

Orbitrap Fusion

Getting Started Guide

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Release history: Revision A, August 2013

Software version: (Thermo) Foundation 3.0 and later, Xcalibur 3.0 and later, Orbitrap Fusion Tune 1.0 and later

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EMC Directive 2004/108/EC

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EN 55011: 2009, A1: 2010

EN 61000-3-2: 2006, A2: 2009

EN 61000-3-3: 2008

EN 61000-4-2: 2009

EN 61000-4-3: 2006, A2: 2010

EN 61000-4-4: 2004, A1: 2010

EN 61000-4-5: 2006

EN 61000-4-6: 2009

EN 61000-4-11: 2004

EN 61326-1: 2006

CISPR 11: 2009, A1: 2010

ICES-003 Issue 5: 2012

CFR 47, FCC Part 15, Subpart B, Class A: 2012

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Preface

The *Orbitrap Fusion Getting Started Guide* describes how to set up and calibrate the Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer (MS).

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- [Installation Kits](#)
- [Cautions and Special Notices](#)
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❖ To suggest changes to documentation or to Help

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



Related Documentation

The software DVD included with the Orbitrap Fusion mass spectrometer provides complete documentation. In addition to this guide, you can also access the following documents as PDF files from the data system computer:


- *Orbitrap Fusion Preinstallation Requirements Guide*
- *Orbitrap Fusion Getting Connected Guide*
- *Orbitrap Fusion Hardware Manual*
- *Ion Max NG and EASY-Max NG Ion Sources User Guide*
- *EASY-ETD and EASY-IC Ion Sources User Guide* (for instruments with the ETD or Internal Calibration configuration)

- *Safety and Regulatory Guide*

You also receive a printed copy of the *Safety and Regulatory Guide* with your mass spectrometer. This guide contains important safety information about Thermo Scientific LC and MS systems. Make sure that all lab personnel have read and have access to this document.

To access the manuals from the Microsoft™ Windows™ taskbar, do the following:

- For the Thermo Scientific mass spectrometer, choose **Start > All Programs > Thermo Instruments > Thermo Orbitrap Fusion**.
- For an LC instrument controlled by a Thermo software application, choose **Start > All Programs > Thermo Instruments > Manuals > LC Devices** and so on.

The Orbitrap Fusion software also provides Help. To access the Help, click the **Options** icon, , and then choose **Tune Help**.

Installation Kits


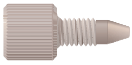
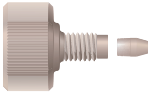
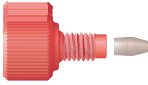
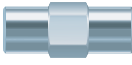


The Orbitrap Fusion mass spectrometer ships with several kits. However, these specific kits provide the necessary components for the procedures in this guide:

- [MS Calibration Kit](#)
- [Performance Specification Kit](#)
- [Orbitrap Fusion Chemicals Kit](#)

For a full list of the Orbitrap Fusion MS kits and their contents, refer to the *Orbitrap Fusion Hardware Manual*.

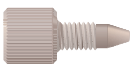


MS Calibration Kit

Table 1. MS Calibration Kit (P/N 80000-62013)

Image	Item	Quantity	Part number
	Ferrule, fingertight, natural PEEK™	2	00101-18196
	Fitting, fingertight, one-piece natural PEEK, 10-32	1	00109-99-00016
	Fitting, fingertight, two-piece natural PEEK, two wings, 10-32	2	00101-18081
	Fitting, fingertight, two-piece, one wing, 10-32	2	00101-18195
	Grounding union, zero-dead-volume (ZDV), stainless steel, 1/16 in. orifice, 0.010 in. (0.25 mm) thru-hole, 10-32	1	00101-18182
	LC union, black PEEK, 10-32, 0.01 in. thru-hole	1	00101-18202
—	Syringe, GC, gas tight, 500 µL, 51 mm long	1	00301-01-00040
—	Tubing, natural PEEK, 1/16 in. OD, 0.0025 in. ID, 28 cm (11 in.) long	2	80000-22032
Note Use this tubing with the calibration solutions and for flow rates less than 50 µL/min.			
—	Tubing, red PEEK, 1/16 in. OD, 0.005 in. ID, 0.6 m (2 ft) long	1	00301-22912
—	Tubing, red PEEK, 1/16 in. OD, 0.005 in. ID, 18 cm (7.1 in.) long	2	80000-22053
Note Use this tubing for flow rates equal to or greater than 50 µL/min.			
—	Tubing, Teflon™ FEP, 1/16 in. OD, 0.03 in. ID, 30.5 mm (0.1 ft) long	1	00301-22915
	Union Tee, PEEK, 1/16 in. orifice, 0.020 in. (0.5 mm) thru-hole, 10-32 (provided with fingertight fittings)	1	00101-18204

Performance Specification Kit

Table 2. Performance Specification Kit (P/N 80100-62008)

Image	Item	Quantity	Part number
—	Column, HPLC, 20 mm × 2.1 mm ID, Hypersil GOLD AQ™ C18, 1.9 µm particles	1	00109-01-00013
	Fitting, fingertight, one-piece natural PEEK, 10-32	6	00109-99-00016
	Needle port, PEEK	1	00110-22030
	Sample loop, 2 µL, PEEK	1	00110-16012
—	Syringe, GC, gas tight, 500 µL, 51 mm long	1	00301-01-00040
—	Tubing, red PEEK, 1/16 in. OD, 0.005 in. ID, 3 m (10 ft) long	1	00301-22912

Orbitrap Fusion Chemicals Kit

IMPORTANT Be aware of the following storage precautions.

- Calibration and reserpine solutions—Refrigerate the containers after opening. For long-term storage, keep refrigerated at 2–8 °C (36–46 °F).
- Enfuvirtide—Refrigerate the container after opening. For long-term storage, keep refrigerated at –25 to –15 °C (–13 to 5 °F).

Table 3. Orbitrap Fusion Chemicals Kit (P/N 80000-62049)

Item	Quantity	Part number
Positive calibration solution (calmix), n-Butylamine, 10 mL (Pierce™ LTQ™ Velos™ ESI Positive Ion Calibration Solution, P/N 88323)	2	HAZMAT-01-00061
Negative calibration solution (calmix), Ultramark 1621, 10 mL (Pierce LTQ ESI Negative Ion Calibration Solution, P/N 88324)	2	HAZMAT-01-00062
Enfuvirtide, 90 mg	1	HAZMAT-01-00083
Reserpine standard solution, 100 pg/µL, 1 mL	5	HAZMAT-01-00081
LCMS Functionality Test Kit (for field service use only)	1	HAZMAT-01-00044

Cautions and Special Notices

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

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



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

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

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

The *Orbitrap Fusion Getting Started Guide* contains the following caution-specific symbols (Table 4).

Table 4. Caution-specific symbols and their meanings (Sheet 1 of 2)






Symbol	Meaning
	Chemical hazard: Wear gloves and other protective equipment, as appropriate, when handling toxic, carcinogenic, mutagenic, corrosive, or irritant chemicals. Use approved containers and proper procedures to dispose of waste oil and when handling wetted parts of the instrument.
	Hot surface: Before touching the API source assembly, allow heated components to cool.
	Risk of electric shock: This instrument uses voltages that can cause electric shock and/or personal injury. Before servicing, shut down the instrument and disconnect it from line power. While operating the instrument, keep covers on.

Table 4. Caution-specific symbols and their meanings (Sheet 2 of 2)

Symbol	Meaning
	Risk of eye injury: Eye injury could occur from splattered chemicals or airborne particles. Wear safety glasses when handling chemicals or servicing the instrument.
	Sharp object: Avoid physical contact with the tip of the syringe needle.

Contacting Us

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

❖ To contact Technical Support

Phone	800-532-4752
Fax	561-688-8736
E-mail	us.techsupport.analyze@thermofisher.com
Knowledge base	www.thermokb.com

Find software updates and utilities to download at mssupport.thermo.com.

❖ To contact Customer Service for ordering information

Phone	800-532-4752
Fax	561-688-8731
E-mail	us.customer-support.analyze@thermofisher.com
Web site	www.thermo.com/ms

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❖ To copy manuals from the Internet

- Visit the Thermo Scientific Web site dedicated to Orbitrap™ systems:
PlanetOrbitrap.com
- Go to mssupport.thermo.com, agree to the Terms and Conditions, and then click **Customer Manuals** in the left margin of the window.

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

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

Introduction

This chapter provides general information about the Orbitrap Fusion Tribrid mass spectrometer. For information about using the Thermo Tune application, see [Appendix A, “Basic Tune Functions.”](#) For information about daily operation, maintenance, and system startup and shutdown, refer to the *Orbitrap Fusion Hardware Manual*.

Note

- The “Glossary” on [page 83](#) defines some of the terms used in this guide.
- To ensure the proper operation of the mass spectrometer, Thermo Fisher Scientific recommends that you perform the daily preventive maintenance described in the *Orbitrap Fusion Hardware Manual*.

Contents

- [Ionization Techniques](#)
- [LC Flow Rate Ranges](#)
- [Types of Buffers](#)
- [Templates in Thermo Xcalibur Instrument Setup \(Method Editor\)](#)

Ionization Techniques

Typically, [heated-electrospray \(H-ESI\)](#) is the preferred ionization mode for polar compounds, [atmospheric pressure chemical ionization \(APCI\)](#) is the preferred ionization mode for non-polar compounds, and [nanoelectrospray ionization \(nanoESI or NSI\)](#) is typically the preferred ionization mode for peptides and proteins.

- [Using H-ESI](#)
- [Using APCI](#)
- [Using NSI](#)

Using H-ESI

H-ESI is a soft gas phase ionization technique. The H-ESI source transfers ions in solution to the gas phase. H-ESI can analyze many samples that previously were not suitable for mass analysis (for example, heat-labile compounds or high molecular mass compounds). You can use H-ESI to analyze any polar compound that is an ion in solution, including adduct ions. Included in this class of compounds are biological polymers (such as proteins, peptides, glycoproteins, and nucleotides), pharmaceuticals and their metabolites, and industrial polymers. For example, you might analyze polyethylene glycols from a solution containing ammonium acetate because of adduct formation between NH_4^+ ions in the solution and oxygen atoms in the polymer. With H-ESI, the range of molecular masses that the Orbitrap Fusion mass spectrometer can analyze can exceed 50 000 Da if there is multiple charging.

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

Use H-ESI in either positive or negative ion polarity mode. The polarity of the ions in solution determines the ion polarity mode: acidic molecules form negative ions in high pH solution and basic molecules form positive ions in low pH solution. The installed ESI spray insert can be either positively or negatively charged. When it is positively charged, it generates positive ions. When it is negatively charged, it generates negative ions.

