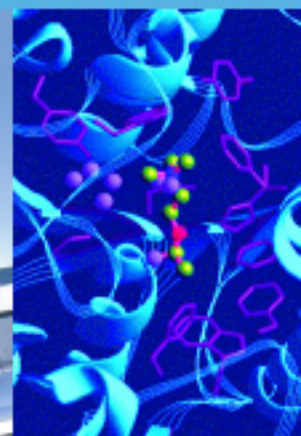
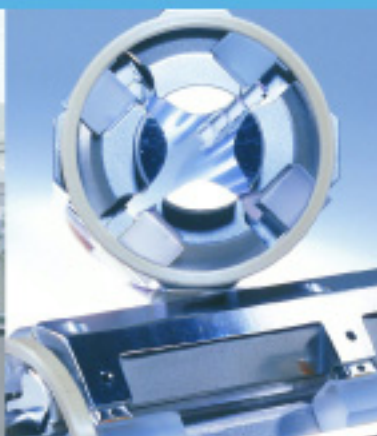


Finnigan™ LXQ™

Getting Started

97055-97042 Revision B

June 2006




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EN 61000-3-2	1995, A1; 1998, A2; 1998, A14; 2000	EN 61000-4-4	1995, A1; 2001, A2; 2001
EN 61000-3-3	1998	EN 61000-4-5	1995, A1; 2001
EN 61326-1	1998	EN 61000-4-6	1996, A1; 2001
EN 61000-4-2	2000	EN 61000-4-11	1994, A1; 2001
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Preface

About This Guide

Welcome to the Thermo Electron, Finnigan™ LXQ™ system. The LXQ is a member of the Finnigan family of MS detectors.

This *Finnigan LXQ Getting Started* manual provides you with information on how to set up, calibrate, and tune the Finnigan LXQ MS detector, and how to acquire LC/MS data. All of these procedures can be performed from the Xcalibur® Tune Plus window.

To perform analyses in ESI mode, see Chapters 2, 3, 4, and 5. To perform analyses in APCI mode, see Chapters 2, 3, 6, 7, and 8.

Related Documentation

In addition to this guide, Thermo Electron provides the following documents for the LXQ system as .PDF files:

- LXQ Preinstallation Guide
- LXQ Getting Connected
- LXQ Hardware Manual

Help is also available from within the software

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Note Highlights information of general interest.

Tip Helpful information that can make a task easier.

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Chapter 1 Introduction

The LXQ™ is a member of the Finnigan™ family of MS detectors. The Finnigan LXQ MS detector is an advanced analytical instrument that includes a syringe pump, a divert/inject valve, an atmospheric pressure ionization (API) source, an MS detector, and the Xcalibur data system. In a typical analysis, a sample can be introduced in any one of the following ways:

- Using the syringe pump (direct infusion)
- Using the inject valve fitted with a loop and an LC pump (flow injection analysis) or loop injection
- Using a valve and an LC system fitted with a column (LC/MS)

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

This introduction answers the following questions:

- [Why Use the Finnigan LXQ MS Detector?](#)
- [Which Ion Source—ESI or APCI—Is Better for Analyzing My Samples?](#)
- [How Can I Introduce My Samples into the MS Source?](#)
- [What Types of Buffers Should I Use? What Types Should I Avoid?](#)
- [How Should I Set Up the MS Detector for Various LC Flow Rates?](#)
- [What is Tuning and Calibration of the MS Detector All About?](#)
- [What Types of Experiments Can I Perform with the Finnigan LXQ MS Detector?](#)

Why Use the Finnigan LXQ MS Detector?

The attribute that sets the Finnigan LXQ MS detector apart from other LC detectors is the high level of analytical specificity that it provides. The Finnigan LXQ MS detector can provide multiple levels of analysis. Each level of analysis adds a new dimension of specificity for unequivocal compound identification. The various levels of analysis are as follows:

- Chromatographic separation and compound detection (non MS technique utilizing chromatographic retention time)
- Mass analysis (molecular mass information)
- Two-stage mass analysis, MS/MS (structural information)
- Multi-stage mass analysis, MSⁿ (structural information)
- ZoomScan™ analysis (charge state information)

Chromatographic separation and compound detection can be obtained by all LC/detector systems. 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, even if a compound is identified correctly by retention time, quantitation results can be in error because other compounds in the sample might co-elute with the compound of interest.

Single stage mass analysis allows for the identification of analytes of interest based on molecular mass information. Atmospheric pressure ionization typically produces mass spectra that provide molecular mass information.

Two-stage mass analysis allows for even more certainty in compound identification. MS/MS analysis monitors how a parent ion fragments when it is exposed to an additional stage of excitation. There are two types of MS/MS analysis: Full Scan MS/MS and Selective Reaction Monitoring (SRM). Full Scan MS/MS monitors the production of all product ions from a specific parent ion. SRM MS/MS analysis monitors a specific reaction path: the production of a specific product ion from a specific parent ion.

Using either type of MS/MS analysis, you can easily 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, quantitative target compound analysis is easily accomplished using the Finnigan LXQ MS detector.

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 Finnigan LXQ MS

detector allow for stepwise fragmentation pathways, making interpretation of MSⁿ spectra relatively straightforward. The Finnigan LXQ MS detector has several advanced features that make its MSⁿ capabilities extremely powerful for qualitative analysis. (Refer to the topic [“What Types of Experiments Can I Perform with the Finnigan LXQ MS Detector?”](#) on page 16.)

ZoomScan analysis provides information about the charge state of one or more mass ions of interest. ZoomScan data are collected by using slower scan rates that give higher resolution. This level of resolution allows the unambiguous determination of charge state. This in turn allows for the correct determination of molecular mass for compounds that have multiple ionization sites.

In addition to the aforementioned levels of analysis, there is an additional technique called Wideband Activation. The Wideband Activation option allows the Finnigan LXQ MS detector to apply collision energy to ions during MS/MS fragmentation over a fixed mass range of 20 u. This option allows collision energy to be applied to both the parent ion, as well as to product ions created as a result of non-specific losses of water (18 u) or ammonia (17 u), or to product ions formed from the loss of fragments less than 20 u. When you want enhanced structural information without resorting to MS³ analysis, choose the Wideband Activation option for qualitative MS/MS. Fragmentation efficiency is somewhat reduced and therefore you will need to increase the value of the collision energy to compensate for this.

Which Ion Source—ESI or APCI—Is Better for Analyzing My Samples?

The Finnigan LXQ MS detector has two atmospheric pressure ionization sources:¹

- Electrospray ionization (ESI)
- Atmospheric pressure chemical ionization (APCI)

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

Using ESI/MS

ESI is considered to be a soft ionization technique. The ESI source transfers ions in solution to the gas phase. Many samples that previously were not suitable for mass analysis (for example, heat-labile compounds or high molecular mass compounds) can be analyzed by ESI. ESI can be used to analyze any polar compound that is an ion in solution. This can include adduct ions. For example, polyethylene glycols can be analyzed 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 can be analyzed by the Finnigan LXQ MS detector can be greater than 50,000 u if there is multiple charging. ESI is especially useful for the mass analysis of polar compounds. Included in this class of compounds are: biological polymers (such as, proteins, peptides, glycoproteins, and nucleotides), pharmaceuticals and their metabolites, and industrial polymers.

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. This mass spectrum can be mathematically manipulated to determine the molecular mass of the sample.

You can use the ESI source in either positive or negative ion polarity mode. The ion polarity mode is determined by the polarity of the ions in solution: acidic molecules form negative ions in high pH solution and basic molecules form positive ions in low pH solution. The ESI needle may be either positively or negatively charged. When it is positively charged it generates positive ions. When it is negatively charged it generates negative ions.

You can vary the flow rate into the mass spectrometer over a range of 0.1 $\mu\text{L}/\text{min}$ to 1000 $\mu\text{L}/\text{min}$. Refer to [Table 2](#).

¹Optional ionization sources [atmospheric pressure photo ionization (APPI), atmospheric pressure matrix assisted laser desorption ionization (AP MALDI), and nanospray] are also available.

In ESI, the buffer type and buffer concentration both have a noticeable effect on sensitivity. Therefore, it is important to choose these variables correctly.

The ESI process is affected by aerosol droplet size, liquid surface tension, solvent volatility, surface charge, ion solvation strength and solution conductivity. Specifically, ESI is not favored by large droplets with high surface tension, low volatility, low surface charge, strong ion solvation and high conductivity. Conversely, ESI is favored by small droplets with low surface tension, high volatility, high surface charge, weak ion solvation and low conductivity.

Mixed organic/aqueous solvent systems that include organic solvents such as methanol, acetonitrile, and isopropyl alcohol are recommended for ESI. Using only water as a solvent is not recommended. Volatile acids and bases give good results. Salt solutions with concentrations above 10 mM are not recommended. Strong mineral acids and bases can damage the instrument and should not be used.

To obtain good ESI follow these rules:

- Keep non-volatile salts and buffers out of the solvent system. For example, avoid the use of salts containing sodium, potassium or phosphate. Use ammonium or acetate salts instead.
- Use organic/aqueous solvent systems and volatile acids and bases.
- If possible, optimize the pH of the solvent system for your analyte of interest. For example, if your analyte of interest contains a primary or secondary amine, your mobile phase should be slightly acidic (pH 2 to 5).

Using APCI/MS

Like ESI, *APCI* is a soft ionization technique. 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.

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

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. The rate of solvent flowing from the LC into the MS detector in APCI mode is typically high (between 0.2 and 2 mL/min). Refer to Table 3, Guidelines for setting operating parameters for LC/APCI/MS.

1 Introduction

Which Ion Source—ESI or APCI—Is Better for Analyzing My Samples?

You can use APCI in 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 which generally produce strong negative ions, with acidic sites such as carboxylic acids and acid alcohols, are an exception to this general rule.

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

Should I Use Sheath, Auxiliary, and/or Sweep Gases?

Nitrogen gas can be applied to the system using any combination of the three gas sources: Auxiliary gas, Sweep gas, and/or Sheath gas. When Auxiliary gas is being used, nitrogen flows through the ion source nozzle and the vapor plume is affected; the spray is focused and desolvation is improved. When Sweep gas is used, the nitrogen flows out from behind the sweep cone and can result in solvent declustering and adduct reduction. When Sheath gas is used, nitrogen is applied as an inner coaxial gas (when used in tandem with Auxiliary gas), helping to nebulize the sample solution into a fine mist as the solution exits the ESI or APCI nozzle. Note that Sheath gas is not used with the nano spray ionization source.

When you are analyzing complex matrices such as plasma or non-volatile salt buffers, Sweep gas is required for ruggedness. In full-scan MS or data dependent scan experiments, the signal-to-noise ratio can be improved by application of Sweep gas. In some cases, signal intensity can be increased by using Auxiliary gas, particularly for higher LC flow rates.

All analyses must be individually optimized with Sheath gas, Sweep gas, and Auxiliary gas to determine the combination that will provide optimum performance. It is especially important to optimize with each gas independently before you perform experiments using MSⁿ techniques and before you perform any quantitative analysis experiments. Refer to [Table 2](#) and [Table 3](#) for additional information on using supplemental gas flows.

How Can I Introduce My Samples into the MS Source?

You can introduce your samples into the MS source in a variety of ways. Refer to [Table 1](#).

The syringe pump is often used to introduce 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 tuning purposes. This would be done to determine the structure of an unknown compound or the fragmentation pattern of a standard.

You can also use a Tee union to direct samples from the syringe pump into an LC flow (without a column), which then enters the MS source. This technique is used to introduce a sample at a steady rate and at higher solvent flow rates. This is done for tuning on an analyte of interest in ESI or APCI.

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

You can also use an LC autosampler to introduce pure samples into an LC flow. This technique is also used in ESI or APCI to introduce analytes automatically into the LC flow and then into the MS detector.

Finally, you can perform LC/MS experiments by using an autosampler to introduce a mixture onto an LC column. This technique is used with ESI or APCI to separate the analytes before they are introduced sequentially into the MS detector.

You can refer to subsequent chapters in this manual and to *Finnigan LXQ Getting Connected* for plumbing diagrams for methods of sample introduction.

Table 1. Sample introduction techniques

	Sample Introduction Technique	Analytical Technique	Figure Reference
Syringe Pump Flow (no LC Flow)	Syringe pump*	ESI automatic tuning and calibrating ESI analysis of a pure analyte solution	<i>Finnigan LXQ Getting Started</i> Figure 2-5
LC Flow Without Chromatographic Separation (no column)	Syringe pump into LC flow (connected by Tee union)*	ESI or APCI automatic optimization of tuning on analyte of interest ESI or APCI analysis of a pure analyte solution	<i>Finnigan LXQ Getting Started</i> Figure 4-1 (ESI) Figure 6-1 (APCI)
	Loop injection into LC flow	ESI or APCI analysis of a pure analyte solution	<i>Finnigan LXQ Getting Started</i> Figure 5-6 (ESI) Figure 8-1 (APCI)
	Autosampler injection into LC flow (one or multiple injections)	ESI or APCI analysis of a pure analyte solution	<i>Finnigan LXQ Getting Connected</i> Figure 11-5 (ESI) Figure 11-8 (APCI)
LC Flow With Chromatographic Separation	Autosampler injections into LC column via LC flow (one or multiple injections)	ESI or APCI analysis of mixtures	

*Provides steady state introduction of sample (direct infusion)

1 Introduction

What Types of Buffers Should I Use? What Types Should I Avoid?

What Types of Buffers Should I Use? What Types Should I Avoid?

Many LC applications use nonvolatile buffers such as phosphate and borate buffers. It is best to avoid the use of nonvolatile buffers with the MS detector because they can cause the following problems:

- Blocking the capillary in the probe
- Causing salt buildup on the spray head and thus compromising the integrity of the spray

Use volatile buffers when you use the MS detector. Many volatile buffer solutions are available that can be used instead of nonvolatile ones. Volatile buffer solutions can include the following:

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



CAUTION Store and handle all chemicals in accordance with standard safety procedures. The Material Safety Data Sheets (MSDS) describing the chemicals being used are to be freely available to lab personnel for them to examine at any time.

How Should I Set Up the MS Detector for Various LC Flow Rates?

The ESI probe can volatilize ions from liquid flows² of 1 $\mu\text{L}/\text{min}$ to 1.0 mL/min. This flow rate range allows you to use a wide range of separation techniques: CE, CEC, capillary LC, microbore LC, and analytical LC.

The APCI probe can volatilize ions from liquid flows³ of 200 $\mu\text{L}/\text{min}$ to 2.0 mL/min. This flow range allows you to use microbore LC, analytical LC, and semi-preparative LC.

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

For ESI, you need to adjust the capillary temperature and adjust the gas flow rates for the Sheath, Auxiliary, and/or Sweep gas.

For APCI, you need to adjust the capillary temperature and vaporizer temperature and adjust the gas flow rates for the Sheath, Auxiliary, and/or Sweep gas.

In general, an increase in the rate of liquid flowing into the MS detector, requires a higher temperature of the ion transfer capillary (and vaporizer) and the higher gas flow rate.

[Table 2](#) provides guidelines for ESI operation for ion transfer capillary temperatures and gas flow rates for various LC solvent flow rates.

[Table 3](#) provides guidelines for APCI operation for the ion transfer capillary temperature, vaporizer temperature, and gas flow rate for a range of LC solvent flow rates.

² The ESI probe can generate ions from liquid flows of 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.

³ For the APCI probe, flows below 200 $\mu\text{L}/\text{min}$ require more care to maintain a stable spray.

1 Introduction

How Should I Set Up the MS Detector for Various LC Flow Rates?

Table 2. Guidelines for setting operating parameters for LC/ESI/MS*

LC Flow Rates	Suggested Column Size	Ion Transfer Capillary Temperature	Sheath Gas	Auxiliary and/or Sweep Gas
Infusion or LC at flow rates of < 10 $\mu\text{L}/\text{min}$	Capillary	Typical setting: 150 to 200 $^{\circ}\text{C}$	Not required Typical setting: 5 to 15 units	Not required Typical setting: 0 units
LC at flow rates from 50 to 200 $\mu\text{L}/\text{min}$	1 mm ID (microbore column)	Typical setting: 200 to 275 $^{\circ}\text{C}$	Required Typical setting: 20 to 40 units	Not required, but might help depending on conditions Typical setting: 0 to 20 units
LC at flow rates from 100 to 500 $\mu\text{L}/\text{min}$	2 to 3 mm ID (narrow bore column)	Typical setting: 250 to 350 $^{\circ}\text{C}$	Required Typical setting: 40 to 60 units	Not required, but usually helps to reduce solvent background ions Typical setting: 0 to 20 units
LC at flow rates from 0.4 to 1 mL/min	4.6 mm ID (standard column)	Typical setting: 300 to 400 $^{\circ}\text{C}$	Required Typical setting: 60 to 100 units	Required Typical setting: 10 to 40 units

* Note: Be sure to choose either Auxiliary gas and/or Sweep gas according to the hints in the topic **Should I Use Sheath, Auxiliary, and/or Sweep Gases?**

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

LC Flow Rate	Ion Transfer Capillary Temperature	Vaporizer Temperature	Sheath Gas	Auxiliary and/or Sweep Gas
LC at flow rates from 0.2 to 2 mL/min	Typical setting: 150 to 350 $^{\circ}\text{C}$	Typical setting: 350 to 500 $^{\circ}\text{C}$	Required Typical setting: 40 to 100 units	Not required, but usually helps to reduce solvent background ions Typical setting: 0 to 20 units

* Note: Be sure to choose either Auxiliary gas and/or Sweep gas according to the hints in the topic [“Should I Use Sheath, Auxiliary, and/or Sweep Gases?”](#) on page 7.

What is Tuning and Calibration of the MS Detector All About?

To optimize the performance of data acquisition on the Finnigan LXQ MS detector, you tune and calibrate in four steps:

- In ESI mode you infuse a calibration solution into the MS detector at a steady rate of 5 $\mu\text{L}/\text{min}$ for several minutes. In Tune Plus you observe the signal at m/z 195, the mass-to-charge ratio of caffeine in the calibration solution. Then, while observing the signal at m/z 195, you adjust probe positions and gas flows to achieve the greatest signal strength while still maintaining a stable spray of ions into the MS detector.
- Once you have established a stable spray of ions into the MS detector, you tune the MS detector. In this step, you use the automatic tuning procedure in Tune Plus to ensure that the transmission of ions into the MS detector is optimum. You observe the Tune Plus window as the Xcalibur data system tunes your Finnigan LXQ MS detector automatically.
- After your tune method is optimized, you calibrate the MS detector. In this step, you want to ensure that the calibration parameters complete automatic calibration successfully. The Calibrate dialog box in Tune Plus provides a readback of the status of the calibration parameters, both during the automatic calibration and when calibration is complete.
- Lastly, if you want to maximize the detection of one or more particular ions, you can optimize the tune of the MS detector with your analyte of interest in the ionization mode that you are going to use to analyze your samples. You choose a mass-to-charge ratio of your analyte of interest. Alternatively, you can choose an ion in the calibration solution that is closest to the mass-to-charge ratio for your ion of interest. (It is sometimes possible to acquire qualitative data without optimizing the parameters, but detection sensitivity might be compromised.)

Calibration parameters are instrument parameters whose values do not vary with the type of experiment. It is recommended that you calibrate the MS detector at least once every three months and that you check the calibration about once a week.

Automatic and semi-automatic calibration (including checking the calibration) require that you introduce calibration solution into the MS detector at a *steady flow rate* while the procedure is running. You introduce the solution directly from the syringe pump into the MS detector in the ESI/MS mode.

Tune parameters are instrument parameters whose values can vary with the type of experiment. For example, if your experiment requires quantitative data on one or more particular ions, you need to tune the MS detector with your analyte. You also have to tune the MS detector if you change any one of the parameters specific to the experiment or analyte.

Automatic and semi-automatic tuning procedures (including optimizing the collision energy) require that you introduce calibration solution, or a tuning solution of your analyte of interest, into the MS detector at a steady rate in either of two ways:

- Introduce the solution directly from the syringe pump. Refer to the topic: [Setting Up the Syringe Pump for Tuning and Calibration](#) in [Chapter 3](#).
- Introduce the sample from the syringe pump into the effluent of the LC by using a Tee union. Refer to the topic: [Setting Up to Introduce Sample by Syringe Pump into Solvent Flow from an LC](#) in [Chapter 4](#).

The first method is good for tuning if you intend to use an experiment type at a low flow rate involving the syringe pump. The second method is useful if you intend to use an experiment type at a higher flow rate involving the LC. However, the second method of introduction puts a comparatively large amount of analyte into the MS detector. Therefore, before you can perform an analytical run to analyze for the analyte, you might need to clean the API spray shield.

Caution Do not use the calibration solution at flow rates above 10 $\mu\text{L}/\text{min}$. Ultramark 1621 can contaminate your system at high concentrations.

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

Note The most important parameters that affect the signal quality during ESI/MS operation are the ion transfer capillary temperature, tube lens voltage, gases, and solution flow rate. For optimum sensitivity, tune with the instrument in the same operational mode as the mode you use for the analytical experiment.

Table 4 summarizes methods of sample introduction for each of the calibration and tuning procedures.

Table 4. Summary of methods of sample introduction for calibration and tuning

Sample/ Sample Intro	Calibrating			Tuning			
	Check	Auto	Semi- auto	Auto	Semi- auto	Manual	Collision Energy
Calibration solution/ Syringe pump	✓	✓	✓	✓	✓	✓	✓
Your tune solution/ Syringe pump				✓	✓	✓	✓
Your tune solution/ Syringe pump into LC flow by using Tee union				✓	✓	✓	✓

What Types of Experiments Can I Perform with the Finnigan LXQ MS Detector?

This topic describes several types of experiments that you can perform with the Finnigan LXQ MS detector. The experiments can be grouped into the following categories:

- General MS or MSⁿ
- Data-Dependent™
- Ion Mapping™
- Ion Tree

You can specify which type of experiment you want to perform in the Xcalibur Instrument Setup window, and then save it in an Xcalibur Instrument Method (*.meth*) file.

Note Procedures for these experiments are beyond the scope of this **Finnigan LXQ Getting Started** manual. For more information you may contact: a) Thermo North American Customer Support Center in West Palm Beach, Florida, USA, at 1-800-765-4532, b) Thermo European Customer Institute, UK, at 44-1442-233-555, c) Thermo Japan, 81-66387-6681, d) Thermo China, 86-10-6621-0839. Customers in other regions please ask your Thermo representative about the Thermo Support Center that serves you. Online Help may also be useful.

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 Instrument Setup so you can get started with a General MS or MSⁿ experiment. For an example of a General MS or MSⁿ experiment template, see [Figure 1](#)

General MS or MSⁿ

In a General *MS* quantitation experiment, you need to specify: a) the mass range of your analyte(s) of interest, b) a parent (precursor ion) that fragments into distinctive product ions, and c) the mass-to-charge ratios of *all* the parent ions of interest. The Finnigan LXQ MS detector can then collect data on the ions in the range or on the product ions of the parent ion(s) that you specify.

If you use a General experiment to collect data for qualitative (structural) analysis, you specify the scan mode (MS through MSⁿ) for which you want data in the Scan Event Settings group box. If you specify MS/MS or MSⁿ,

you then choose the parent ion(s) for which you want data in the MSⁿ Settings table. The Finnigan LXQ MS detector can then collect distinct qualitative information for structural analysis or for spectral reference.

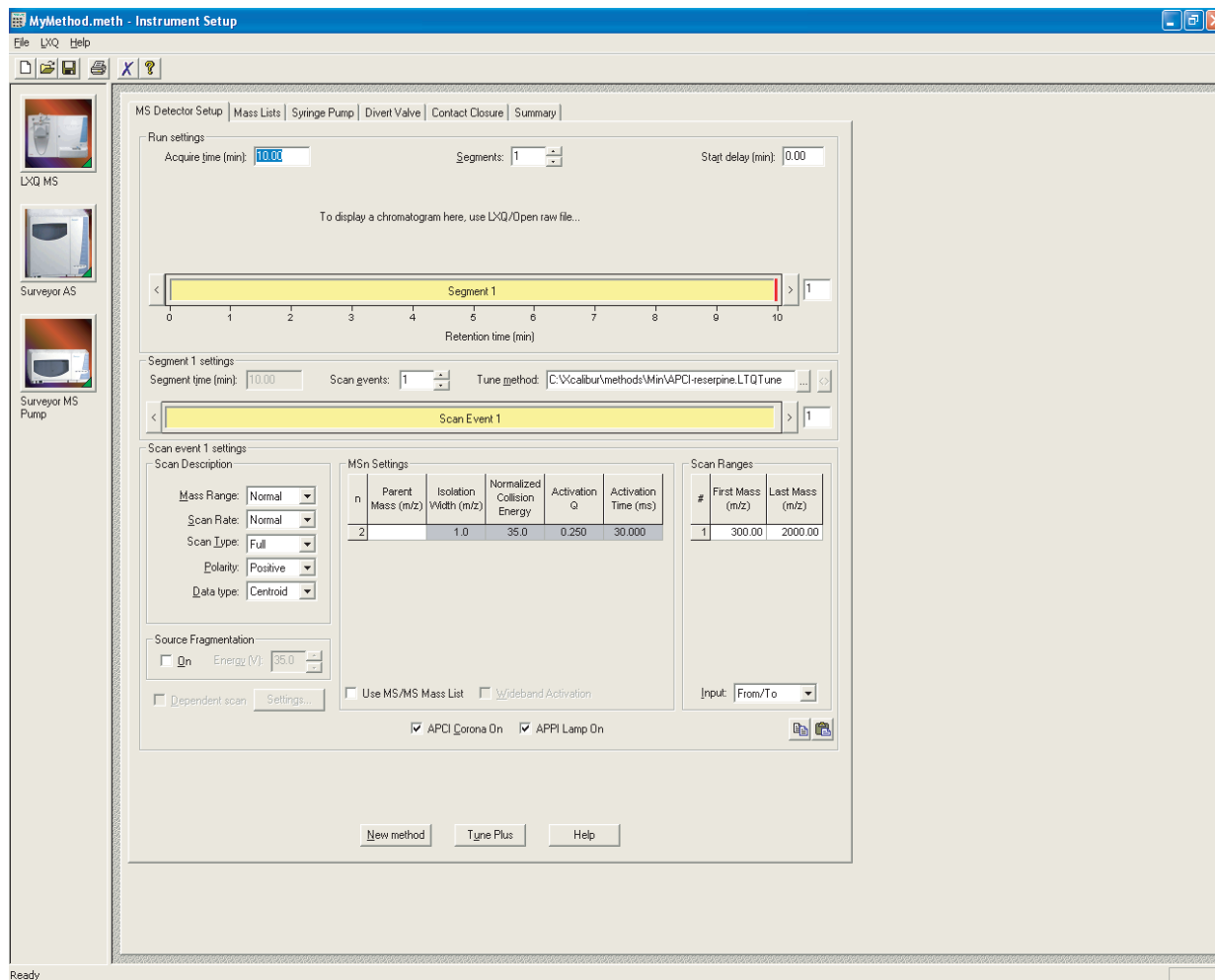


Figure 1. MS Detector Setup page in Instrument Setup, showing a template for a General MS experiment

The Finnigan LXQ MS detector can generate reproducible, analyte-specific spectra, even from laboratory to laboratory. Consequently, reference spectra that are generated with the Finnigan LXQ MS detector can be used to confirm structures of compounds generated with other Finnigan LXQ systems.

Data-Dependent Experiments

A Data-Dependent experiment is best used for the qualitative analysis of unknown compounds for structure elucidation or confirmation. These experiments are designed to maximize the data obtained while requiring minimal user input. The Finnigan LXQ MS detector uses the information

in a Data-Dependent experiment to make decisions about subsequent experiments automatically. Xcalibur Instrument Setup contains the Instrument Method templates that you need to get started with Data-Dependent experiments.

A Data-Dependent experiment produces a great deal of data from a single sample analysis. In a Data-Dependent experiment, you can specify parent ions for fragmentation or you can let the Finnigan LXQ MS detector automatically select the ions for fragmentation. The Finnigan LXQ MS detector can collect the structural information for every parent ion in the sample automatically, even if the sample is a mixture of compounds.

A Data-Dependent experiment requires that the user specify that one or more scan events of an experiment segment are to be run as Data-Dependent. For example, in a Data-Dependent Triple Play experiment for a mixture of compounds, the Finnigan LXQ MS detector decides which parent ion to isolate, estimates the charge state of the parent ion (zoom scan) and performs MS/MS for the appropriate product ion mass range. For an example of a Data-Dependent Triple Play experiment template, see [Figure 2](#).

Ion Mapping experiments can be Data-Dependent. (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.

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.

You can approach the setup of Data-Dependent experiments in either of two ways:

- If you have some idea of the parent ion, or if you expect a certain kind of parent, you can set up a list of possible parent ions. Then, when one of the specified parent ions is detected, you can acquire product spectra and analyze the information.

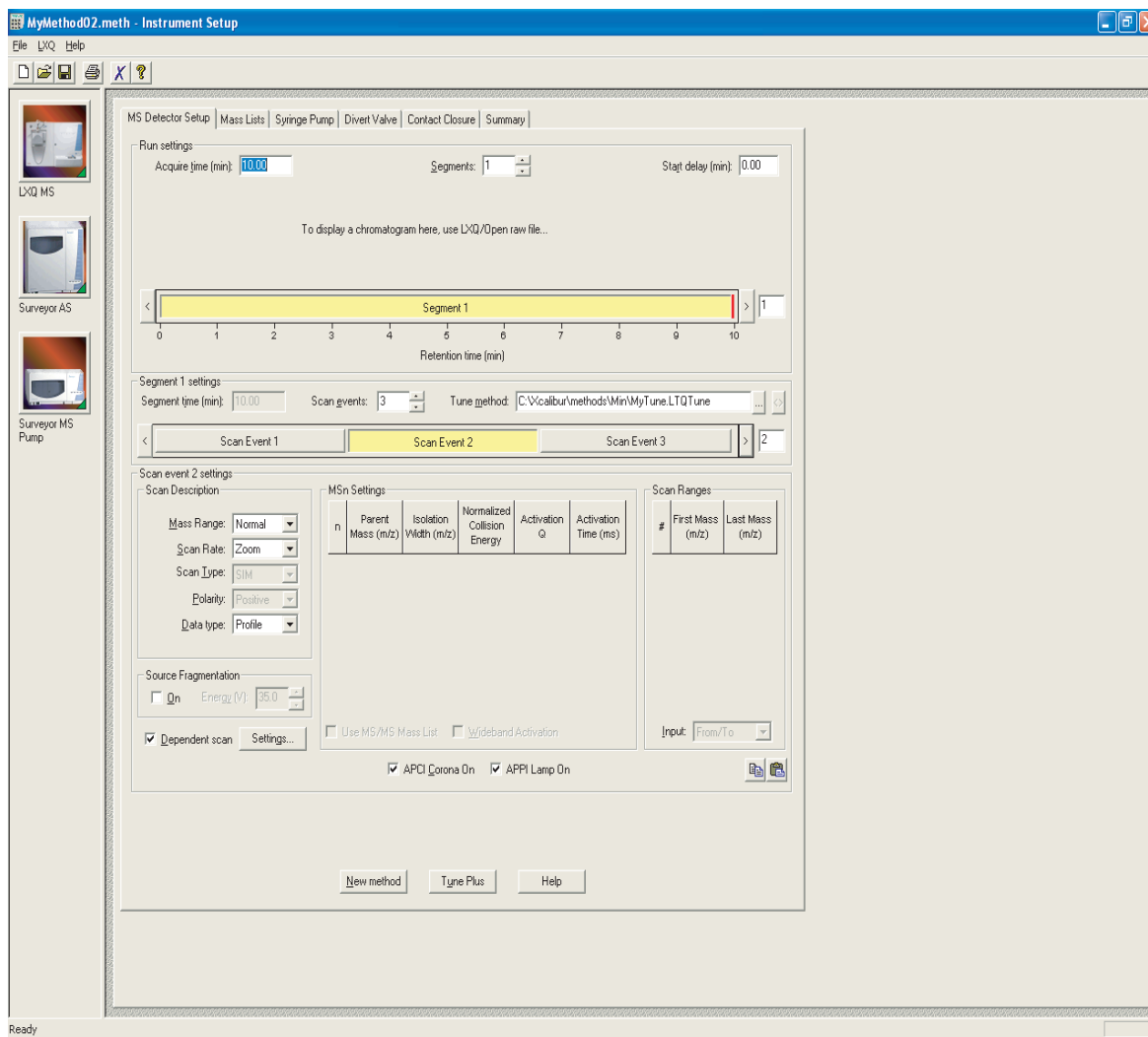


Figure 2. MS Detector Setup page in Instrument Setup, showing a template for a Data-Dependent Triple Play experiment. (To select a scan event that makes active the Dependent Scan check box, you click on either the Scan Event 2 or Scan Event 3 button.)

- If you have little information about your 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 Finnigan LXQ MS detector generates product spectra. Parameters that you might specify, for example, include threshold values for the intensity of the MS or MSⁿ ion signal.

1 Introduction

What Types of Experiments Can I Perform with the Finnigan LXQ MS Detector?

You can find useful structural information about your compound automatically with the simplest Data-Dependent experiment, Data-Dependent MS/MS. In this experiment you specify only the MS scan range, you do not need to specify a parent ion. The Finnigan LXQ MS detector can then collect full scan MS data, pick the most intense parent ion in the spectrum, and then fragment the ion to generate product ions.

You can use a Data-Dependent experiment (from templates in Instrument Setup) to do the following:

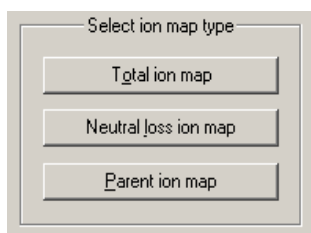
- Identify low-level impurities in high-purity compounds (Data-Dependent MS/MS)
- Identify metabolites in a complex mixture (Chromatographic Separation with Data-Dependent MS/MS)
- Build a custom library of composite MSⁿ spectra (Ion Tree)

You can use a Data-Dependent MSⁿ experiment to identify process impurities. In the quality assurance process for aspirin, for example, the Finnigan LXQ MS detector can identify impurities of less than 0.1%.

A Data-Dependent MS/MS experiment of a complex mixture of drug metabolites can provide highly specific structural information. Characteristic masses along the metabolic pathways of a drug, for example, can produce MS/MS spectra that are specific to the structure of the drug. These spectra are essential in metabolite identification.

A Data-Dependent experiment can produce a composite spectrum of, for example, MS₂, MS₃, and MS₄ data. The Finnigan LXQ MS detector can store the MSⁿ fingerprint data in a custom MSⁿ library spectrum. The data are valuable for use in process control, quality assurance, or research.

Ion Mapping Experiments

A rectangular button with a light gray background and a thin black border. The text "Ion mapping..." is centered in a dark gray font.

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

The Finnigan LXQ MS detector includes the following Ion Mapping templates in Instrument Setup so you can get started with an Ion Mapping experiment:

- Total (or full scan) Ion Map

- Neutral Loss Ion Map
- Parent Ion Map

These Ion Mapping experiments, in general, require that sample solution enter the MS Detector at a composition that is constant throughout. Therefore, you use infusion to introduce your sample for these Ion Mapping experiments. See Figure 1-3 for an example of an Ion Mapping experiment template.

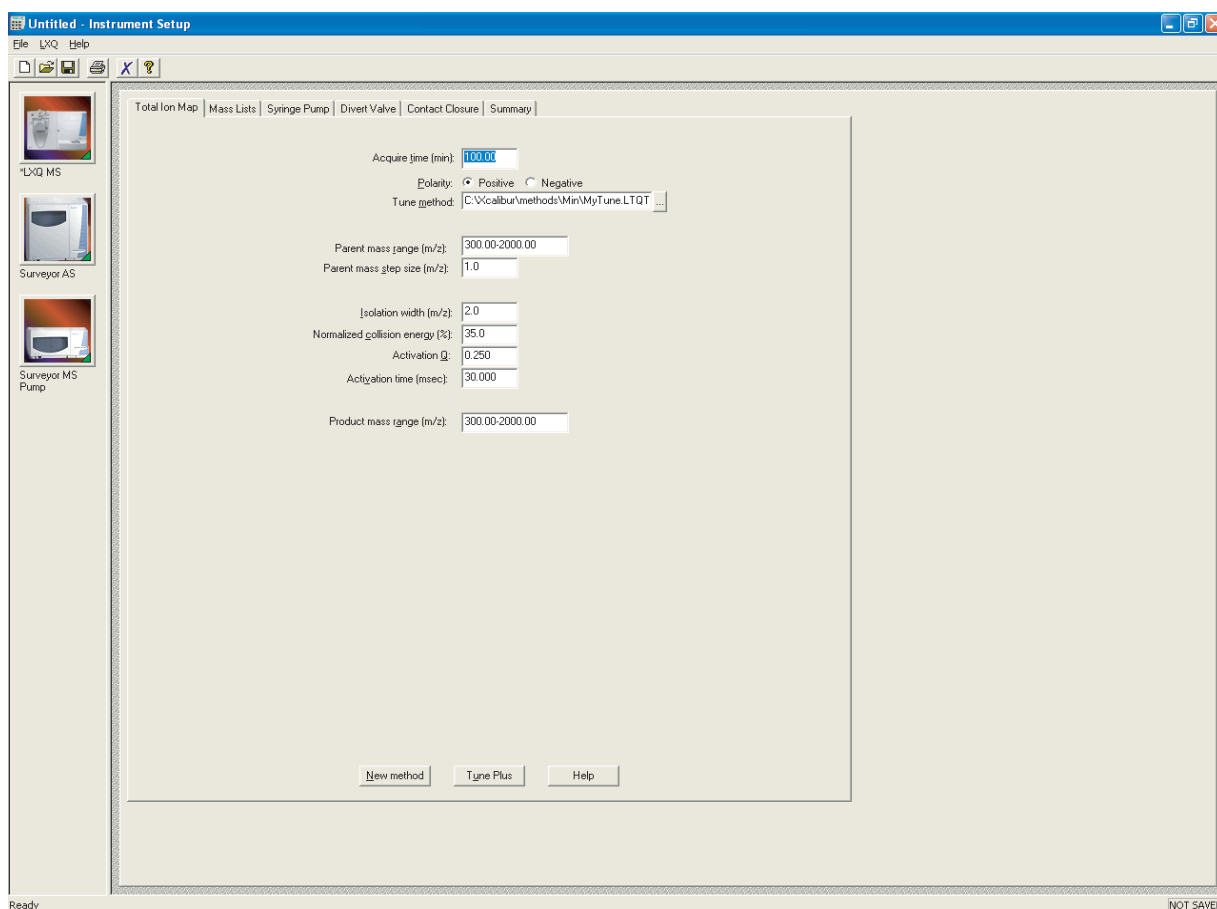


Figure 3. Total Ion Map page in Instrument Setup, showing a template that contains parameters for an Ion Mapping experiment

In a Total (or full scan) Ion Mapping experiment, you get product ion scans for each parent ion, so you can determine which parent ions lost a particular fragment to yield a particular product ion. Furthermore, you can determine which parent ions are related to specific product ions. For example, you can map the spectral peaks in a mass range from m/z 400 to m/z 2000 and

1 Introduction

What Types of Experiments Can I Perform with the Finnigan LXQ MS Detector?

specify to scan 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.

