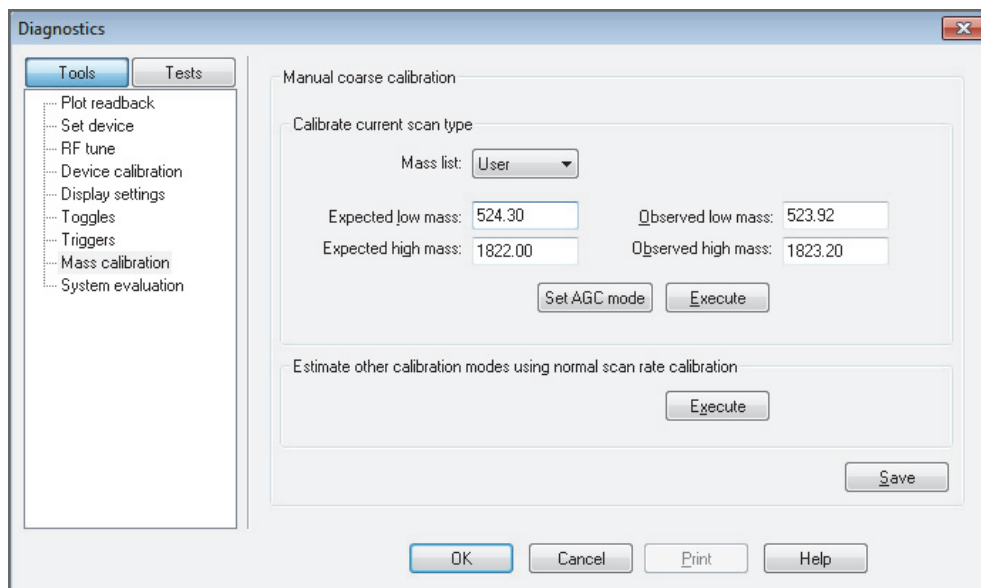
- In the Tune Plus window, choose **Diagnostics > Diagnostics** to open the Diagnostics dialog box (Figure 95).

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 (Velos Pro MS)



10. Under Calibrate Current Scan Type, do the following:
 - In the Expected Low Mass box, do one of the following:
 - For Velos Pro MS, type **524.3**.
 - For LTQ XL and LXQ MSs, type **195.10**.
 - In the Expected High Mass box, type **1822.00**.
 - In the Observed Low Mass box, do one of the following:
 - For Velos Pro MS, type the observed mass closest to MRFA (**524.3**).
 - For LTQ XL and LXQ MSs, type the mass closest to caffeine (**195.10**).
 - 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 148.
 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 91](#) on page 147.
 14. Do one of the following:
 - If any of the midrange Ultramark peaks (1122, 1222, 1322, 1422, 1522, or 1622) are more than ± 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 (~ 1725), or if they are greater than 100 Da apart, pick the next higher peak (~ 1925). Enter this peak as the observed mass and repeat [step 11](#) through [step 13](#) before proceeding.
 - If the masses are now ± 3 Da of those specified above, click **Execute** under Estimate Other Calibration Modes Using Normal Scan Rate Calibration, and then click **Save**.
- IMPORTANT** Make sure 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 instrument](#)
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 instrument

1. Install new red PEEK tubing.
2. Load a clean, 500 μ L Unimetrics syringe with 450 μ L of the PPG 2700 calibration solution.
3. Turn on the instrument.
4. Turn the syringe pump on at 5 μ L/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**