Vary the flow rate into the mass spectrometer over a range of 1–1000 $\mu\text{L}/\text{min}$. See [Table 5](#) on [page 5](#) for guidelines.

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

Large droplets with high surface tension, low volatility, low surface charge, strong ion solvation, and high conductivity negatively affect the H-ESI process. Conversely, H-ESI favors 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 superior to water alone for H-ESI. Volatile acids and bases are good, but for best results do not use salts above 10 mM. Be aware that strong mineral acids and bases are extremely detrimental to the instrument.

IMPORTANT To obtain good H-ESI results, follow these guidelines:

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

Using APCI

Like H-ESI, APCI is a soft gas phase ionization technique. Therefore, the gas phase acidities and basicities of the analyte and solvent vapor play an important role in the APCI process. APCI provides molecular mass information for compounds of medium polarity that have some volatility. APCI is typically used to analyze small molecules with molecular masses up to about 1000 Da.

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

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

The rate of solvent flowing from the LC into the mass spectrometer in APCI mode is typically high (200–2000 $\mu\text{L}/\text{min}$). See [Table 6 on page 5](#) for guidelines.

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

Using NSI

Conventional electrospray (ESI) employs flow rates from 1 $\mu\text{L}/\text{min}$ to 1 mL/min . Due to the high volume of liquid exiting the emitter, a drying gas, thermal heating, or both are often required to expedite desolvation and droplet shrinkage. NSI (or nanoESI) is a form of ESI that employs low flow rates of 10–1000 nL/min . NSI generally does not require a drying gas or thermal heating. Compared with ESI or H-ESI, NSI tolerates a wider range of liquid compositions including pure water.

As you lower the flow rate, a lower volume of mobile phase passes through the emitter, producing smaller aerosol droplets. This makes NSI more effective than conventional ESI or H-ESI at concentrating the analyte at the emitter tip, producing significant increases in sensitivity demonstrated by the signal response of the mass spectrometer. See [Table 7](#) on [page 5](#) for guidelines.

LC Flow Rate Ranges

The ESI spray insert can volatilize ions from liquid flows¹ of 1–1000 $\mu\text{L}/\text{min}$. This flow rate range provides for a wide range of separation techniques: CE, CEC, analytical LC, capillary LC, and microbore LC.

The APCI spray insert can volatilize ions from liquid flows² of 200–2000 $\mu\text{L}/\text{min}$. This flow range provides for the use of separation techniques: analytical LC, microbore LC, and semi-preparative LC.

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

- For H-ESI mode, adjust the ion transfer tube temperature and the flow rates for the sheath, auxiliary, and sweep gases.
- For APCI mode, adjust the ion transfer tube and vaporizer temperatures, and the flow rates for the sheath, auxiliary, and sweep gases.
- For NSI mode, adjust the ion transfer tube temperature.

The following tables list the guidelines and default values for system operation using H-ESI ([Table 5](#)), APCI ([Table 6](#)), and NSI ([Table 7](#)) for a range of LC solvent flow rates.

¹ The ESI spray insert can generate ions from liquid flows as low as 1 $\mu\text{L}/\text{min}$. However, flows below 5 $\mu\text{L}/\text{min}$ require more care.

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

Table 5. Guidelines for setting operating parameters for LC/**H-ESI**/MS

LC flow rate (μL/min)	Spray voltage (V) ^a	Sheath gas (arb ^b units)	Auxiliary gas (arb units)	Sweep gas (arb units)	Ion transfer tube temperature (°C)	Vaporizer temperature (°C)	Typical nitrogen gas consumption (L/min)
5	Pos: 3500 Neg: -2500	5	5	0	275	50	<1
200	Pos: 3500 Neg: -2500	35	10	0	325	275	8
500	Pos: 3500 Neg: -2500	50	20	2	350	300	13
1000	Pos: 3500 Neg: -2500	60	20	2	380	325	17

^a positive and negative polarity modes

^b arbitrary

Table 6. Guidelines for setting operating parameters for LC/**APCI**/MS

LC flow rate (μL/min)	Sheath gas (arb ^a units)	Auxiliary gas (arb units)	Sweep gas (arb units)	Ion transfer tube temperature (°C)	Vaporizer temperature (°C)	Corona discharge current (μA) ^b
200	25	5	0	250	325	Pos: 4 Neg: -10
1000	45	5	2	275	375	Pos: 4 Neg: -10

^a arbitrary

^b positive and negative polarity modes

Table 7. Guidelines for setting operating parameters for LC/**NSI**/MS

Spray voltage (V)	Sweep gas (arbitrary units)	Ion transfer tube temperature (°C)
Positive mode: 1200	2	275
Negative mode: -600		

Types of Buffers

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

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

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

For a list of recommended solvents, refer to the *Orbitrap Fusion Preinstallation Requirements Guide*.



CAUTION Avoid exposure to potentially harmful materials.

By law, producers and suppliers of chemical compounds are required to provide their customers with the most current health and safety information in the form of Material Safety Data Sheets (MSDSs) or Safety Data Sheets (SDSs). The MSDSs and SDSs must be freely available to lab personnel to examine at any time. These data sheets describe the chemicals and summarize information on the hazard and toxicity of specific chemical compounds. They also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures to remedy spills or leaks.

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

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

- Optimize the spray insert position.
- Install the mass spectrometer's optional ion sweep cone.
- Reduce the concentration of buffers to an absolute minimum.

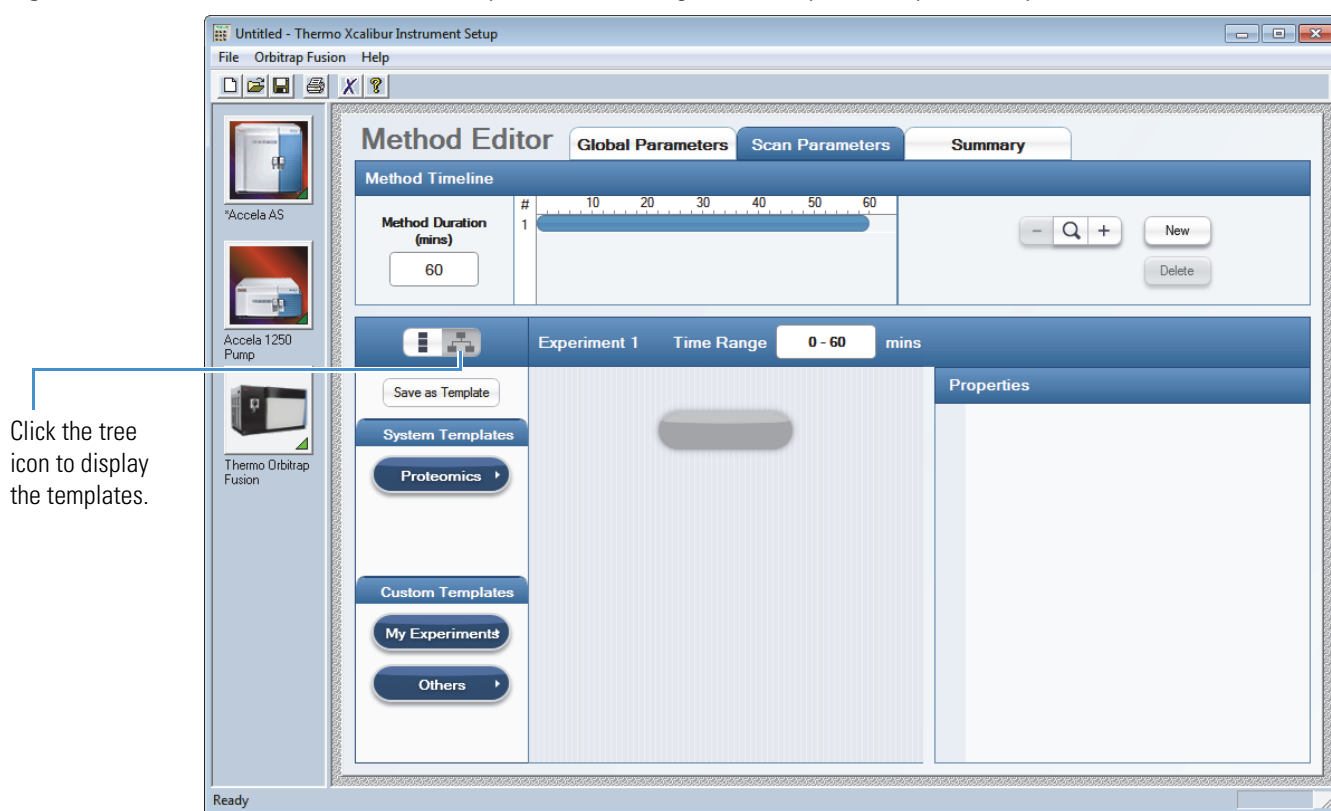
Note You might need to increase the frequency of API source maintenance when you use nonvolatile buffers.

Templates in Thermo Xcalibur Instrument Setup (Method Editor)

Use the Method Editor (Figure 1) that opens in the Thermo Xcalibur™ Instrument Setup window to create the instrument methods for your experiments. To save time entering the parameters for an instrument method, open the system template designed for the experiment type that you want to perform, enter the parameters specific to the experiment, and then save the entries as part of an Xcalibur instrument method (.meth file extension). For additional information, refer to the Xcalibur Instrument Setup Help.

Method Editor provides default system templates for several types of experiments: metabolomics, proteomics, and small molecules.

Figure 1. Thermo Xcalibur Instrument Setup window showing the Orbitrap Fusion system templates



Setting Up the API Source

This chapter provides information about setting up the API source. Use the EASY-Max NG™ API source for H-ESI and APCI experiments. For NSI experiments, use one of the compatible Thermo Scientific nanospray sources.

The EASY-Max NG source ships with the Orbitrap Fusion mass spectrometer and consists of the source housing, a heater assembly, and the ESI spray insert. For APCI experiments, order the APCI Installation Kit (P/N 80000-62060), which includes the APCI spray insert.

Contents

- [Preparing the Mass Spectrometer](#)
- [Installing or Removing the API Source](#)
- [Preparing the Spray Insert for the EASY-Max NG API Source](#)

Preparing the Mass Spectrometer

Before you install the API source, install or remove the ion sweep cone as specified in the following procedure.

IMPORTANT For best results, wear clean gloves before you handle the API source's spray insert or the mass spectrometer's ion sweep cone.

❖ To prepare the mass spectrometer

1. Complete all data acquisition, if any.
2. Open the Tune window (see [page 70](#)).
3. Place the mass spectrometer in **Off** mode (see [page 71](#)).