A Neutral Loss Ion Mapping Experiment collects scans for masses that have lost neutral fragments. As with Full Scan Ion Mapping, you can get product ion scans on every parent ion. However, a Neutral Loss Ion Map identifies which parent ions lost a neutral fragment of a particular mass. For example, you can specify a neutral loss of 80 u (as in the case of a phosphorylated peptide). A Neutral Loss Ion Mapping experiment can step through each product mass in the mixture. The experiment searches for evidence of the loss of a neutral moiety of mass 80 u.

A Parent Ion Mapping experiment identifies all the ions that produce a particular molecular ion that you specify. For example, if you specify a product ion mass of m/z 50, a Parent Ion Map includes all the parent ions that yielded the specified product ion, m/z 50.

A Data-Dependent Zoom Map is an Ion Mapping experiment that collects ZoomScan data on every scan interval in a mass range that you specify, as well as Data-Dependent MS/MS product spectra on every mass above an intensity threshold.

The results of any of the Ion Mapping experiments can be viewed in the Xcalibur Qual Browser window.

Ion Tree Experiments

Data dependent ion tree

In an Ion Tree experiment, the Finnigan LXQ MS detector can collect MSⁿ data automatically. You can specify a particular parent ion for fragmentation, or you can let the Finnigan LXQ MS detector find the parent ions automatically and fragment them to any level between MS₂ and MS₁₀. The Finnigan LXQ MS detector automates the collection of data by deciding what actions need to occur next for the experiment to progress. See [Figure 4](#) for an example of an Ion Tree experiment template.

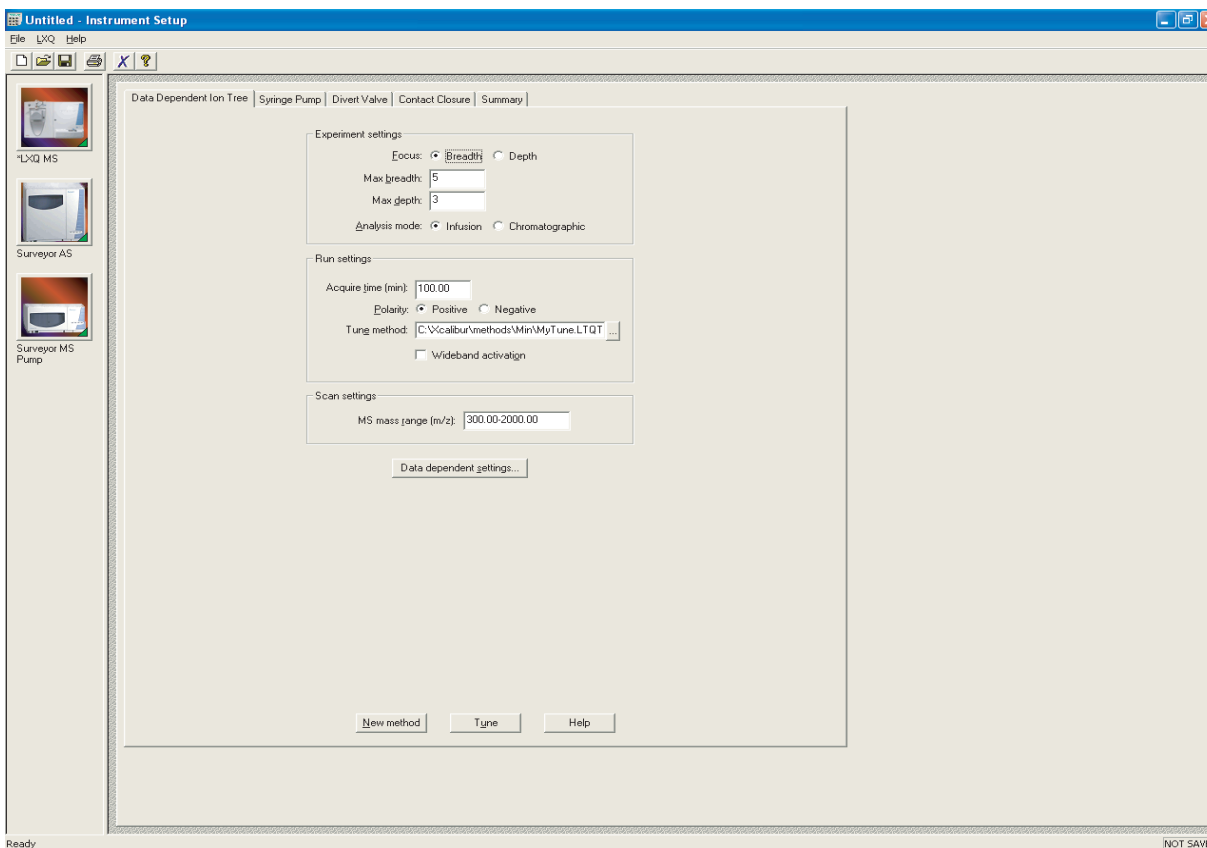


Figure 4. Data-Dependent Ion Tree page in Instrument Setup, showing a template for an Ion Tree experiment

In an Ion Tree experiment, you can specify either of two options that prioritize how the Finnigan LXQ MS detector gathers information:

Depth Focus and *Breadth Focus*.

- *Depth Focus* characterizes an ion by performing a series of MS^n -level fragmentations (for example, MS/MS , MS^3 , MS^4 , etc.) before characterizing the next most intense ion in the MS^n series.
- *Breadth Focus* characterizes all ions to the same MS^n level before advancing to the next MS^n 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.

With either *Depth* or *Breadth Focus*, the Finnigan LXQ MS detector scans for parent ions (MS) over the specified mass range. The most intense ion of the MS spectrum is selected for fragmentation (MS/MS).

1 Introduction

What Types of Experiments Can I Perform with the Finnigan LXQ MS Detector?

- Then, if you chose the Depth Focus, after the most intense ion of the MS spectrum is fragmented—producing an MS/MS spectrum—the Finnigan LXQ MS detector 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 Finnigan LXQ MS detector 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 parent ion. This process is then repeated for the second most intense ion in the MS spectrum.
- If you chose the Breadth Focus, after the most intense ion of the MS spectrum is fragmented—producing an MS/MS spectrum—the Finnigan LXQ MS detector selects and fragments the second-most intense ion of the *same* MS spectrum. The fragmentation of parent ions continues to the *Max Breadth* level that you specified (2, for this example). After the two most intense peaks on the MS level are fragmented, the Finnigan LXQ MS detector scans the first MS/MS spectrum to select and fragment the two most intense ions. This results in product ions on the level of MS3, the level specified as the maximum depth for this example. This process is then repeated for the second most intense ion in the MS spectrum.

The results of a Data-Dependent Ion Tree experiment can be viewed in the Xcalibur Qual Browser window. The results are displayed as a structure tree that originates from a particular parent ion.

Chapter 2 **Setting Up the Ion Source for Tuning and Calibrating the MS Detector**

This chapter provides information on setting up the hardware for tuning and calibrating your Finnigan LXQ MS detector. You tune and calibrate the MS detector in the ESI mode before you acquire data in either the ESI or APCI mode.

This chapter contains the following sections:

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

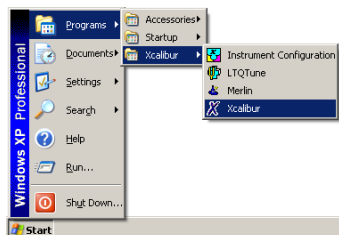
2 Setting Up the Ion Source for Tuning and Calibrating the MS Detector

Placing the LC/MS System in Standby

Placing the LC/MS System in Standby

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

To place the LC/MS system in Standby:



OnOffStandby

1. If necessary, stop the flow of solvent to the API source:
 - a. If the Xcalibur data system is not already open, choose **Start > Programs > Xcalibur > Xcalibur** from the Windows® taskbar to open the Xcalibur window.
 - b. In the Xcalibur Home Page window – Roadmap view, choose **GoTo > Instrument Setup** to open the Instrument Setup window.
 - c. Click on the Surveyor® MS Pump button on the view bar in the Instrument Setup window to display the Surveyor MS Pump view.
 - d. Choose **Surveyor MS Pump > Direct Control** to open the Surveyor MS Pump Direct Control dialog box.
 - e. In the Direct Control dialog box, click on the Pump Off button to stop the MS pump.
2. If Tune Plus is not already open, choose **Start > Programs > Xcalibur > LTQTune** from the taskbar to open Tune Plus.

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

3. If the MS detector is On, click on the On/Standby button to place the MS detector in the Standby mode. When the MS detector is in Standby, the Finnigan LXQ MS detector turns off the ion source sheath gas, auxiliary gas, high voltage, and syringe pump.

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

If the ESI probe is already installed in the Ion Max™ ion source housing, leave the LC/MS system in Standby and go to: [Chapter 3, “Tuning and Calibrating Automatically in the ESI/MS Mode.”](#)

If the ESI probe is not already installed in the Ion Max ion source housing, go to the next topic: [Removing the APCI Probe.](#)

Removing the APCI Probe

This topic describes how to remove the APCI probe from the Ion Max ion source housing.

Note The following procedures assume that you are familiar with your instrument and software. If you need additional guidance, refer to Finnigan LXQ online Help, *Finnigan LTQ Getting Connected*, *Finnigan Ion Max API Source Hardware Manual*, or the *Finnigan LTQ Hardware Manual*



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

To remove the APCI probe:

1. Unplug the vaporizer heater cable from the vaporizer heater cable socket on the APCI probe. See Figure 5.
2. Disconnect the sample transfer line from the APCI probe. (See Figure 5.)
3. Remove the auxiliary gas line (green colored fitting) from the APCI probe. (Figure 5)
4. Remove the sheath gas line (blue colored fitting) from the APCI probe.
5. Remove the APCI probe as follows:
 - a. Connect the vaporizer heater cable to the ESI interlock socket on the ion source housing. See Figure 6.
 - b. Release the probe locking lever to loosen the probe collar. You might need to unscrew the lever a few turns to permit probe movement.
 - c. Carefully pull the probe straight back in the port in the housing until it meets with the slot in the API interlock block. The guide pin on the probe manifold will prevent you from rotating the probe until the pin is aligned with the slot in the API interlock block. Once the probe is all the way back and aligned with the slot, turn the probe 45 degrees counter-clockwise to free the probe from the alignment notch.
 - d. Pull the probe straight out to remove it from the ion source housing.
 - e. Store the APCI probe in its original shipping container.

2 Setting Up the Ion Source for Tuning and Calibrating the MS Detector

Removing the APCI Probe

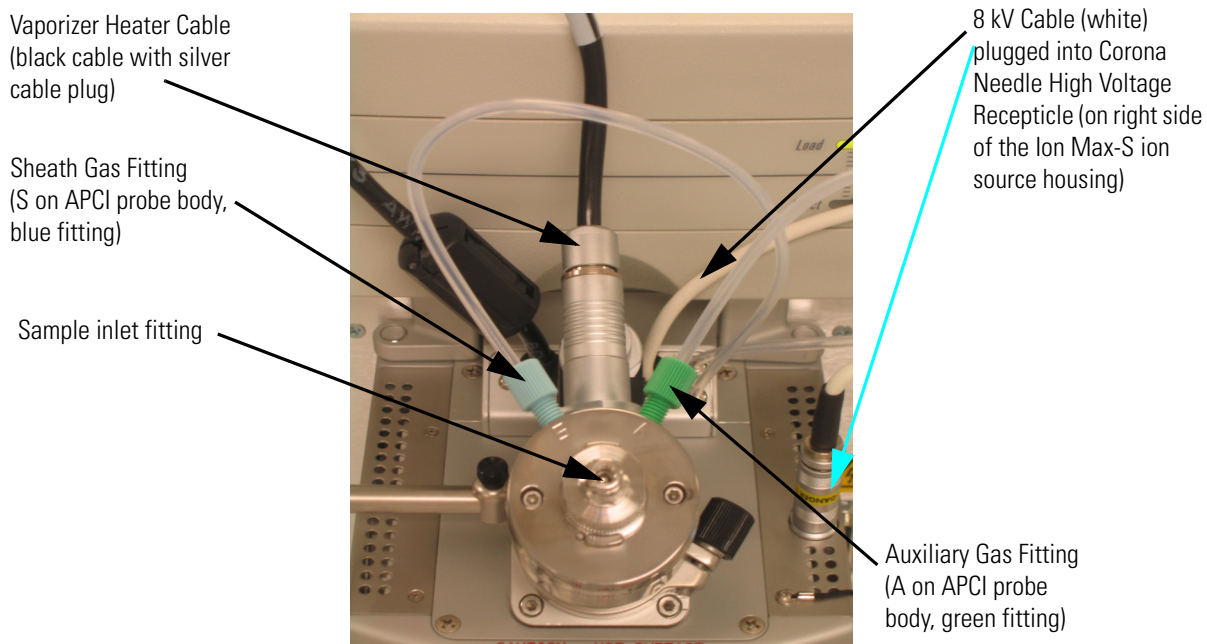


Figure 5. Ion Max ion source housing with APCI probe installed

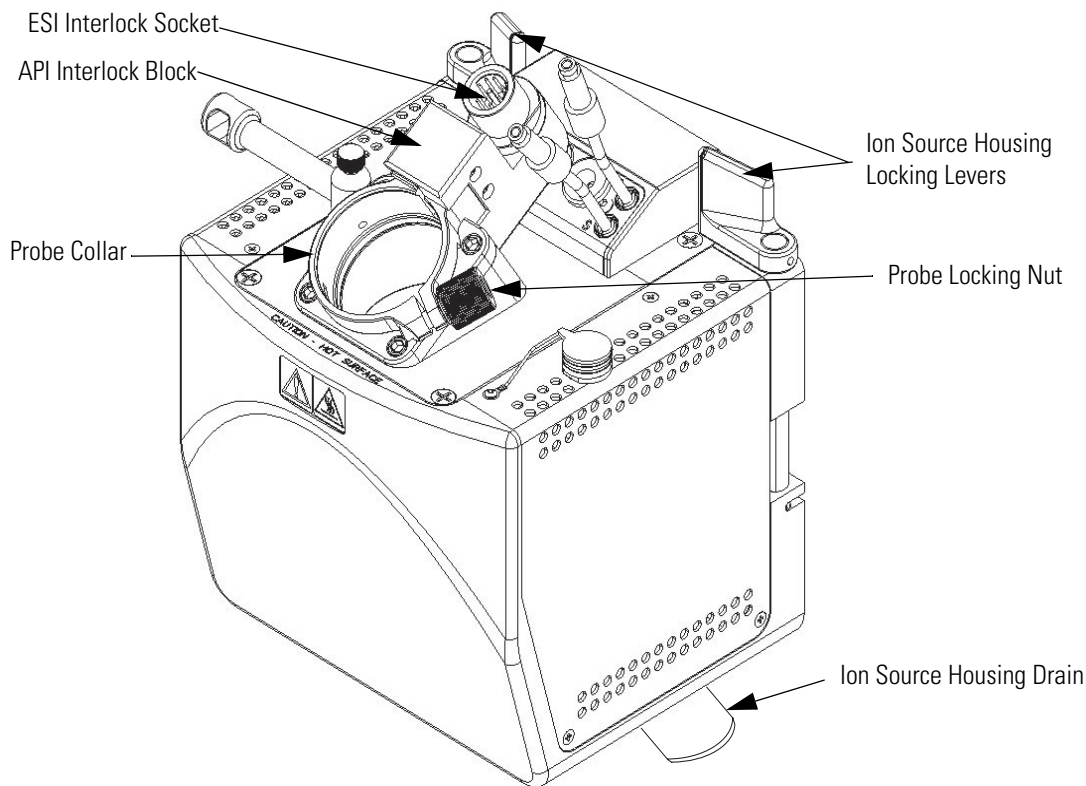


Figure 6. Ion Max-S ion source housing, detail of components

6. Remove the 8 kV cable from the corona needle high voltage receptacle as follows:
 - a. Unlock the cable by rotating the locking ring counter-clockwise.
 - b. Unplug the 8 kV cable from the corona needle high voltage receptacle.



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

7. Remove the corona needle as follows:
 - a. Unlock the ion source housing by turning the ion source locking levers 90 degrees (see [Figure 6](#)). Also see the next Section, [Removing the Ion Max Ion Source Housing](#) and [Figure 8](#).
 - b. Remove the ion source housing by pulling the housing straight off of the ion source assembly.
 - c. The corona needle is in the corona assembly which is inside of the ion source housing. Using pliers, grasp the corona needle and pull it straight out of the corona needle contact (see [Figure 7](#)).
 - d. Remount the ion source housing per the Section [“Installing the Ion Max Ion Source Housing”](#) on [page 34](#) and [Figure 11](#). Or, place the ion source housing in a safe location for temporary storage.
8. Store the corona needle in its original shipping container.

The APCI probe and the corona needle are now properly removed from the Ion Max ion source housing.

If you want to install the optional ion sweep cone, go to the next topic: [Removing the Ion Max Ion Source Housing](#).

If you do not want to install the ion sweep cone, go to the topic: [Installing the ESI Probe](#).

2 Setting Up the Ion Source for Tuning and Calibrating the MS Detector

Removing the APCI Probe

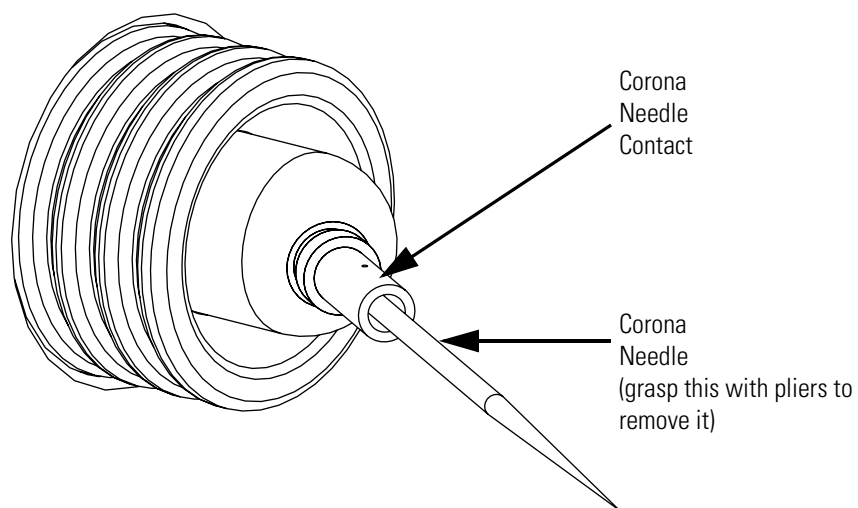


Figure 7. Corona needle, view from inside of the Ion Max housing

Removing the Ion Max Ion Source Housing

The Ion Max ion source housing is removed to access the ion sweep cone.

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

To remove the ion source housing:

1. Detach the drain tube from the ion source housing drain. See [Figure 8](#).
2. Rotate the ion source housing locking levers 90 degrees to release the ion source housing from the ion source mount assembly.
3. Remove the ion source housing by pulling the housing straight off of the ion source mount assembly
4. Place the ion source housing in a safe location for temporary storage.

The Ion Max ion source housing is now properly removed.

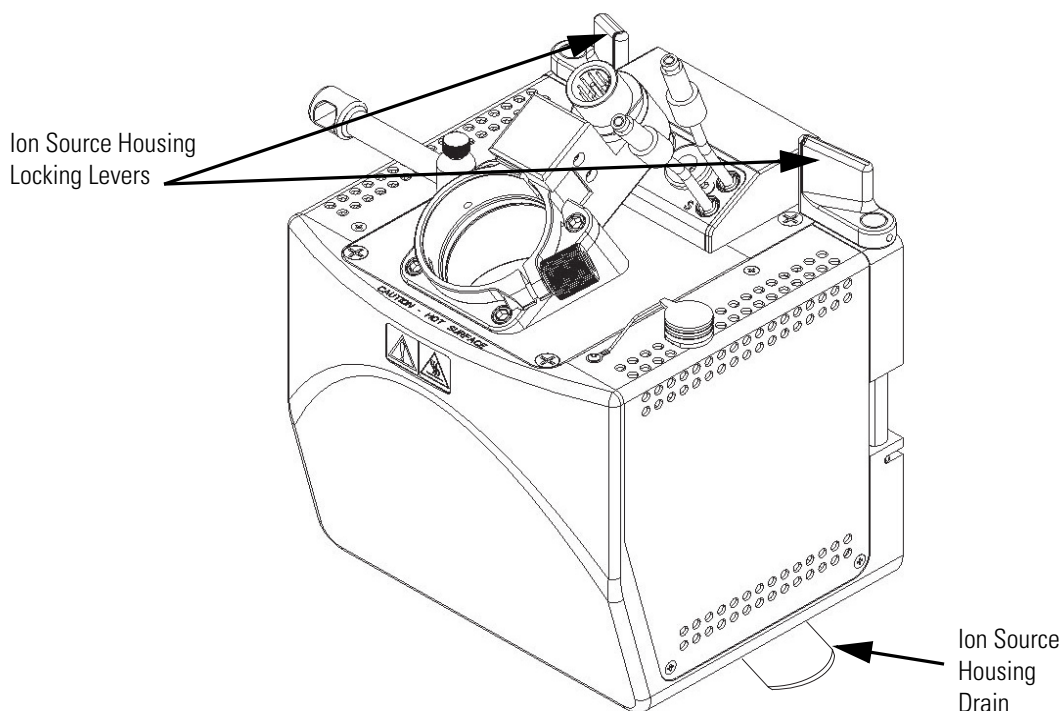


Figure 8. Ion Max-S ion source housing, detail of components

Installing the Ion Sweep Cone

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

Install the ion sweep cone as follows:

1. Remove the ion sweep cone from its storage container. Inspect and clean it if necessary.
2. Note the location of the sweep gas supply port in the API cone seal. The gas inlet on the ion sweep cone is placed in this port. See [Figure 9](#) and [Figure 10](#).

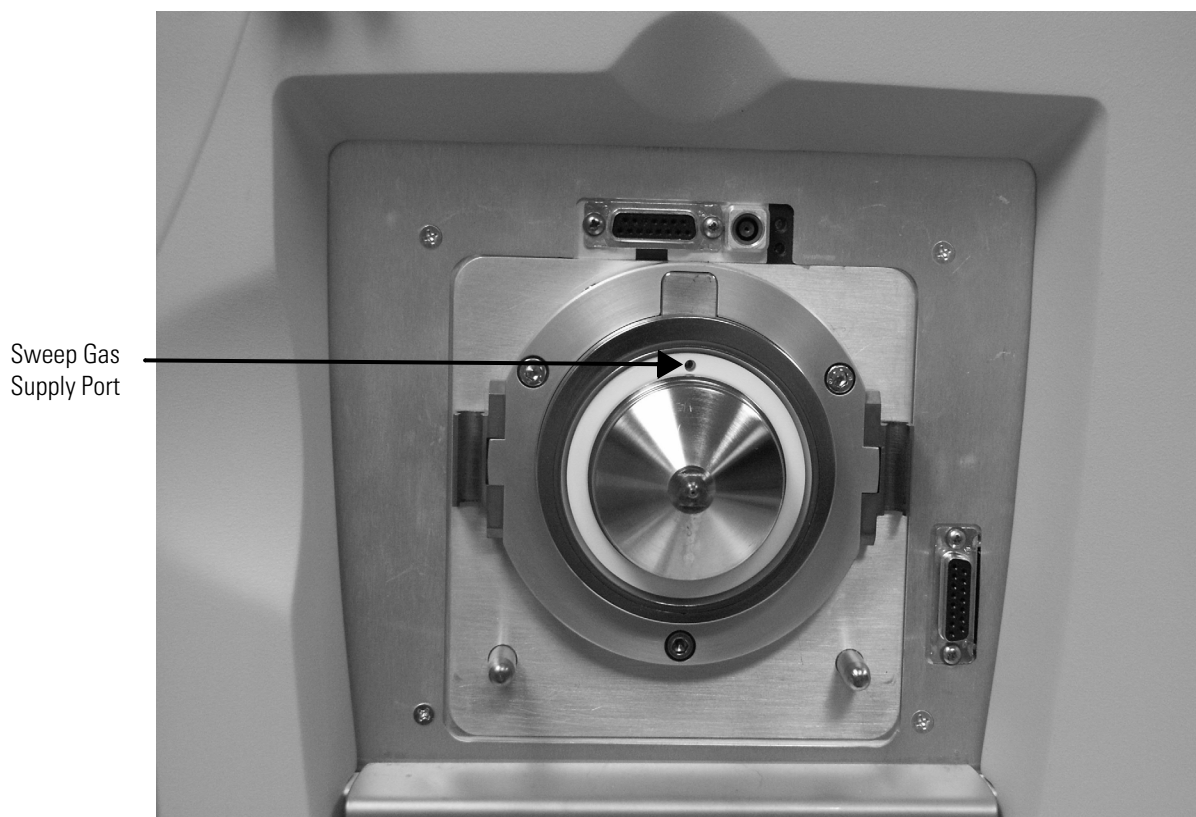


Figure 9. Sweep gas supply port in the API cone seal

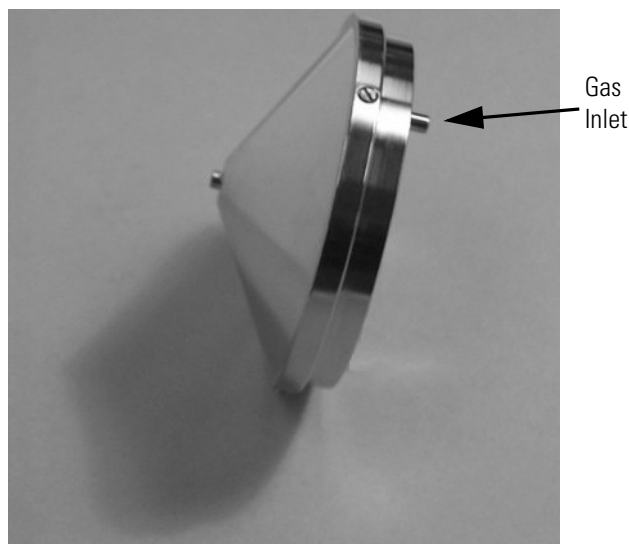


Figure 10. Ion sweep cone, showing the gas inlet



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

3. After the ion transfer tube has cooled to room temperature, carefully align the gas inlet on the ion sweep cone with the sweep gas supply port in the API cone seal. Firmly press the ion sweep cone into position.
4. If necessary to achieve a proper ion sweep cone installation, you might adjust the set screws around the perimeter of the ion sweep cone.

The ion sweep cone is now properly installed on the MS detector.

Installing the Ion Max Ion Source Housing

Reinstall the Ion Max ion source housing as follows:

1. Carefully align the two guide pin holes on the rear of the ion source housing with the ion source housing guide pins on the MS detector, and carefully press the ion source housing onto the ion source mount. See [Figure 11](#) and [Figure 12](#).

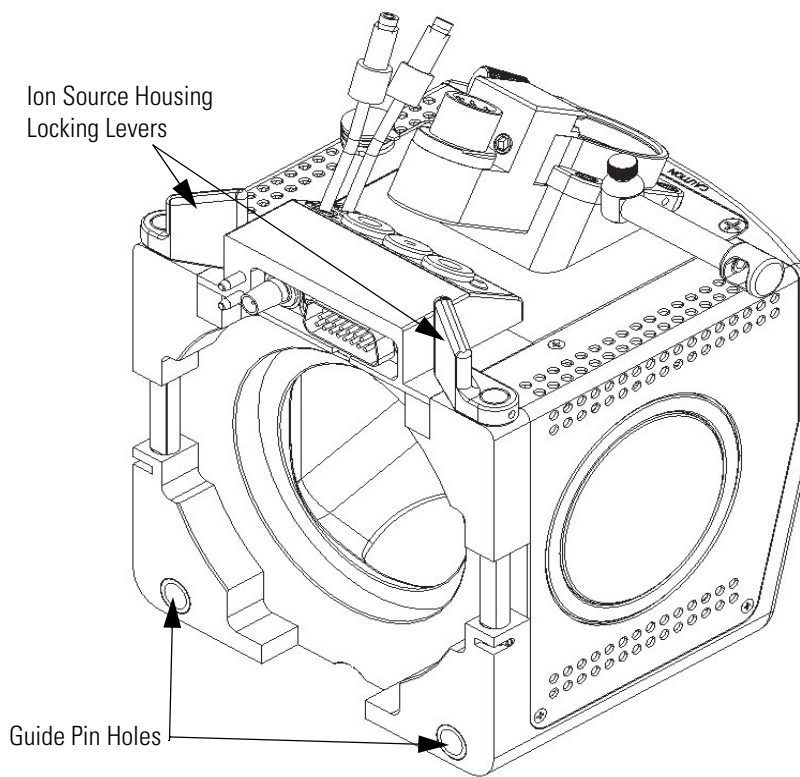


Figure 11. Rear view of the Ion Max-S ion source housing

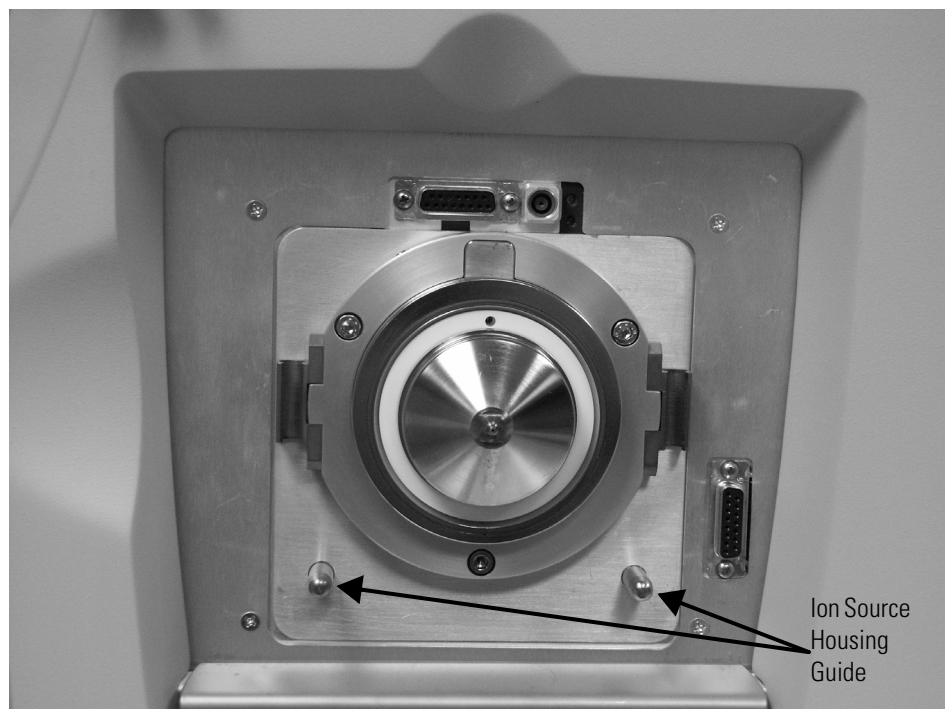


Figure 12. Ion source mount showing ion source housing guide pins

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



CAUTION Prevent solvent waste from backing up into the ion source and MS detector. Always ensure that liquid in the drain tube is able to drain to a waste container.

3. Reattach the ion source drain tube as follows:

2 Setting Up the Ion Source for Tuning and Calibrating the MS Detector

Installing the Ion Max Ion Source Housing



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

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

- a. Connect the 1-in. ID Tygon® tubing to the ion source housing drain.
- b. Attach the free end of the hose to a dedicated drain system. Ideally, the drain system should be vented to a fume exhaust system.

The Ion Max ion source housing is now properly installed on the MS detector.

Installing the ESI Probe

To install the ESI probe:

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

Note If your ESI probe does not already have a sample tube (fused-silica capillary or metal needle) and safety sleeve attached, you need to follow the procedure for installing a sample tube and PEEK safety sleeve that is outlined in the topic Installing a New Fused-Silica Sample Tube and PEEK Safety Sleeve in the *Finnigan Ion Max API Source Hardware Manual*.

2. Ensure that the probe locking nut on the ion source housing (Ion Max or Ion Max-S) is loosened. The probe locking nut is shown in [Figure 13](#).
3. Insert the ESI probe into the port in the ion source housing, align the guide pin on the probe body at a minus 45 degree angle from the API interlock block. See [Figure 14](#)

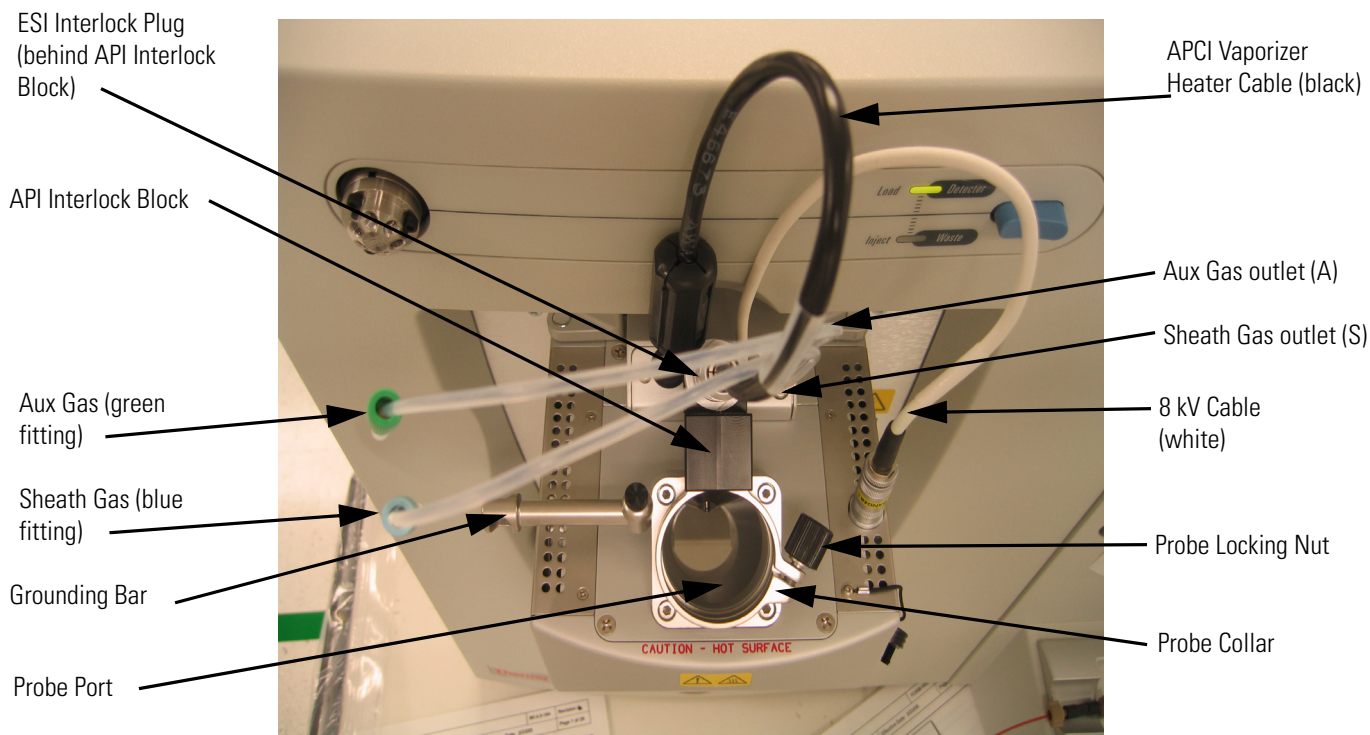


Figure 13. Ion Max-S ion source housing with some components labelled.

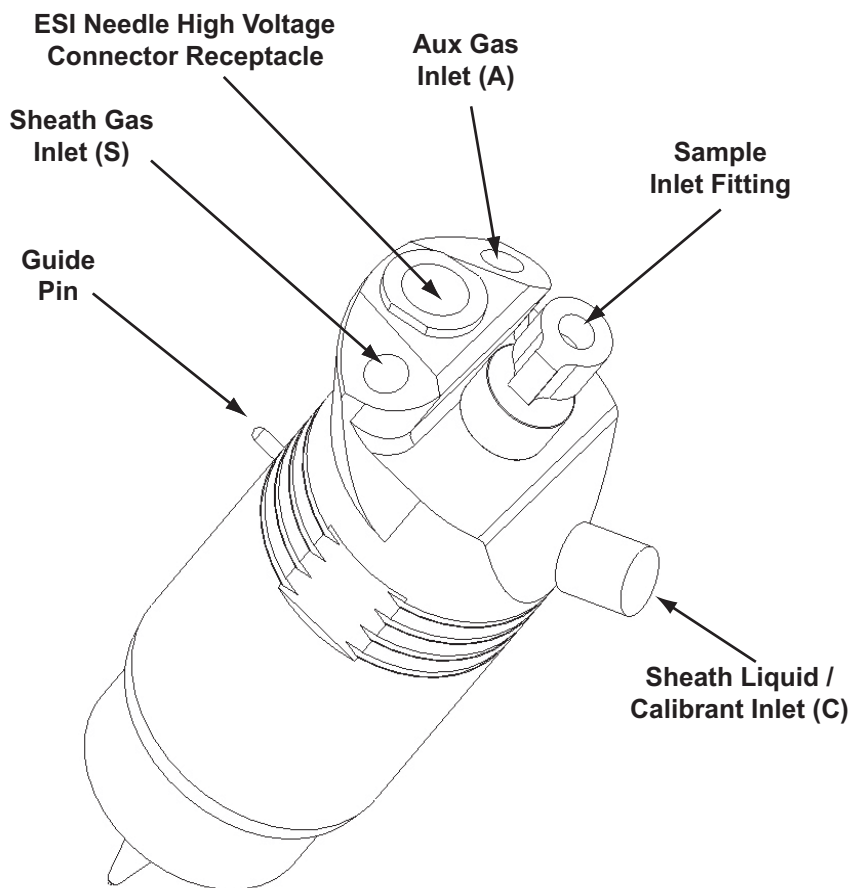


Figure 14. ESI probe, side view

4. Push the probe into the port until the guide pin meets with the probe collar on the ion source housing.
5. Turn the probe 45 degrees clockwise and align the guide pin with the slot in the API interlock block (you might need to pull the probe towards you slightly to properly align the pin with the notch). Once you have turned the probe far enough to align the pin with the alignment notch at the rear of the port, push the probe straight in until the guide pin stops at the bottom of the alignment notch.
6. Lock the probe in place by rotating the probe locking lever towards the front of the housing; closing the probe locking lever towards the rear of the ion source housing might make it difficult to unlock. You might first need to tighten the locking lever threaded shaft by rotating it clockwise a few turns if rotating the lever does not tighten the probe collar enough.
7. Insert the APCI vaporizer heater cable into the API interlock socket.