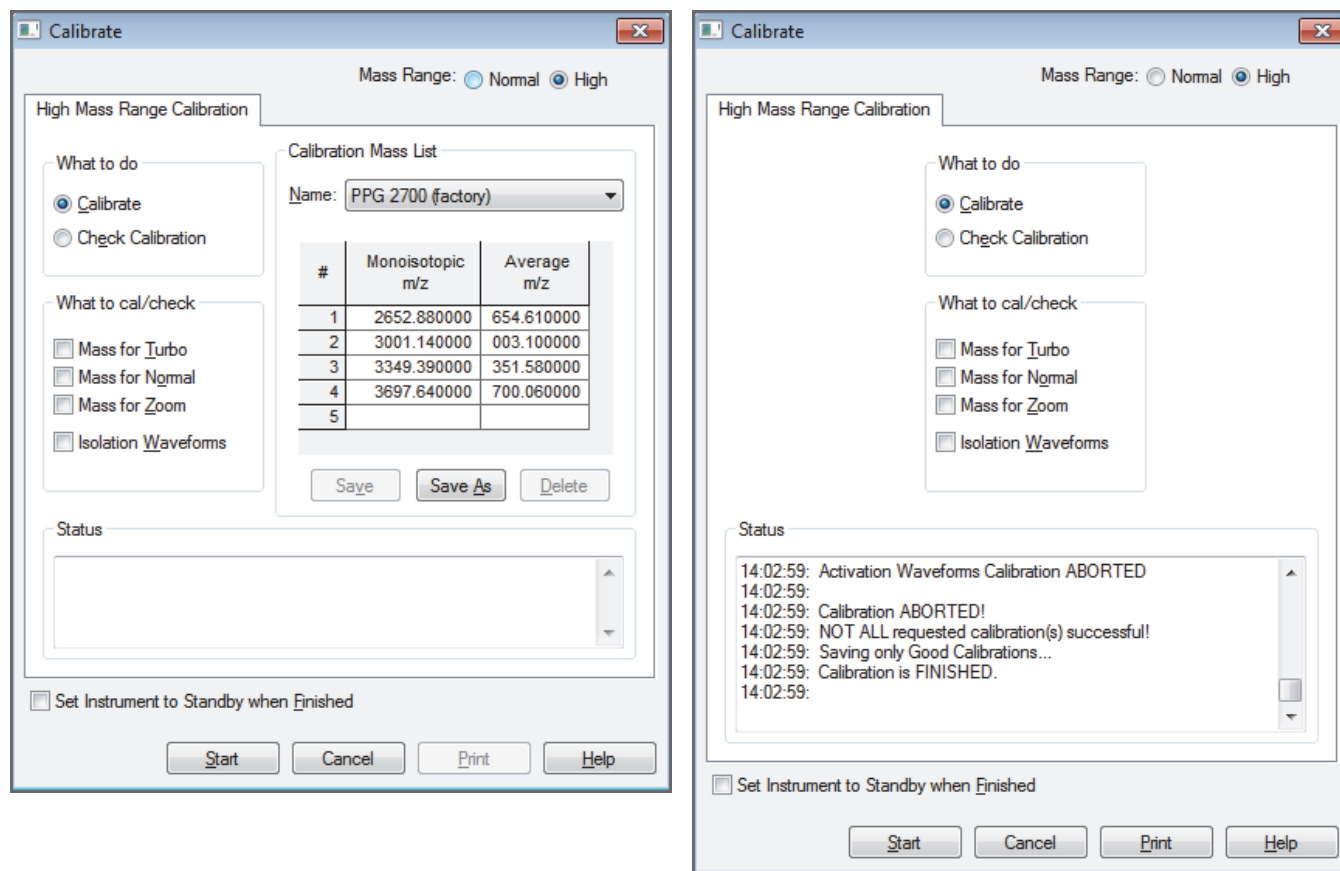


1. In the Tune Plus window, click the **Calibrate** button to open the Calibrate dialog box.
2. For the Mass Range, select the **High** option (Figure 97).

Figure 97. Calibrate dialog boxes showing the High Mass Range Calibration page

LTQ XL and LXQ MSs

Velos Pro MS



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**
 - **Isolation Waveforms**
5. (For the LTQ XL and LXQ MSs only) Under Calibration Mass List, in the Name list, select **PPG 2700 (factory)**.

- 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.

- Click **Start**.

A message appears:

Please ensure that the 500 microliter syringe is full.

- Make sure that the syringe contains at least 450 μL of the high mass range calibration solution, that the syringe pump is on, and that it is set to 5 $\mu\text{L}/\text{min}$.
- Click **OK**.
- 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, ZoomScan, 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 LTQ XL and LXQ 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 liquid chromatograph 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 API 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.

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 ions are accelerated to high-kinetic energy and then allowed to collide with neutral gas molecules such as helium. 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 loop injector or as a divert valve.

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 (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.

electrospray ionization (ESI) *See* [electrospray \(ESI\)](#).

F

full-scan type Provides a full mass spectrum of each analyte or precursor ion. With the full-scan type, the mass analyzer is scanned from the first mass to the last mass without interruption. Also known as single stage full-scan type.

H

heated-electrospray (H-ESI) Converts ions in solution into ions in the gas phase by using electrospray ionization (ESI) in combination with heated auxiliary gas.

heated-electrospray ionization (H-ESI) *See* [heated-electrospray \(H-ESI\)](#).

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 precursor 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 A high sensitivity, off-axis system for detecting ions. It produces a high [signal-to-noise ratio \(S/N\)](#) and allows for switching of the voltage polarity between positive ion and negative ion modes of operation. The ion detection system includes a ± 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 LC that provides the pressure on the input side of a column to drive the eluent and sample through the column.

lens An element that provides focusing of the ion beam.

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 Da) by the number of charges carried by the ion. For example, for the ion $C_7H_7^{2+}$, $m/z = 45.5$.

mass analyzer A device that determines the mass-to-charge ratios (m/z) of the ions to produce a mass spectrum.

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

nanoelectrospray (nanoESI or NSI) A type of electrospray ionization (ESI) that accommodates very low flow rates of sample and solvent at 1–20 nL/min (for static nanospray) or 100–1000 nL/min (for dynamic nanospray).

nanospray ionization (nanoESI or NSI) *See* [nanoelectrospray \(nanoESI or NSI\)](#).

P

precursor ion An electrically charged molecular species that can dissociate to form fragments. The fragments can be electrically charged or neutral species. A precursor 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 fragment of an isolated precursor 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.

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.

source See [API source](#).

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 precursor 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-Da scan-range windows are monitored to determine the charge-state of up to ten ions.



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