The LC/MS system is now in off mode. After the API source housing, spray insert, or both have cooled to room temperature, you can safely remove these components.

Note Always place the system in off mode before removing the spray insert or the API source housing. When the system is in off mode, the API gases, high voltage, and syringe pump are off.

4. If you want to change the installed API source, wait until it has cooled to room temperature.

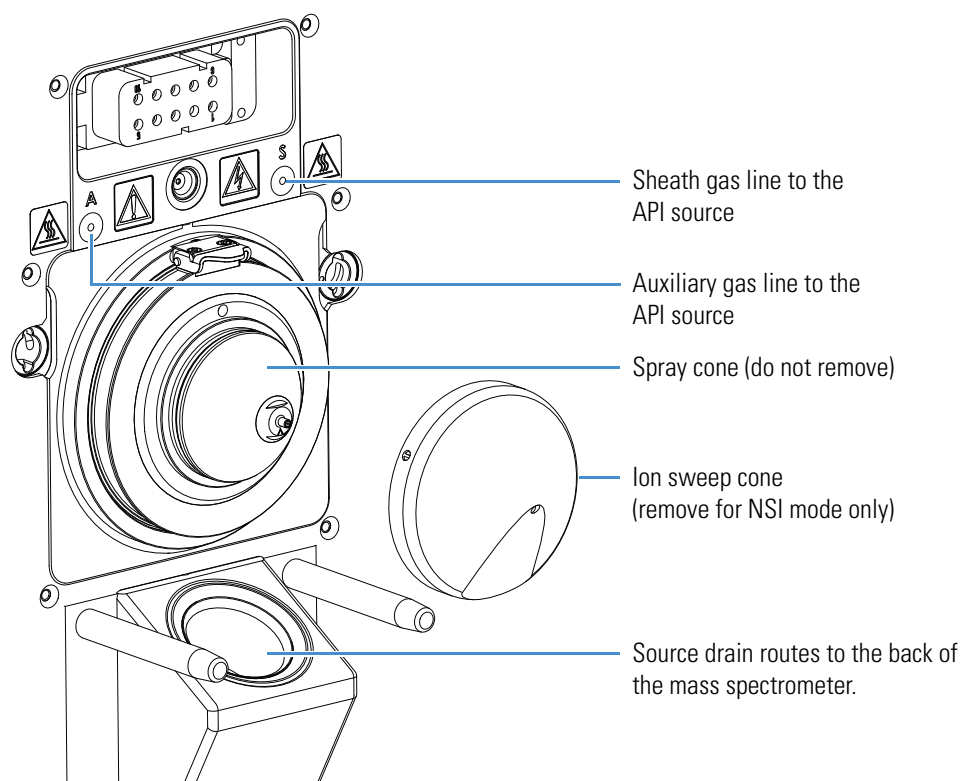
For instructions on how to remove the EASY-Max NG API source, refer to the *Ion Max NG and EASY-Max NG Ion Sources User Guide*.



CAUTION Hot surface. Avoid touching the API source housing when the mass spectrometer is in operation. The external surface of the EASY-Max NG API source housing can become hot enough to burn your skin.

5. Depending on the ionization mode, do the following (Figure 2):
 - For H-ESI or APCI mode, install the ion sweep cone over the mass spectrometer's spray cone (Figure 2).
 - For NSI mode, remove the ion sweep cone from the mass spectrometer by grasping its outer ridges and pulling it off.

Figure 2. MS API source mount assembly and ion sweep cone



Installing or Removing the API Source

The EASY-Max NG API source holds the ESI or APCI spray insert. All the wiring and gas plumbing for this API source are internal. This means you can install or remove the API source or change the ionization mode by changing the spray insert—all without the use of tools.

The Orbitrap Fusion MS internally routes the solvent waste from the bottom of the API source to the back Drain/Waste port.

This section provides the following procedures:

- “[Installing the API Source](#)” on [page 11](#)
- “[Removing the API Source](#)” on [page 14](#)

Installing the API Source

Complete the appropriate procedure:

- [To install the EASY-Max NG API source](#) (instrument calibration, and H-ESI or APCI experiments)
- [To install the NSI source](#), on [page 13](#)



CAUTION

Use these guidelines for the API source drain:

- Use the Tygon™ tubing provided with the solvent waste container to connect the solvent waste container to a fume exhaust system.
- To prevent solvent waste from backing up into the mass spectrometer, make sure that all Tygon tubing is above the level of liquid in the waste container as follows:
 - From the mass spectrometer to the solvent waste container
 - From the waste container to the exhaust system

Equip your lab with at least two fume exhaust systems:

- The analyzer optics become contaminated if the drain/waste tubing and the exhaust tubing from the forepump connect to the same fume exhaust system. Route the exhaust tubing from the forepump to a dedicated fume exhaust system.
- Do not vent the Tygon drain tube (or any vent tubing connected to the waste container) to the same fume exhaust system that connects to the forepump. Vent the waste container to a dedicated fume exhaust system. The exhaust system for the API source must accommodate a flow rate of up to 30 L/min (64 ft³/h).

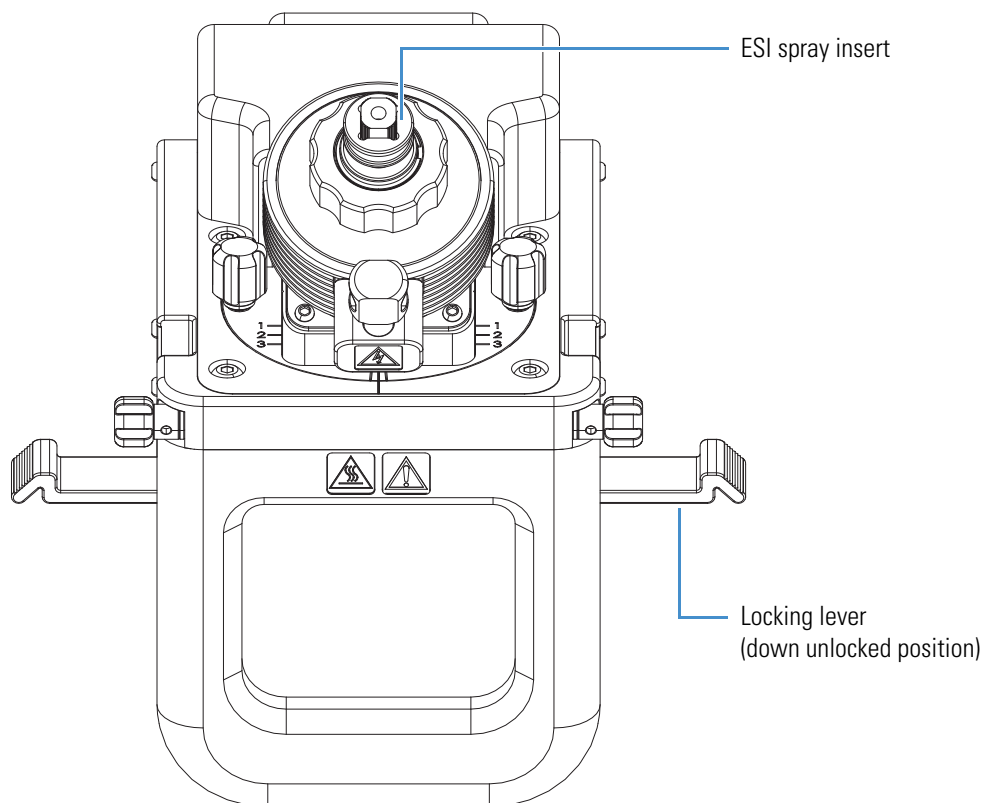
❖ **To install the EASY-Max NG API source**

1. Follow the procedure “To prepare the mass spectrometer” on page 9.
2. For APCI mode, check that the corona discharge needle assembly is installed in the API source housing.

For instructions, refer to the *Ion Max NG and EASY-Max NG Ion Sources User Guide*.

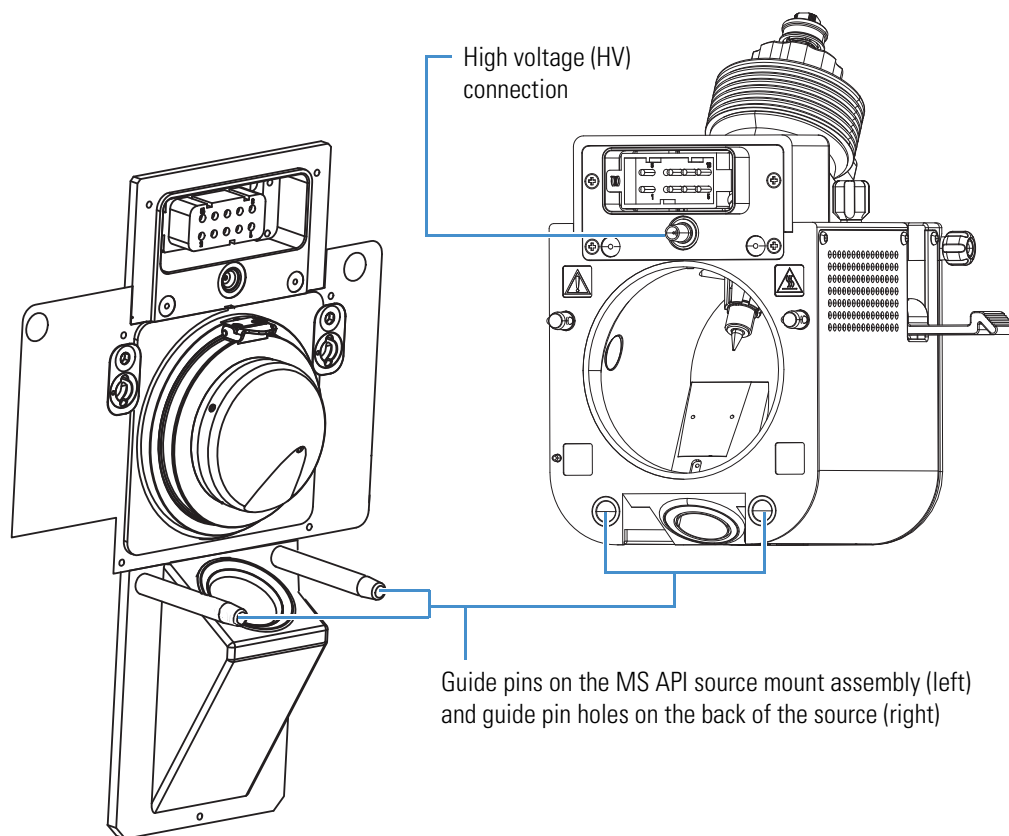
3. Unlock the source’s locking levers (down position, [Figure 3](#)).