8. Insert the stainless steel ZDV fitting (grounding union) into the grounding bar on the ion source housing. See [Figure 15](#).

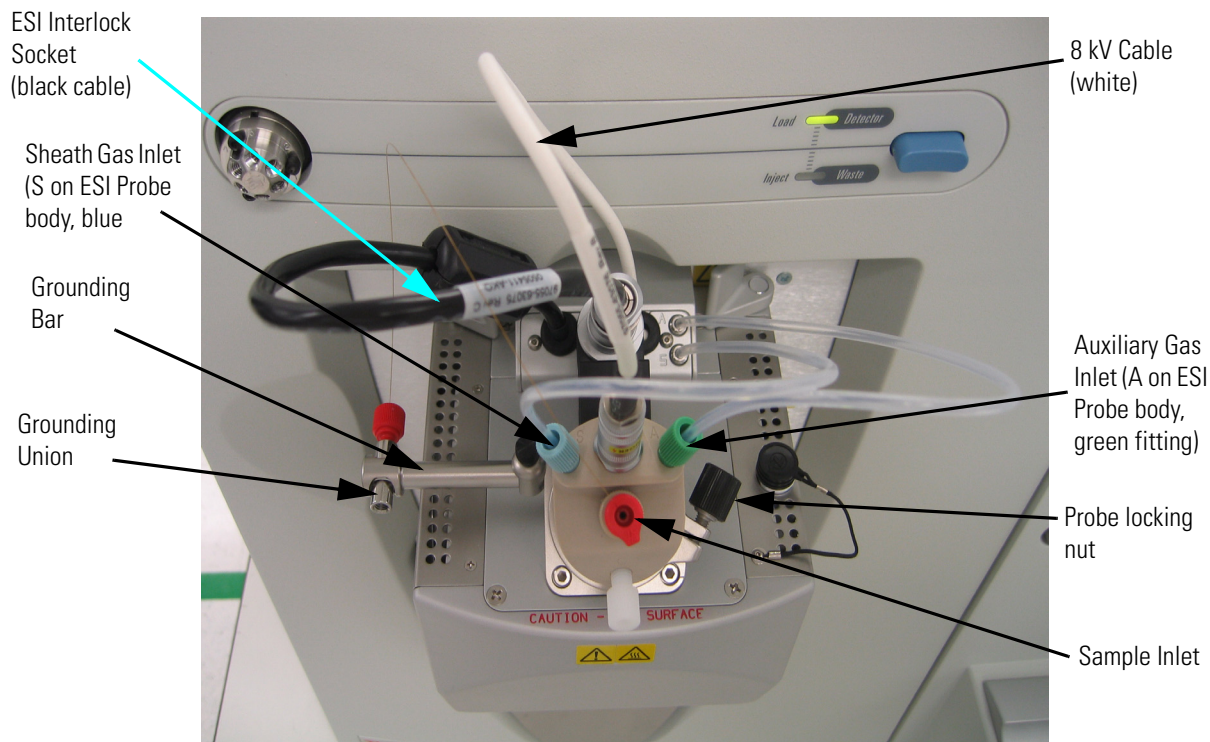


Figure 15. Ion Max-S ion source housing with ESI probe installed

9. Connect the sheath gas fitting (blue) to the sheath gas inlet (S) on the probe. (See [Figure 15](#).)
10. Connect the auxiliary gas fitting (green) to the auxiliary gas inlet (A) on the probe. ([Figure 15](#).)
11. Connect the 8 kV cable to the ESI needle high voltage receptacle on the ESI probe. Tighten the locking ring on the 8 kV connector.
12. Connect the sample transfer tubing to the grounding union.

The ESI probe is now properly installed in the Ion Max ion source housing.

Leave the LC/MS system in Standby and go to: [Chapter 3, “Tuning and Calibrating Automatically in the ESI/MS Mode.”](#)

Chapter 3 Tuning and Calibrating Automatically in the ESI/MS Mode

This chapter provides information on how to tune and calibrate the Finnigan LXQ MS detector in the ESI/MS mode. For most applications, you tune and calibrate in the ESI mode through automatic procedures. The procedures use a calibration solution that is introduced into the MS detector in low flow mode (refer to Table 2 on page 1-12.). You need to calibrate the MS detector every one to three months of operation for optimum performance over the entire mass range of the detector.

To tune and calibrate your MS detector automatically in the ESI/MS mode, you do the following:

Infuse a low concentration calibration solution containing caffeine, MRFA, and Ultramark 1621 into the ESI source by using the syringe pump. (Refer to the topic: [Setting Up the Syringe Pump for Tuning and Calibration.](#))

- Test the efficiency and stability of the spray of calibration solution into the MS detector. You can observe the following singly-charged, positive ions for caffeine, MRFA, and Ultramark 1621 in the Tune Plus window: m/z 195, 524, 1222, 1522, and 1822.
- Tune the MS detector from the Tune Plus window to optimize automatically the lenses.
- Calibrate the MS detector to adjust automatically the voltages of the linear trap.

This chapter contains the following topics:

- [Setting Up the Syringe Pump for Tuning and Calibration](#)
- [Setting Up the MS Detector in the Xcalibur Data System for Tuning and Calibration](#)
- [Testing the Operation of the MS Detector in the ESI/MS Mode](#)
- [Tuning the MS Detector Automatically in the ESI/MS Mode](#)

3 Tuning and Calibrating Automatically in the ESI/MS Mode

- [Saving Your ESI/MS Tune Method](#)
- [Calibrating the MS Detector Automatically](#)
- [Cleaning the MS Detector after Tuning and Calibrating](#)

Setting Up the Syringe Pump for Tuning and Calibration

You introduce tuning and calibration solution into the ESI source with a syringe infusion pump. A syringe pump allows you to infuse a sample solution into the ESI source for extended periods of time.

The syringe pump and syringe are located on the front panel of your Finnigan LXQ MS detector. To infuse solution for tuning and calibration, you install a 500-mL Unimetrics® syringe containing the calibration solution.

Note To minimize the possibility of cross-contamination, use a different syringe and section of fused silica tubing for the calibration solution than you do for your sample solution.

To set up the syringe pump for infusion:

1. Connect a 4 cm (1.5 in.) segment of Teflon® tube with a (brown) fingertight fitting and a (brown) ferrule to the (black) LC union. See [Figure 16](#).

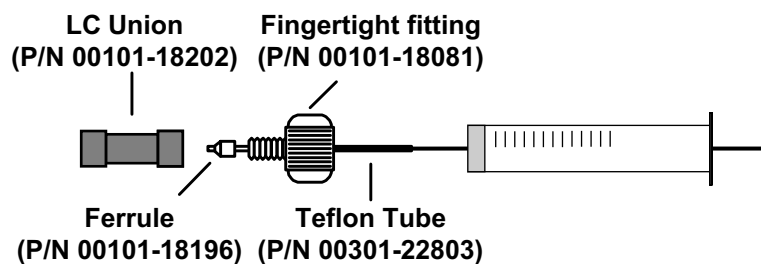


Figure 16. Plumbing connections for the syringe

2. Load a clean, 500- μ L Unimetrics syringe with 450 μ L of the calibration solution. (Refer to [Appendix A, "Sample Formulations."](#) for the calibration solution formulation procedure.)
3. Insert the syringe needle into the segment of Teflon tube.
4. Place the syringe into the syringe holder of the syringe pump.
5. While squeezing the blue release button on the syringe pump handle, push the handle forward until it just contacts the syringe plunger.
6. Connect a fused-silica infusion line from the LC union to the (stainless steel) grounding union as follows. See [Figure 17](#).

3 Tuning and Calibrating Automatically in the ESI/MS Mode

Setting Up the Syringe Pump for Tuning and Calibration

7. Connect the infusion line with a (brown) fingertight fitting and a (brown) ferrule to the free end of the LC union.
8. Connect the other end of the infusion line with a (red) fingertight fitting and a (brown) ferrule to the grounding union.

The syringe pump is now properly set up for infusing solution into the MS detector.

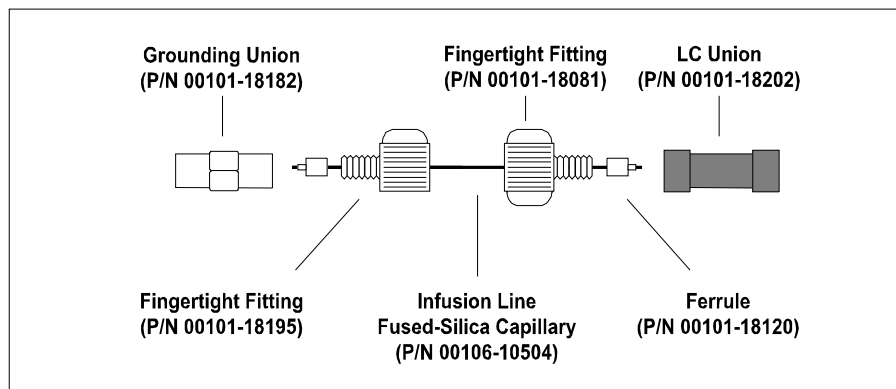


Figure 17. ESI/MS plumbing connections for the fused-silica infusion line

Setting Up the MS Detector in the Xcalibur Data System for Tuning and Calibration

You first tune manually with calibration solution to establish a stable spray of solution and to ensure that enough ions are detected to calibrate the MS detector. You then calibrate the MS detector automatically to optimize the parameters that affect ion detection.

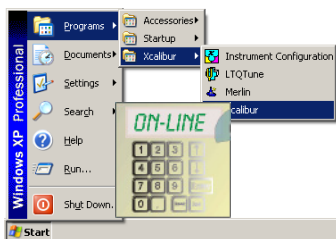
Note The following procedures assume that you are familiar with your Finnigan LXQ instrument and the Tune Plus window. If you need additional guidance, refer to: Finnigan LXQ online Help, *Finnigan LXQ Getting Connected*, and/or the *Finnigan LXQ Hardware Manual*.

CAUTION Before you begin normal operation each day, ensure that you have sufficient nitrogen for your ESI source. If you run out of nitrogen, the Finnigan LXQ MS detector automatically turns Off to prevent atmospheric oxygen from entering the ion source. The presence of oxygen in the ion source when the MS detector is On could be unsafe. (In addition, if the Finnigan LXQ MS detector automatically turns Off during an analytical run, you could lose data.)



Use the following procedure to set up the MS detector in the Xcalibur data system for tuning and calibration in the ESI/MS mode:

1. If you have not already done so, open the Tune Plus window from the Start button on your Windows XP task bar, as follows:



- a. Choose **Start > Programs > Xcalibur > Xcalibur** to display the Xcalibur Home Page – Roadmap view.
- b. Click on the Instrument Setup button to display the Instrument Setup window.
- c. Click the Finnigan LXQ button to display the New Method page.
- d. Click the Tune Plus button to display the Tune Plus window. See [Figure 18](#).



2. In the Tune Plus window, on the Control/Scan Mode toolbar, click on the On/Standby button to take the MS detector out of the Standby (or Off) mode and turn it On. When you turn the MS detector to On, you initiate the following events:

- The MS detector begins scanning.

3 Tuning and Calibrating Automatically in the ESI/MS Mode

Setting Up the MS Detector in the Xcalibur Data System for Tuning and Calibration

- Nitrogen flows into the ESI ion source.
- The Finnigan LXQ MS system applies high voltage to the ESI ion source.
- The Xcalibur data system shows a real-time mass spectrum display in the Spectrum view.

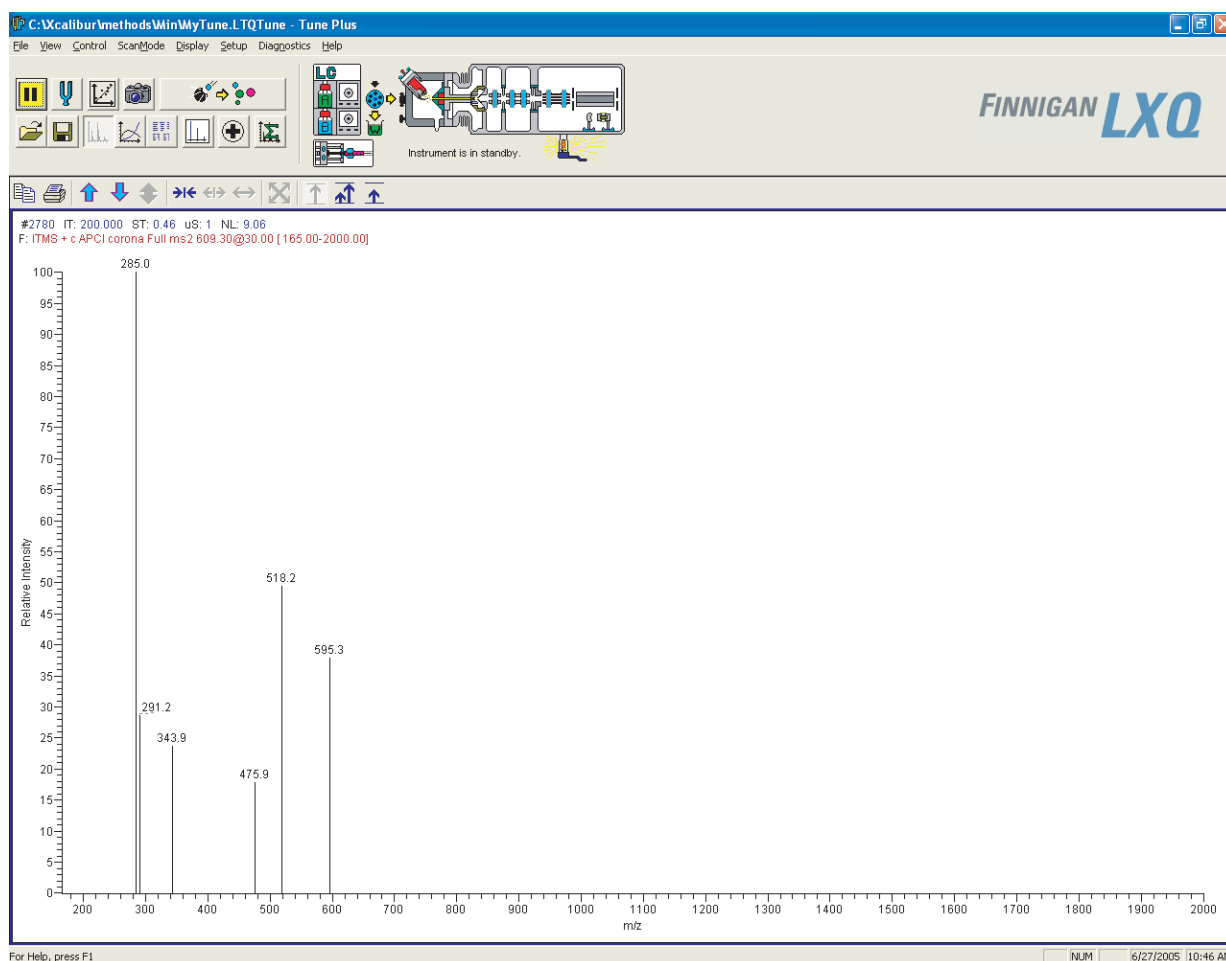


Figure 18. Tune Plus window, showing the MS detector in the Standby mode

Note The Xcalibur data system contains customized tune files for different applications in the folder *C:\Xcalibur\methods*, including one for low flow LC/ESI/MS operation.

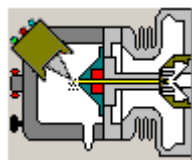
3. Open the Tune Method file that stores the factory default tune settings for low-flow ESI operation, as follows:

3 Tuning and Calibrating Automatically in the ESI/MS Mode

Setting Up the MS Detector in the Xcalibur Data System for Tuning and Calibration

- a. Choose **File > Open** to display the Open dialog box.
- b. Browse for the folder *C:\Xcalibur\methods*. Then, select the file *AutoTune.LTQTune*.
- c. Click **Open** to open the file. Tune Plus downloads the Tune Method parameters to the MS detector.

4. Examine the pre-tune ESI source settings as follows:



- a. From the Instrument Setup toolbar, click on the API Source button to open the ESI Source dialog box. Verify that the settings in your dialog box are the same as those shown in [Figure 19](#).
- b. Click **OK** to return to the Tune Plus window.

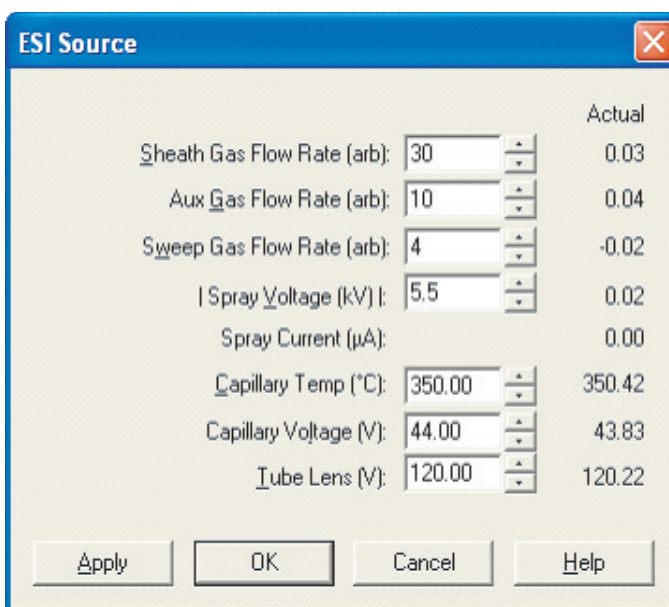


Figure 19. ESI Source dialog box, showing the settings to start a typical low flow experiment

5. Set the scan parameters for tuning and calibration, as follows:



- a. On the Control/Scan Mode toolbar, click on the Define Scan button to open the Define Scan dialog box. See [Figure 20](#). (If your dialog box appears different from the one shown in the figure, it is probably because the advanced settings are not displayed. You can turn on the advanced settings as follows: In Tune Plus, choose **ScanMode**, and then click on *Advanced Scan Features* to select the option.)

3 Tuning and Calibrating Automatically in the ESI/MS Mode

Setting Up the MS Detector in the Xcalibur Data System for Tuning and Calibration

- b. In the Scan Description group box, in the Mass Range list box, select *Normal* to allow for a selection of mass ranges between m/z 150 to 2000.
- c. In the Scan Rate list box, select *Normal* to specify a normal scan rate.
- d. In the Scan Type list box, select *Full* to specify a full scan.
- e. In the Scan Time group box, in the Microscans spin box, enter 1 to set the total number of microscans to 1.
- f. In the Max. Inject Time spin box, enter 200.000 to specify a 200 ms maximum injection time.
- g. In the Source Fragmentation group box, confirm that the On check box is not selected () to specify that the ion source fragmentation option is turned off.
- h. In the Scan Ranges group box, in the Input list box, select *From/To* to make available the First Mass and Last Mass text boxes in the Scan Ranges table.

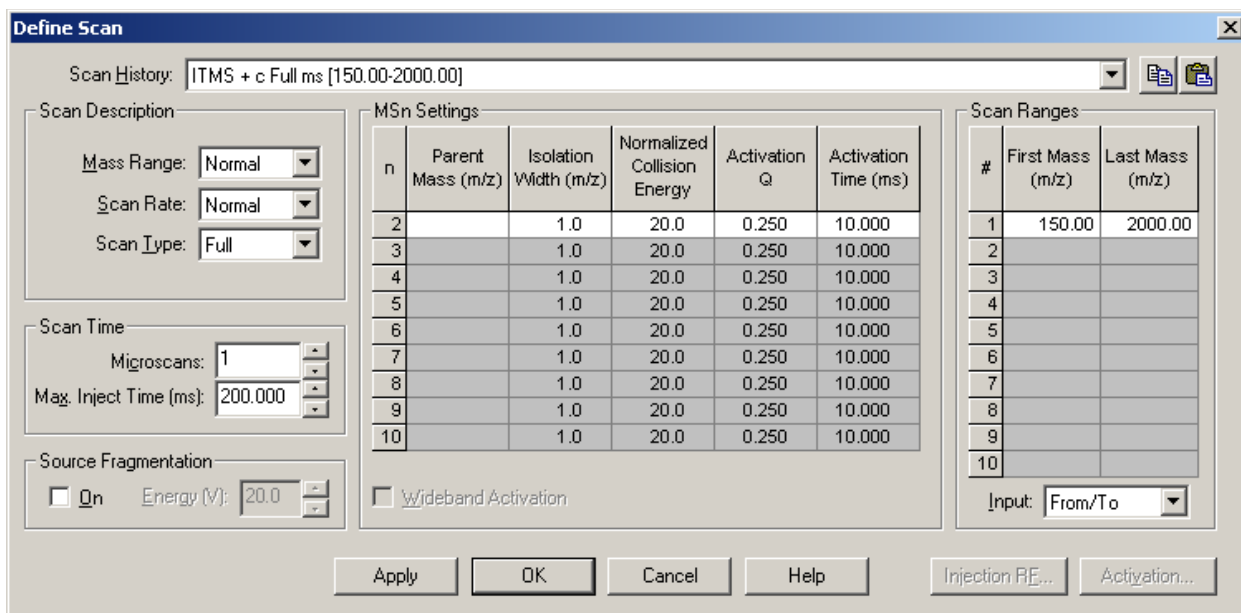


Figure 20. Define Scan dialog box, showing the default settings for ESI/MS operation

- i. In the Scan Ranges group box, in the Scan Ranges table, in the First Mass text box, enter 150 to set the first mass for the scan range to m/z 150.
- j. In the Last Mass text box, enter 2000 to set the last mass for the scan range to m/z 2000.
- k. Ensure that the settings in your Define Scan dialog box are the same as those shown in [Figure 20](#).

3 Tuning and Calibrating Automatically in the ESI/MS Mode

Setting Up the MS Detector in the Xcalibur Data System for Tuning and Calibration

1. Click **OK** to apply the MS detector scan parameters and to close the Define Scan dialog box.



6. On the Control/Scan Mode toolbar, click on the Centroid/Profile button to toggle the data type to profile. (The picture on the button should be the same as that shown here.)



7. Click on the Positive/Negative button to toggle the ion polarity mode to positive. (The picture on the button should be the same as that shown here).

The MS detector is now properly set up in the Xcalibur data system for tuning and calibration in the ESI/MS mode.

3 Tuning and Calibrating Automatically in the ESI/MS Mode

Testing the Operation of the MS Detector in the ESI/MS Mode

Testing the Operation of the MS Detector in the ESI/MS Mode

You are now ready to test if your MS detector is operating properly. To test for proper operation, you infuse the calibration solution into the ESI source, and then you monitor the real-time display of the mass spectrum of the calibration solution.

The procedure to test the operation of the MS detector in the ESI/MS mode is:



1. Click on the Syringe Pump button to display the Syringe Pump dialog box. See Figure 21.

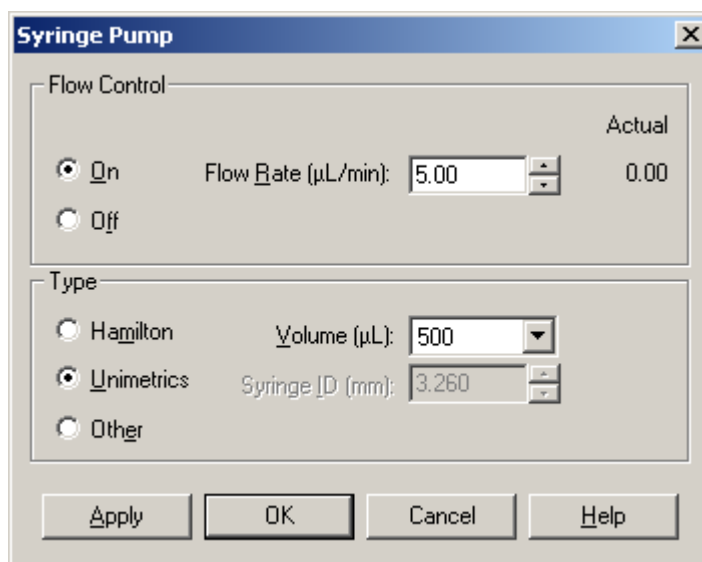


Figure 21. Syringe Pump dialog box

2. Turn on the syringe pump and set an infusion flow rate of 5 $\mu\text{L}/\text{min}$, as follows:
 - a. In the Flow Control group box click the On option button. This will: i) make active the Flow Rate spin box and, ii) the pump will start when the dialog box is completed and **OK** is clicked.
 - b. Enter 5 in the Flow Rate spin box to specify a rate of 5 $\mu\text{L}/\text{min}$.

Note This procedure assumes that you are using the 500- μL Unimetrics syringe that is provided with your Finnigan LXQ system. If you are using another type of syringe, follow the procedures in (c) or (d) below.

- c. If you are using the Unimetrics syringe supplied with the LXQ, or a Hamilton syringe, set up the syringe parameters as follows:

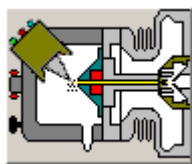
- i. In the Type group box, click on the Unimetrics or Hamilton option button to specify the syringe that you are using.
 - ii. Click on the Volume list box arrow to display the list of available volumes, and then select 500 (or your syringe size) from the list to set the proper syringe volume. Note that, if you are using a Unimetrics syringe, the Finnigan LXQ MS detector automatically sets the syringe ID to its proper value of 3.260 mm.
- d. If you are using a syringe that is not a Unimetrics or a Hamilton syringe, set up the syringe parameters as follows:
- i. In the Type group box, click on the Other option button to make active the syringe ID spin box.
 - ii. Enter the inner diameter of your syringe in the Syringe ID spin box.
- e. Click **APPLY**.
- f. Click **OK**. This will start the syringe pump and close the Syringe Pump dialog box.



3. On the File/Display toolbar, click on the Display Spectrum View button to ensure that the Spectrum view is displayed.

4. Monitor the data for the calibration solution, as follows:

- a. In the Spectrum view of the Tune Plus window, observe the mass spectra of the singly-charged ions of calibration solution. The ions are as follows. See [Figure 22](#).
 - Caffeine: m/z 195
 - MRFA: m/z 524
 - Ultramark 1621: m/z 1022, 1122, 1222, 1322, 1422, 1522, 1622, 1722, 1822
- b. At the top of the Spectrum view, notice the values for the ionization time (IT) and normalization level (NL). See [Figure 22](#).
- c. Click on the API Source button to open the ESI Source dialog box. (See the Spray Current readback shown in [Figure 19](#).)
- d. Observe the value for the Spray Current readback and the values for NL and IT in the Spectrum view. As calibration solution infuses, and the readback values fluctuate, ask yourself the following questions about the ion current signal:



3 Tuning and Calibrating Automatically in the ESI/MS Mode

Testing the Operation of the MS Detector in the ESI/MS Mode

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

If you answered “yes” to the questions in step 4.d, then your MS detector is operating properly.

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

- Ensure that the fused-silica sample tube does not extend beyond the tip of the ESI needle.
- Ensure that the entrance to the ion transfer capillary is clean, and is not covered with a piece of septum.
- Ensure that the solution entering the probe is free of air bubbles and that the tubing and connectors are free of leaks.
- Ensure that the sheath gas flow rate is optimal.

Congratulations! You have demonstrated that your MS detector is operating properly in the ESI mode. You are now ready to tune and calibrate the MS detector. Leave your Finnigan LXQ MS detector as it is, and go to the next topic: [Tuning the MS Detector Automatically in the ESI/MS Mode](#).

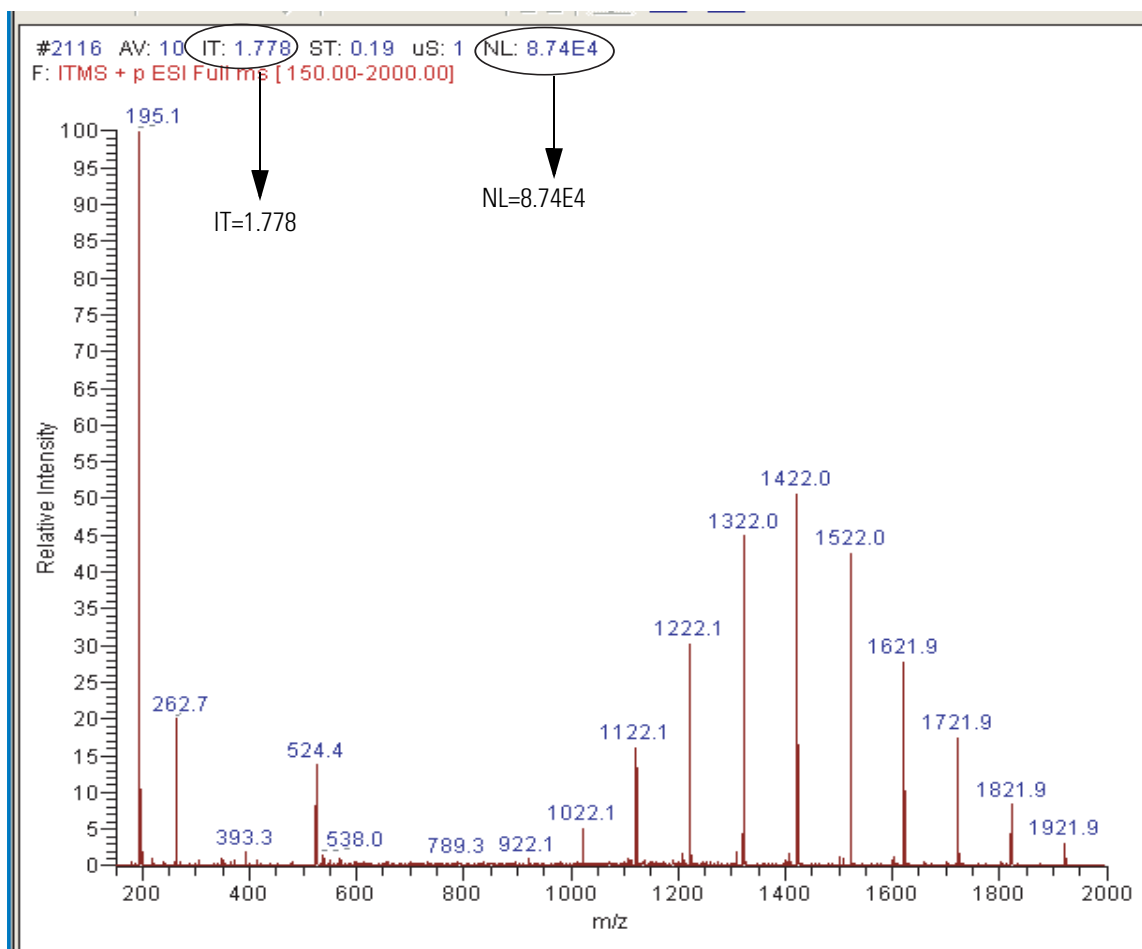


Figure 22. Spectrum view of the Tune Plus window, showing ionization time (IT) and normalization level (NL) of the calibration solution

Tuning the MS Detector Automatically in the ESI/MS Mode



You tune the MS detector automatically in the ESI/MS mode to optimize important parameters, including heated capillary voltage and tube lens voltage.

To tune the MS detector automatically:

1. On the Control/Scan Mode toolbar, click on the Tune button to display the Tune dialog box.
2. If necessary, click on the Automatic tab to display the Automatic tuning page. See [Figure 23](#).
3. In the What to Optimize On group box, select the Mass option button to make active the Mass spin box.

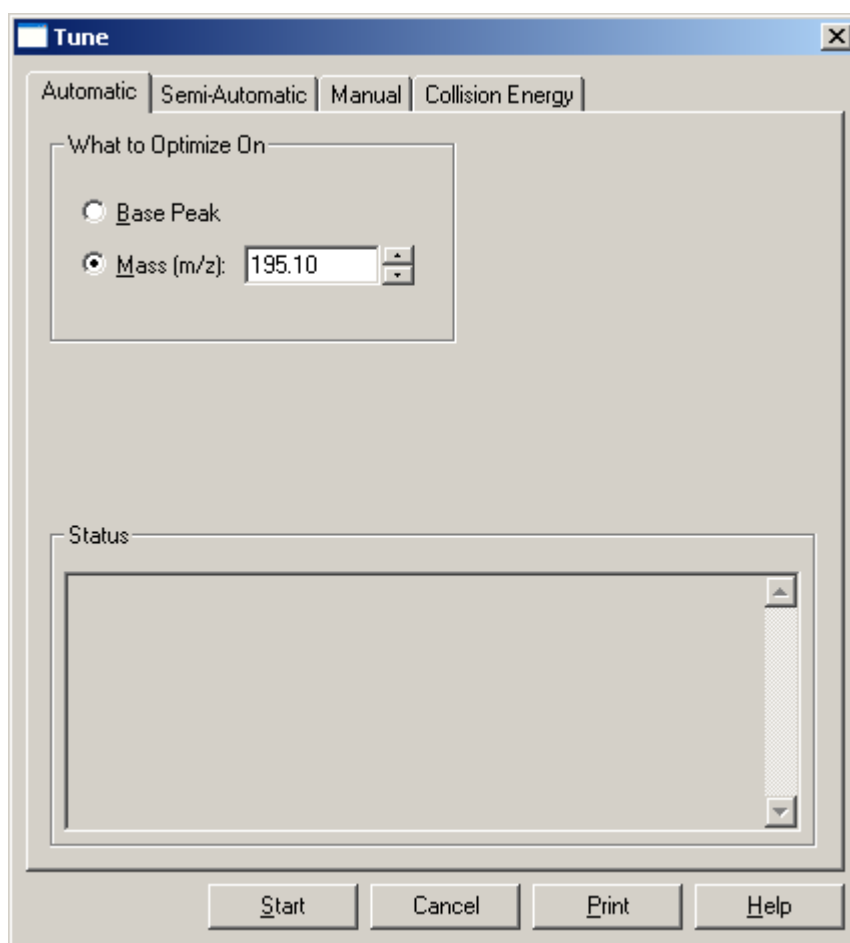


Figure 23. Tune dialog box, showing the Automatic tuning page

4. In the Mass spin box, enter 195.1 to specify that the Finnigan LXQ MS detector optimize your Tune Method on the peak at m/z 195.1.

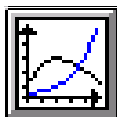
Note In this example, you use the mass peak at m/z 195.1 to optimize the Tune Method. However, you can optimize the tune on any mass peak of the calibration solution.

5. Start the automatic tuning procedure, as follows:
 - a. Click **Start**. A message box displays the following message:

Please ensure that the 500 microliter syringe is full.

Ensure that the syringe contains at least 450 μL of calibration solution (this solution is specified in “[ESI Calibration Solution: Caffeine, MRFA, Ultramark 1621](#)” on [page 137](#)).

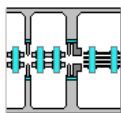
- b. Click **OK** to close the message box, and return to the Tune dialog box.



6. On the File/Display toolbar, click on the Graph View button to display the Graph view. See [Figure 24](#).

7. Observe the Tune Plus window and the Tune dialog box. While automatic tuning is in progress, the Finnigan LXQ MS detector displays various tests in the Spectrum and Graph views in Tune Plus and displays various messages in the Status group box in the Tune dialog box. Your Tune Plus window should now look similar to the one shown in [Figure 24](#).

8. Click on the ESI Source dialog box to examine the ESI source parameters after tuning. Compare the settings shown in [Figure 25](#) with the pre-tune settings shown in [Figure 19](#) on [page 3-47](#).



9. Click on the Ion Optics toolbar button to display the Ion Optics dialog box. The parameters in the Ion Optics dialog box are optimized automatically by the Finnigan LXQ MS detector. See [Figure 26](#).

You have now successfully tuned the MS detector in ESI/MS mode using the calibration solution.