Figure 3. EASY-Max NG API source with ESI spray insert (top, front view)



4. Align the two guide pin holes on the back of the source with the guide pins on the front of the mass spectrometer (Figure 4), and then carefully press the source onto the mass spectrometer.

Figure 4. API source connection to the MS mount assembly (installed ion sweep cone)



5. Lock the source's locking levers (up position).
6. To switch between H-ESI and APCI modes, refer to the *Ion Max NG and EASY-Max NG Ion Sources User Guide*.
7. Verify that the solvent waste system connects to the back Drain/Waste port.

During the initial installation of the mass spectrometer, a Thermo Fisher Scientific field service engineer installs the solvent waste system. For instructions, refer to the *Orbitrap Fusion Getting Connected Guide*.

❖ **To install the NSI source**

1. Follow the procedure “[To prepare the mass spectrometer](#)” on [page 9](#).
2. For additional instructions, refer to the NSI source manual.

Removing the API Source

To access the ion sweep cone, API source interface, ion transfer tube, or internal APCI corona needle (APCI source housing only), you must remove the API source from the mass spectrometer.

❖ To remove the API source

1. Complete all data acquisition, if any.
2. Turn off the liquid flow from the LC (or other sample introduction device) to the API source.
3. In the Tune window, place the mass spectrometer in **Off** mode (see [page 71](#)).



CAUTION Hot surface. The maximum safety limit for heated surfaces is 70 °C (158 °F). Although the source falls below this maximum, it can still severely burn you. Allow the source to cool to room temperature (approximately 20 minutes) before you touch it.

4. Disconnect the sample line from the grounding union or spray insert, as applicable.
5. Unlock the source's locking levers.
For the EASY-Max NG API source, see [Figure 3](#) on [page 12](#).
6. Pull the source straight off of the mass spectrometer.
7. Place the source in a safe location for temporary storage.

Preparing the Spray Insert for the EASY-Max NG API Source

For detailed instructions, refer to the *Ion Max NG and EASY-Max NG Ion Sources User Guide*.

- [Installing the Spray Insert](#)
- [Adjusting the Spray Direction](#)

Installing the Spray Insert

For H-ESI mode, install the ESI spray insert and turn on the source heater. For APCI mode, install the APCI spray insert.

❖ To install the spray insert

1. Follow the procedure “[Installing the API Source](#)” on [page 11](#).
2. To switch between H-ESI and APCI modes, refer to the *Ion Max NG and EASY-Max NG Ion Sources User Guide*.

Adjusting the Spray Direction

To maximize sensitivity or robustness, you can adjust the spray direction by a few millimeters. Typically, you adjust the spray direction while optimizing the API source parameters for the analytes. Use the adjustment guidelines listed in [Table 8](#).

Note The depth and angle of the spray insert and heater assembly are not adjustable.

Table 8. Guidelines for adjusting the heater position

Adjustment control	Description
Rotational position	
Center	Use the center mark to position the spray closest to the entrance of the mass spectrometer.
Front-to-back position	
1	For H-ESI mode, use position 1 for calibrating the mass spectrometer and for low liquid flow rates (less than 50 $\mu\text{L}/\text{min}$). In position 1, the spray is closest to the entrance of the mass spectrometer.
2	Use position 2 (default) for liquid flow rates greater than 50 $\mu\text{L}/\text{min}$.
3	Use position 3 for enhanced robustness, for example, when you use a biological matrix. In position 3, the spray is farthest from the entrance of the mass spectrometer.

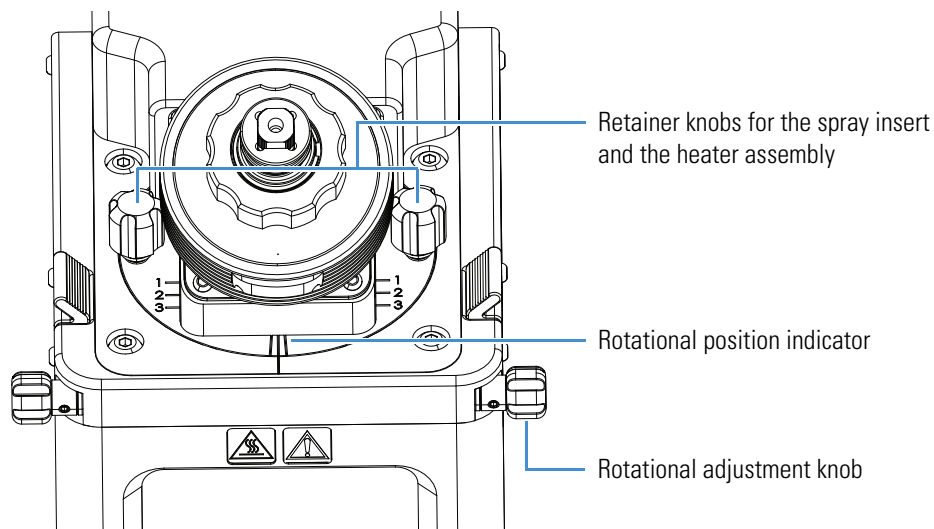
2 Setting Up the API Source

Preparing the Spray Insert for the EASY-Max NG API Source

❖ To adjust the spray direction

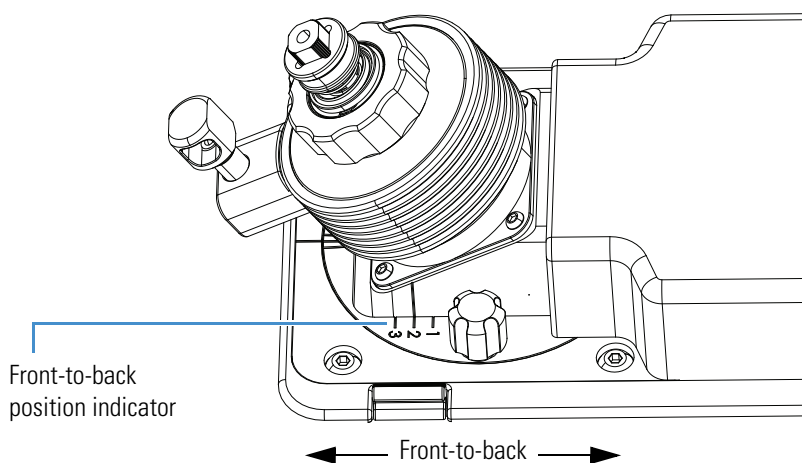
1. Use your fingers to turn the top two retainer knobs counterclockwise.
2. Turn the side rotational adjustment knobs (Figure 5) to achieve the desired position.

Figure 5. Rotational adjustment



3. Grasp the heater assembly, move it front-to-back (Figure 6) to achieve the desired position.

Figure 6. Front-to-back adjustment



4. Use your fingers to tighten the two retainer knobs that secure the heater assembly.

Inlet Plumbing Connections

This chapter provides information about the sample introduction techniques and how to set up the inlet plumbing for these techniques. For operational information about the syringe pump and divert/inject valve, see [Chapter 4, “Syringe Pump and Divert/Inject Valve.”](#)

The MS Calibration Kit (see [Table 1 on page xv](#)) and the Performance Specification Kit (see [Table 2 on page xvi](#)) contain the required components for the inlet plumbing connections.

Contents

- [Sample Introduction Techniques](#)
- [Plumbing Connections](#)
- [Setting Up the Syringe Pump](#)
- [Setting Up the Inlet Plumbing](#)
- [Setting Up the Inlet for an LC/MS System with an Autosampler](#)
- [Connecting the Grounding Union to the ESI Spray Insert](#)

Sample Introduction Techniques

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

- [Direct Infusion](#)
- [High-Flow Infusion](#)
- [Loop Injection \(Flow-Injection Analysis\)](#)
- [High-Performance Liquid Chromatography \(HPLC\) with an Autosampler](#)

[Figure 7 on page 20](#) shows schematic drawings of these sample introduction techniques.

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

Direct Infusion

The direct infusion technique uses the syringe pump to infuse sample directly into the API source. Use this technique to introduce the calibration solution for calibrating in H-ESI mode. You can also use this technique to introduce a solution of pure analyte at a steady rate in H-ESI mode for qualitative analyses, and perform experiments at a low flow rate with the syringe pump.

For plumbing instructions, see [“Setting Up the Inlet for Direct Infusion”](#) on page 23.

High-Flow Infusion

The high-flow infusion technique uses an LC union Tee to direct the solvent flow from the syringe pump into the solvent flow produced by an [LC pump](#). The combined solvent flow goes through the divert/inject valve into the API source. Use this infusion method to perform experiments at a higher flow rate with an LC system. The high-flow infusion method puts a comparatively large amount of solvent into the mass spectrometer, which means you might need to clean the ion [spray cone](#) more frequently.

When the divert/inject valve is in the Load position, solvent flow from the LC pump enters the valve through port 6 and exits the valve through port 5, which connects to the API source. When the divert/inject valve is in the Inject position, solvent flow from the LC pump enters the valve through port 6 and exits the valve through port 1 to waste.

For plumbing instructions, see [“Setting Up the Inlet for High-Flow Infusion”](#) on page 24. For information about the valve configurations, see [“Configurations”](#) on page 35.

Loop Injection (Flow-Injection Analysis)

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

Additionally, follow these guidelines:

- Use a manual loop injection without chromatographic separation for qualitative or quantitative analysis when there is a limited amount of a pure sample.
- Use a manual loop injection with chromatographic separation for qualitative or quantitative analysis when there is a limited amount of a sample mixture. Requires an LC column between the injection valve and the API source.
- Use an automatic loop injection to optimize the mass spectrometer's sensitivity to a compound for an MS/MS experiment.

For plumbing instructions, see [“Setting Up the Inlet for Manual or Auto-Loop Injections”](#) on [page 28](#).

High-Performance Liquid Chromatography (HPLC) with an Autosampler

To perform loop injection by using the liquid chromatography (LC) technique in either H-ESI or APCI mode, install an LC column between the sample inlet of the API source and port 6 of the divert/inject valve, or connect an LC system with an [auxiliary gas](#) to the Orbitrap Fusion mass spectrometer.

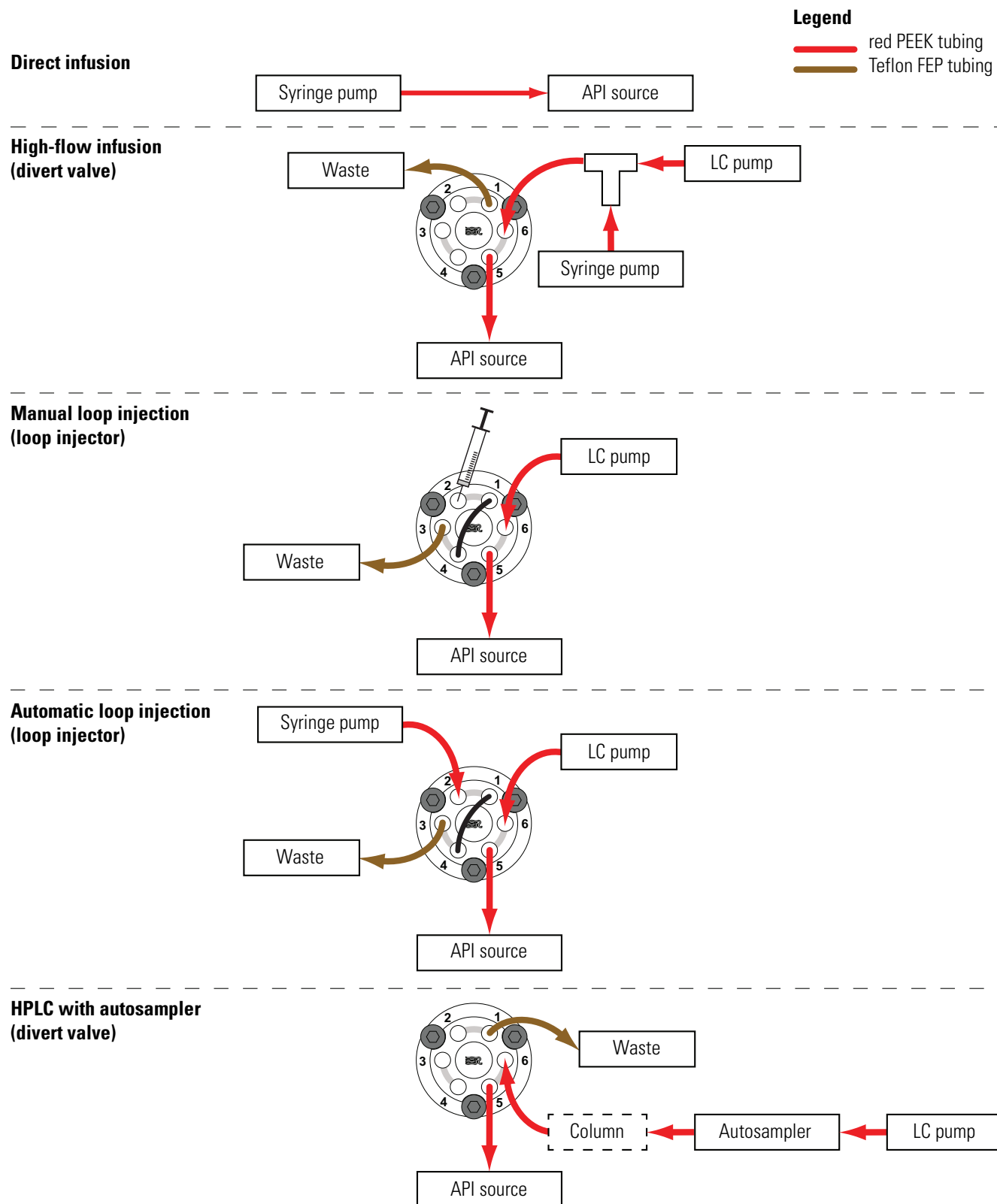
To automatically inject a set of samples, connect an LC system with an autosampler to the divert/inject valve and connect the divert/inject valve to the API source. Use the autosampler to inject sample solution into the flow from an LC pump. In a typical LC/MS experiment, direct the solvent flow through an LC column to separate the compounds of a mixture before they are directed into the API source.

For plumbing instructions, see [“Setting Up the Inlet for an LC/MS System with an Autosampler”](#) on [page 30](#).

3 Inlet Plumbing Connections

Sample Introduction Techniques

Figure 7. Schematics of the sample introduction techniques



Plumbing Connections

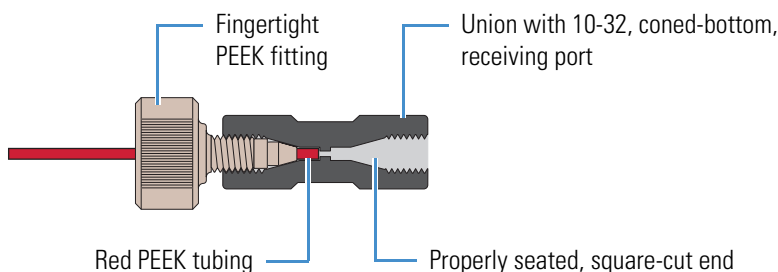
The modular divert/inject valve shipped with your order is a six-port, two-position, Rheodyne™ injection valve. The ports use standard 10-32 fittings for high-pressure and 1/16 in. OD tubing. To connect the high-pressure tubing to the valve, use the one-piece fingertight fittings provided in the MS Calibration Kit (see [Table 1](#) on [page xv](#)).

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

Ensure the following when you make the plumbing connections:

- The ends of the PEEK tubing are squarely cut ([Figure 8](#)). For best results, use a polymeric tubing cutter to ensure square cuts. Poorly cut tubing can cause flow restrictions.
- The PEEK tubing makes contact with the bottom of the receiving port ([Figure 8](#)). Tubing that is not properly seated can add dead volume to a chromatographic system.
- The fittings are not overtightened. Tighten the PEEK fittings by using your fingers only not a wrench. Overtightening the PEEK fittings can cause leaks.

Figure 8. Proper connection for the PEEK tubing and fitting (syringe adapter assembly)



Setting Up the Syringe Pump

Use the syringe pump to directly infuse sample into the API source, to infuse sample into the solvent stream that is produced by an LC pump, or to automatically load sample into the divert/inject valve.

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

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

❖ **To set up the syringe for infusion or high-flow infusion experiments**

1. Load a clean, 500 μ L syringe with the sample solution.

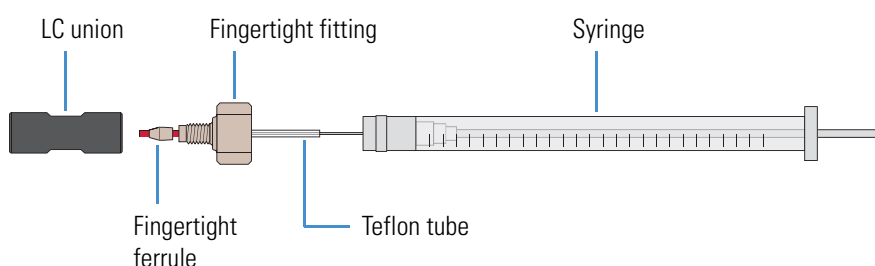


CAUTION Sharp object. The syringe needle can puncture your skin. Handle it with care.

2. Using one of the two-piece, fingertight fittings, connect a 4 cm (1.5 in.) length of Teflon tubing to the (black) LC union (Figure 9).

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

Figure 9. Plumbing connection for the syringe



3. Hold the plunger of the syringe in place and carefully insert the tip of the syringe needle into the free end of the tubing.

Note If necessary, use the syringe needle tip to enlarge the opening slightly in the end of the tubing.

4. Place the syringe into the syringe holder of the syringe pump.
5. Squeeze the release button on the syringe pump's pusher block and slowly move the pusher block until it contacts the syringe plunger.

Setting Up the Inlet Plumbing

This section describes how to set up the inlet plumbing for the following techniques:

- [Setting Up the Inlet for Direct Infusion](#)
- [Setting Up the Inlet for High-Flow Infusion](#)
- [Setting Up the Inlet for Manual or Auto-Loop Injections](#)

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

Setting Up the Inlet for Direct Infusion

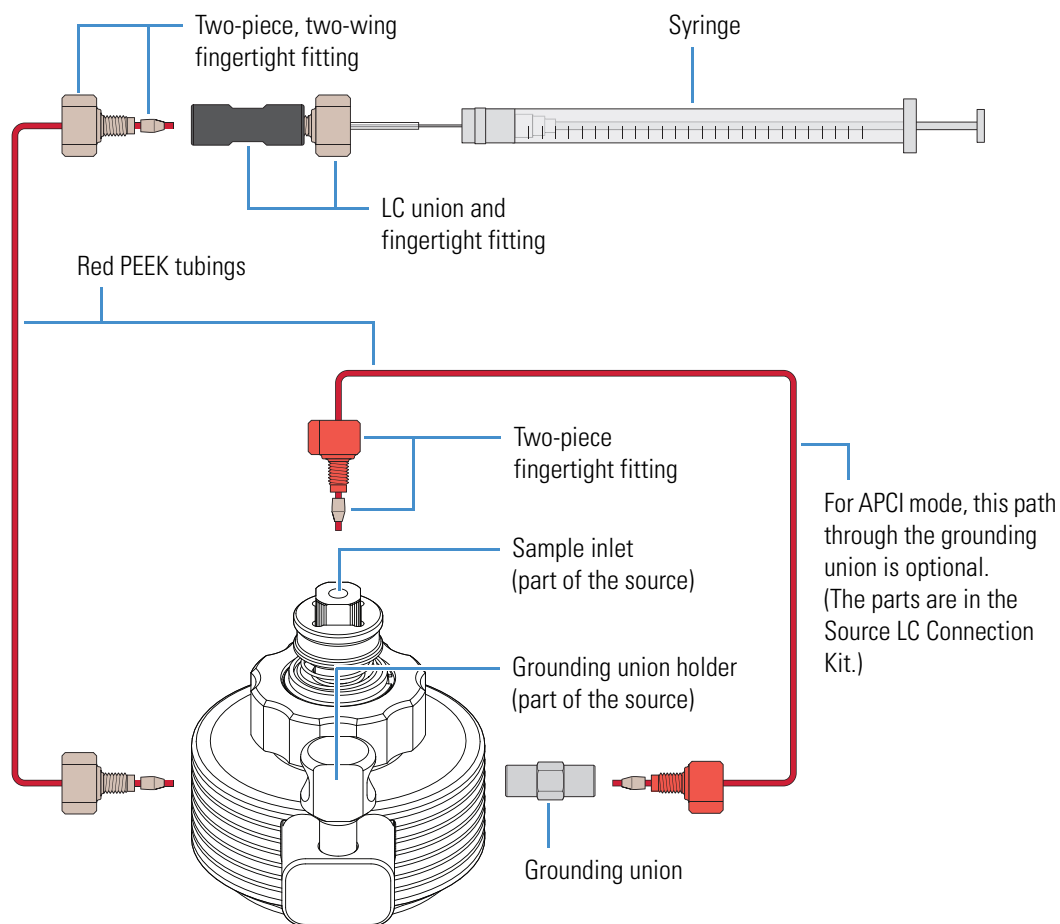
Figure 10 shows the inlet plumbing connections to introduce sample into the API source by using direct infusion. For instrument calibration, remember to use the natural PEEK tubing.