3 Tuning and Calibrating Automatically in the ESI/MS Mode

Tuning the MS Detector Automatically in the ESI/MS Mode

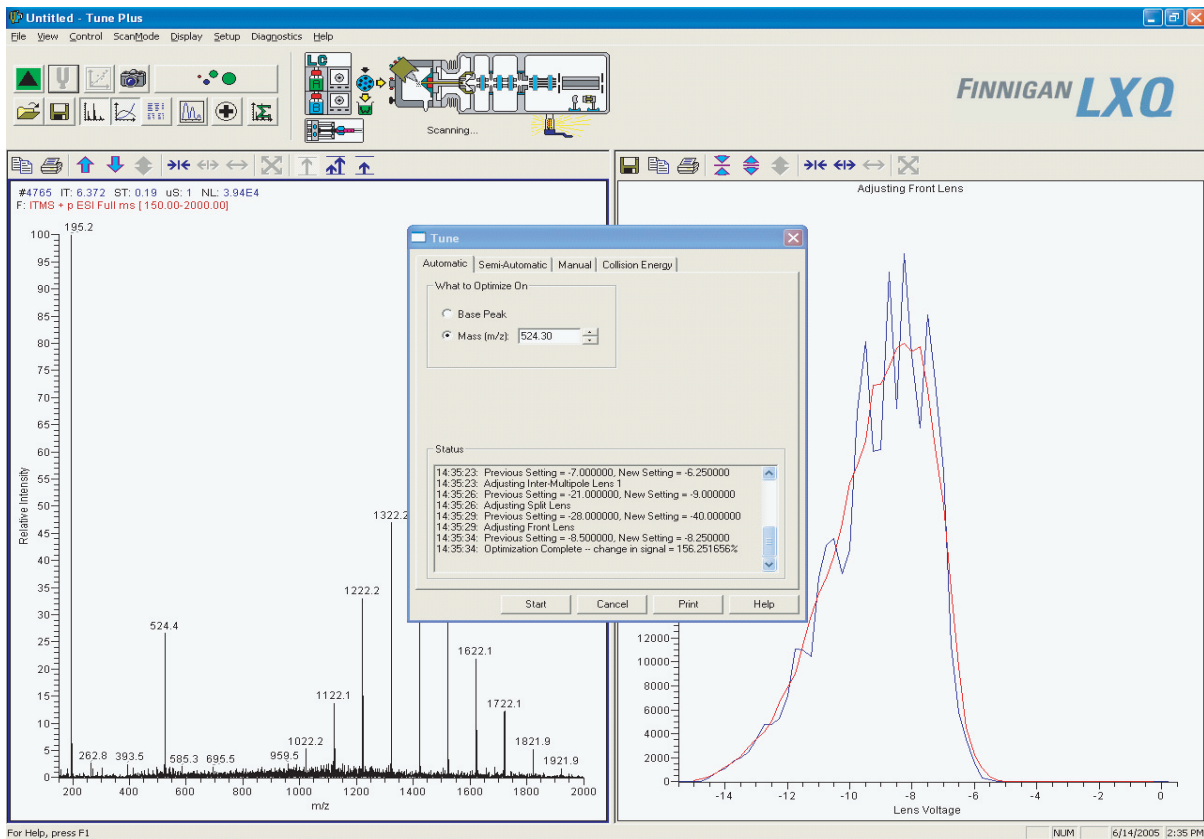


Figure 24. Tune Plus window, showing the windows obtained from a typical automatic tune procedure

3 Tuning and Calibrating Automatically in the ESI/MS Mode

Tuning the MS Detector Automatically in the ESI/MS Mode

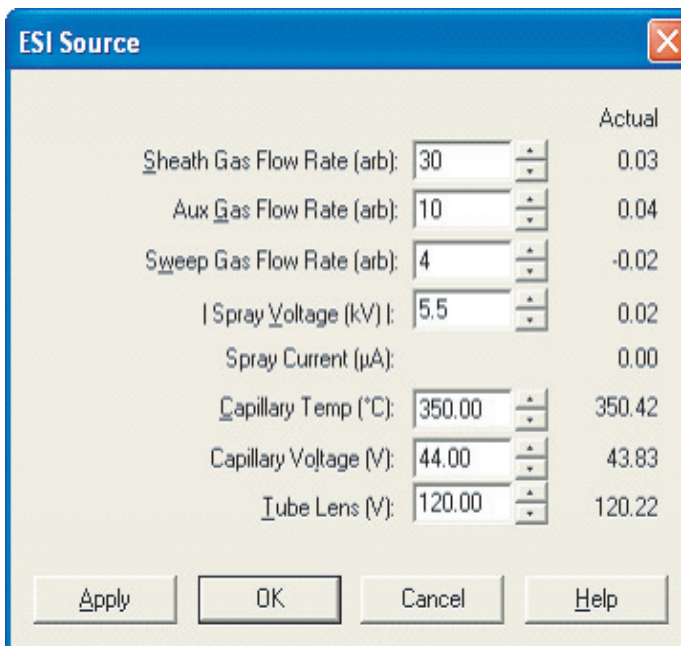


Figure 25. ESI Source dialog box, showing typical parameters after automatic tuning

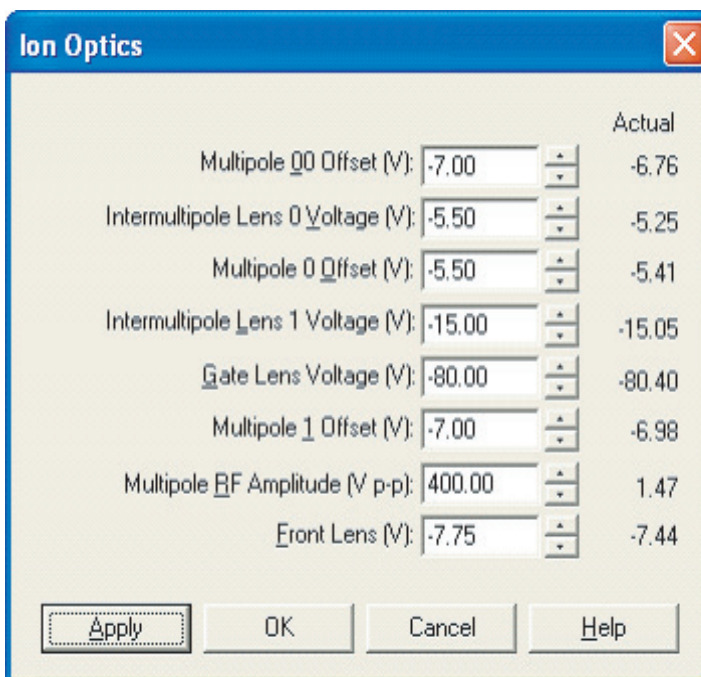


Figure 26. Ion Optics dialog box, showing examples of voltages of lenses and Intermultipoles, which are optimized by the Finnigan LXQ automatic tuning procedure

Saving Your ESI/MS Tune Method

You can save the parameters you just set in a Tune Method specific to your particular analyte and solvent flow rate. (In this case, you save the settings obtained using the calibration solution.) You can recall the Tune Method and use it as a starting point for optimizing the MS detector on a different analyte of interest or at a different flow rate

Note You must save the Tune Method while the MS detector is On.

Save your ESI/MS Tune Method (for low-flow operation) when automatic tuning is complete, as follows:

1. Choose **File > Save As** to display the Save As dialog box. See [Figure 27](#).

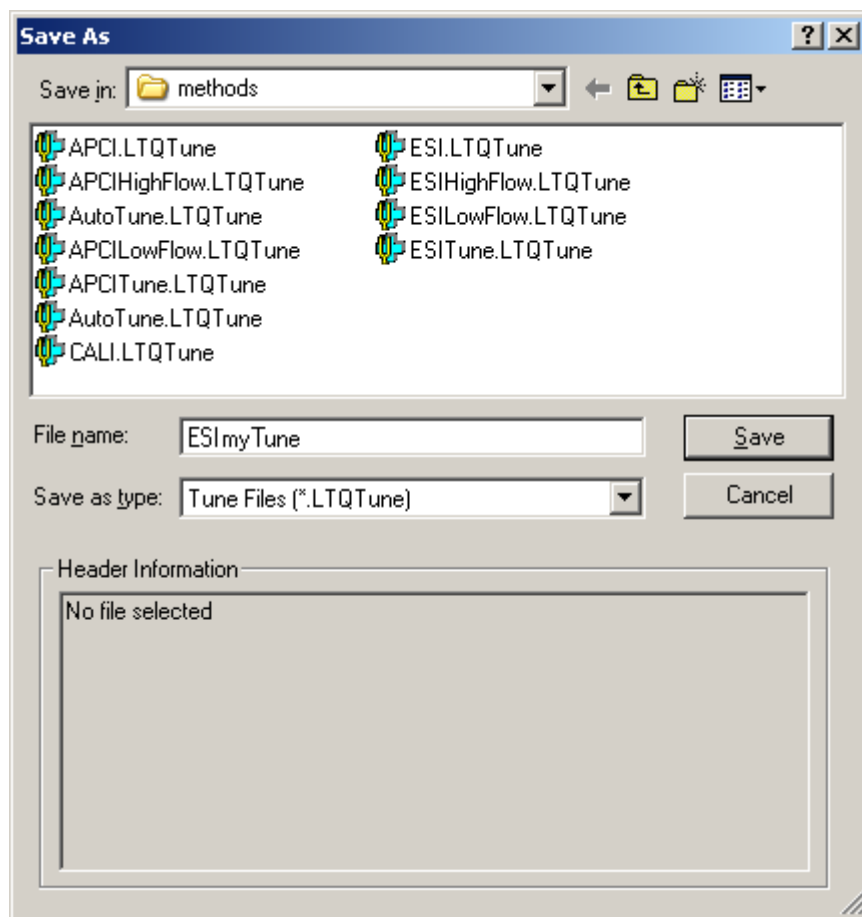


Figure 27. Save As dialog box, showing files in the folder *C:\Xcalibur\methods*

2. Select the *C:\Xcalibur\methods* folder.

3. Click on the File Name text box, and then enter the name under which you want to save the tune method. As an example, you could enter **ESImyTune** to name the Tune Method *ESImyTune.LTQTune*.
4. Click on **Save** to save the Tune Method and return to the Tune Plus window. If you entered the name given in the example above, the Tune Method is named *ESImyTune.LTQTune*.

Once you have tuned the MS detector, you are ready to calibrate.

Calibrating the MS Detector Automatically

Use the following procedure to calibrate the MS detector automatically from the Tune Plus window:

1. Choose **Control > Calibrate** to display the Calibrate dialog box.
2. If necessary, click on the Automatic tab to display the Automatic calibration page. See [Figure 28](#).

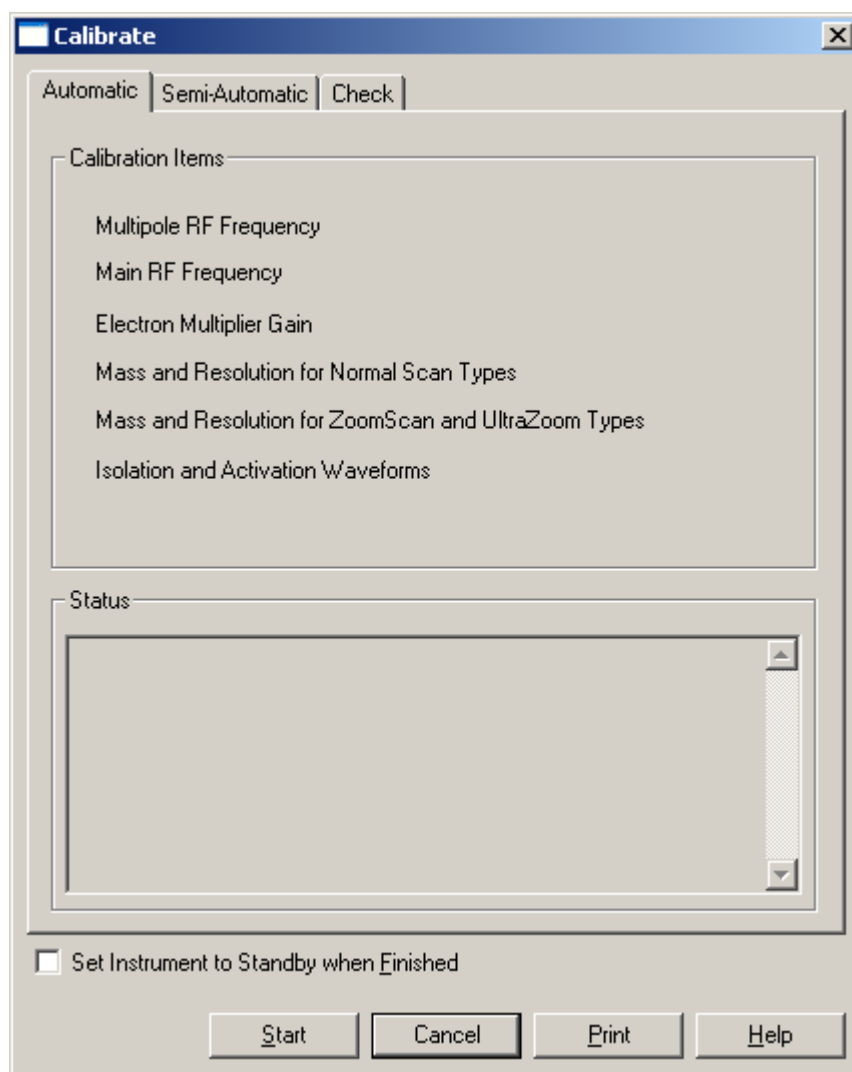


Figure 28. Calibrate dialog box, showing the Automatic calibration page

3. Start the automatic calibration procedure, as follows:
 - a. Click on **Start**. A message box displays the following message:

Please ensure that the 500 microliter syringe is full.

Ensure that the syringe contains at least 450 μL of calibration solution (this solution is specified in “[ESI Calibration Solution: Caffeine, MRFA, Ultramark 1621](#)” on [page 137](#)).

- b. Click on **OK** to close the message box, and return to the Calibrate dialog box.
4. Observe the Tune Plus window and the Calibrate dialog box. While the automatic calibration is in progress, the Finnigan LXQ MS detector displays a variety of test results in the Spectrum and Graph views and displays a variety of messages in the Status box of the Calibrate dialog box.

The automatic calibration procedure typically takes about 40 min.

When the Finnigan LXQ MS detector completes the calibration procedure it restores the full scan ESI mass spectrum in the Spectrum view. The Instrument Messages dialog box is displayed, which indicates whether or not the calibration procedure for an item is successful.

- If a calibration item is successful, the Finnigan LXQ MS detector saves the new calibration parameter automatically to the hard disk.
- If a calibration item fails, you can try calibrating on that item again after you ensure the following: 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. If the sensitivity of the ions is low, increase the solution flow rate somewhat, and then use the *semi-automatic* calibration procedure to calibrate the specific parameter that failed. See [Figure 29](#). Consider deselecting the ZoomScan Mode option if repeated failures occur.

When all calibration items are successful, your MS detector is properly tuned and calibrated for low-flow experiments. A successful calibration exhibits adequate intensities of the following calibrant ions: m/z 195, 524, 1222, 1522, and 1822. In many cases, fine tuning on your particular analyte is not necessary if the intensity of these ions is sufficient.

You are ready to analyze samples if you do not need to maximize the intensity of the ion signals for your analyte.

Later you can change the solution flow rate and optimize the MS detector parameters on reserpine or on your particular analyte. See “[Optimizing the MS Detector Tune Automatically](#)” on [page 4-75](#).

3 Tuning and Calibrating Automatically in the ESI/MS Mode

Calibrating the MS Detector Automatically

Before you tune with your analyte, go to the next topic: [Cleaning the MS Detector after Tuning and Calibrating.](#)

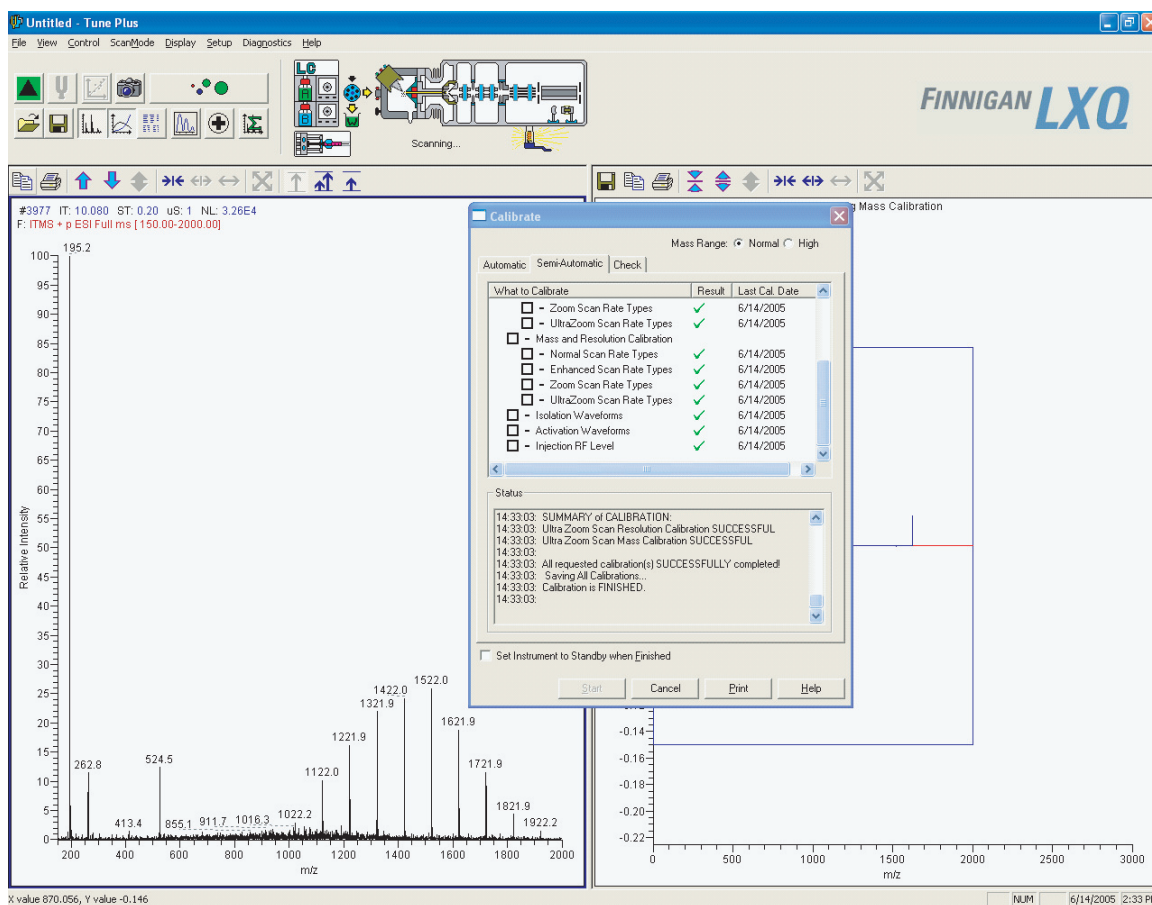


Figure 29. Tune Plus window with Calibrate dialog box, showing the results of a successful semi-automatic calibration procedure

Cleaning the MS Detector after Tuning and Calibrating

This topic describes how to clean your MS detector after using the calibration solution, in preparation for acquiring data on your analyte of interest.

Use the following procedure to clean the MS detector after calibrating:



OnStandby



1. Click the **On/Standby** button to put the MS detector in Standby mode. When the MS detector is in Standby, the Finnigan LXQ MS detector turns off the sheath gas, Auxiliary gas, Sweep gas, ESI high voltage, and syringe pump. The MS detector stops scanning, and the Finnigan LXQ MS detector freezes the displays for the Spectrum and Graph views.

CAUTION Always place the MS detector in Standby (or Off) before you open the API source to atmospheric oxygen. The presence of oxygen in the ion source when the MS detector is On could be unsafe. (The Finnigan LXQ MS detector automatically turns the MS detector Off when you open the API source, however, it is best to take this added precaution.)

2. Remove the syringe from the syringe pump holder, as follows:
 - a. Squeeze the blue buttons, and pull back on the syringe pump handle to free the syringe.
 - b. Remove the syringe from the holder.
 - c. Disconnect the tip of the syringe needle from the Teflon tubing.
3. Clean the syringe thoroughly, as follows:
 - a. Clean the syringe with a solution of 5% formic acid in water.
 - b. Rinse the syringe with a solution of 50:50 methanol:water.
 - c. Use acetone to rinse the syringe. (Repeat this step several times.)
4. To gain access to the ion transfer capillary, the Ion Max ion source housing and the ion sweep cone need to be removed. Refer to the topic [“Removing the Ion Max Ion Source Housing”](#) on [page 31](#) for instructions for removing the Ion Max ion source housing.
5. Remove the ion sweep cone as follows:
 - a. Put on a pair of talc-free gloves.

3 Tuning and Calibrating Automatically in the ESI/MS Mode

Cleaning the MS Detector after Tuning and Calibrating



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

- b. Grasp the outer ridges of the ion sweep cone and pull the cone straight off of the API cone seal. Note, you might need to loosen the set screws on the ion sweep cone in order to remove it.
6. Remove the ion transfer capillary by using the custom tool provided.
7. Clean the ion sweep cone and the ion transfer capillary as follows:
 - a. Place the ion sweep cone and the ion capillary tube in a beaker of 50:50 methanol/water.
 - b. Sonicate the components for 15 min.
8. Reinstall the ion transfer capillary.
9. Reinstall the ion sweep cone as described in topic “[Installing the Ion Sweep Cone](#)” on [page 32](#).
10. Place a small Teflon coated septum over the entrance end of the ion transfer capillary to seal the vacuum chamber of the MS detector.
11. Flush the sample transfer line, sample tube, and ESI probe thoroughly with a solution of 5% formic acid in water (or with another appropriate solvent), as follows:

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

- a. Fill a clean, 250 μ L Unimetrics syringe with a solution an appropriate solvent.
- b. While holding the plunger of the syringe in place, carefully insert the needle of the syringe into the free end of the Teflon tube.
- c. Flush the sample transfer line, sample tube, and ESI probe with the solution by slowly depressing the syringe plunger. Visually check that the solution is exiting the tip of the ESI probe on the inside of

the probe assembly. Use a lint-free tissue to gently remove the excess solution as it exits the probe.

- d. Remove the needle of the syringe from the Teflon tube.
12. Repeat step 11 with a solution of 50:50 methanol:water.
 13. Repeat step 11 with acetone.
 14. Clean the spray shield as follows:
 - a. Fill a spray bottle with solvent solution.
 - b. Temporarily place a large Kimwipe (or other lint-free tissue) beneath of the spray shield. (The Kimwipe is required to absorb the solution used to flush the ion transfer capillary and spray shield.)
 - c. Use the spray bottle to flush contaminants from the exterior surface of the spray shield.
 - d. Remove the Kimwipe you used to absorb the solution. Swab the surface of the spray shield with a dry Kimwipe.
 - e. Repeat step 14.a through step 14.d with acetone to remove the (high molecular weight) Ultramark 1621.
 15. Being careful not to touch the ion transfer capillary with your hand, remove the septum from the entrance end of the ion transfer capillary.
 16. Reinstall the Ion Max ion source housing as described in [“Installing the Ion Max Ion Source Housing”](#) on [page 34](#).

The MS detector is now clean and ready for acquiring data on your analyte of interest.

If you plan to run analytical samples in high-flow ESI mode (using flow rates between 50 and 1000 $\mu\text{L}/\text{min}$), optimize the tune further by following the procedures in the next chapter: [Tuning with Your Analyte in LC/ESI/MS Mode](#).

Chapter 4 Tuning with Your Analyte in LC/ESI/MS Mode

This chapter provides information on tuning the MS detector in the LC/ESI/MS mode using your analyte. You will optimize the sensitivity of the MS detector to your analyte through an automatic procedure. The customized Tune Methods contained in your Finnigan LXQ data system are optimized for a wide range of applications, and they can often be used without further tuning of your MS detector.

The most important parameters that affect signal quality with the ESI interface are as follows:

- Electrospray voltage
- Heated capillary temperature (voltage)
- Tube lens voltage
- Capillary voltage
- Sheath gas flow rate
- Auxiliary gas flow rate
- Sweep gas flow rate

The settings for these parameters depend on the solvent flow rate and target analyte composition. In general, you should fine tune your MS detector whenever you change the solvent flow rate conditions of your particular application. In this procedure, you use the ESI low-flow Tune Method *ESI_{myTune}.LTQTune* as a starting point, and then further optimize the MS detector parameters using an automatic procedure. The automatic procedure adjusts the tube lens voltage, capillary voltage, and voltages applied to the ion optics until the ion transmission of your analyte is maximized.

Note Based on the LC flow rate of your experiment, you can specify the value of each of the following tuning parameters on the Finnigan LXQ MS detector: sheath, Auxiliary, and Sweep gas pressures, ESI needle (or “spray”) voltage, ion transfer capillary temperature, and probe position. With the exception of ESI needle voltage and probe position, recommended tuning parameter values can be obtained from Table 2, page 1-12. Automatic tuning sets the values of the parameters not mentioned here.

The capillary is heated to maximize the ion transmission to the MS detector. For ESI only, you set the ion transfer capillary temperature proportional to the flow rate of your solution. Refer to Table 1-2 for guidelines for setting operating parameters for LC/ESI/MS.

Note If your experiment is performed at a flow rate below 10 $\mu\text{L}/\text{min}$, and the results you want can be obtained without optimizing the MS detector on your particular analyte, you can go to [Chapter 5, “Acquiring ESI Sample Data Using the Tune Plus Window.”](#) to acquire sample data.

Before you optimize the tune for your analyte of interest, ensure that the Finnigan LXQ MS detector has been calibrated within the previous three months. If the system needs to be calibrated, refer to the procedures in the [Chapter 3, “Tuning and Calibrating Automatically in the ESI/MS Mode.”](#)

To tune the MS detector in the ESI/MS (high-flow) mode using your analyte, you perform the following tasks:

- Set up the MS detector for your specific analyte.
- Infuse your analyte into the MS detector using a syringe pump connected to the LC with a Tee union.
- Optimize the MS detector parameters for your analyte.

This chapter contains the following topics:

- [Setting Up to Introduce Sample by Syringe Pump into Solvent Flow from an LC](#)
- [Setting Up to Tune the MS Detector with Your Analyte](#)
- [Optimizing the MS Detector Tune Automatically](#)
- [Saving the ESI/MS Tune Method](#)

Setting Up to Introduce Sample by Syringe Pump into Solvent Flow from an LC

This topic describes setting up the MS detector to introduce your analyte by syringe pump into solvent flow from an LC.

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

1. Connect a 4 cm (1.5 in.) segment of Teflon tubing with a (brown) fingertight fitting and a (brown) ferrule to the (black) LC union. (See Figure 17 on page 3-44.)
2. Fill a clean, 500- μ L Unimetrics syringe with the 10 pg/ μ L solution of reserpine or your analyte of interest. If you are using the reserpine tuning solution refer to [Appendix A, "Sample Formulations."](#) for a procedure to make this solution. Use the dilution made in [step 9](#) of the [Reserpine Tuning Solution and Reserpine APCI Sample Solution](#) procedure on [page 138](#).
3. Insert the needle of the syringe into the segment of Teflon tube. Check that the needle tip of the syringe fits readily into the opening in the free end of the Teflon tubing. If necessary, you can enlarge the opening in the end of the tubing slightly.
4. Place the syringe into the syringe holder of the syringe pump.
5. While squeezing the blue release buttons on the syringe pump handle, push the handle forward until it just contacts the syringe plunger.
6. Connect the fused-silica infusion line from the (black) LC union to the (black) LC Tee union, as follows. See [Figure 30](#).
 - a. Connect the infusion line with a (brown) fingertight fitting and a (brown) ferrule to the free end of the LC union.
 - b. Connect the other end of the infusion line with a (red) fingertight fitting and a (brown) ferrule to the side arm of the LC Tee union.
7. Connect an appropriate length of (red) PEEK tubing from the (stainless steel) grounding union to the (black) LC Tee union, as follows.
 - a. Use a PEEK tubing cutter to cut a 4 cm (1.5 in.) length of the PEEK tubing.
 - b. Connect the PEEK tubing with a (brown) fingertight fitting and a (brown) ferrule to the grounding union.
 - c. Connect the other end of the PEEK tubing with a (natural color) fingertight fitting and a (natural color) ferrule to the LC Tee union.

4 Tuning with Your Analyte in LC/ESI/MS Mode

Setting Up to Introduce Sample by Syringe Pump into Solvent Flow from an LC

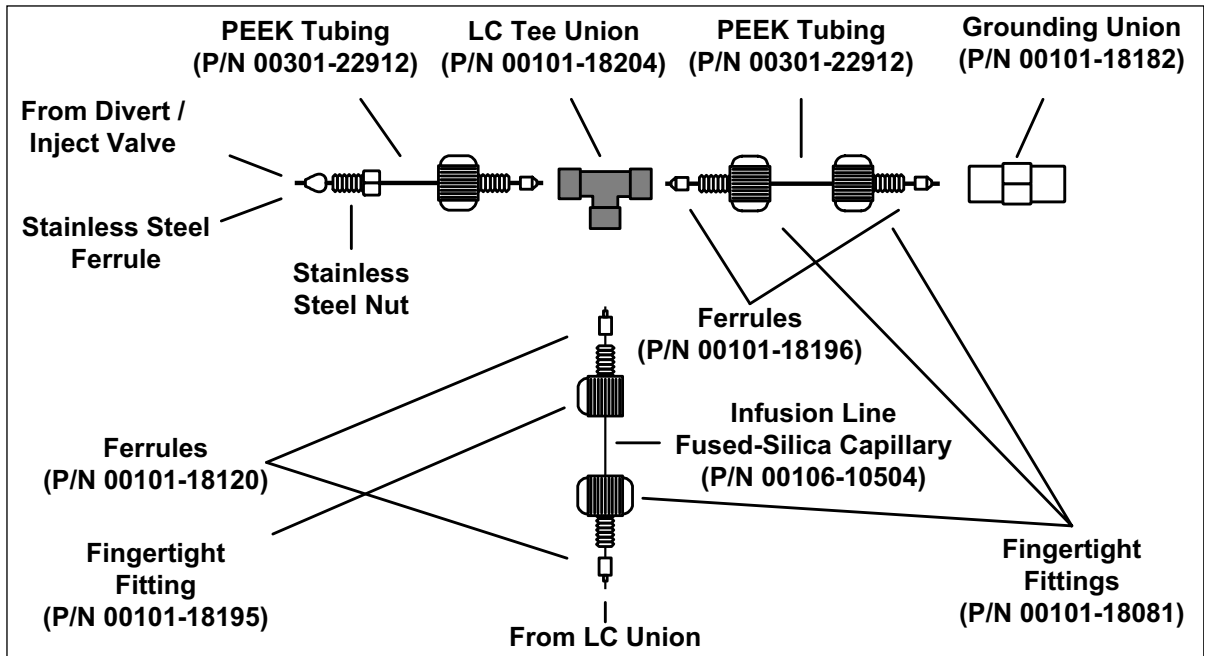


Figure 30. ESI/MS plumbing connections for the LC Tee union

8. If you have not already done so, connect the PEEK safety sleeve and fused-silica sample tube from the grounding union to the ESI probe sample inlet as described in the topic: *Installing a New Fused-Silica Sample Tube and PEEK Safety Sleeve in the Finnigan Ion Max API Source Hardware Manual.*

If you have installed the stainless steel needle in the ESI probe, connect the PEEK safety sleeve and fused-silica capillary tube from the grounding union to the ESI probe sample inlet as described in the topic: *Installing a New Stainless Steel Needle in the ESI Probe and Installing a New Fused-Silica Sample Tube and PEEK Safety Sleeve in the Finnigan Ion Max API Source Hardware Manual.*

9. Connect an appropriate length of PEEK tubing (transfer line from the divert/inject valve) from the divert/inject valve to the free end of the (black) LC Tee union, as follows.
 - a. Connect a length of PEEK tubing with a (stainless steel) nut and a (stainless steel) ferrule to port 3 of the divert/inject valve. See [Figure 31](#).

- b. Connect the other end of the PEEK tubing with a (brown) fingertight fitting and a (brown) ferrule to the free end of the LC Tee union. (See [Figure 30](#).)

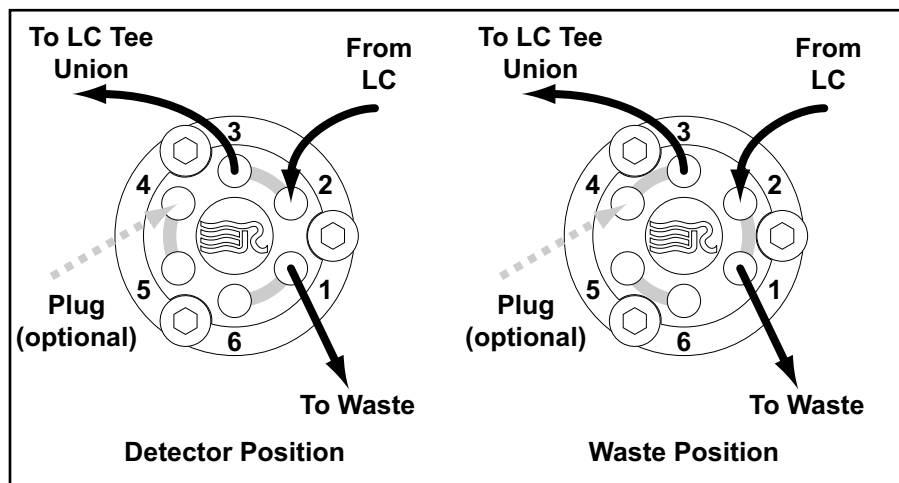


Figure 31. Divert/Inject valve, showing the correct setup for tuning by syringe infusion and showing the flow of liquid through the valve in the Detector and Waste positions

10. Connect an appropriate length of PEEK tubing (transfer line from the LC) from the divert/inject valve to the LC, as follows:
 - a. Connect a length of PEEK tubing with a (stainless steel) nut and a (stainless steel) ferrule to port 2 of the divert/inject valve.
 - b. Connect the other end of the PEEK tubing with a proper fitting and a ferrule to the outlet of the LC.
11. Connect an appropriate length of PEEK tubing (waste line) from the divert/inject valve to a waste container, as follows:
 - a. Connect a length of PEEK tubing with a (stainless steel) nut and a (stainless steel) ferrule to port 1 of the divert/inject valve.
 - b. Insert the other end of the PEEK tubing in a suitable waste container.

The MS detector is now properly set up to introduce your analyte by syringe pump into solvent flow from an LC.

Setting Up to Tune the MS Detector with Your Analyte

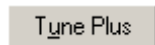
Use the following procedure to set up the MS detector to tune automatically on your analyte in ESI/MS mode. (In this example, you can use the reserpine solution described in **Appendix A: Sample Formulations**, or you can use a solution of your analyte of interest.)



CAUTION Do not infuse calibration solution at syringe pump flow rates above 10 $\mu\text{L}/\text{min}$. Your system could be contaminated by high concentrations of the Ultramark 1621 component of the calibration solution.

Note The following procedures assume that you are familiar with your Finnigan LXQ instrument and the Tune Plus window. If you need additional guidance, refer to: Finnigan LXQ online Help and/or *Finnigan LXQ Hardware Manual*.

1. Open the Tune Plus window from the Start button on your Windows XP Desktop, as follows:



- a. Choose **Start > Programs > Xcalibur > Xcalibur** to display the Xcalibur Home Page – Roadmap view.
- b. Click on the Instrument Setup button to display the Instrument Setup window.
- c. Click on the Finnigan LXQ button to display the New Method page.
- d. Click on the Tune Plus button to display the Tune Plus window.



On



Standby

2. In Tune Plus, click on the On/Standby button to take the MS detector out of Standby mode and turn it On. The MS detector begins scanning, the Finnigan LXQ MS detector applies high voltage to the ESI probe, and the Finnigan LXQ MS detector shows a real-time display in the Spectrum view.

3. Open the *ESImyTune.LTQTune* Tune Method, the Tune Method you saved in Chapter 3, as follows:



- a. On the File/Display toolbar, click on the Open File icon to display the Open dialog box.

- b. Browse to the folder *C:\Xcalibur\methods*. Then, select the file *ESImyTune.LTQTune*.
 - c. Click **Open** to open the file. Tune Plus downloads the Tune Method parameters to the MS detector, and the title bar in the Tune Plus window should read as follows:
C:\Xcalibur\methods\ESImyTune.LTQTune – Tune Plus
4. Define the scan parameters for tuning with your analyte in the ESI/MS mode, as follows:



- a. In the Instrument Control toolbar, click on the Define Scan button to open the Define Scan dialog box. See [Figure 32](#).
- b. In the Scan Description group box, in the Mass Range list box, select *Normal* to allow for a selection of mass ranges between *m/z* 150 to 2000.
- c. In the Scan Rate list box, select *Normal* to specify a normal scan rate.
- d. In the Scan Type list box, select *SIM* specify a Selected Ion Monitoring experiment.
- e. In the Scan Time group box, in the Number of Microscans spin box, enter **1** to set the total number of microscans to 1.
- f. In the Max. Inject Time spin box, enter *200.000* to specify a 200 ms maximum injection time.
- g. In the Scan Ranges group box, in the Input list box, select *Center/Width* to make available the Center Mass and Width text boxes in the Scan Ranges table.
- h. In the Source Fragmentation group box, confirm that the On check box is not selected () to specify that the ion source fragmentation option is turned off.
- i. In the Scan Ranges group box (Scan Ranges table), in the Center Mass text box, enter the mass of your analyte to set the center of mass for the scan range. If reserpine is the analyte you would enter **609.20** to set the center mass for the scan range to *m/z* 609.20. If you are using another analyte enter its mass value into the Center Mass text box. See [Figure 32](#).
- j. In the Width text box, enter **2.0** to set the width of the scan range to 2.0 daltons. See [Figure 32](#).
- k. Ensure that the settings in your Define Scan dialog box are the same as those shown in [Figure 32](#) if reserpine is the analyte being used. If you are using another analyte the Define Scan dialog box settings will be appropriate for the analyte.