❖ To connect an infusion line between the LC union and the grounding union

1. Set up the syringe pump (see “Setting Up the Syringe Pump” on page 21).
2. Use red PEEK tubing (infusion line) to connect the LC union to the grounding union as follows (Figure 10):
 - Using a two-piece fingertight fitting, connect one end of the tubing to the free end of the LC union that connects to the syringe.
 - Using a two-piece fingertight fitting, connect the other end to the grounding union.
3. Follow the procedure in “Connecting the Grounding Union to the ESI Spray Insert” on page 31.

This completes the inlet setup for the direct infusion technique.

Figure 10. Plumbing connections for direct infusion (H-ESI mode)



Setting Up the Inlet for High-Flow Infusion

Table 9 lists the plumbing connections required to set up the system for a high-flow infusion experiment. (You can make the connections in any order.)

Table 9. Connections for high-flow infusion

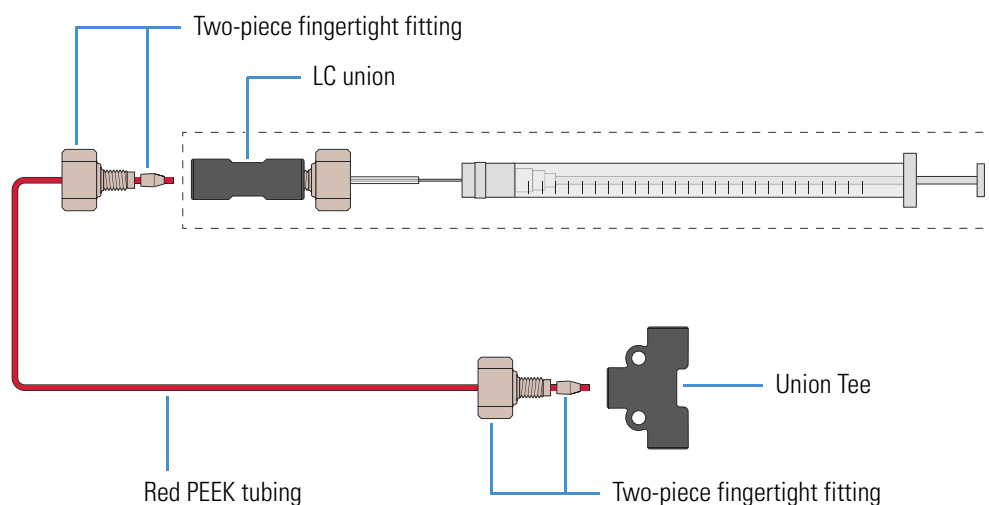
Connection	What to connect	Reference
1	Connect the syringe to the union Tee.	“Connecting the Syringe to the Union Tee” on page 25
2	Connect the LC pump to the union Tee.	“Connecting the LC pump to the Union Tee” on page 26
3	Connect the union Tee to the divert/inject valve.	“Connecting the Union Tee to the Divert/Inject Valve” on page 26
4	Connect port 1 of the divert/inject valve to a waste container.	“Connecting the Divert/Inject Valve to a Waste Container” on page 26
5	For H-ESI mode, connect the union Tee to the grounding union. For APCI mode, connect the union Tee directly to the sample inlet.	“Connecting the Union Tee to the API Source” on page 27
6	For H-ESI mode, connect the grounding union to the sample inlet of the ESI spray insert.	“Connecting the Grounding Union to the ESI Spray Insert” on page 31

Connecting the Syringe to the Union Tee

❖ To connect the syringe to the union Tee

1. Set up the syringe pump (see “[Setting Up the Syringe Pump](#)” on [page 21](#)).
2. Use red PEEK tubing (infusion line) to connect the LC union to the union Tee as follows ([Figure 11](#)):
 - Using a two-piece fingertight fitting, connect one end of the tubing to the free end of the LC union that connects to the syringe.
 - Using a two-piece fingertight fitting, connect the other end to the union Tee.

Figure 11. Plumbing connection between the LC union and the union Tee

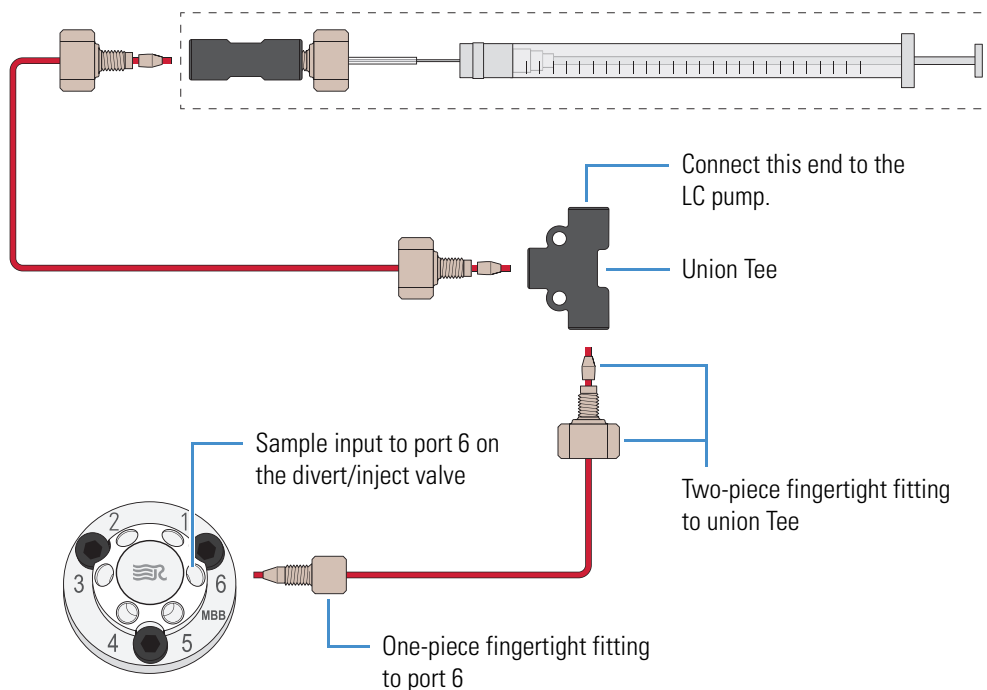


Connecting the LC pump to the Union Tee

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

- Using an appropriate fitting, connect a length of red PEEK tubing to the outlet of the LC pump.
- Using a two-piece fingertight fitting, connect the other end of the tubing to the union Tee (Figure 12).

Figure 12. Plumbing connection between the union Tee and the divert/inject valve



Connecting the Union Tee to the Divert/Inject Valve

❖ To connect the union Tee to the divert/inject valve

- Using a one-piece fingertight fitting, connect a length of red PEEK tubing to the union Tee (Figure 12).
- Using a one-piece fingertight fitting, connect the other end of the tubing to port 6 of the divert/inject valve.

Connecting the Divert/Inject Valve to a Waste Container

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

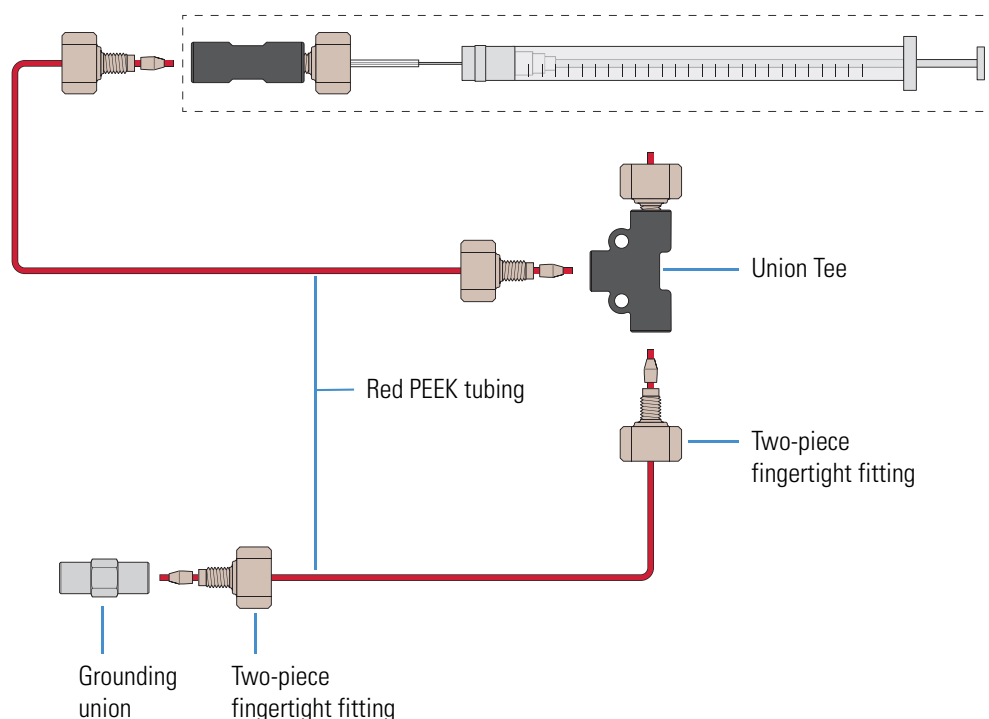
- Using a one-piece fingertight fitting, connect a length of the Teflon tubing to port 1 of the divert/inject valve.
- Insert the other end of the tubing into a suitable waste container.

Connecting the Union Tee to the API Source

❖ To connect the union Tee to the API source

1. Using a fingertight fitting and a ferrule, connect a length of red PEEK tubing to the union Tee (Figure 13).

Figure 13. Plumbing connection between the union Tee and the grounding union



2. Do one of the following to connect the other end of the tubing:
 - For H-ESI mode, use a two-piece fingertight fitting to connect the other end of the tubing to the grounding union (Figure 10 on page 23).

For instructions on how to connect the other end of the grounding union, see “Connecting the Grounding Union to the ESI Spray Insert” on page 31.

- For APCI mode, use a two-piece fingertight fitting to connect the other end of the tubing directly to the sample inlet of the APCI spray insert.

Note The plumbing path through the grounding union of the API source is optional for APCI mode. A knurled nut secures the grounding bar to the API source housing. You do not need to remove the grounding bar if you choose not to use that plumbing path in the APCI mode.

This completes the inlet setup for the high-flow infusion technique.