4 Tuning with Your Analyte in LC/ESI/MS Mode

Setting Up to Tune the MS Detector with Your Analyte

1. Click **OK** to apply the MS detector scan parameters and to close the Define Scan dialog box.

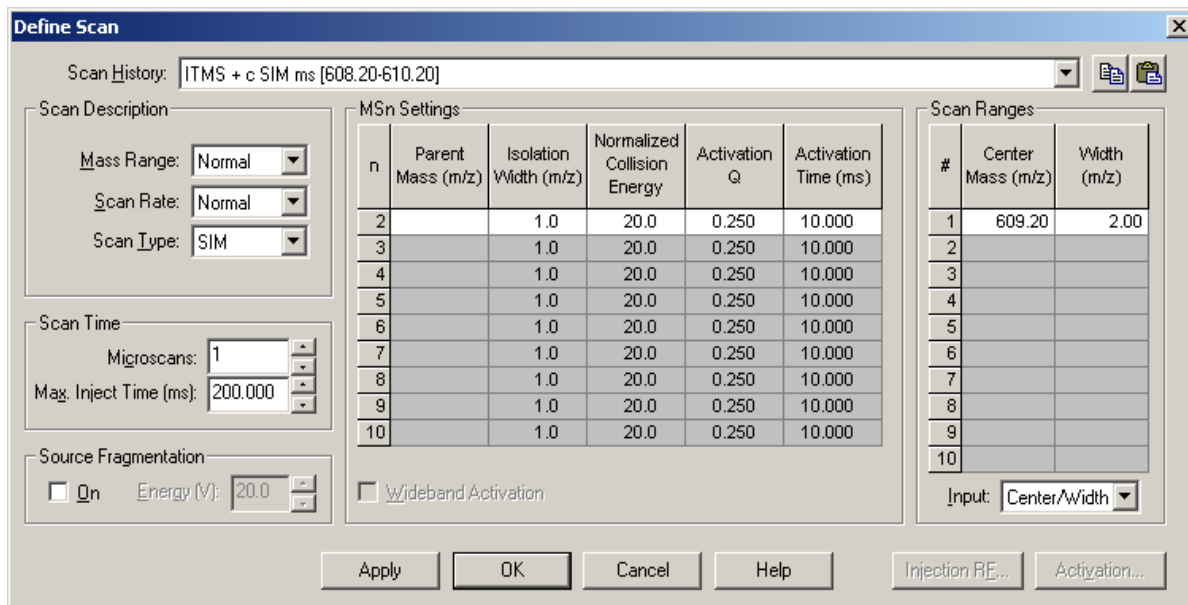


Figure 32. Define Scan dialog box, showing typical settings for acquiring reserpine data of the SIM type



5. On the Control/Scan Mode toolbar, click on the Centroid/Profile button to toggle the data type to Profile (the picture on the button should be as shown here).



6. Click on the Positive/Negative button to toggle the ion polarity mode to positive (the picture on the button should be the same as that shown here).

You have completed setting up to tune your MS detector with your analyte in ESI/MS mode.

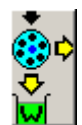
Optimizing the MS Detector Tune Automatically

Optimize the MS detector tune automatically to maximize the ion transmission of reserpine (or your analyte of interest) for a high-flow experiment. It is recommended that you begin optimizing after you have successfully passed an automatic tuning procedure and an automatic calibration procedure with the calibration solution infused at 5 $\mu\text{L}/\text{min}$.

The following procedure describes how to optimize the MS detector Tune Method on the reserpine m/z 609.2 at an LC flow rate of 400 $\mu\text{L}/\text{min}$. You can follow the same procedure with your analyte of interest and at your particular LC flow rate. (Refer to [Table 2](#) for guidelines about setting flow rates and temperatures.)



1. On the Control/Scan Mode toolbar, click on the Tune button to display the Tune dialog box.
2. If necessary, click on the Automatic tab to display the Automatic tuning page. See [Figure 33](#).
3. In the What to Optimize On group box, select the Mass option button to make active the Mass spin box.
4. In the Mass spin box, enter **609.2** (or the appropriate mass of your analyte of interest) to specify that the Finnigan LXQ MS detector is to use the peak at m/z 609.2 to optimize your tune.



5. Ensure that the Divert/Inject valve is in the Detector position, as follows:
 - a. Click on the Divert/Inject button to open the Divert/Inject Valve dialog box. See [Figure 34](#).
 - b. Select the Detector option button.
 - c. Click **Close**.
6. Start the automatic tuning procedure from the Tune dialog box, as follows:
 - a. Click **Start**. A message box displays the following message:
Please ensure that the 500 microliter syringe is full.

Ensure that the syringe pump contains at least 450 μL of the 10 $\text{pg}/\mu\text{L}$ reserpine tuning solution. See [Appendix A, "Sample Formulations."](#) for a procedure for making the reserpine tuning

4 Tuning with Your Analyte in LC/ESI/MS Mode

Optimizing the MS Detector Tune Automatically

solution. Use the dilution made in [step 9](#) of the [Reserpine Tuning Solution and Reserpine APCI Sample Solution](#) procedure on [page 138](#)

- b. Click **OK** to close the message box, and return to the Tune Plus window.



7. In the File/Display toolbar, click on the Graph View button to display the Graph view.

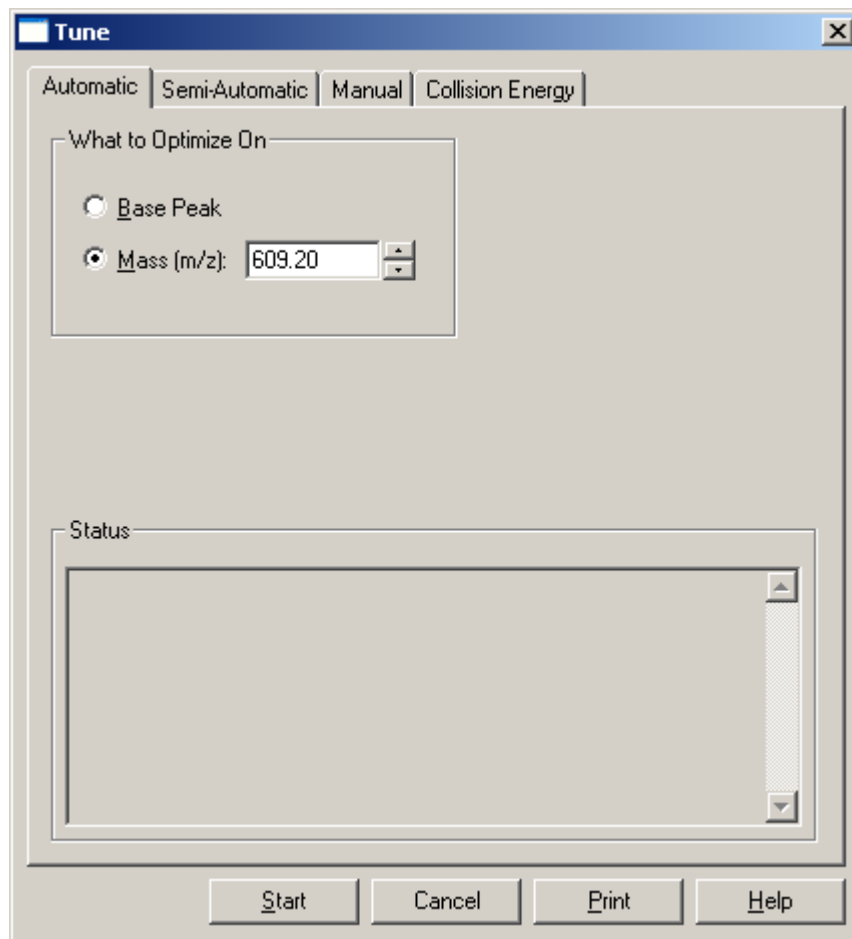


Figure 33. Tune dialog box, showing the Automatic tuning page

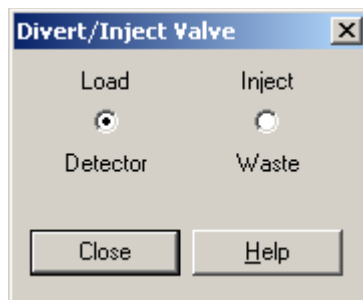


Figure 34. Divert/Inject Valve dialog box

8. Observe the Tune Plus window and the Tune dialog box. While automatic tuning is in progress, the Finnigan LXQ MS detector displays various tests in the Spectrum and Graph views in the Tune Plus window and displays various messages in the Status group box in the Tune dialog box.

Your Tune Plus window should now look similar to the one shown in [Figure 35](#).

Note The most important parameters that affect the signal quality during ESI/MS operation are the electrospray voltage, ion transfer capillary temperature, heated capillary voltage, tube lens voltage, gases, and solution flow rate. If any one of these parameters is changed, you need to reoptimize MS detector parameters. You can use the Semi-Automatic tune procedure to tune the MS detector on individual parameters.

You have now successfully tuned the MS detector in ESI/MS mode for the compound reserpine (or your analyte of interest).

4 Tuning with Your Analyte in LC/ESI/MS Mode

Optimizing the MS Detector Tune Automatically

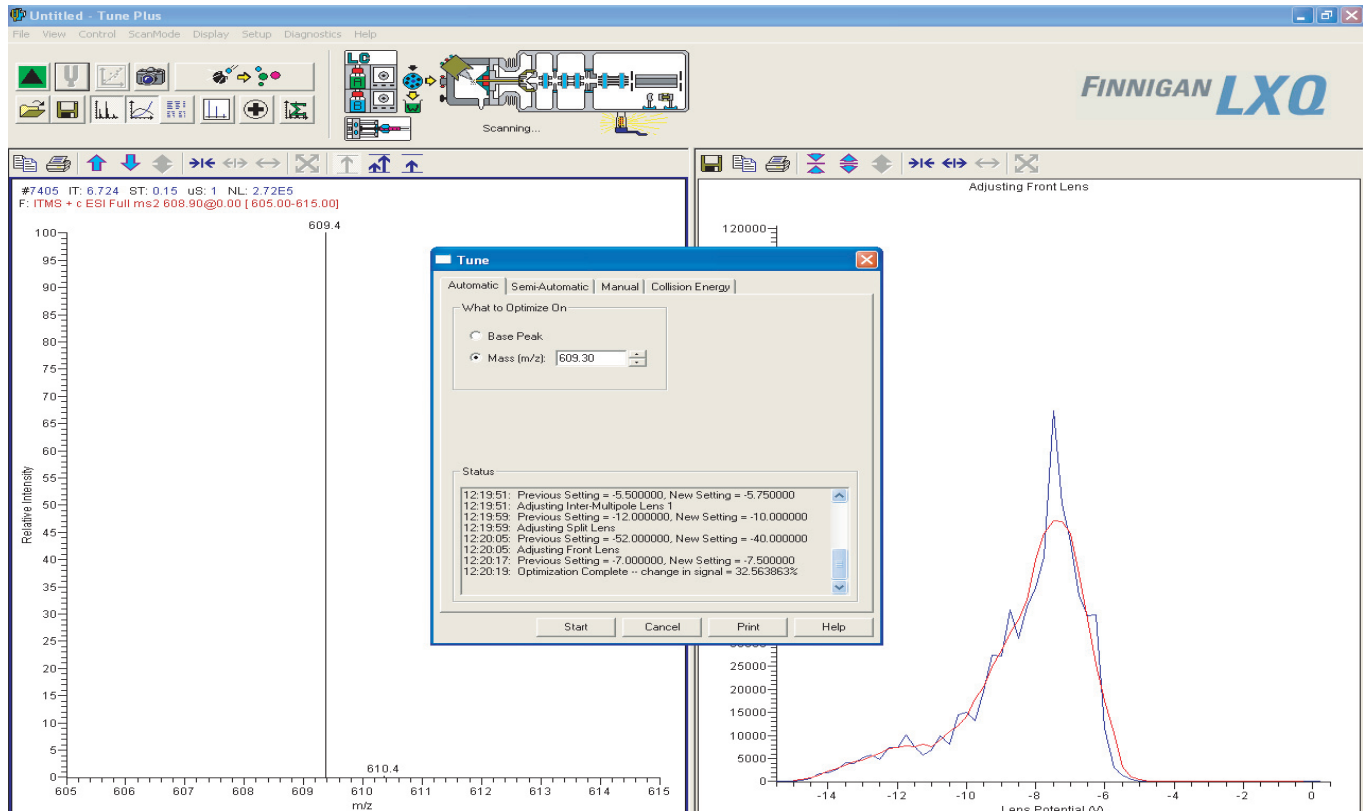


Figure 35. Tune Plus window with the Tune dialog box, showing the Automatic tuning page

Saving the ESI/MS Tune Method

To save your ESI/MS Tune Method (for a high-flow experiment using your analyte) when automatic tuning is complete:

Note Save the Tune Method while the MS detector is On.

1. Choose **File > Save As** to display the Save As dialog box. See [Figure 36](#).

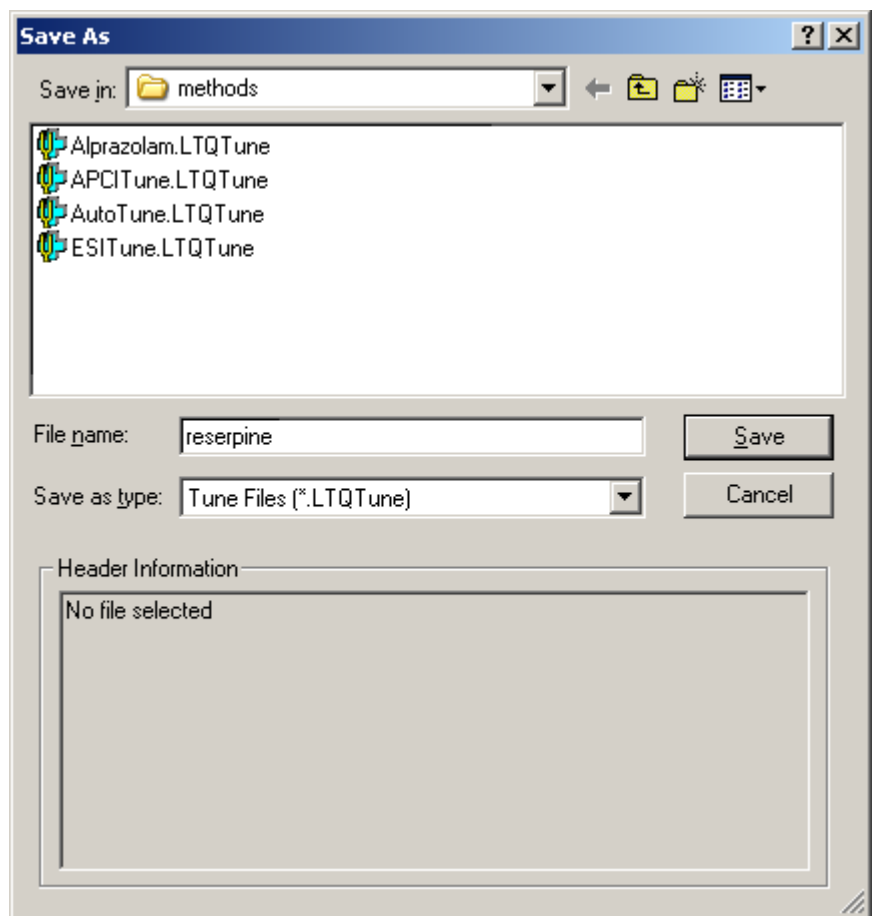


Figure 36. Save As dialog box, showing files in the folder *C:\Xcalibur\methods*

2. Select the *C:\Xcalibur\methods* folder.
3. Click on the File Name text box, and enter **reserpine** (or the name of your analyte of interest).
4. Click **Save** to save the Tune Method, and return to the Tune Plus window. Note that the Tune Method is named *reserpine.LTQTune*.

The Tune Method is now properly saved and you are ready to acquire data on your analyte of interest.

Chapter 5 Acquiring ESI Sample Data Using the Tune Plus Window

This chapter describes how to acquire LC/ESI/MS sample data using the Tune Plus window. This experiment uses reserpine but you can follow the same procedure with your analyte of interest.

Note The following procedures assume that you are familiar with your Finnigan LXQ instrument and the Tune Plus window. If you need information, refer to the Finnigan LXQ online Help, *Finnigan LXQ Getting Connected*, and/or *Finnigan LXQ Hardware Manual*.

Ensure that you have completed the procedures in the topics [Tuning and Calibrating Automatically in the ESI/MS Mode](#) and [Tuning with Your Analyte in LC/ESI/MS Mode](#).

This chapter contains the following topics:

- [Setting Up to Acquire MS/MS Data in the Full Scan Type](#)
- [Setting Up to Introduce Sample by Loop Injection into Solvent Flow from an LC](#)
- [Acquiring MS Data in the SIM Scan Type](#)

Setting Up to Acquire MS/MS Data in the Full Scan Type

Prepare to acquire MS/MS data in the Full scan type on reserpine (or on your analyte of interest). You need to optimize the isolation width and the relative collision energy parameters before you acquire MS/MS data.

You first optimize the isolation width to ensure that the ion of interest is isolated effectively, and then you optimize the collision energy to ensure that fragmentation of the parent ion is efficient. The relative collision energy for a particular analysis depends on the type of sample you are analyzing.

The information in this topic applies to operation of the Finnigan LXQ MS detector in either the ESI or the APCI mode.

This topic contains the following subtopics:

- [Optimizing the Isolation Width and Setting Up to Optimize the Collision Energy](#)
- [Optimizing the Collision Energy Automatically for an MS/MS Experiment](#)

Optimizing the Isolation Width and Setting Up to Optimize the Collision Energy

Optimize the isolation width and set up to optimize the collision energy for an MS/MS experiment, as follows:

Note The collision energy is optimized on the Finnigan LXQ MS detector by changing the values for the parameter *Normalized Collision Energy* in the MSⁿ Settings group box of the Define Scan dialog box. *For this experiment, and for most applications, leave the parameters Activation Q and Activation Time set to their default values.* For more information about these parameters, refer to the online Help.

The following procedure is based on the use of a reserpine solution. A procedure for making a suitable reserpine solution is given in [Appendix A, “Sample Formulations.”](#) Use the dilution made in [step 9 of the Reserpine Tuning Solution and Reserpine APCI Sample Solution procedure on page 138](#) (10 pg/mL). A different analyte may be used with appropriate changes made to the procedure (Define Scan dialog box settings, m/z values, etc).



OnStandby



1. From the Tune Plus window, click **On/Standby** to take the MS detector out of Standby mode and turn it On.
2. Ensure that Centroid data type is selected. (The picture on the button should be the same as that shown here.)

3. Ensure that the scan parameters are defined to acquire MS/MS Full scan data for reserpine (or your analyte of interest), as follows:



- a. Click on the Define Scan button to open the Define Scan dialog box. See [Figure 37](#).

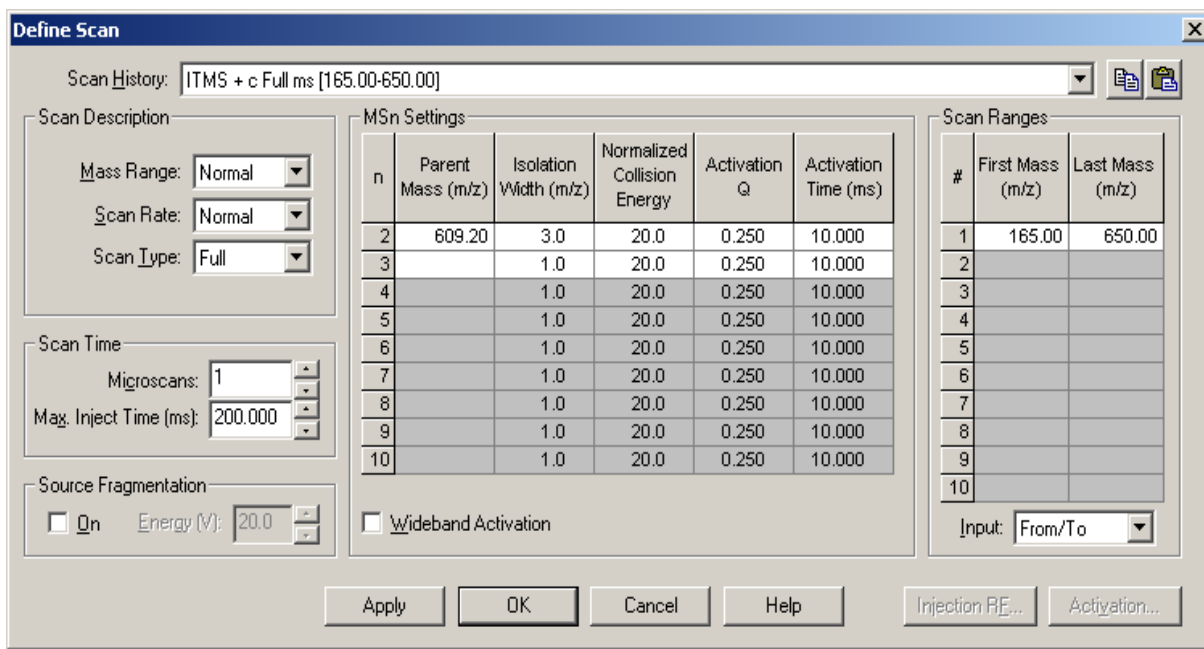


Figure 37. Define Scan dialog box, showing initial settings to optimize the isolation width of an MS/MS experiment for reserpine

- b. Verify that the values in your dialog box are the same as those shown in [Figure 37](#). Start with a relatively wide Isolation Width of 3 u (MSⁿ Settings group box, Isolation Width text box, [Figure 37](#)). Click **Apply** when you are finished entering the values. Leave the Define Scan dialog box open.
4. At this time you might want to turn on your LC pump and specify a flow rate of 0.4 mL/min. Verify that your system does not leak.



5. Click on the Syringe Pump button to display the Syringe Pump dialog box. See [Figure 38](#).
6. Turn on the syringe pump and set an infusion flow rate of 5 µL/min, as follows:
 - a. In the Flow Control group box, click on the On option button to make active the Flow Rate spin box.
 - b. Type 5 in the Flow Rate box to specify a rate of 5 µL/min.

5 Acquiring ESI Sample Data Using the Tune Plus Window

Setting Up to Acquire MS/MS Data in the Full Scan Type

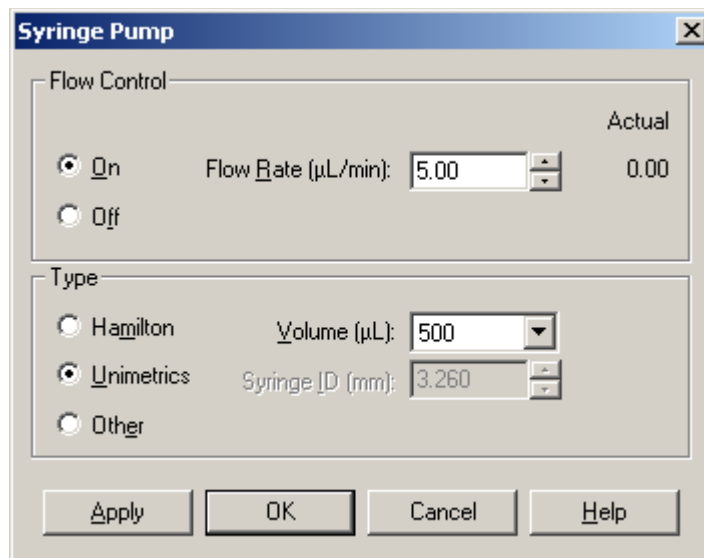


Figure 38. Syringe Pump dialog box

- c. If you are using a standard Unimetrics or Hamilton syringe, set up the syringe parameters as follows:
 - i. In the Type group box, click on the Unimetrics or Hamilton syringe click the option button to specify the proper syringe type.
 - ii. Click the Volume list box arrow to display the list of available volumes, and then select 500 (or your syringe size) from the list to set the proper syringe volume. Note that, if you are using a Unimetrics syringe, the Finnigan LXQ MS detector automatically sets the syringe ID to its proper value of 3.260 mm.
 - d. If you are not using a Unimetrics or Hamilton syringe, set up the syringe parameters as follows:
 - i. In the Type group box, click the Other option button to make active the syringe ID spin box.
 - ii. Enter the inner diameter of your syringe in the Syringe ID spin box.
 - e. Click **Apply** to apply the syringe parameters and start the syringe pump.
 - f. Finally, move the Syringe Pump dialog box out of the way, to the top of the monitor screen.
7. In the Tune Plus window, observe the mass spectrum of reserpine (or your analyte of interest). Also observe the values for NL and IT

(*Normalization Level* and *Ion Time*), while you optimize the value of the Isolation Width in the Define Scan dialog box, as follows:

- a. In the Define Scan dialog box, in the MSⁿ Settings group box, in the Isolation Width text box, verify that an isolation width of m/z 3 is being used, per [step 3b](#).
- b. In the Tune Plus window, observe the mass spectrum for the parent ion of reserpine, m/z 609.2. Ensure that the readback values for NL and IT are relatively stable.
- c. Again, in the Define Scan dialog box, in the MSⁿ Settings group box, in the Isolation Width box, type 2.8 to specify an isolation width of m/z 2.8. Then, click **Apply**.

Note The optimum value for the Isolation Width is the smallest m/z width (instrument minimum width = m/z 0.4) that gives a mass spectrum of maximum intensity for only the ions of interest. When the optimum Isolation Width is obtained the values for *NL* and *IT* are stable and the mass peak for the parent ion is at its maximum intensity and appears symmetrical. An Isolation Width value that is less than the optimum value causes a substantial drop in the *NL* reading. A significant drop in *sensitivity* indicates that the ions of interest are not effectively isolated.

- d. Repeat steps b and c above, entering successively smaller values for Isolation Width. Continue to observe the intensity of the mass spectrum of the parent ion, and ensure that the values for NL and IT are stable with each change you make to the Isolation Width.

Note The Isolation Width is typically m/z -1-3. After the Isolation Width is optimized you may compensate for minor changes in tune stability by increasing the Isolation Width value a small amount. This adjustment should be no larger than m/z =1.

8. In the Define Scan dialog box, in the MSⁿ Settings group box, in the Normalized Collision Energy spin box, enter 20 to specify an initial value of 20 for the collision energy. Then, click **Apply**.
9. In the Tune Plus window, observe the mass spectrum of the product ions of reserpine (or your analyte of interest). If necessary, increase the value for the Normalized Collision Energy in increments of 5% to cause the clear display of product ion mass spectrum. (After each change in value, click **Apply** to implement the change.) See [Figure 39](#).
10. When you have clearly identified a product ion mass-to-charge ratio for reserpine (or your analyte of interest), click on the Tune button to display the Tune dialog box.



5 Acquiring ESI Sample Data Using the Tune Plus Window

Setting Up to Acquire MS/MS Data in the Full Scan Type

11. In the Tune dialog box, click on the Collision Energy tab to display the page. See [Figure 40](#).

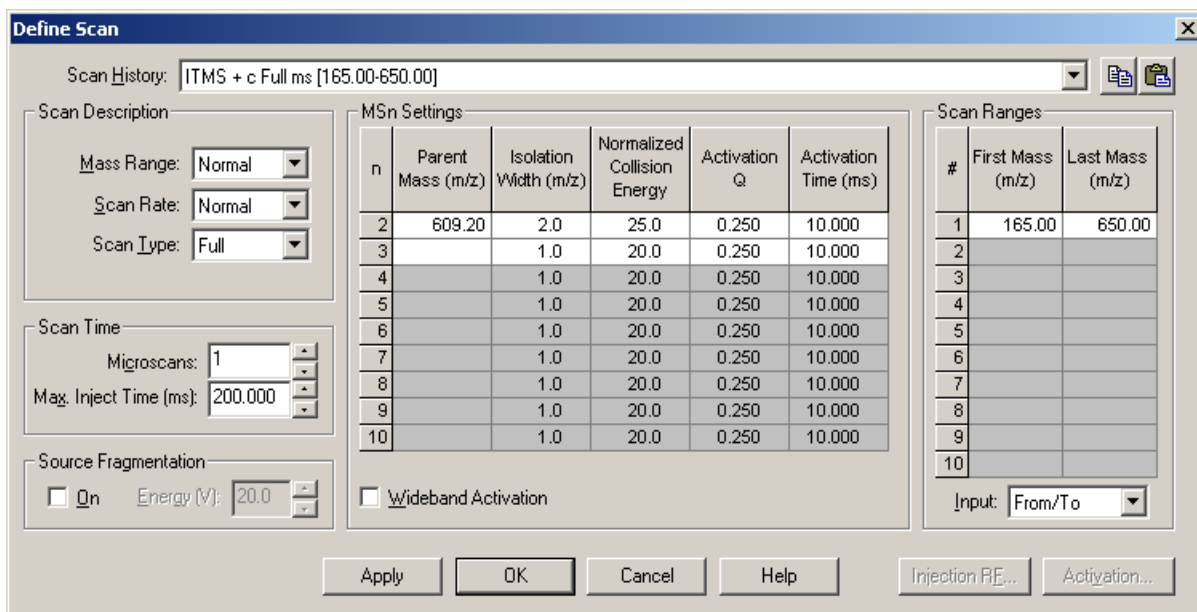


Figure 39. Define Scan dialog box, showing typical settings for acquiring MS/MS data in the Full scan type on reserpine

12. Click the **Product Ion Mass** option button to make the adjacent box active. Type 397.2 to specify the product ion at m/z 397.2 for reserpine. The Finnigan LXQ MS detector can optimize collision energy automatically by using this product ion of reserpine.

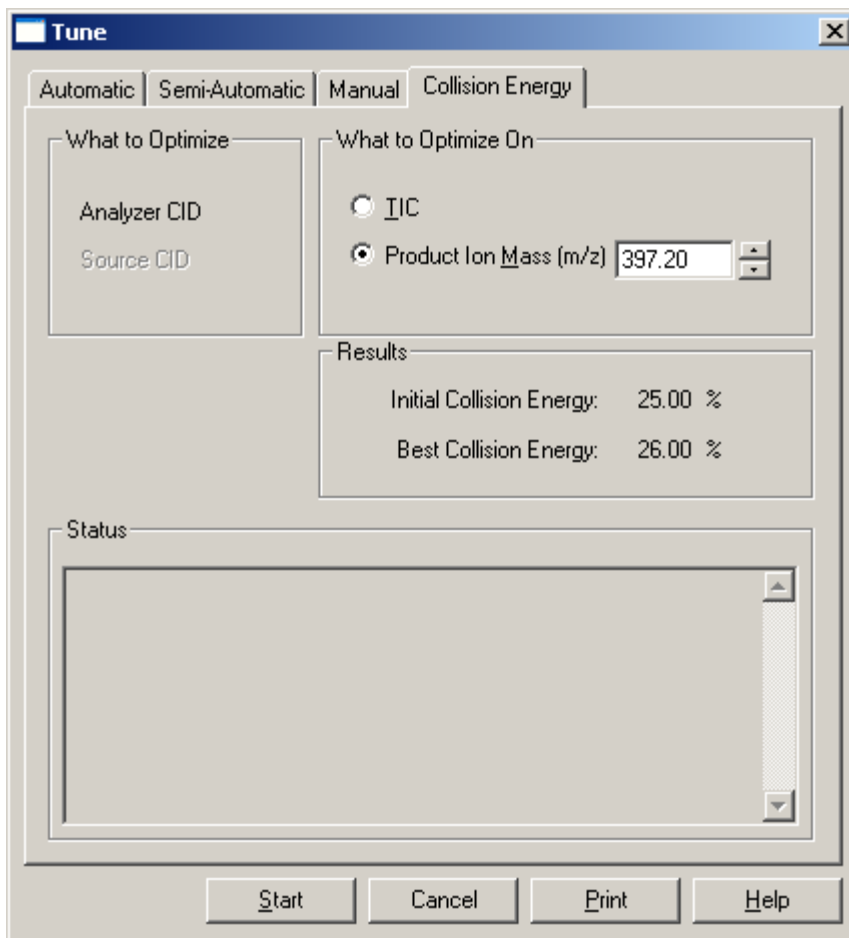


Figure 40. Tune dialog box, showing the Collision Energy page

Optimizing the Collision Energy Automatically for an MS/MS Experiment

The optimum relative collision energy is the one that produces the maximum product ion intensity. Optimize the relative collision energy automatically for the ESI/MS/MS analysis of reserpine (or your analyte of interest), as follows:

1. In the Tune dialog box, on the Collision Energy page (Figure 40), click **Start** to start the optimization procedure. A message box displays the following message:

Ensure that the 500 microliter syringe is full.

Ensure that the syringe contains at least 450 mL of the 10 pg/mL reserpine tuning solution (see Note about reserpine solution at the beginning of the subtopic “[Optimizing the Isolation Width and Setting Up to Optimize the Collision Energy](#)” on page 82).

5 Acquiring ESI Sample Data Using the Tune Plus Window

Setting Up to Acquire MS/MS Data in the Full Scan Type

2. Click **OK** to close the message box, and leave the Tune dialog box open. Your Tune Plus window should now look similar to the one shown in [Figure 41](#).
3. In the Spectrum view of Tune Plus, observe the MS/MS Full scan spectrum of reserpine (or that of your analyte of interest).
4. When the collision energy is optimized, the Accept Optimized Value dialog box appears. See [Figure 42](#).
5. Click **Accept** to accept the new collision energy value, and return to Tune Plus. The new value is displayed in the Define Scan dialog box.
6. In the Syringe Pump dialog box, select the Off option button to turn off the syringe pump. Click **Close** to close the Syringe Pump dialog box.
7. Click **Cancel** to close the Tune dialog box.

After you optimize the relative collision energy, the Finnigan LXQ MS detector is ready to acquire MS/MS data on your analyte of interest.

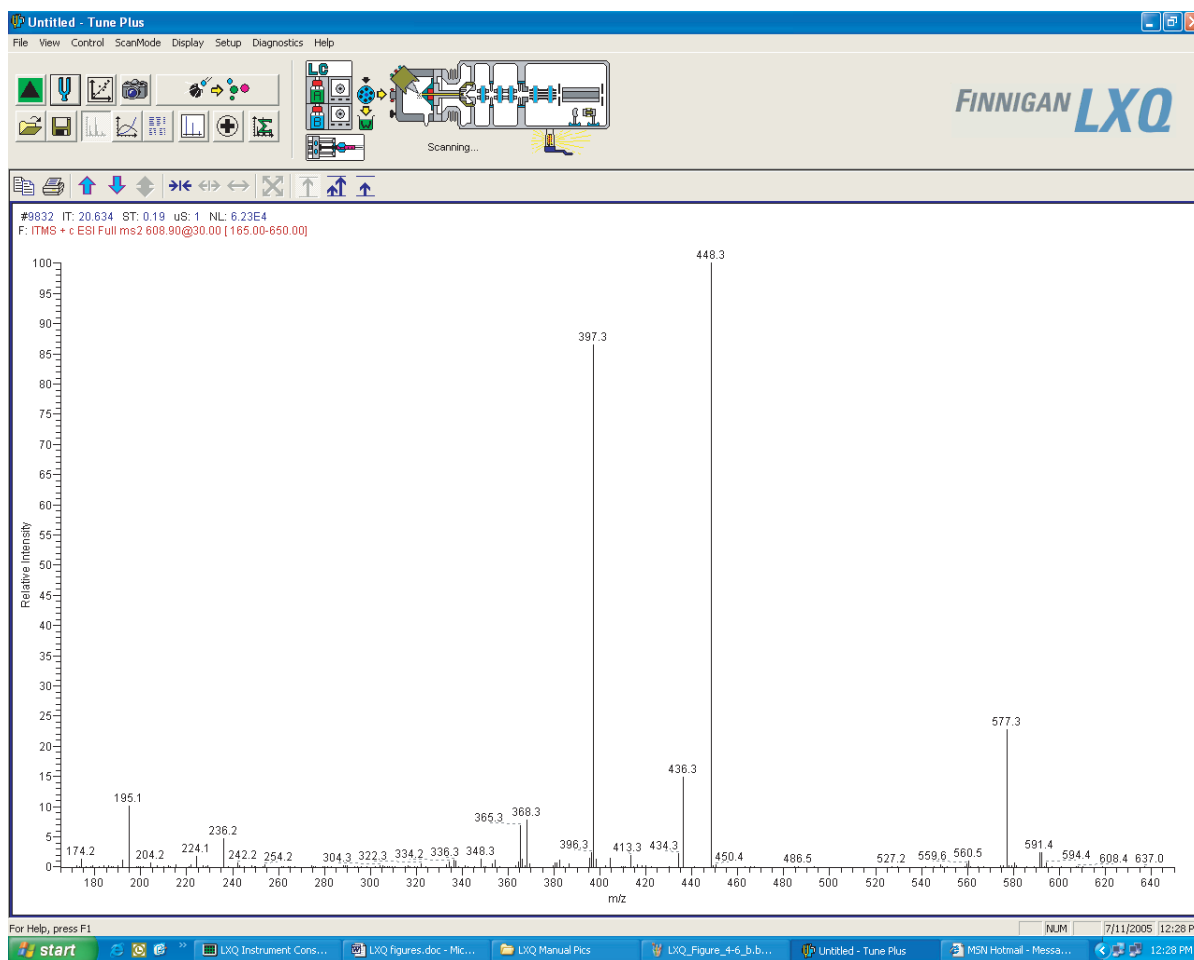


Figure 41. Tune Plus window, showing MS/MS product ions of reserpine in the Spectrum view. The Tune dialog box from Figure 40 is not shown.

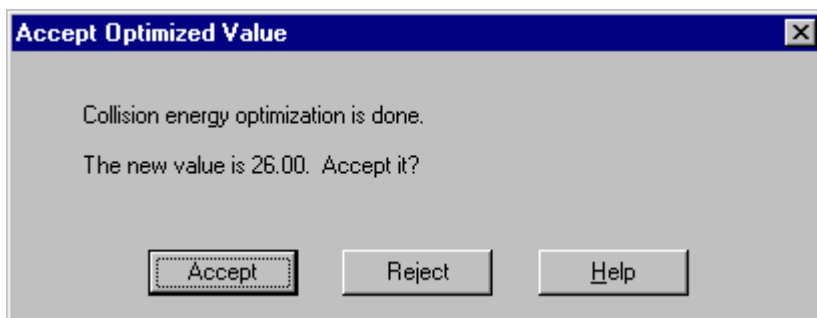


Figure 42. Accept Optimized Value dialog box

Setting Up to Introduce Sample by Loop Injection into Solvent Flow from an LC

Set up to introduce sample by loop injection into solvent flow from an LC.