Setting Up the Inlet for Manual or Auto-Loop Injections

This section describes how to connect the inlet plumbing to introduce sample into the API source by using manual or auto-loop injection.

❖ To set up the inlet for loop injections

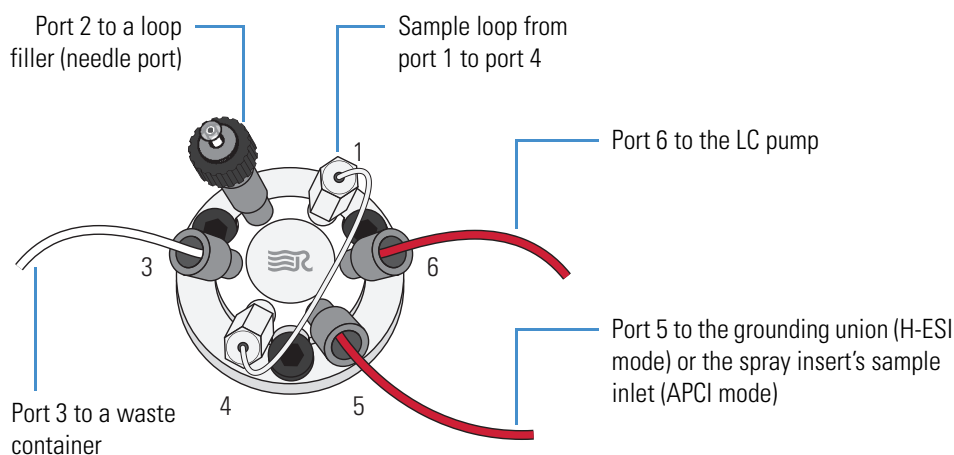
1. Do one of the following:

- To load sample automatically with the syringe pump, set up the syringe pump (see “Setting Up the Syringe Pump” on page 21). Using a red PEEK infusion line, make the following connections:
 - Using a two-piece fingertight fitting, connect one end of the infusion line to the free end of the LC union that connects to the syringe.
 - Using a one-piece fingertight fitting, connect the other end to port 2 of the divert/inject valve.

–or–

- To load sample manually with a hand-held syringe, connect the **needle port** to port 2 of the divert/inject valve (Figure 14).

Figure 14. Divert/inject valve setup for manual loop injection



2. Connect a sample loop from port 1 to port 4 of the divert/inject valve.
3. Use red PEEK tubing to connect port 6 of the divert/inject valve to the LC pump as follows:
 - Using an appropriate fitting and ferrule, connect one end of the tubing to the outlet of the LC pump.
 - Using a one-piece fingertight fitting, connect the other end to port 6 of the divert/inject valve.

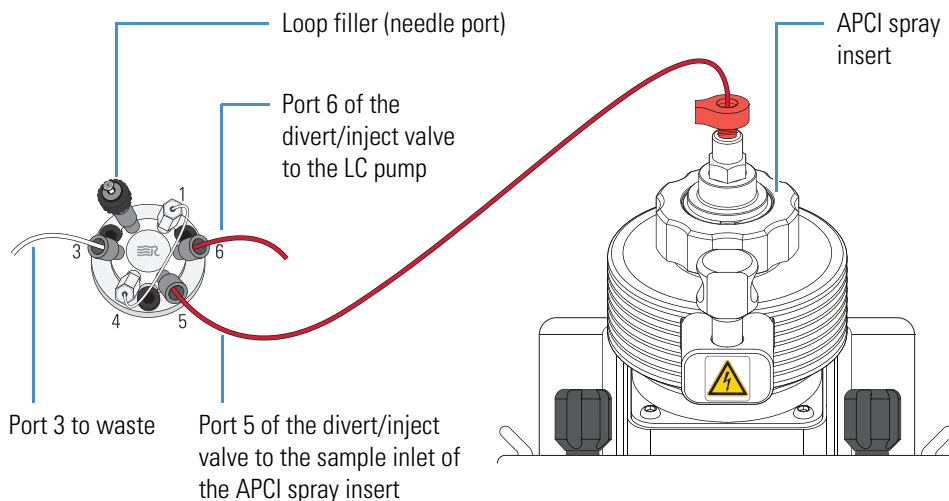
4. Connect port 5 of the divert/inject valve to the API source:
 - a. Using a one-piece fingertight fitting, connect a length of red PEEK tubing to port 5 of the divert/inject valve.
 - b. Depending on whether you installed the ESI or APCI spray insert, do one of the following:
 - For H-ESI mode, use a two-piece fingertight fitting to connect the other end of the red PEEK tubing that connects to port 5 of the divert/inject valve to the grounding union.

For instructions on how to connect the other end of the grounding union, see [“Connecting the Grounding Union to the ESI Spray Insert”](#) on [page 31](#).

—or—

- For APCI mode, connect the other end of the red PEEK tubing to the sample inlet of the APCI spray insert ([Figure 15](#)). Or, you can connect the tubing to the installed grounding union and associated flow path ([Figure 10](#) on [page 23](#), or [Figure 16](#) on [page 31](#)).

Figure 15. Plumbing connections for manual loop injection (APCI mode)



5. Use Teflon tubing to connect port 3 of the divert/inject valve to a waste container as follows:
 - Using a Rheodyne fitting, connect one end of the tubing to port 3 of the divert/inject valve.
 - Place the other end into an appropriate waste container.

This completes the inlet setup for the manual and auto-loop injection techniques.

Setting Up the Inlet for an LC/MS System with an Autosampler

This section describes how to connect the inlet plumbing to introduce sample into the API source from the autosampler in an LC system.

❖ To connect the inlet plumbing for an LC/MS system with an autosampler

1. Use red PEEK tubing to connect port 6 of the divert/inject valve to the outlet of the LC system as follows:
 - Using an appropriate fitting and ferrule, connect one end of the tubing to the outlet of the LC system.
 - Using a one-piece fingertight fitting, connect the other end to port 6 of the divert/inject valve.
2. Use Teflon tubing to connect port 1 of the divert/inject valve to a waste container as follows:
 - Using a Rheodyne fitting, connect one end of the tubing to port 1 of the divert/inject valve.
 - Place the other end into an appropriate waste container.
3. Do one of the following to connect port 5 of the divert/inject valve to the API source:
 - For H-ESI mode, use a one-piece fingertight fitting to connect a length of red PEEK tubing between port 5 of the divert/inject valve and the grounding union.

For instructions on how to connect the other end of the grounding union, see [Connecting the Grounding Union to the ESI Spray Insert](#).

—or—

 - For APCI mode, use a one-piece fingertight fitting to connect a length of red PEEK tubing between port 5 of the divert/inject valve and the sample inlet of the APCI spray insert.

This completes the inlet setup for using an autosampler in an LC/MS system.

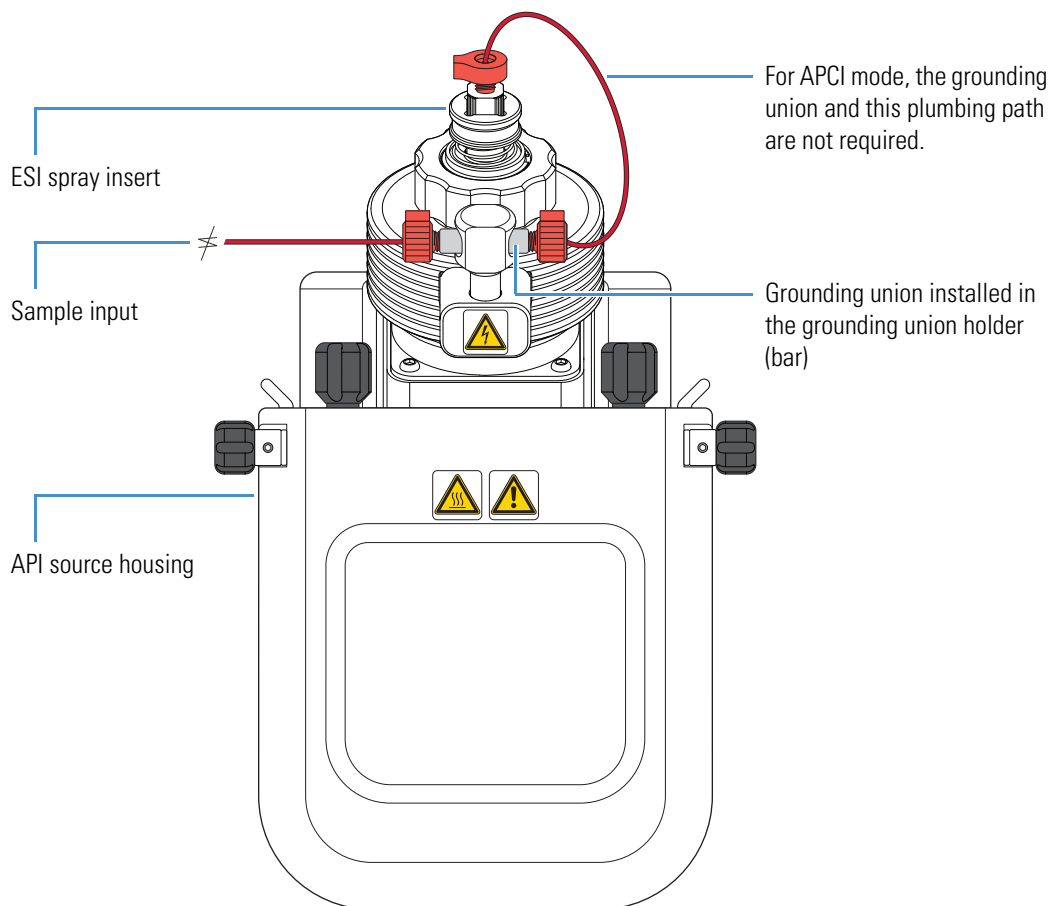
Connecting the Grounding Union to the ESI Spray Insert

Figure 16 shows the connection between the grounding union and the sample inlet of the ESI spray insert. The grounding union is not required for the APCI mode plumbing.



CAUTION To prevent electric shock, verify that the grounding union is made of stainless steel. A grounding union made of a nonconductive material, such as PEEK, creates an electric shock hazard.

Figure 16. Plumbing connections for the grounding union (H-ESI mode)



Syringe Pump and Divert/Inject Valve

This chapter describes the external syringe pump and divert/inject valve that ship with the Orbitrap Fusion mass spectrometer. For information about installing these components, refer to the *Orbitrap Fusion Getting Connected Guide*.