To make the plumbing connections for ESI/MS sample introduction by loop injection into solvent flow from an LC, do the following:

If you have not already done so, connect the PEEK safety sleeve and fused-silica sample tube from the grounding union to the ESI probe sample inlet as described in the topic: *Installing a New Fused-Silica Sample Tube and PEEK Safety Sleeve in Finnigan Ion Max API Source Hardware Manual.*

If you have installed the stainless steel needle in the ESI probe, connect the PEEK safety sleeve and fused-silica capillary tube from the grounding union to the ESI probe sample inlet as described in the topic: *Installing a New Stainless Steel Needle in the ESI Probe and Installing a New Fused-Silica Sample Tube and PEEK Safety Sleeve in Finnigan Ion Max API Source Hardware Manual.*

8. Connect an appropriate length of (red) PEEK tubing (transfer line from the divert/inject valve) from the divert/inject valve to the (stainless steel) grounding union, as follows. See [Figure 43](#).
 - a. Connect a length of PEEK tubing fitted with a (stainless steel) nut and a (stainless steel) ferrule to port 3 of the divert/inject valve. See [Figure 44](#).
 - b. Connect the other end of the PEEK tubing with a (natural color) fingertight fitting and a (natural color) ferrule to the free end of the grounding union.
9. Connect a 2 μ L sample loop with (stainless steel) nuts and (stainless steel) ferrules to ports 1 and 4 of the divert/inject valve. (See [Figure 44](#))

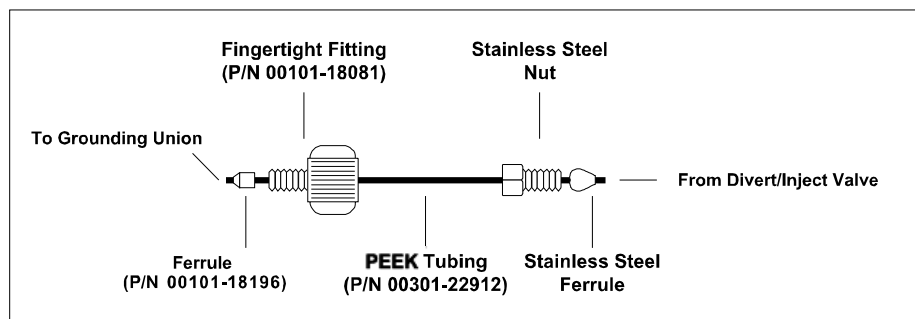


Figure 43. ESI/MS plumbing connections for the divert/inject valve and grounding union

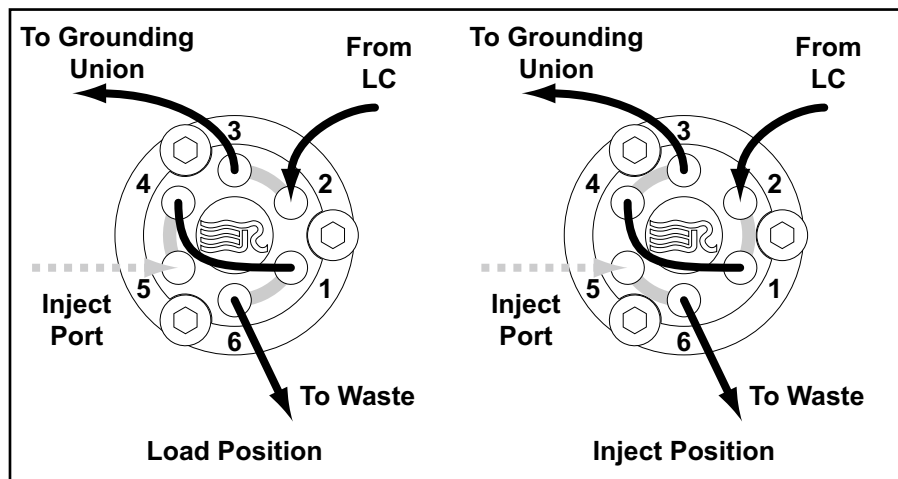


Figure 44. Divert/Inject valve, showing the correct setup for analysis by loop injection and showing the flow of liquid through the valve in the Load and Inject positions

10. Connect an appropriate length of PEEK tubing (transfer line from the LC) from the divert/inject valve to the LC, as follows:
 - a. Connect a length of the PEEK tubing with a (stainless steel) nut and (stainless steel) ferrule to port 2 of the divert/inject valve.
 - b. Connect the other end of the PEEK tubing with a proper fitting and a ferrule to the outlet of the LC.
11. Connect an appropriate length of PEEK tubing (waste line) from the divert/inject valve to a waste container, as follows:
 - a. Connect a length of PEEK tubing with a (stainless steel) nut and (stainless steel) ferrule to port 6 of the divert/inject valve.
 - b. Insert the other end of the PEEK tubing into a suitable waste container.

5 Acquiring ESI Sample Data Using the Tune Plus Window

Acquiring MS Data in the SIM Scan Type

Acquiring MS Data in the SIM Scan Type

Use the following procedure to acquire a file of reserpine data in the selected ion monitoring (SIM) type from the Tune Plus window. The Finnigan LXQ MS detector automatically saves the data you acquire on your hard disk.



OnStandby



1. On the Control/Scan Mode toolbar, click the **On/Standby** button to take the MS detector out of Standby mode and turn it On. The MS detector begins scanning, the Finnigan LXQ MS detector applies high voltage to the ESI probe, and a real-time display shows in the Spectrum view.
2. Verify that the Centroid data type is selected. (The picture on the button should be the same as that shown here.)
3. Verify that the scan parameters are defined to acquire SIM data for reserpine (or your analyte of interest), as follows:
 - a. Click on the Define Scan button to open the Define Scan dialog box. See [Figure 45](#).
 - b. Compare the values in your dialog box to those in [Figure 45](#) and click **OK**.

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

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

Figure 45. Define Scan dialog box, showing typical settings for acquiring reserpine data in the SIM scan type

4. Turn on the LC pump and specify your flow rate of 400 $\mu\text{L}/\text{min}$. Ensure that your system is free of leaks.



5. On the Control/Scan Mode toolbar, click **Acquire** Data to open the Acquire Data dialog box. See [Figure 46](#).
6. Specify the acquisition parameters, as follows:
 - a. In the File Name text box, enter the name of the analyte. Enter **reserpine** if you are using the reserpine solution mentioned in the Note at the beginning of the subtopic “[Optimizing the Isolation Width and Setting Up to Optimize the Collision Energy](#)” on [page 82](#).
 - b. In the Sample Name text box, enter **reserpine** to specify the sample identity. If you are not using reserpine, type the name of your particular analyte.
 - c. Type a comment about your 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 on hard copies of your data.
 - d. In the Acquire Time group box, select the Continuously option button to acquire data continuously (until you stop the acquisition).
7. Leave the Acquire Data dialog box open during data acquisition, but move it to a corner of the Tune Plus window.
8. Click on **Start** in the Acquire Data dialog box to begin acquiring data. The Acquisition Status group box displays the following message.

State: Acquiring

Time (min):



9. Click the **Divert/Inject Valve** to open the Divert/Inject Valve dialog box. See [Figure 47](#).

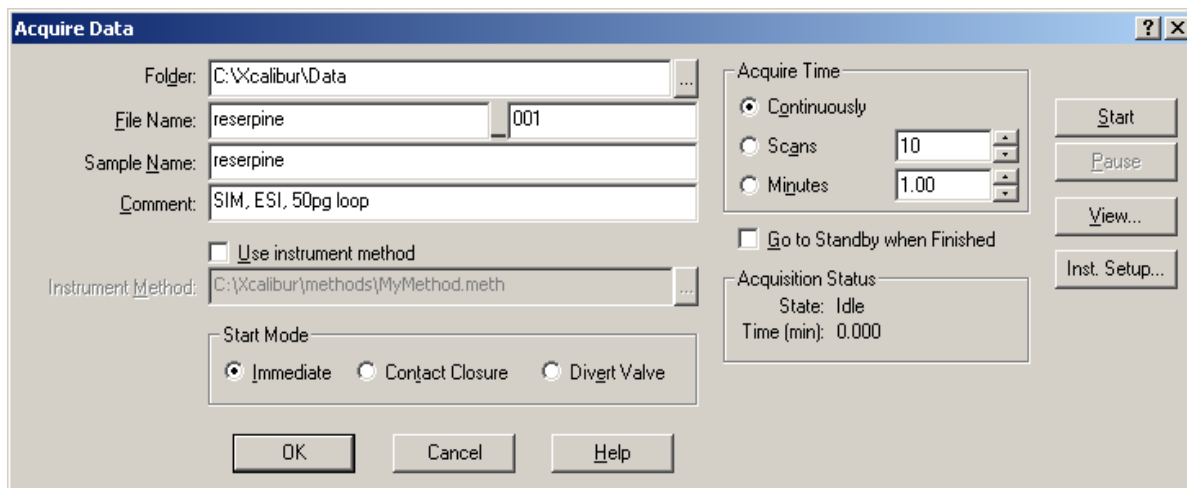


Figure 46. Acquire Data dialog box, showing typical settings for acquiring data

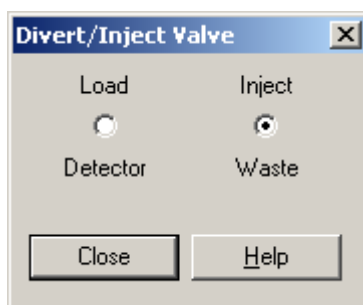


Figure 47. Divert/Inject Valve dialog box

10. Select the Load option button, and overfill the 2 μL injector loop with the 250 $\text{fg}/\mu\text{L}$ solution of reserpine (see “Reserpine” on page 138 in Appendix A, or use a solution of your analyte of interest).
11. Select the Inject option button to inject the reserpine solution into the ESI source. Leave the Divert/Inject Valve dialog box open.
12. Observe the reserpine peak (m/z 609.2), or that of your analyte of interest, in the Spectrum view, see Figure 48, right side. Wait about 1 min before you inject again (step 13.b, below).
13. Perform the following repetitive sequence to obtain a total of four consecutive loop injections of reserpine.
 - a. Select the Load option button, and overfill the injector loop with the 125 $\text{fg}/\mu\text{L}$ solution of reserpine.

- b. Select the Inject option button to inject the reserpine solution into the ESI source, and then observe the Spectrum view.
 - c. Wait 1 min before performing the next injection.
 - d. Perform [step 13.a](#) through [step 13.c](#) three more times.
14. Click **Close** in the Divert/Inject Valve dialog box to return to the Tune Plus window.
 15. Click **Stop** in the Acquire Data dialog box to end the data acquisition. Click **Cancel** to close the Acquire Data dialog box.

Review the mass spectrum and chromatogram in the Xcalibur Qual Browser window. See [Figure 48](#).

For more information about reviewing the data you acquired using the Finnigan LXQ MS detector with the Xcalibur data system, refer to the manual: *Xcalibur Getting Productive: Qualitative Analysis*.

5 Acquiring ESI Sample Data Using the Tune Plus Window

Acquiring MS Data in the SIM Scan Type

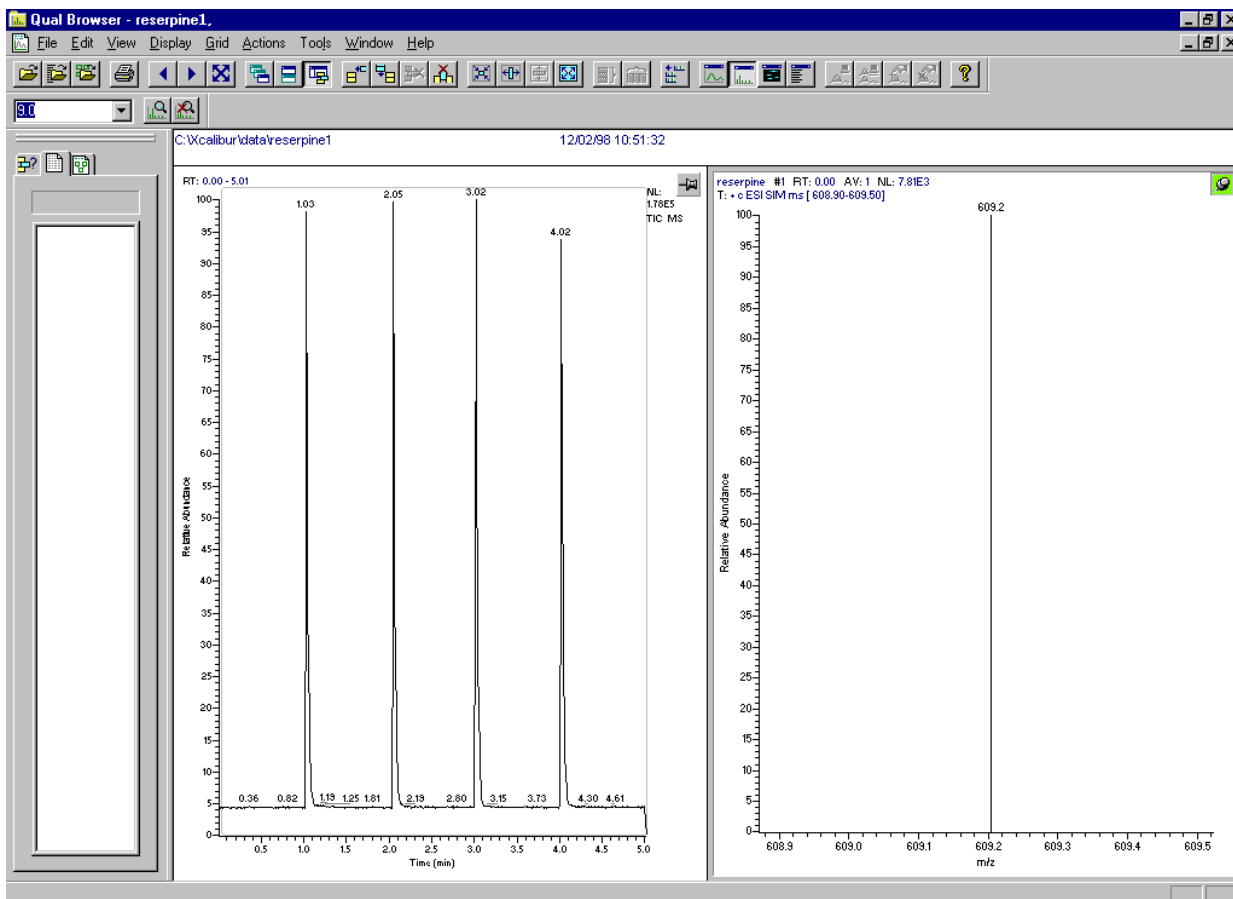


Figure 48. Qual Browser window showing loop injections of reserpine in the Chromatogram view (left) and showing m/z 609 in the Spectrum view (right, centroid data type). Note that the loop injections occur at intervals of approximately 1 min

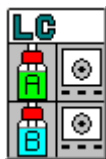
Chapter 6 **Setting Up the Ion Source for Acquiring Data in APCI/MS/MS Mode**

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

This chapter contains the following sections:

- [Removing the ESI Probe](#)
- [Removing the Ion Max Ion Source Housing](#)
- [Removing the Ion Sweep Cone \(optional\)](#)
- [Installing the Corona Needle](#)
- [Installing the Ion Max Ion Source Housing](#)
- [Installing the APCI Probe](#)

Removing the ESI Probe



OnStandby

Remove the ESI probe from the Ion Max ion source housing as follows:

1. Place the LC/MS system in Standby:
 - a. Stop the flow of solvent to the ESI source:
 - i. On the Control / Scan Mode toolbar, click **Inlet Direct Control** to display the Inlet Direct Control dialog box.
 - ii. In the Direct Control Panel group box, click on the Pump Off button to stop the flow of solvent.
 - b. Click **On/Standby** on the Control / Scan Mode toolbar to place the MS detector in Standby.
2. Disconnect the sample transfer tubing from the stainless steel ZDV fitting (grounding union). See [Figure 49](#).
3. Remove the 8 kV cable from the ESI needle high voltage receptacle as shown in [Figure 49](#).
 - a. Unlock the cable by rotating the locking ring counter-clockwise.
 - b. Unplug the 8 kV cable from the ESI needle high voltage receptacle.



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

4. Disconnect the Auxiliary Gas fitting (green) from the auxiliary gas inlet (A) on the probe manifold. (Figure 49.)
5. Disconnect the Sheath Gas fitting (blue) from the sheath gas inlet (S) on the probe manifold.
6. Remove the stainless steel ZDV fitting (Grounding Union) from the grounding bar on the ion source housing.
7. Unlock the probe locking lever by loosening the probe locking nut.
8. Carefully pull the probe straight back in the port in the housing until it meets with the slot in the API interlock block. The guide pin on the probe manifold will prevent you from rotating the probe until the pin is aligned with the slot in the API interlock block. Once the probe is all the way back and aligned with the slot, turn the probe 45 degrees

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

Removing the ESI Probe

counter-clockwise to free the probe from the alignment notch. Be careful not to break the fused-silica sample tube or PEEK safety sleeve.

9. Pull the probe straight out to remove it from the ion source housing.
10. Store the ESI probe in its original shipping container.

The ESI probe is properly removed from the Ion Max ion source housing.

Removing the Ion Max Ion Source Housing

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

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

Remove the Ion Max ion source housing as follows:

1. Remove the drain tube from the ion source housing drain. See [Figure 50](#).
2. Rotate the ion source housing locking levers 90 degrees to release the ion source housing from the ion source mount assembly.
3. Remove the ion source housing by pulling the housing straight off of the ion source mount assembly.
4. Place the housing in a safe location for temporary storage.

The Ion Max ion source housing is now properly removed. If you want to remove the ion sweep cone, go to the next topic: [Removing the Ion Sweep Cone](#). Otherwise, go to the topic “[Installing the Corona Needle](#)” on [page 104](#).

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

Removing the Ion Max Ion Source Housing

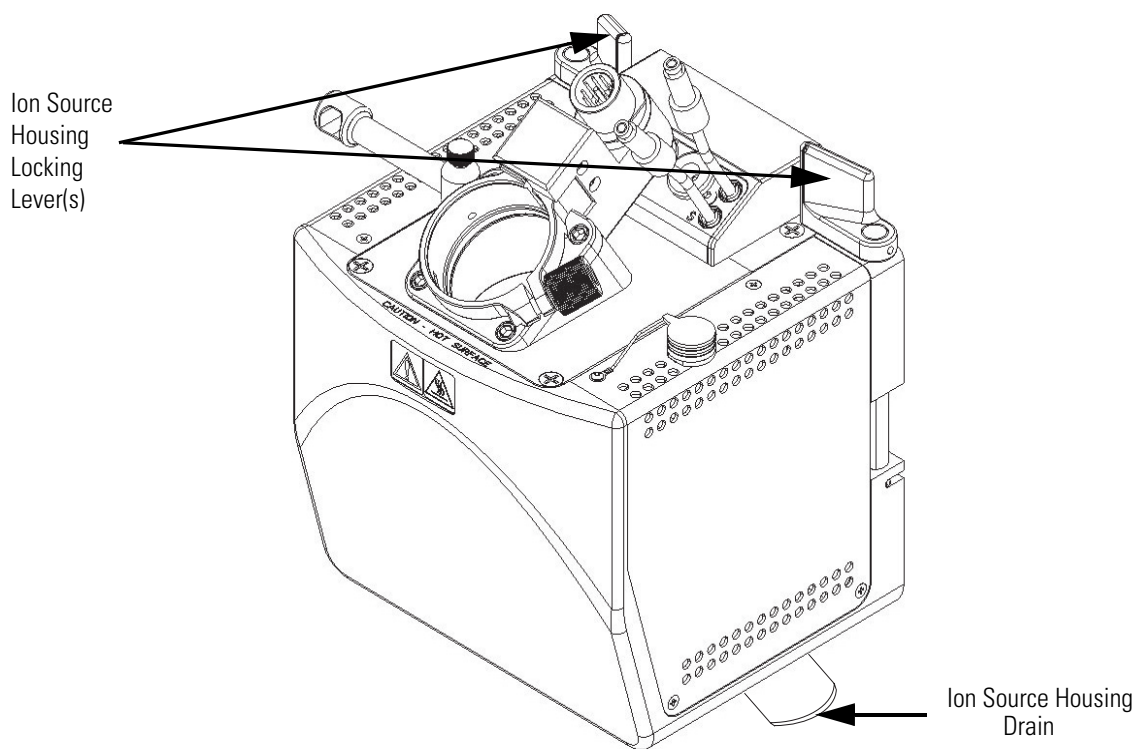


Figure 50. Ion Max-S ion source housing, detail of components (this component detail also applies to the Ion Max ion source housing)

Removing the Ion Sweep Cone

Use of the ion sweep cone is optional. If you do not need to use the ion sweep cone, remove the ion sweep cone as follows:

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



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

2. Grasp the outer ridges of the ion sweep cone and pull the cone straight off of the API cone seal.
3. Store the ion sweep cone in its original shipping container.

The ion sweep cone is now properly removed.

If you need to install the corona needle, go to the next topic: [Installing the Corona Needle](#). Otherwise, go to the topic: [“Installing the Ion Max Ion Source Housing”](#) on [page 105](#).

Installing the Corona Needle

Install the corona needle, from the rear of the Ion Max ion source housing, as follows:



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

1. Using pliers, grasp the needle by the corona needle contact and push the needle straight into the needle socket in the Ion Max ion source housing. See [Figure 51](#).

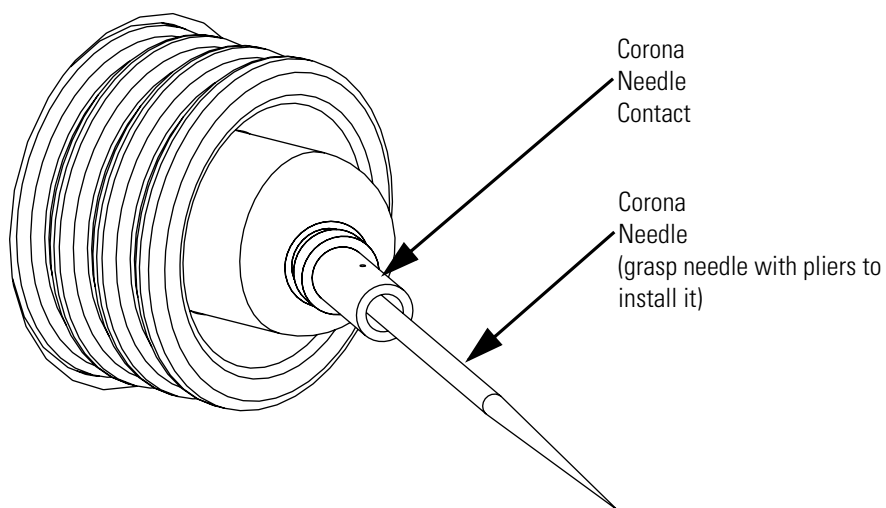


Figure 51. Corona needle, view from inside of the Ion Max housing

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

The corona needle is now properly installed.

Installing the Ion Max Ion Source Housing

To reinstall the Ion Max ion source housing

1. Carefully align the two guide pin holes on the rear of the ion source housing with the ion source housing guide pins on the MS detector, and carefully press the ion source housing onto the ion source mount. See [Figure 52](#) and [Figure 53](#).

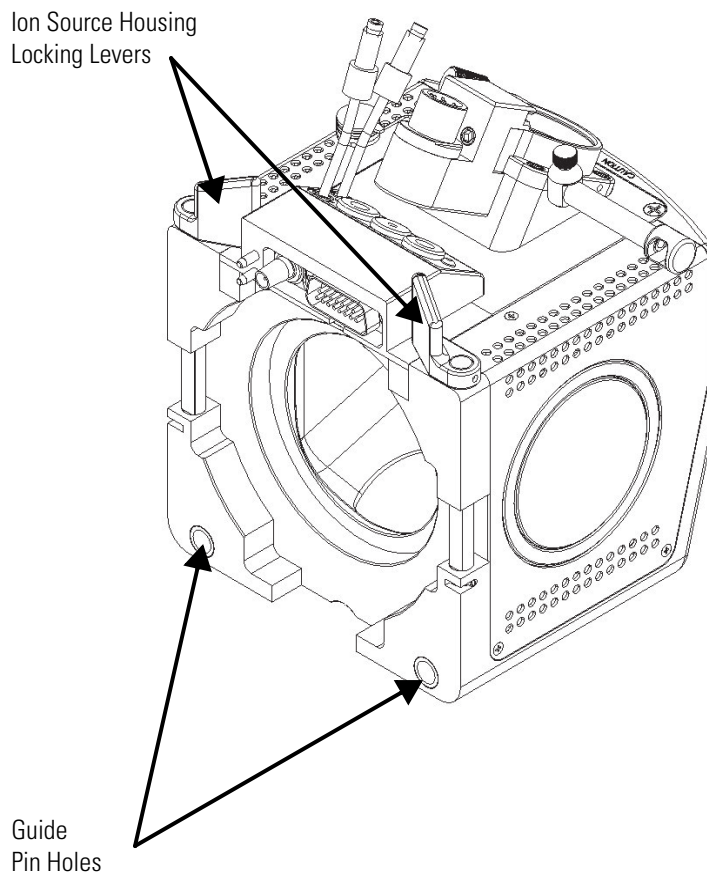


Figure 52. Rear view of the Ion Max-S ion source housing

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

Installing the Ion Max Ion Source Housing

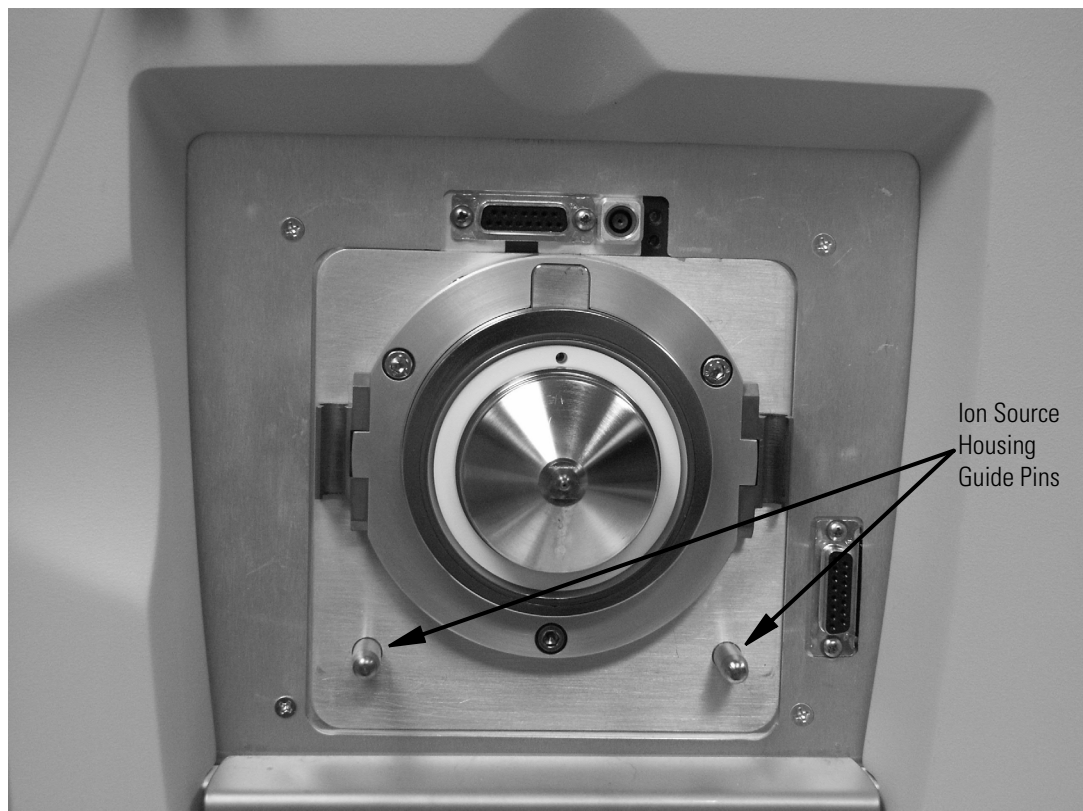


Figure 53. Ion source mount showing ion source housing guide pins

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

CAUTION Prevent solvent waste from backing up into the ion source housing and MS detector. Always ensure that liquid in the drain tube is able to drain to a waste container and that the outlet of the drain tube is above the level of liquid in the waste container.



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

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

3. Reinstall the ion source drain tube as follows:
 - a. Connect the 1-in. ID Tygon tubing to the ion source housing drain fitting.
 - b. Attach the free end of the hose to a waste container. Ideally, the waste container should be vented to a fume exhaust system.

The Ion Max ion source housing is now properly installed on the MS detector.

Installing the APCI Probe

Install the APCI probe into the Ion Max ion source housing, as follows:

1. Connect the 8 kV cable to the corona needle high voltage receptacle as follows:
 - a. Plug the 8 kV cable into the corona needle high voltage receptacle on the right side of the top of the ion source housing.
 - b. Lock the cable by rotating the locking ring clockwise.
2. Be sure to unlock the probe locking lever (widest open position) before attempting to install the probe.
3. Insert the APCI probe into the port in the ion source housing, align the guide pin on the probe body at a 45 degree angle from the API interlock block. See [Figure 54](#)
4. Push the probe into the port until the guide pin meets with the probe collar on the ion source housing.
5. Turn the probe 45 degrees clockwise and align the guide pin with the slot in the API interlock block (you might need to pull the probe towards you slightly to properly align the pin with the notch). Once you have turned the probe far enough to align the pin with the alignment notch at the rear of the port, push the probe straight in until the guide pin stops at the bottom of the alignment notch.
6. Seat the probe all the way down into the alignment notch.

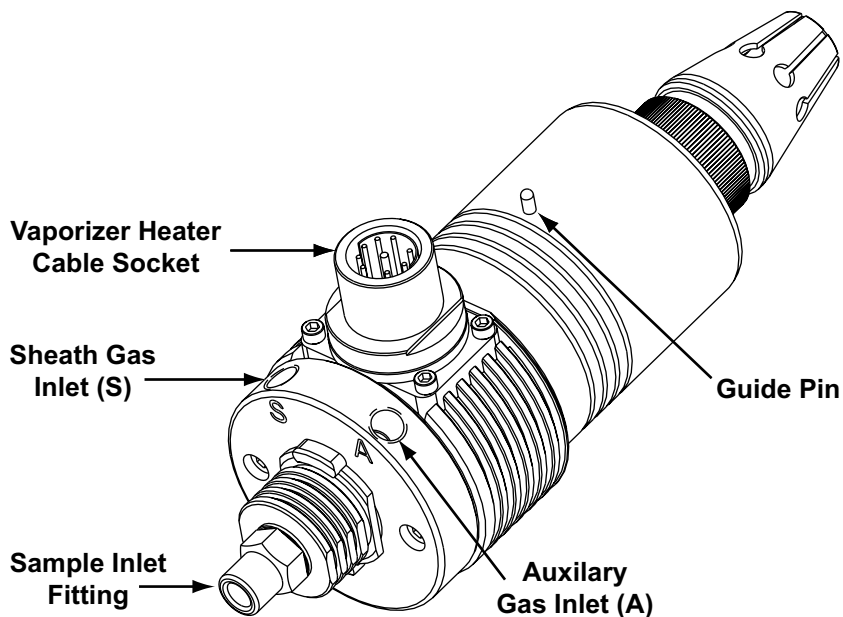


Figure 54. APCI probe

7. Lock the probe in place by rotating the probe locking lever towards the front of the housing; closing the probe locking lever towards the rear of the ion source housing might make it difficult to unlock.
8. Unplug the vaporizer heater cable from the ESI interlock plug on the ion source housing.
9. Connect the vaporizer heater cable to the vaporizer heater cable socket on the APCI probe.
10. Connect the sheath gas fitting (blue) to the sheath gas inlet (S) on the probe. (See Figure 54)
11. Connect the auxiliary gas fitting (green) to the auxiliary gas inlet (A) on the probe. (Figure 54)
12. Connect the sample transfer line to the APCI probe inlet.

The APCI probe is now properly installed in the Ion Max ion source housing.



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

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

Installing the APCI Probe

Leave the LC/MS system in Standby and go to the next chapter: [Optimizing the MS Detector with Your Analyte in APCI/MS Mode](#).

Chapter 7 Optimizing the MS Detector with Your Analyte in APCI/MS Mode

This chapter provides information on optimizing the tuning of your MS detector in the APCI/MS high flow mode. It is not necessary to recalibrate the MS detector when you switch to APCI/MS operation. You can use the calibration settings you obtained from the successful automatic calibration procedure you performed in the ESI/MS mode.

For APCI/MS operation you simply open a default Tune Method located in your C:\Xcalibur\methods folder, in this case APCIhighflow.LTQTune. From this starting point, you optimize automatically the tube lens voltage for your particular analyte. The capillary voltage and ion transfer capillary temperature may then be optimized manually to enhance ion transmission.

Note The following procedures assume that you are familiar with your Finnigan LXQ instrument and the Tune Plus window. If you need to become familiar with your LXQ refer to the Finnigan LXQ online Help, *Finnigan LXQ Getting Connected Manual*, and/or the *Finnigan LXQ Hardware Manual*.

Ensure that you have performed and completed the procedures in the topics Tuning and Calibrating Automatically in the ESI/MS Mode and Setting Up to Acquire Data in the APCI/MS Mode.

This chapter includes the following sections:

- [Setting Up the Inlet for Tuning Using High-Flow Infusion](#)
- [Setting Up the MS Detector for APCI/MS Operation](#)
- [Optimizing the Tune of the MS Detector Automatically in APCI/MS Mode](#)
- [Saving the APCI/MS Tune Method](#)
- [Cleaning the MS Detector after Tuning in APCI Mode](#)

Setting Up the Inlet for Tuning Using High-Flow Infusion

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

1. Connect a 4 cm (1.5 in) segment of Teflon tubing with a fingertight fitting (natural color) and a ferrule to the (black) LC union. See [Figure 55](#).

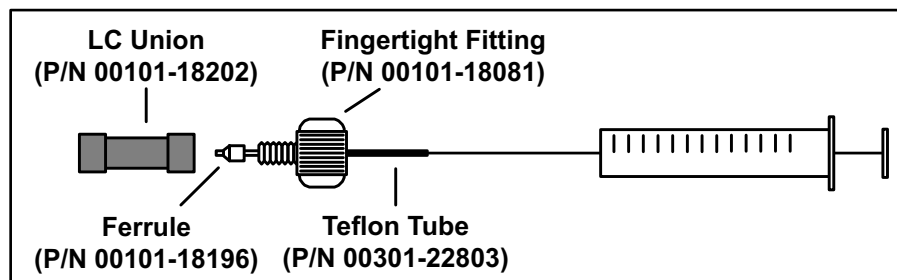


Figure 55. APCI/MS plumbing connections for the syringe pump

2. Load a clean, 500- μ L Unimetrics syringe with 450 μ L of a 10 pg/ μ L solution of reserpine or your analyte of interest. See [“Reserpine” on page 138](#) for the procedure to make a 10 pg/ μ L reserpine tuning solution (use the dilution at [step 9](#) of the procedure).
3. Insert the needle of a syringe into the segment of Teflon tubing and place the syringe in the syringe holder of the syringe pump.
4. Connect a fused-silica infusion line from the (black) LC union to the (black) LC Tee union, as follows. See [Figure 56](#).
 - a. Connect the infusion line with a fingertight fitting (natural color) and a (brown) ferrule to the free end of the LC union.
 - b. Connect the other end of the infusion line with a (red) fingertight fitting and a ferrule to the side arm of the LC Tee union.

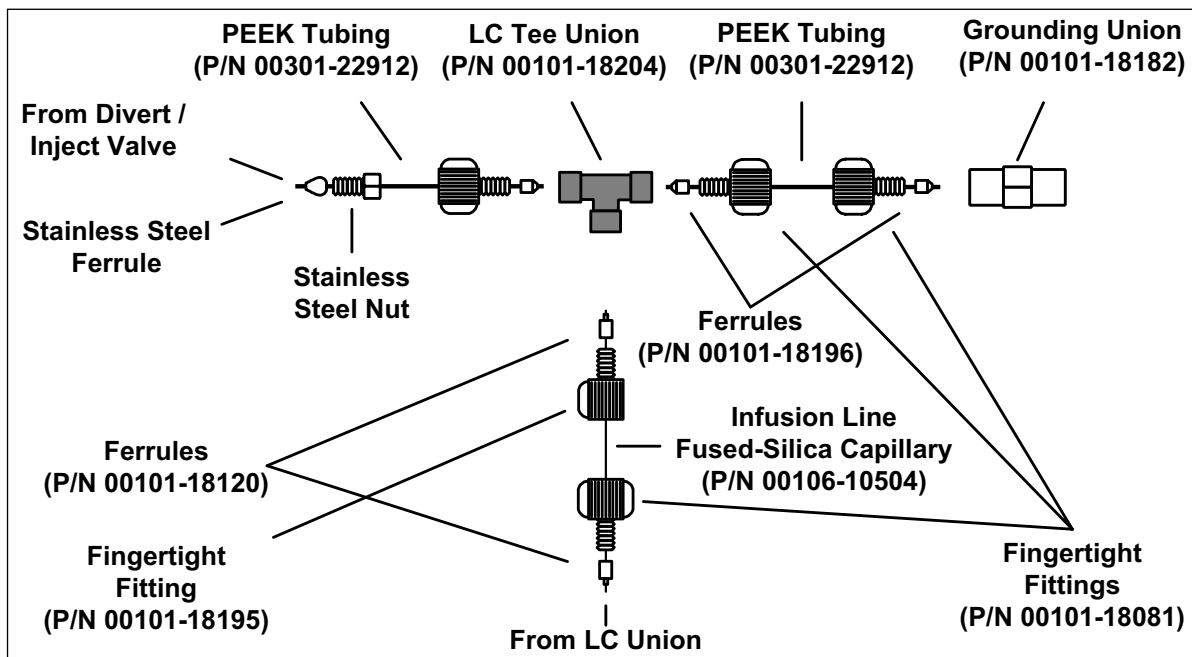


Figure 56. APCI/MS plumbing connections for the LC Tee union

Note . To cut the PEEK tubing used to connect your LC to the divert/inject valve and the divert/inject valve to the APCI source, use a PEEK tubing cutter. This ensures that the tubing is cut straight. In addition, make sure your LC fittings, ferrules, and PEEK tubing are installed properly. By using these precautions, you prevent void (dead) volumes. The exclusion of void volumes is critical to microbore LC. Also, void volumes affect the quality of the MS detector signal.

5. Connect a segment of PEEK tubing from the (black) LC Tee union to the APCI LC inlet, as follows. (See [Figure 56](#).)
 - a. Use a PEEK tubing cutter to cut a 4 cm (1.5 in.) length of the PEEK tubing.
 - b. Connect the PEEK tubing with a fingertight fitting and a (brown) ferrule to a free end of the (black) LC Tee union.
 - c. Connect the other end of the PEEK tubing with a (red) fingertight fitting and a ferrule to the LC inlet located on the APCI probe.
6. Connect an appropriate length of PEEK tubing (transfer line from the divert/inject valve) from the divert/inject valve to the LC Tee union, as follows. (See [Figure 56](#).)

7 Optimizing the MS Detector with Your Analyte in APCI/MS Mode

Setting Up the Inlet for Tuning Using High-Flow Infusion

- a. Connect a length of PEEK tubing with a (stainless steel) nut and a (stainless steel) ferrule to port 3 of the divert/inject valve.
 - b. Connect the other end of the PEEK tubing with a (brown) fingertight fitting and a (brown) ferrule to the free end of the LC Tee union.
7. Connect an appropriate length of PEEK tubing (transfer line from the LC) from the divert/inject valve to the LC, as follows:
- a. Connect a length of PEEK tubing with a (stainless steel) nut and a (stainless steel) ferrule to port 2 of the divert/inject valve.
 - b. Connect the other end of the PEEK tubing with a proper fitting and a ferrule to the outlet of the LC.
8. Connect an appropriate length of PEEK tubing (waste line) from the divert/inject valve to a waste container, as follows:
- a. Connect a length of PEEK tubing with a (stainless steel) nut and a (stainless steel) ferrule to port 1 of the divert/inject valve.
 - b. Insert the other end of the PEEK tubing in a suitable waste container.

The LC plumbing connections are now properly made for APCI/MS sample introduction from the syringe pump into solvent flow from an LC.

Setting Up the MS Detector for APCI/MS Operation



On



Standby

Set up the MS detector for APCI/MS operation

- In Tune Plus, click **On/Standby** to take the MS detector out of Standby mode and turn it On. The MS detector begins scanning, and the Finnigan LXQ MS detector applies high voltage to the corona needle and shows a real-time display in the Spectrum view.
- Open the *APCIhighflow.LTQTune* Tune Method, the Tune Method for high-flow APCI operation, as follows:
 - Choose **File > Open** to display the Open dialog box.
 - Scroll down until you see the folder *C:\Xcalibur\methods*. Then, select the file *APCIhighflow.LTQTune*.
 - Click **OK** to open the file. Finnigan LXQ MS detector downloads the Tune Method parameters to the MS detector.
- Verify that the Finnigan LXQ MS detector opened the Tune Method, as follows:
 - On the Instrument Setup toolbar, click **API Source** to open the APCI Source dialog box. See [Figure 57](#).

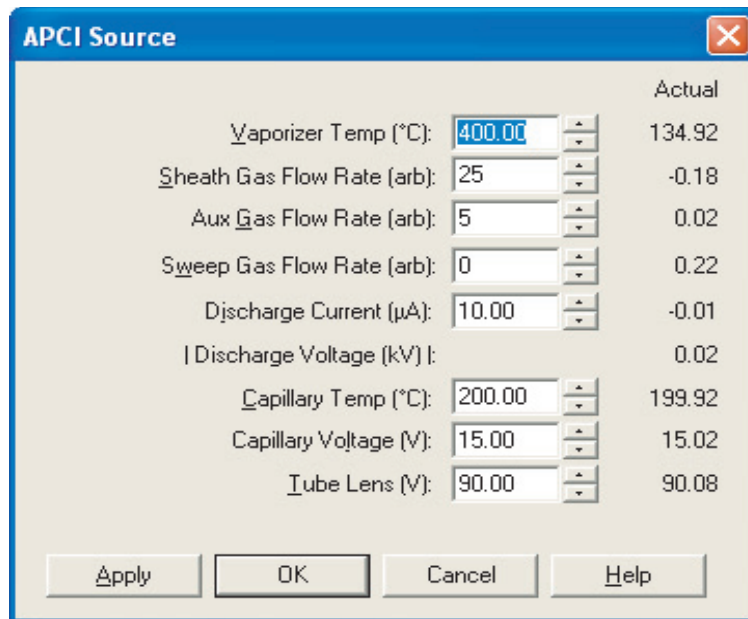
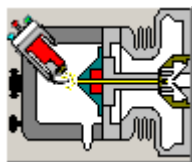


Figure 57. APCI Source dialog box, showing the proper settings for a typical high flow experiment

- Verify that the settings in your dialog box are similar to those shown in [Figure 57](#).

7 Optimizing the MS Detector with Your Analyte in APCI/MS Mode

Setting Up the MS Detector for APCI/MS Operation

- c. Click on **OK** to close the APCI Source dialog box.
4. Define the scan parameters for tuning the MS detector in the APCI/MS mode, as follows:



- a. On the Control/Scan Mode toolbar, click **Define Scan** to open the Define Scan dialog box. **Figure 58** (If your dialog box appears different from the one shown in the figure, it is probably because the advanced settings are not displayed. You can turn on the advanced settings as follows: In Tune Plus, choose **ScanMode**, and then click **Advanced Scan Features** to select the option.)
- b. In the Scan Description group box, in the Mass Range list box, select **Normal** to allow for a selection of mass ranges between m/z 150 to 2000.
- c. In the Scan Rate list box, select *Normal* to specify a normal scan rate.
- d. In the Scan Type list box, select *SIM* to specify a selected ion monitoring scan.
- e. In the Scan Time group box, in the Microscans spin box, type **1** to set the total number of microscans to 1.
- f. In the Max. Inject Time spin box, type *200.000* to specify a 200 ms maximum injection time.

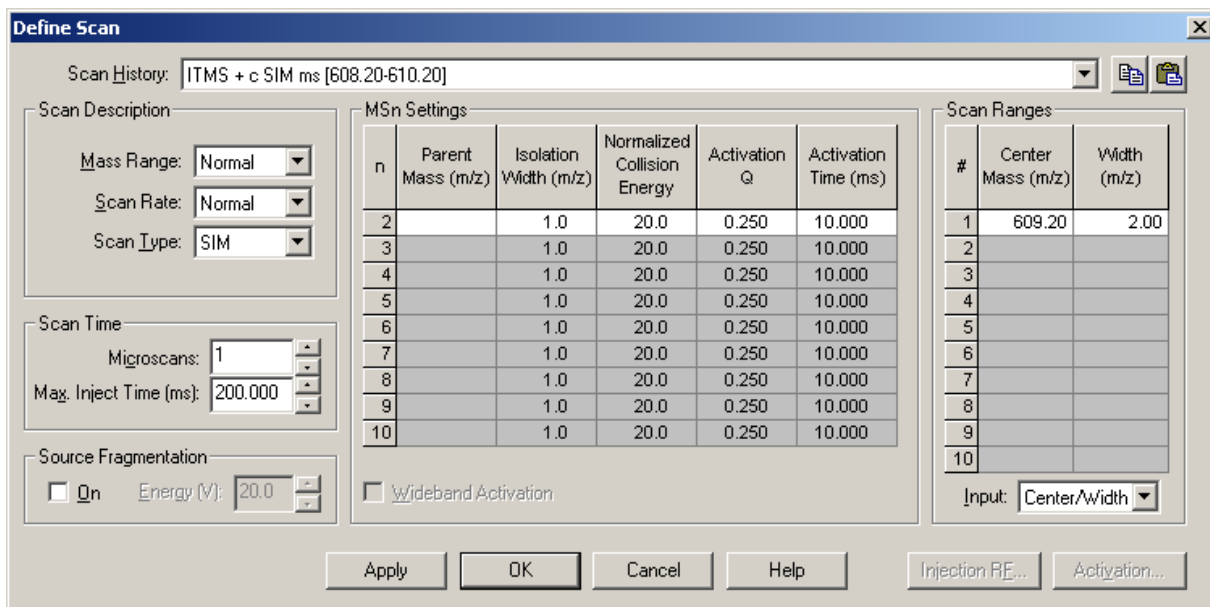


Figure 58. Define Scan dialog box, showing typical settings for APCI/MS operation

- g. In the Source Fragmentation group box, confirm that the On check box is clear () to specify that the ion source fragmentation option is turned off.
- h. In the Scan Ranges group box, in the Input list box, select *Center/Width* to make available the Center Mass and Width text boxes in the Scan Ranges table.
- i. In the Scan Ranges group box, in the Scan Ranges table, in the Center Mass text box, type **609.20** to set the center mass for the scan range to *m/z* 609.20.
- j. In the Width text box, type **2.00** to set the width of the scan range to *m/z* 2.00.
- k. Verify that the settings in your Define Scan dialog box are the same as those shown in [Figure 58](#).
- l. Click **OK** to apply the MS detector scan parameters and to close the Define Scan dialog box.



5. On the Control/Scan Mode toolbar, click **Centroid/Profile** to toggle the data type to profile (the picture on the button should be the same as that shown here).



6. Click **Positive/Negative** to toggle the ion polarity mode to positive (the picture on the button should be the same as that shown here).

You have now completed setting up your MS detector for APCI/MS operation.

Optimizing the Tune of the MS Detector Automatically in APCI/MS Mode

You can optimize the tune of the MS detector automatically for APCI operation.

The most important parameters that affect the signal quality during APCI/MS operation are vaporizer temperature, ion transfer tube temperature, API gas flows, and solution flow rate. If any one of these parameters changes, you must re-optimize MS detector parameters. (You can use the Semi-Automatic tune procedure to tune the MS detector on individual parameters.)

Use the following procedure to optimize the MS detector automatically on the reserpine peak at m/z 609.2 at your particular flow rate, for example, 400 $\mu\text{L}/\text{min}$. (See [Table 3 on page 12](#) for guidelines about flow rates and temperatures.)

Automatically Optimize the MS detector



1. On the Control/Scan Mode toolbar, click **Tune** to display the Automatic tuning page. See [Figure 59](#).
2. In the What to Optimize On group box, select the Mass option button to make active the Mass spin box.
3. In the Mass spin box, type **609.2** to specify that you want to tune on the peak at m/z 609.2.
4. Verify that the Divert/Inject valve is in the Detector position, as follows:
 - a. Click on the Divert/Inject Valve button to open the Divert/Inject Valve dialog box.
 - b. Select the Detector option button, and then click **Close** to return to Tune Plus.
5. Start the automatic tuning procedure from the Tune dialog box, as follows:
 - a. Click **Start**. A message box displays the following message:
Please ensure that the 500 microliter syringe is full.



Ensure that the syringe pump contains at least 450 mL of a 10 pg/mL reserpine tuning solution. See [Appendix A, "Sample Formulations."](#) for a procedure for making the reserpine tuning solution. Use the dilution made in [step 9](#) of the [Reserpine Tuning Solution and Reserpine APCI Sample Solution](#) procedure on [page 138](#).

- b. Click **OK** to close the message box, and return to the Tune Plus window.



6. On the File/Display toolbar, click on the Graph View button to display the view.

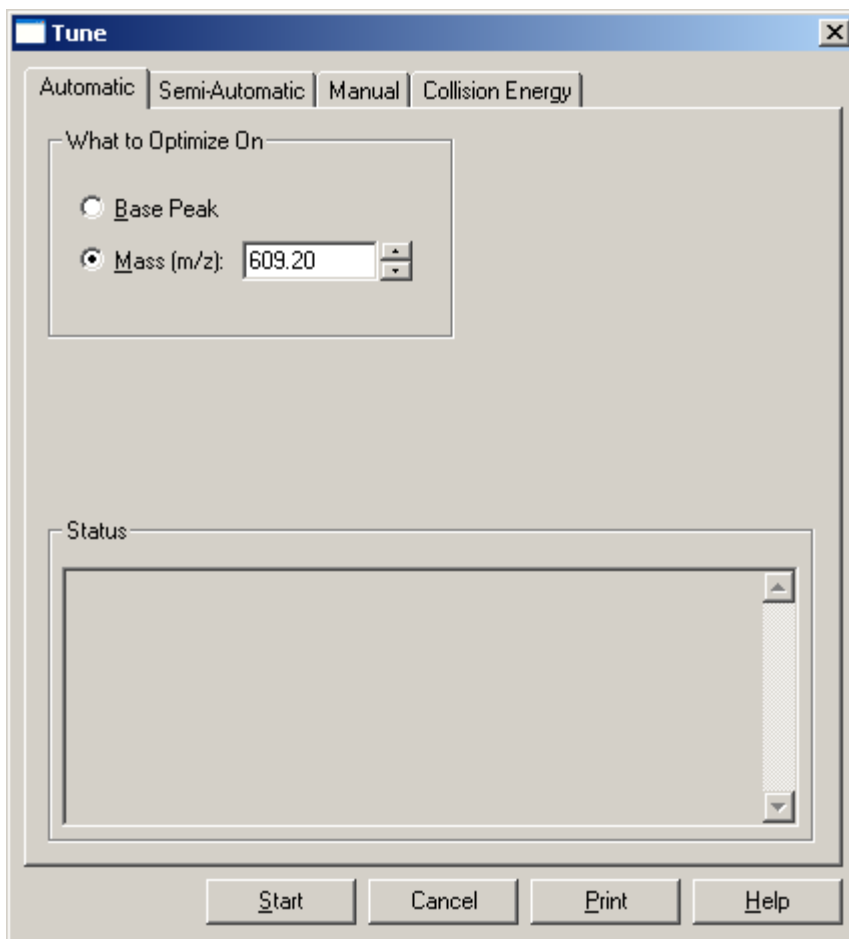


Figure 59. Tune dialog box, showing the Automatic tuning page

7. Observe the Tune Plus window and the Tune dialog box. While automatic tuning is in progress, the Finnigan LXQ MS detector displays various tests in the Spectrum and Graph views in the Tune Plus window and displays various messages in the Status group box in the Tune dialog box. Your Tune Plus window should now look similar to the one shown in [Figure 60](#).

You have now successfully tuned the MS detector in APCI/MS mode for the compound reserpine (or your analyte of interest). Leave the LC pumps on (with a flow rate of approximately 400 $\mu\text{L}/\text{min}$), and leave the Tune Plus window open with *APCIhighflow.LTQTune* to complete the next topic: [Saving the APCI/MS Tune Method](#).

7 Optimizing the MS Detector with Your Analyte in APCI/MS Mode

Optimizing the Tune of the MS Detector Automatically in APCI/MS Mode

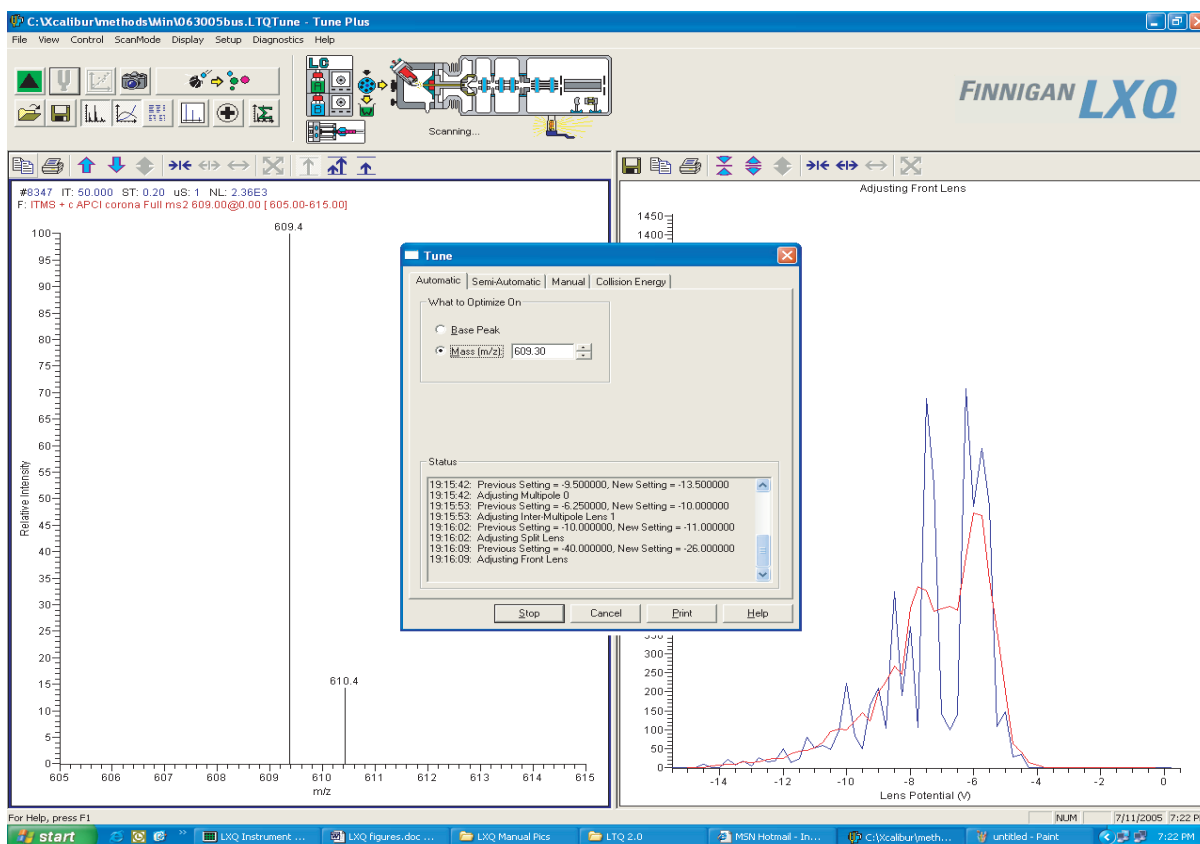


Figure 60. Tune Plus window with the Tune dialog box, showing the Automatic tuning page

Saving the APCI/MS Tune Method

You can save the settings you just obtained in a Tune Method specific to your particular analyte and solvent flow rate. (In this case, you save settings obtained using reserpine.) You can recall the Tune Method and use it as a starting point for optimizing the MS detector on reserpine at a different flow rate.

Note Save the Tune Method while the MS detector is still On.

Save the APCI/MS Tune Method

1. Choose **File > Save As** to display the Save As dialog box. See [Figure 61](#).
2. Select the C:\Xcalibur\methods folder.
3. Click on the File Name text box, and then enter **APCImyTune** to name the Tune Method APCImyTune.LTQTune.

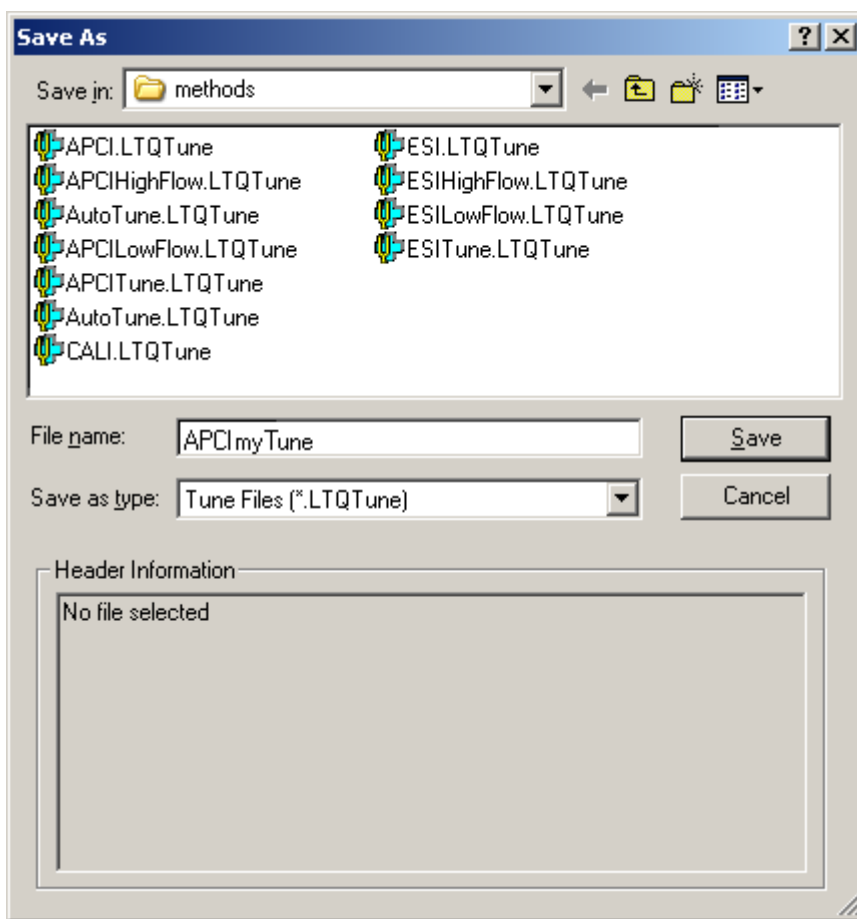


Figure 61. Save As dialog box, showing files in the folder *C:\Xcalibur\methods*

7 Optimizing the MS Detector with Your Analyte in APCI/MS Mode

Saving the APCI/MS Tune Method

4. Click **Save** to save the Tune Method and close the Save As dialog box. Note that the Tune Method is named APCImyTune.LTQTune.

Before you acquire data, go to the next section: [Cleaning the MS Detector after Tuning in APCI Mode](#).

Cleaning the MS Detector after Tuning in APCI Mode



On



Standby



Clean the MS detector after tuning on your analyte of interest

1. Click **On/Standby** to put the MS detector in Standby mode. When the MS detector is in Standby, the Finnigan LXQ MS detector turns off the vaporizer heater, corona discharge voltage, and syringe pump. The MS detector stops scanning, and the Finnigan LXQ MS detector freezes the displays for the Spectrum and Graph views.

CAUTION Always place the MS detector in Standby (or Off) before you open the API source to atmospheric oxygen. The presence of oxygen in the ion source when the MS detector is On could be unsafe. (The Finnigan LXQ MS detector automatically turns Off when you open the API source, however, it is best to take this added precaution.)

2. Remove the syringe from the syringe pump holder, as follows:
 - a. Squeeze the blue buttons, and pull back on the syringe pump handle to free the syringe.
 - b. Remove the syringe from the holder.
 - c. Disconnect the tip of the syringe needle from the Teflon tubing.
3. Clean the syringe thoroughly, as follows:
 - a. Clean the syringe with a solution of 5% formic acid in water.
 - b. Rinse the syringe with a solution of 50:50 methanol:water.
 - c. Use acetone to rinse the syringe. (Repeat this step several times.)



CAUTION AVOID BURNS. The APCI vaporizer heater can reach temperatures of 600 °C. Always allow the APCI probe to cool to ambient temperature, for approximately 20 min, before handling or removing the APCI probe from the APCI flange.



CAUTION AVOID INJURY. The corona discharge needle is very sharp and can puncture your skin if you handle it without caution.

4. Remove the Ion Max ion source housing as described in the topic [“Removing the Ion Max Ion Source Housing”](#) on page 31.

5. Flush the sample transfer line, sample tube, and APCI probe thoroughly with a solution of 5% formic acid in water (or with another appropriate solvent), as follows:

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

- a. Fill a clean, 250 μL Unimetrics syringe with an appropriate solvent.
 - b. While holding the plunger of the syringe in place, carefully insert the needle of the syringe into the free end of the Teflon tube.
 - c. Flush the sample transfer line, sample tube, and APCI probe with the solution by slowly depressing the syringe plunger. Visually check that the solution is exiting the tip of the APCI probe on the inside of the probe assembly. Use a lint-free tissue to gently remove the excess solution as it exits the probe.
 - d. Remove the needle of the syringe from the Teflon tube.
6. Repeat step 5 with a solution of 50:50 methanol:water.
 7. Reinstall the Ion Max ion source housing as described in [“Installing the Ion Max Ion Source Housing”](#) on [page 34](#).

If you plan to run analytical samples in APCI mode, go to the next chapter: [Acquiring APCI Sample Data Using the Tune Plus Window](#).

Chapter 8 Acquiring APCI Sample Data Using the Tune Plus Window

This chapter provides information on acquiring LC/APCI/MS sample data using the Tune Plus window. This experiment uses reserpine but you can follow the same procedure with your analyte of interest.

Note The following procedures assume that you are familiar with your Finnigan LXQ instrument and the Tune Plus window. If you need information, refer to the Finnigan LXQ online Help, *Finnigan LXQ Getting Connected*, and/or *Finnigan LXQ Hardware Manual*. Verify that the procedures in [Chapter 3, “Tuning and Calibrating Automatically in the ESI/MS Mode.”](#) and [Chapter 7, “Optimizing the MS Detector with Your Analyte in APCI/MS Mode.”](#) are completed.

This chapter contains the following topics:

- [Setting Up to Introduce Sample by Loop Injection into Solvent Flow from an LC](#)
- [Acquiring APCI Data in the SIM Scan Mode](#)

Setting Up to Introduce Sample by Loop Injection into Solvent Flow from an LC

This topic provides information on how to introduce sample by loop injection into solvent flow from an LC.

Make the plumbing connections as follows:

1. Connect an appropriate length of (red) PEEK tubing (transfer line from the divert/inject valve) from port 3 of the divert/inject valve to the (stainless steel) sample inlet fitting on the APCI probe. See [Figure 62](#) and [Figure 63](#).
2. Connect a 2 μ L sample loop with (stainless steel) set nuts and (stainless steel) ferrules to ports 1 and 4 of the divert/inject valve.
3. Connect an appropriate length of PEEK tubing (transfer line from the LC) from the divert/inject valve to the LC, as follows:
 - a. Connect a length of the PEEK tubing with a (stainless steel) nut and (stainless steel) ferrule to port 2 of the divert/inject valve.
 - b. Connect the other end of the PEEK tubing with a proper fitting and a ferrule to the outlet of the LC.

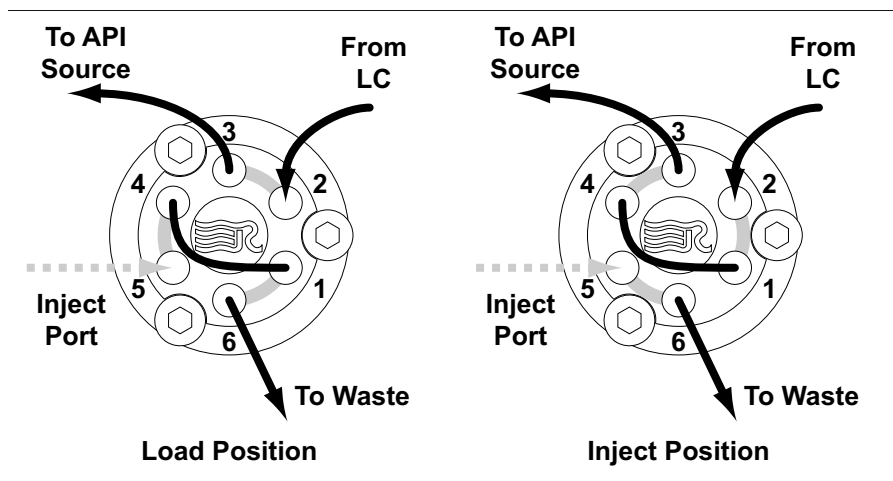


Figure 62. Divert/Inject valve, showing the correct setup for analysis by loop injection and showing the flow of liquid through the valve in the Load and Inject positions

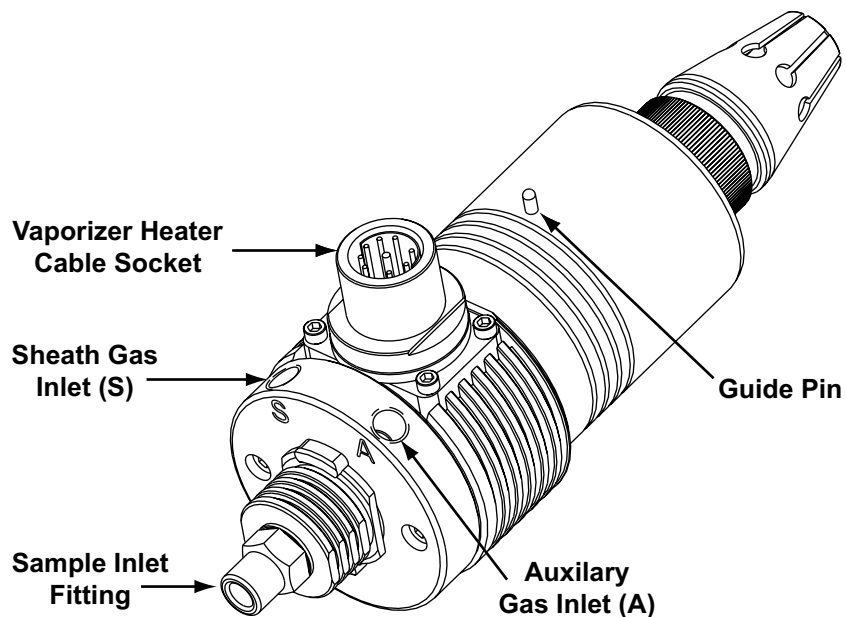


Figure 63. APCI probe

4. Connect an appropriate length of PEEK tubing (waste line) from the divert/inject valve to a waste container, as follows:
 - a. Connect a length of PEEK tubing with a (stainless steel) nut and (stainless steel) ferrule to port 6 of the divert/inject valve.
 - b. Insert the other end of the PEEK tubing into a suitable waste container.

The MS detector is now set up to introduce sample by loop injection into solvent flow from an LC.

Acquiring APCI Data in the SIM Scan Mode

Use the following procedure to acquire a file of reserpine data in the selected ion monitoring (SIM) mode. The Finnigan LXQ MS detector automatically saves the data you acquire on your hard disk.



1. If you have not already done so, in Tune Plus, click **On/Standby** to take the MS detector out of Standby mode and turn it On. The MS detector begins scanning, and the Finnigan LXQ MS detector applies high voltage to the corona needle and shows a real-time display in the Spectrum view.



2. Ensure that the Centroid data type is selected. (The picture on the button should be the same as that shown here.)

3. Ensure that the scan parameters are defined to acquire SIM data for reserpine (or your analyte of interest), as follows:



- Click **Define Scan** to open the Define Scan dialog box. [Figure 64](#)
- Verify that the values in your dialog box are the same as those in [Figure 8-3](#). Then, click **OK**.

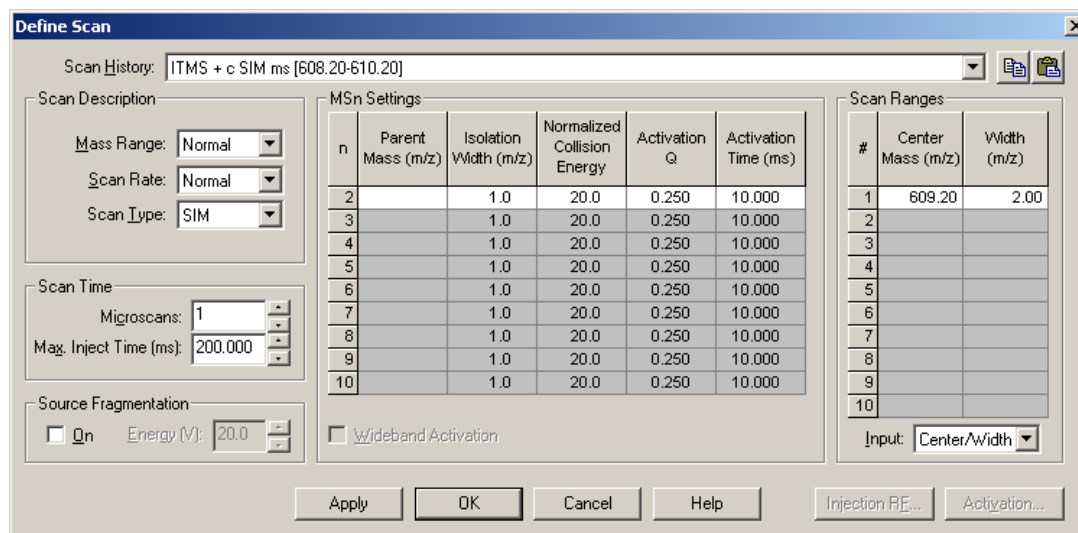


Figure 64. Define Scan dialog box, showing typical settings for acquiring data in the SIM scan mode

4. Turn on your LC pump, and specify an appropriate flow rate of 400 $\mu\text{L}/\text{min}$, for example. Verify that your system is free of leaks.



5. On the Control/Scan Mode toolbar, click **Acquire Data** to open the Acquire Data dialog box. See [Figure 65](#).

6. Specify the acquisition parameters, as follows:

- a. In the File Name text box, type reserpine to specify a filename.
 - b. In the Sample Name text box, type reserpine to specify the sample identity. If you are not using reserpine, type the name of your particular analyte.
 - c. In the Comment text box, you may type a comment about the experiment. Comments usually specify attributes of the experiment such as the scan mode, ionization mode, sample amount, and method of sample introduction (an example would be the entry: **SIM, APCI, 50 pg, loop**). Comments may include any other descriptive information about the experiment. Information entered in the Comment text box of the Xcalibur data system will be included on hard copies of the data.
 - d. In the Acquire Time group box, select the Continuously option button to specify the continuous acquisition of data (until you stop the acquisition).
7. Leave the Acquire Data dialog box open during data acquisition, but move it to a corner of the Tune Plus window.
 8. Click **Start** in the Acquire Data dialog box to begin acquiring data to the file reserpine3.raw. See [Figure 65](#). The Acquisition Status group box displays the following message.

State: Acquiring

Time (min):

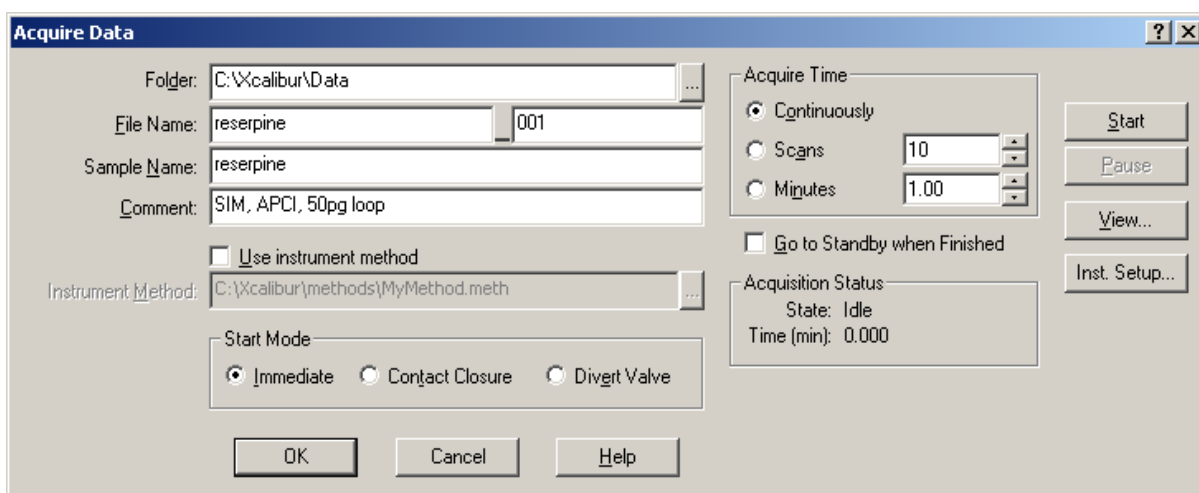


Figure 65. Acquire Data dialog box, showing the acquisition status of the raw data file

8 Acquiring APCI Sample Data Using the Tune Plus Window

Acquiring APCI Data in the SIM Scan Mode



9. Inject the reserpine solution into the APCI source from the Instrument Setup toolbar, as follows:
 - a. Click **Divert/Inject Valve** to display the Divert/Inject Valve dialog box. See [Figure 66](#).

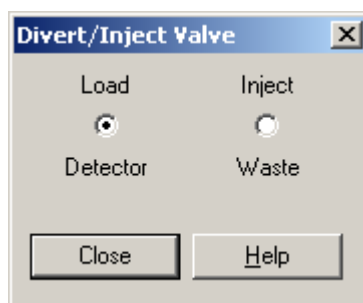


Figure 66. Divert/Inject Valve dialog box

- b. Select the **Load** option button, and overfill the 2 μL injector loop with a 250 $\text{fg}/\mu\text{L}$ solution of reserpine or a solution of your analyte of interest. See “[Reserpine Tuning Solution and Reserpine APCI Sample Solution](#)” on [page 138](#) in Appendix A for the 250 $\text{fg}/\mu\text{L}$ reserpine sample solution recipe.
 - c. Select the Inject option button.
10. Observe the reserpine peak (m/z 609.2), or that of your analyte of interest, in the Spectrum view (for an example see [Figure 67](#), right side).
11. Perform the following repetitive sequence to obtain a total of four consecutive loop injections of reserpine in the SIM scan mode. Wait about 1 min between injections.
 - a. Select the Load option button to put the divert/inject valve in the Load position. Overfill the injector loop with the 125 $\text{fg}/\mu\text{L}$ solution of reserpine.
 - b. Select the Inject option button to inject the reserpine solution into the APCI source. Then, observe the Spectrum view.
 - c. Wait 1 min before the next injection.
 - d. Repeat [step 11.a](#) through [step 11.c](#) three times
12. Click **Stop** in the Acquire Data dialog box to end the data acquisition.

Review the mass spectrum and chromatogram in the raw file you just acquired using the Xcalibur Qual Browser window. See [Figure 67](#).

For more information about reviewing the data you acquire using the Finnigan LXQ MS detector with the Xcalibur data system, refer to the *Xcalibur Getting Productive: Qualitative Analysis* manual.

Note If you want to acquire MS/MS Full scan data in APCI mode, for information about setting up the Finnigan LXQ MS detector see [Setting Up to Acquire MS/MS Data in the Full Scan Type](#) on [page 82](#).

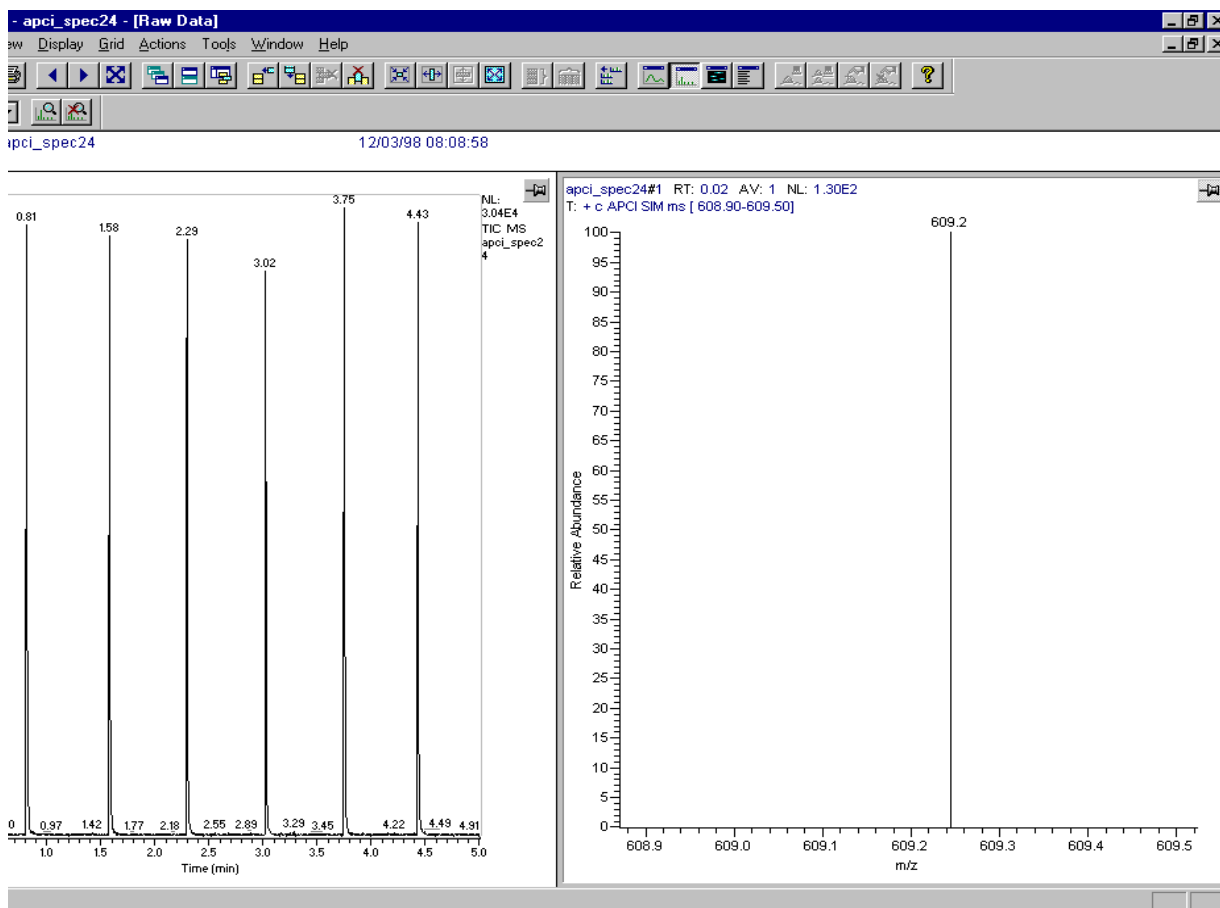


Figure 67. Qual Browser window, showing loop injections of reserpine in the Chromatogram view (left) and m/z 609 in the Spectrum view

Appendix A Sample Formulations

This appendix provides instructions for the preparation of several stock solutions. These solutions are used for tuning, calibrating, and demonstrating applications of the APCI / ESI system. Formulations for sample solutions in this appendix include:

- [Caffeine, MRFA, and Ultramark 1621 Stock Solutions](#)
- [ESI Calibration Solution: Caffeine, MRFA, Ultramark 1621](#)
- [Reserpine](#)

The caffeine, MRFA, Ultramark 1621, and Reserpine needed to make the solutions are supplied with your chemical accessory kit. You can order replacement chemical accessory kits from Thermo Electron (Thermo Electron part #97000-62042).



CAUTION Store and handle all chemicals in accordance with standard safety procedures. The Material Safety Data Sheets (MSDS) describing the chemicals being used are to be freely available to lab personnel for them to examine at any time. Material Safety Data Sheets (MSDS) provide summarized information on the hazard and toxicity of specific chemical compounds. MSDSs also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures for the remedy of spills or leaks. Producers and suppliers of chemical compounds are required by law to provide their customers with the most current health and safety information in the form of an MSDS. Read the material safety data sheets for each chemical you use.

Potentially hazardous chemicals used in procedures throughout this manual include:

- Acetic acid
- Acetonitrile
- Methanol
- Reserpine
- Formic Acid

Caffeine, MRFA, and Ultramark 1621 Stock Solutions

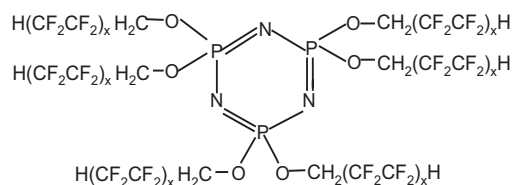
For tuning and calibrating the ESI system, you use a calibration solution of caffeine, MRFA, and Ultramark 1621 in an acetonitrile:methanol:water solution containing 1% acetic acid. You prepare the calibration solution from the following stock solutions:

- Caffeine stock solution (prepared stock solution is in the kit)
- MRFA stock solution (you prepare using MRFA supplied in the kit)
- Ultramark 1621 stock solution (you prepare using Ultramark 1621 supplied in the kit)

Note Vials of caffeine and MRFA are included in the API accessory kit. These compounds are available from Sigma Chemical Co. The Sigma caffeine Product Number is C6035 (Caffeine Solution, drug standard 1mg/mL \pm 5% in methanol). The Sigma MRFA Product Number is M1170 (MRFA=Met-Arg-Phe-Ala acetate salt). To order more of these compounds from Sigma, write or call:

Sigma Chemical Company
P. O. Box 14508
St. Louis, Missouri, USA 63178-9916
(800) 325-3010 (in the USA)
(905) 829-9500 (in Canada)
(314) 771-3750 (outside the USA or Canada)
www.sigmaaldrich.com

Note A vial of Ultramark 1621 (neat liquid) is included in the API accessory kit. This compound is available from Lancaster Synthesis. The structure of Ultramark 1621 is shown below (x is 1, 2, or 3).



The Lancaster Ultramark 1621 Catalog Number is 16698 (Ultramark 1621, Mass Spec Std.). To order more of this compound from Lancaster Synthesis, write or call:

Lancaster Synthesis, Inc.
 P.O. Box 1000
 Windham, NH USA 03087-9977
 (603) 889-3306, (800)-238-2324 (in the USA & Canada)
 +44 (0)1524 36101 (UK & International)
www.lancastersynthesis.com



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



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

Stock Solution: Caffeine

A 1 mg/mL stock solution of caffeine in 100% methanol is provided with your Finnigan LXQ system. This is also the concentration of the Sigma replacement mentioned in the Note above (Sigma #C6035).

Stock Solution: MRFA

Prepare a 1.5 mL stock solution of 166.7 pmol/mL MRFA in 50:50 methanol:water as follows:

1. Obtain the vial of L-methionyl-arginyl-phenylalanyl-alanine acetate•H₂O (MRFA) in your accessory kit. In this form, the MRFA sample has an average molecular weight of 523.7 u. Carefully weigh 2.6 mg of the MRFA sample.
2. Dissolve the MRFA sample in a total volume of 1.0 mL of 50:50 methanol:water.
3. Mix the solution (5.0 nmol/μL) thoroughly.
4. Transfer 50 μL of the 5 nmol/μL solution into a clean polypropylene tube.
5. Add 1.45 mL of 50:50 methanol:water to the tube.
6. Mix this solution (166.7 pmol/μL) thoroughly.
7. Label the tube MRFA stock solution.

Stock Solution: Ultramark 1621

Prepare a 10 mL stock solution of 0.1% Ultramark 1621 in acetonitrile as follows:

1. Obtain the vial of Ultramark 1621 (neat liquid) in your accessory kit.
2. Using a gas-tight syringe, measure out 10 μL of Ultramark 1621. Special care in taking the sample may be needed as the Ultramark 1621 has a high viscosity. Dissolve the 10 μL of Ultramark 1621 in 10 mL of acetonitrile.
3. Mix the solution thoroughly.
4. Label the vial *Ultramark 1621 stock solution*.

ESI Calibration Solution: Caffeine, MRFA, Ultramark 1621

Prepare 10 mL of the calibration solution, as follows:

1. Pipet 200 μL of the stock solution of caffeine into a clean, dry 10 mL volumetric flask.
2. Pipet 100 μL of the stock solution of MRFA into the flask.
3. Pipet 100 μL of the stock solution of Ultramark 1621 into the flask.

Note Use only glass pipets or stainless steel syringes when measuring glacial acetic acid. Using plastic pipet tips causes contamination of acid stock solutions that can introduce contaminants in the calibration solution.

4. Pipet 100 μL of glacial acetic acid into the flask.
5. Pipet 5 mL of acetonitrile into the flask.
6. Bring the volume of the solution up to the 10 mL-mark on the flask with 50:50 methanol:water.
7. Mix the calibration solution thoroughly.
8. Transfer the solution to a clean, dry vial.
9. Label the vial ESI Calibration Solution and store it in a refrigerator until it is needed.

Reserpine

Use the following directions to prepare a stock solution of reserpine. Use serial dilutions of this stock solution to make a sample solution.

Stock Solution: Reserpine

Prepare a stock solution of 1 $\mu\text{g}/\mu\text{L}$ reserpine in 1% acetic acid-methanol

1. Obtain the 1 gram vial of reserpine in your accessory kit. (The average molecular weight of reserpine is 608.7 u). Weigh out 10 mg of reserpine and transfer the sample to a 10 mL volumetric flask.
2. Dilute the reserpine up to the 10 mL mark with a solvent of 1% acetic acid in methanol.
3. Ensure the sample is thoroughly dissolved in solution.
4. Transfer the solution to a clean and dry light resistant vial. Label this vial as *Reserpine Stock Solution* ($1 \mu\text{g}/\mu\text{L} = 1.64 \text{ nmol}/\mu\text{L}$). It is best to use the Reserpine Stock solution as soon as it is made. If it is necessary to store the solution, keep it in a refrigerator until it is needed.

Reserpine Tuning Solution and Reserpine APCI Sample Solution

Prepare 1 mL of either the Reserpine Tuning Solution of 10 $\text{pg}/\mu\text{L}$ (16.4 $\text{fmol}/\mu\text{L}$, [step 9](#)) or the Reserpine APCI Sample Solution of 250 $\text{fg}/\mu\text{L}$ (411 $\text{amol}/\mu\text{L}$, [step 16](#)) in 1% acetic acid-50:50 methanol:water.

Prepare the appropriate Reserpine solution

1. Pipet 100 μL of the Stock Solution (1 $\mu\text{g}/\mu\text{L}$) of reserpine into a clean polypropylene microcentrifuge tube.
2. Add 900 μL of 1% acetic acid in 50:50 methanol:water to the tube.
3. Mix this solution (100 $\text{ng}/\mu\text{L} = 164 \text{ pmol}/\mu\text{L}$) thoroughly.
4. Transfer 10 μL of the 100 $\text{ng}/\mu\text{L}$ solution into a clean polypropylene tube.
5. Add 990 μL of 1% acetic acid in 50:50 methanol:water to the tube.
6. Mix this solution (1 $\text{ng}/\mu\text{L} = 1.64 \text{ pmol}/\mu\text{L}$) thoroughly.
7. Transfer 10 μL of the 1 $\text{ng}/\mu\text{L}$ solution into a clean polypropylene tube.
8. Add 990 μL of 1% acetic acid in 50:50 methanol:water to the tube.

9. Mix this solution ($10 \text{ pg}/\mu\text{L}=16.4 \text{ fmol}/\mu\text{L}$) thoroughly. This is the final dilution for the Reserpine Tuning Solution. If you are stopping at this dilution, label it as *Reserpine Tuning Solution (10 pg/μL)*. It best to use this solution immediately after it is made. Keep it in a light resistant container in a refrigerator if it must be stored. If you are making a Reserpine APCI Sample Solution, continue with [step 10](#).
10. Transfer 100 μL of the 10 pg/μL solution into a clean polypropylene tube.
11. Add 900 μL of 1% acetic acid in 50:50 methanol:water to the tube.
12. Mix this solution ($1 \text{ pg}/\mu\text{L}=1.64 \text{ fmol}/\mu\text{L}$) thoroughly.
13. Transfer 100 μL of the 1 pg/μL solution into a clean polypropylene tube.
14. Add 300 μL of 1% acetic acid in 50:50 methanol:water to the tube.
15. Mix this solution ($250 \text{ fg}/\mu\text{L}=411 \text{ amol}/\mu\text{L}$) thoroughly.
16. Label the tube *Reserpine APCI Sample Solution (250 fg/μL)* . It is best to use this solution immediately after it is made. Keep it in a light-resistant container in a refrigerator if it must be stored.

Appendix B LXQ High Mass Range Calibration

A high mass range calibration is necessary if you want to use your LXQ to analyze masses in the 2000 u-4000 u range. This appendix provides instructions for the high mass range calibration of the LXQ. The presentation in this Appendix assumes that you are familiar with the contents of [Chapter 3, “Tuning and Calibrating Automatically in the ESI/MS Mode.”](#) and [Appendix A, “Sample Formulations.”](#) .

This Appendix Contains the following topics:

- [High Mass Range Calibration Solution](#)
- [Normal Mass Range Calibration](#)
- [Enter Normal Mass Range Data into Tune Plus](#)
- [Tune on m/z 524.3](#)
- [High Mass Range Calibration Procedure](#)
- [Notes](#)

High Mass Range Calibration Solution

The high mass range calibration solution is 70 ng/ μ L polypropylene glycol (PPG) in a solvent of 65/35 methanol/10mM sodium acetate. The PPG used for the calibration procedure is Aldrich #202347 as specified in the second Note below.

Note One possible formulation for this solution is 3.5mg of PPG diluted to 50mL with the 65/35 methanol/10mM sodium acetate solvent.

Note The procedures that follow assume that the high mass range calibrant is polypropylene glycol, number average molecular weight about 2700 ($M_n \sim 2700$), Aldrich product number 202347. To order this compound from Sigma-Aldrich, write or call:

Sigma Chemical Company
P. O. Box 14508
St. Louis, Missouri, USA 63178-9916
(800) 325-3010 (in the USA)
(905) 829-9500 (in Canada)
(314) 771-3750 (outside the USA or Canada)
www.sigmaaldrich.com



CAUTION Store and handle all chemicals in accordance with standard safety procedures. The Material Safety Data Sheets (MSDS) describing the chemicals being used are to be freely available to lab personnel for them to examine at any time. Material Safety Data Sheets (MSDS) provide summarized information on the hazard and toxicity of specific chemical compounds. MSDSs also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures for the remedy of spills or leaks. Producers and suppliers of chemical compounds are required by law to provide their customers with the most current health and safety information in the form of an MSDS. Read the material safety data sheets for each chemical you use.

Normal Mass Range Calibration

Before calibrating for the high mass range you must calibrate for the normal mass range. Use the calibration solution (ESI calibration solution) described in [Appendix A, “Sample Formulations.”](#) and the procedures described in [Chapter 3, “Tuning and Calibrating Automatically in the ESI/MS Mode.”](#) to perform the normal mass range calibration. The calibration solution contains caffeine as the low molecular mass component, MRFA as the mid-molecular mass component, and Ultramark 1621 as the high molecular mass component. Four pieces of information from this calibration are needed for the high mass range calibration:

1. The expected low mass (caffeine component=195.10)
2. The expected high mass (1822.00 peak of the Ultramark 1621 component)
3. Observed low mass (observed mass of the caffeine component)
4. Observed high mass (observed mass of the 1822 peak of the Ultramark 1621 component).

B

Enter Normal Mass Range Data into Tune Plus

Enter Normal Mass Range Data into Tune Plus

Enter Nominal Range Data into Tune Plus

1. After carrying out the normal mass range calibration open the Tune Plus window (**Instrument Set Up > LXQ > Start Tune Plus**).
2. In the Tune Plus window, open the Diagnostics dialog box (**Diagnostics > Diagnostics**) shown in [Figure 68](#).

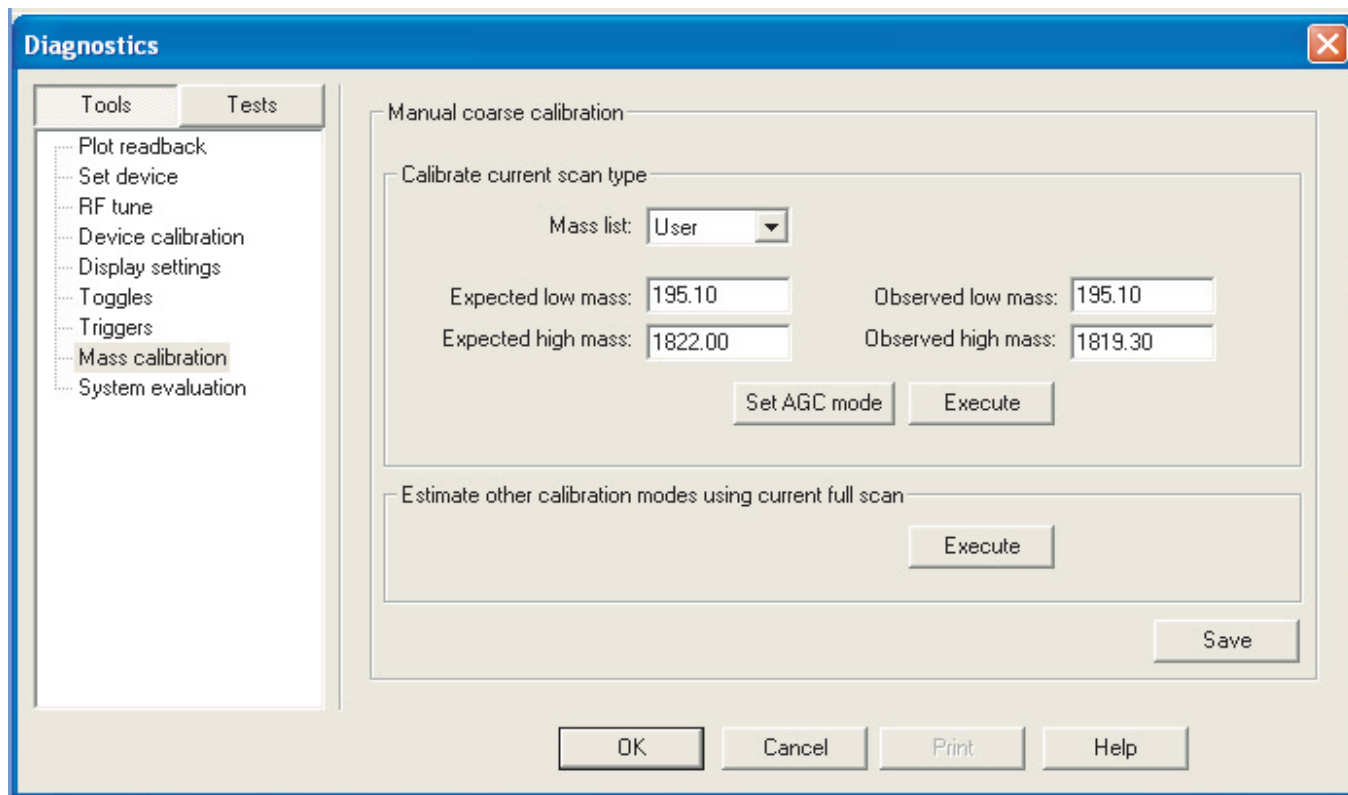


Figure 68. Tune Plus Diagnostics dialog box, normal mass range data

3. Click **Tools** in the upper left of the window. Click on Mass Calibration in the pane below the **Tools** button as shown in [Figure 68](#).
4. Items mentioned in [step 5](#) through [step 7](#) below are inside of the Manual Coarse Calibration group box.
5. In the Calibrate Current Scan Type group box, choose User in the Mass list box. In the Expected Low Mass text box type 195.10 and type 1822.00 in the Expected High Mass text box. Type the Observed Low Mass and the Observed High Mass values in their respective text boxes.

These latter values are obtained as discussed in the preceding section: [Normal Mass Range Calibration](#). After entering the observed mass values click the **Execute** button.

6. In the Estimate Other Calibration Modes Using Current Full Scan group box click **Execute**.
7. Click **Save** at the bottom right of the Manual Coarse Calibration window.
8. Click **OK** at the bottom of the Diagnostics dialog box.

Tune on m/z 524.3



Optimize the MS detector Tune on m/z 524.3u

1. On the Control/Scan Mode toolbar, click **Tune** to display the Tune dialog box.
2. If necessary, click the **Automatic** tab to display the Automatic tuning page. See [Figure 69](#).
3. In the What to Optimize On group box, select the Mass option button to make active the Mass spin box.
4. In the Mass spin box, type 524.3 to specify that the Finnigan LXQ MS detector uses the peak at m/z 524.3 to optimize your tune.

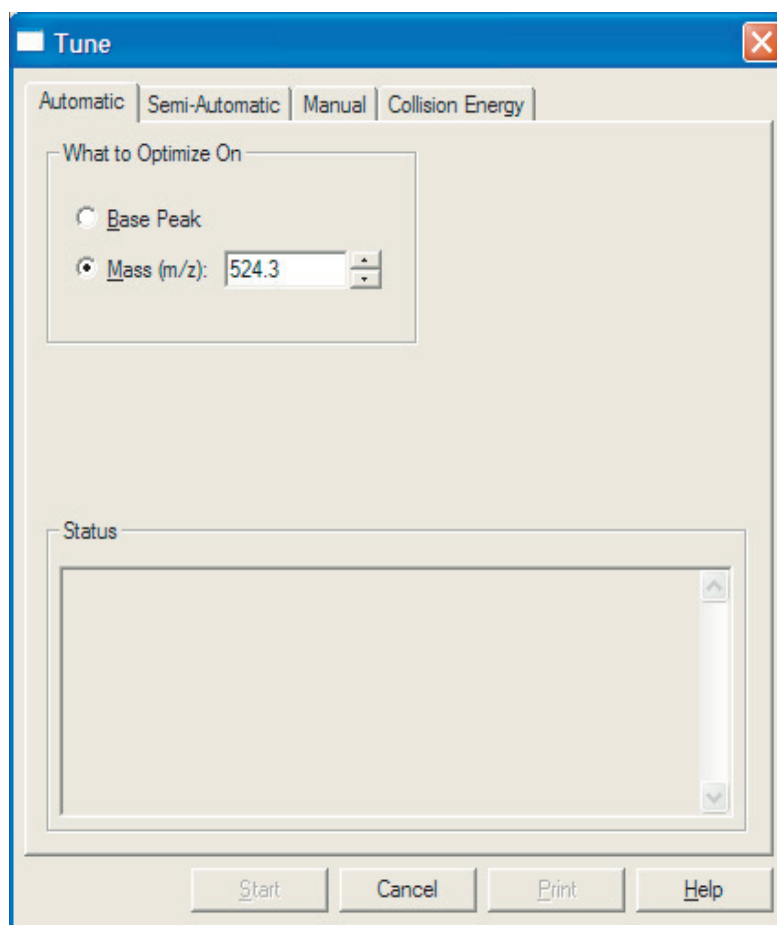


Figure 69. Tune window showing the MS detector Tune on m/z 524.3u

5. Click **Start**. A message box displays the following message:

Please ensure that the 500 microliter syringe is full.

Ensure that the syringe pump contains at least 450 μL of the ESI calibration solution as mentioned in the topic “[Normal Mass Range Calibration](#)” on [page 143](#).

6. Click **OK** to close the message box, and return to the Tune Plus window.



7. In the File/Display toolbar, click **Graph View** to display the Graph view.
8. Observe the Tune Plus window and the Tune dialog box. While automatic tuning is in progress, the Finnigan LXQ MS detector displays various tests in the Spectrum and Graph views in the Tune Plus window and displays various messages in the Status group box in the Tune dialog box.

High Mass Range Calibration Procedure

Calibrate the high mass range automatically in ESI mode

1. Load the syringe with PPG2700 solution.
2. From the Tune page go to Define Scan dialog box (see [Figure 32](#) on [page 74](#)). In the Scan Description group box: a) choose **High** in the Mass Range spin box, b) choose **Normal** in the Scan Rate spin box, and c) choose **Full** in the Scan Type spin box. In the Scan Ranges box, type 2000 for the First Mass and 4000 for the Last Mass. Click **Apply**. The spectrum should look like that shown in [Figure 70](#).
3. Do a two point-mass calibration. Use the Tune Plus Diagnostics dialog box ([Figure 71](#)). Access the Diagnostics dialog box as instructed in “[Enter Normal Mass Range Data into Tune Plus](#)” on [page 144](#). Type the high mass values as shown in [Figure 71](#). Click **Execute**, click **Save** and close the window.
4. In the Tune Plus window, choose **Control>Calibrate** to open the Calibrate dialog box ([Figure 72](#)).

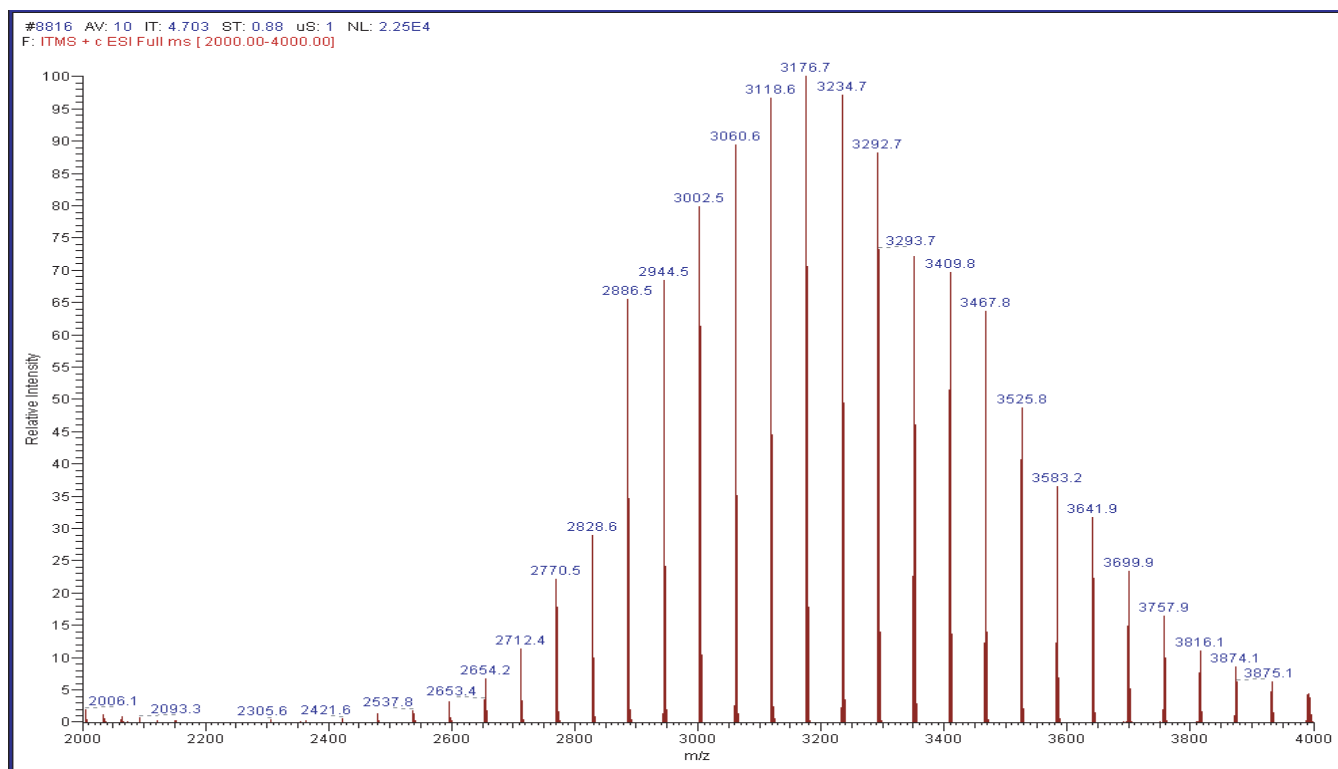


Figure 70. PPG2700 mass spectrum

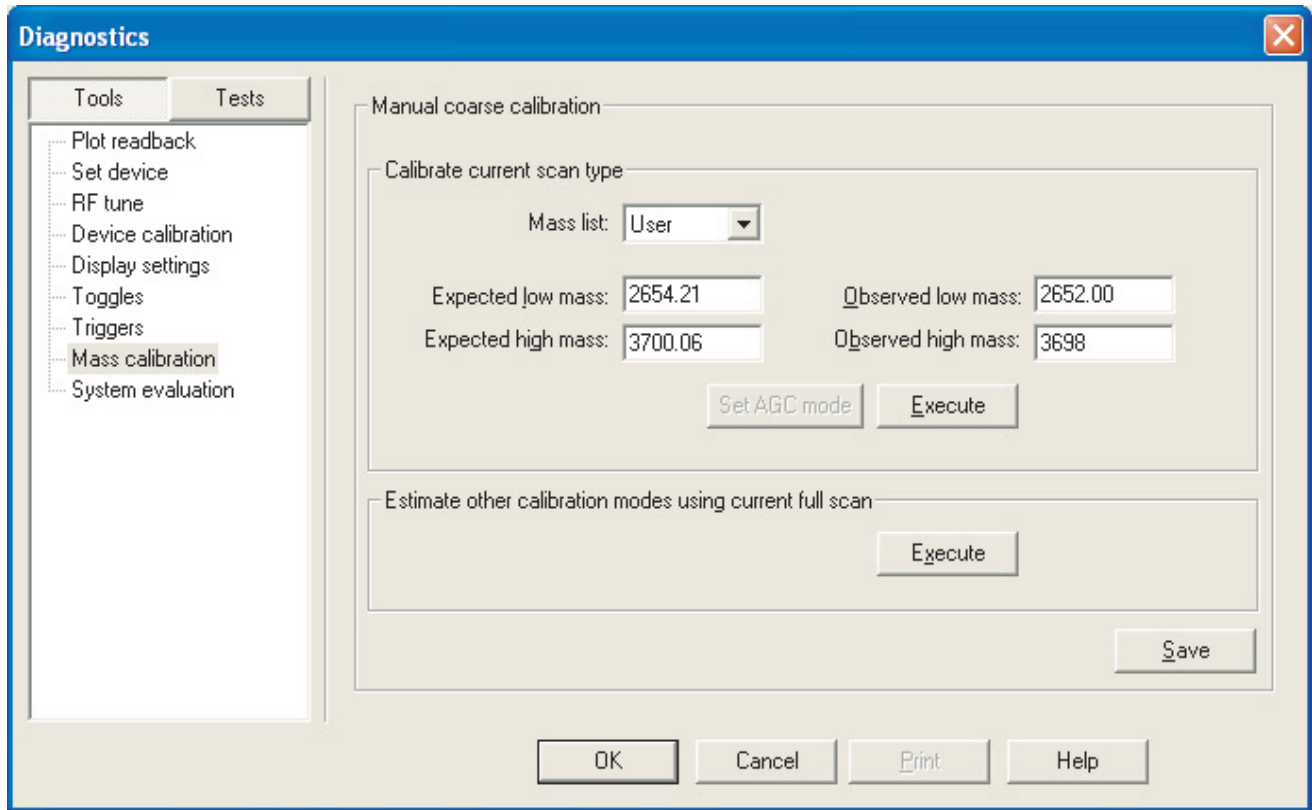


Figure 71. Tune Plus Diagnostics dialog box, two point high mass calibration

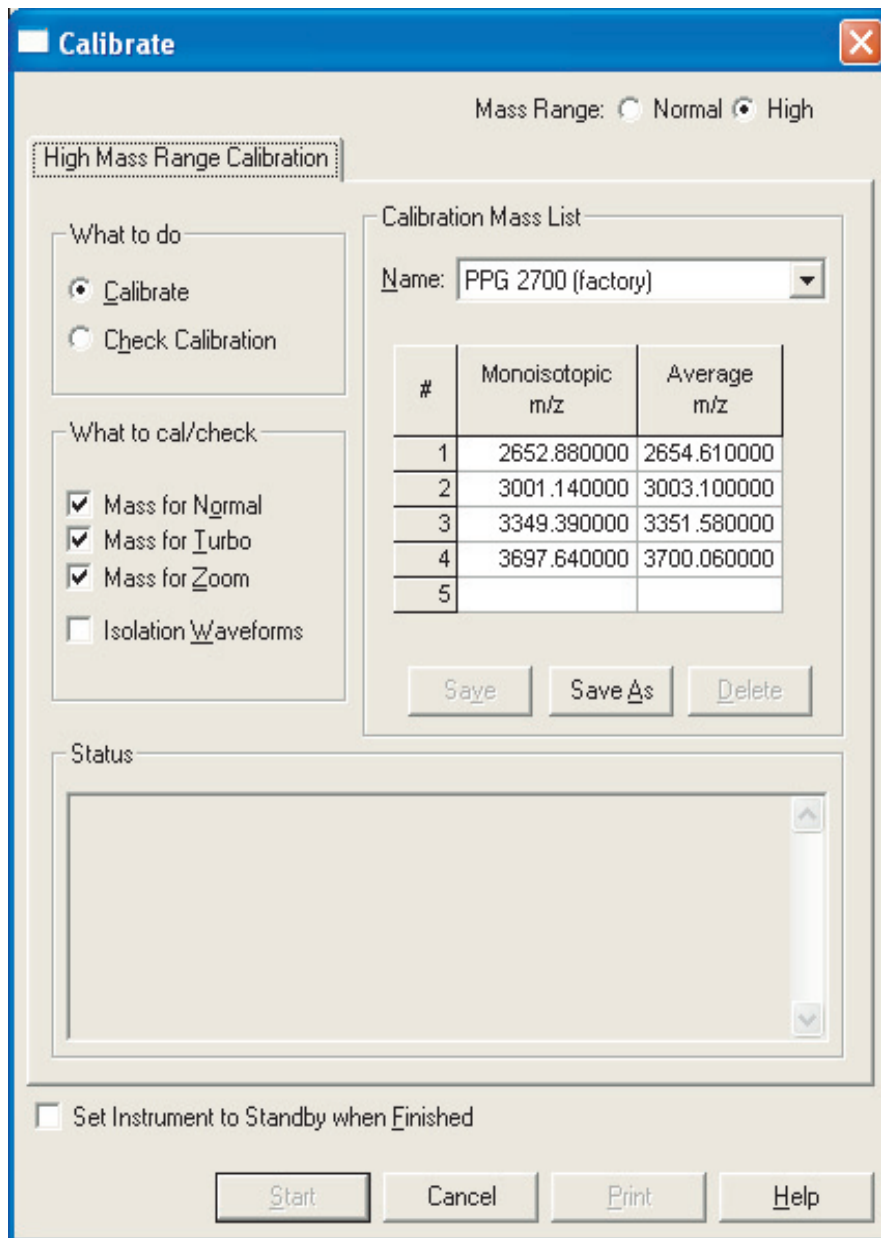


Figure 72. Calibrate dialog box

5. For Mass Range select the High button (upper right of the Calibrate dialog box). The High Mass Range Calibration tabbed page will appear (as shown in [Figure 72](#)).
6. In the What To Do group box select the Calibrate button. Then enter a calibration mass list name in the Name list box inside the Calibration Mass List group box.

- If you want to use a previously saved mass list Name in the Calibration Mass List group box go to [step 7](#).
 - If you want to use a new mass list Name in the Calibration Mass List group box go to [step 8](#).
7. To use a previously saved calibration mass list Name in the Calibration Mass List group box,
 - a. Select from the name of a previously saved calibration mass list the Name box. The calibration masses saved in the selected list appear in the table below the Name list box (in the Monoisotopic m/z and the Average m/z columns). The example in [Figure 72](#) shows the calibration masses saved for the calibration mass list: PPG 2700 (factory).
 - b. Go to [step 9](#).
 8. To assign a new calibration mass list name,
 - a. You can use the factory default PPG2700 name that is in the Name list box or you may assign a new name for this mass list.
 - b. To assign a new name click **Save As**. The Unique Mass List Name dialog box appears.
 - c. Type the new name in the Unique Mass List Name dialog box and click **OK** to accept the name. The dialog box closes and the new name appears in the calibration mass list Name list box.
 - d. Do not change the masses in the Calibration Mass List table.
 9. In the 'What To Cal/Check' area, click the Mass for Normal, Mass for Turbo, and the Mass for Zoom check boxes as shown in [Figure 72](#).
 10. Specify the instrument state when the calibration is completed. Use the 'Set Instrument to Standby When Finished' check box at the bottom left of the Calibrate dialog box.
 - If you want the LXQ to switch to Standby mode after the calibration, click the check box so that a check mark is present in the box.
 - If you want the LXQ to remain in the On mode after the calibration, clear the check box.
 11. Start the calibration procedure.

- a. At the bottom of the Calibrate dialog box, click **Start**. A message box appears with the message: Please ensure that the 500 microliter syringe is full.
 - b. Verify that the syringe contains at least 420 μ L of the high mass range calibration solution.
 - c. Click **OK** to close the message box and return to the Calibrate dialog box. Start the calibration.
12. Observe the Tune Plus window and the Calibrate dialog box. While the automatic calibration is in progress the Spectrum and Graph views display a variety of test results and the Status pane in the Calibrate dialog box displays status messages. The system will automatically use the average mass values for the Normal and Turbo Scan calibrations. Monoisotopic masses will automatically be used for the Zoom Scan calibration.

When all calibration items are completed, your LXQ is properly tuned and calibrated in the high mass range. At the conclusion of the procedure, you must clean the syringe and API source (change the tubing and syringe, clean the API source with acetone to remove the polypropylene glycol).

Notes

1. Two-point mass calibration is available for all high mass range modes accessed through the Estimate Other Calibration Modes Using Current Full Scan group box (in the Diagnostics dialog box, [Figure 68](#)).
2. Use the **Execute** button in the box Estimate Other Calibration Modes Using Current Full Scan to execute estimation for all other calibrations from the normal mass range-normal scan rate mass calibration. The other modes for which the calibrations get estimated include Zoom Scan, Turbo Scan, Enhanced Scan, Isolation Waveform, and High Mass Isolation Waveform calibrations.

This button changes when the High Mass Scan mode is selected. In this case, clicking on the **Execute** button causes the High Mass Isolation to be estimated from the Current Normal Mass Range Isolation calibration (assuming that it has been calibrated). This feature has been added to extrapolate the High Mass Isolation calibration for Initial calibration. As noted above (item #1), a two point mass calibration is available for all other scan modes. This makes it possible to get sufficient convergence of various mass values to allow automatic calibration.

3. In order to calibrate with High Mass Range Zoom Scan it may be necessary to reduce the Zoom Scan target from 3000 u to 1000u.

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