Contents

- [Syringe Pump](#)
- [Divert/Inject Valve](#)

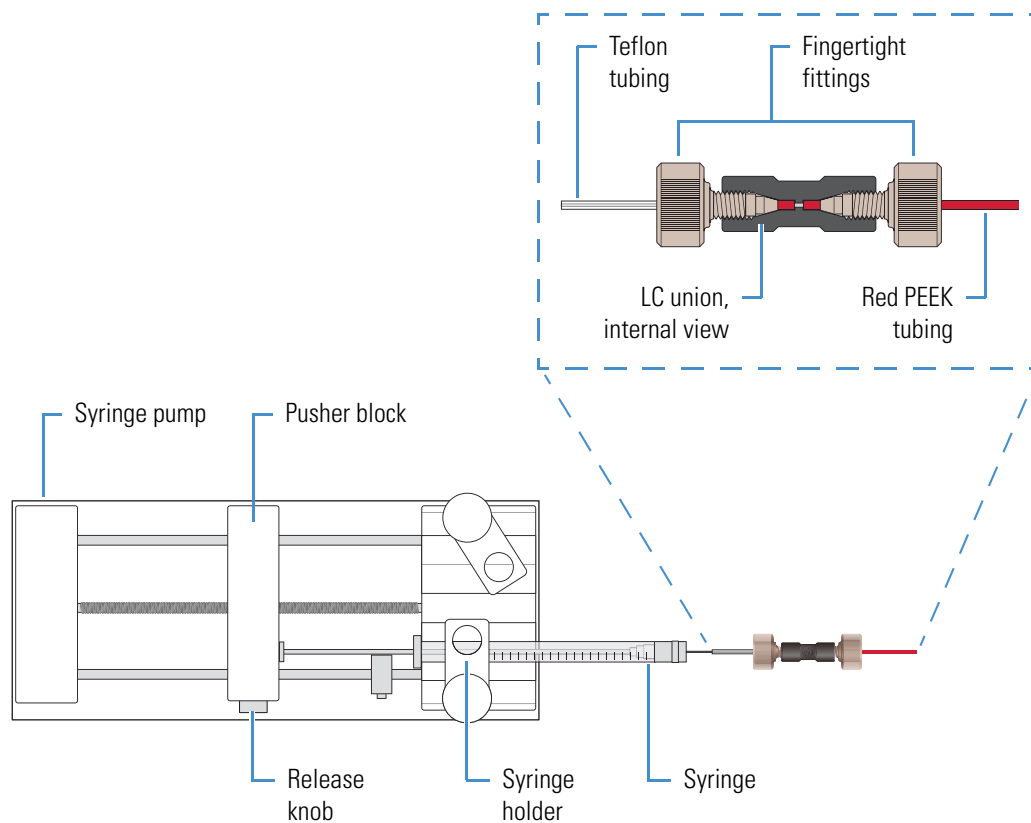
Syringe Pump

The external Chemyx™ Fusion 100T syringe pump delivers sample solution from an installed syringe, through the sample transfer line (red PEEK), and into the API source. The motorized pusher block ([Figure 17](#)) depresses the syringe plunger at the flow rate specified in the data system. (The default flow rate for calibration is 3 $\mu\text{L}/\text{min}$.)

You can start and stop the syringe pump from the data system; refer to the data system Help for instructions. You can also start and stop the syringe pump by pressing the syringe pump buttons.

Note If you choose to provide a syringe pump other than the Fusion 100T, ensure that it can provide a steady, continuous flow of 1–5 $\mu\text{L}/\text{min}$.

Figure 17. Syringe pump setup (top view)

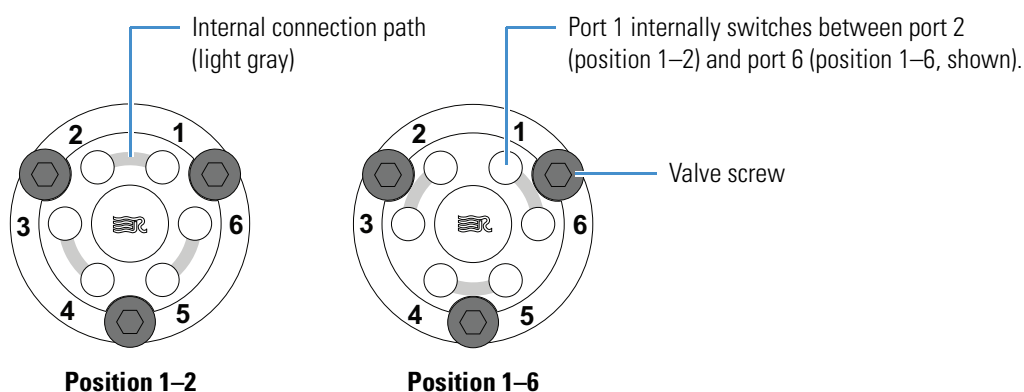


Divert/Inject Valve

The external Rheodyne MX Series II™ divert/inject valve is a 6-port motorized valve that switches between two positions. In the first position, port one connects internally to port two, port three connects to port four, and port five connects to port six. In the second position, the valve rotates one position so that port one connects internally to port six, port two connects to port three, and port four connects to port five. [Figure 18](#) shows the valve's internal flow paths for both positions.

The Method Editor in the Xcalibur application identifies the valve's two positions as “1–2” (port 1 to 2) and “1–6” (port 1 to 6).

Figure 18. Divert/inject valve positions



Configurations

You can configure (plumb) the divert/inject valve as a loop injector (for flow injection analysis) or as a divert valve. The divert valve can switch the solvent front, gradient endpoint, or any portion of the LC run to waste. [Figure 19](#) shows both of these configurations.

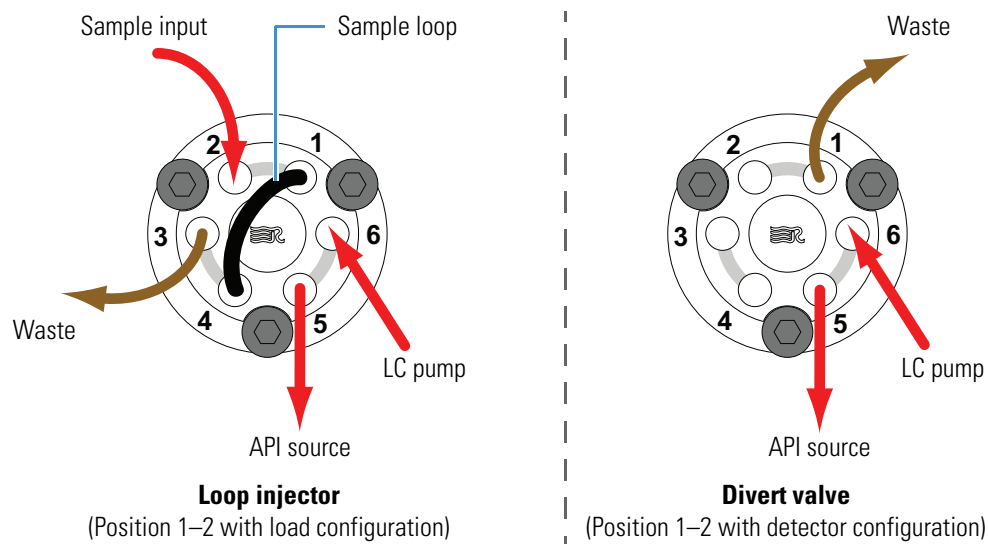
In the loop injector valve configuration, the valve switches between these two positions:

- **Load (position 1–2)**—The sample loop is isolated from the solvent stream. Solvent flow from the LC pump enters and exits the valve through ports six and five, respectively. When you load the sample into port two, the sample enters and exits the sample loop through ports one and four, respectively. As you overfill the sample loop, the excess sample exits the valve through port three to waste.
- **Inject (position 1–6)**—The sample loop is open to the solvent stream. The solvent flow from the LC pump flushes sample out of the sample loop, and then exits through port five into the API source.

In the divert valve configuration, the valve switches between these two positions:

- Detector (position 1–2)—Solvent flow from the LC pump enters the valve through port six and exits through port five into the API source.
- Waste (position 1–6)—Solvent flow from the LC pump enters the valve through port six and exits through port one to waste.

Figure 19. Divert/inject valve plumbed as a loop injector and as a divert valve

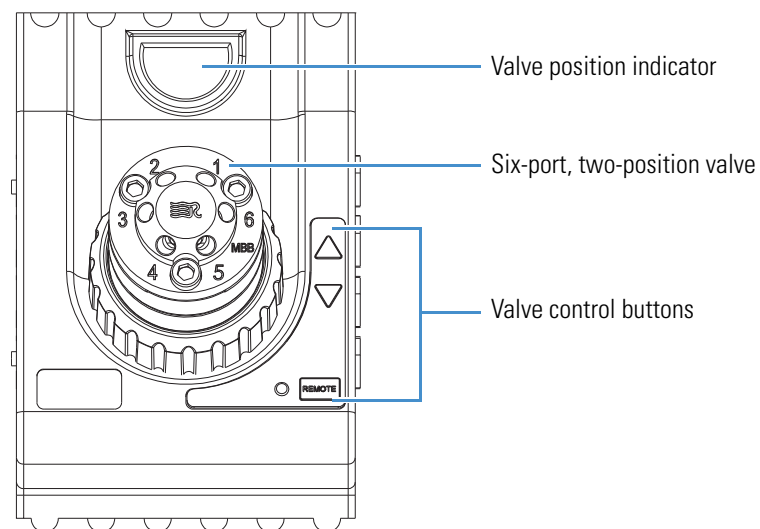


Controlling the Divert/Inject Valve

You can control the divert/inject valve as follows:

- Use the mass spectrometer's data system to specify the parameters in the Divert Valve Properties pane in the Method Editor. For instructions, refer to the Xcalibur Method Editor Help.
- Use the valve's control buttons (Figure 20) to divert the LC flow between the mass spectrometer and waste when the valve is in the divert valve configuration, or switch between load and inject modes when the valve is in the loop injector configuration. For instructions, refer to the manufacturer's manual.

Figure 20. Divert/inject valve (front view)



Preparing the System for Calibration

This chapter describes how to prepare the Orbitrap Fusion mass spectrometer system before you calibrate the mass spectrometer.

Note

- You must pump down the instrument for the full 15 hours and complete the bakeout period before you start the instrument calibration process.
- The figures shown in this chapter exclude the features for the ETD and Internal Calibration (IC) options. If the optional EASY-ETD or EASY-IC ion source is installed in your mass spectrometer, refer to the *EASY-ETD and EASY-IC Ion Sources User Guide* for the applicable figures.

Contents

- [Pumping Down the Mass Spectrometer](#)
- [Setting Up the Syringe Pump for Direct Infusion](#)
- [Setting Up the Mass Spectrometer for Calibration](#)

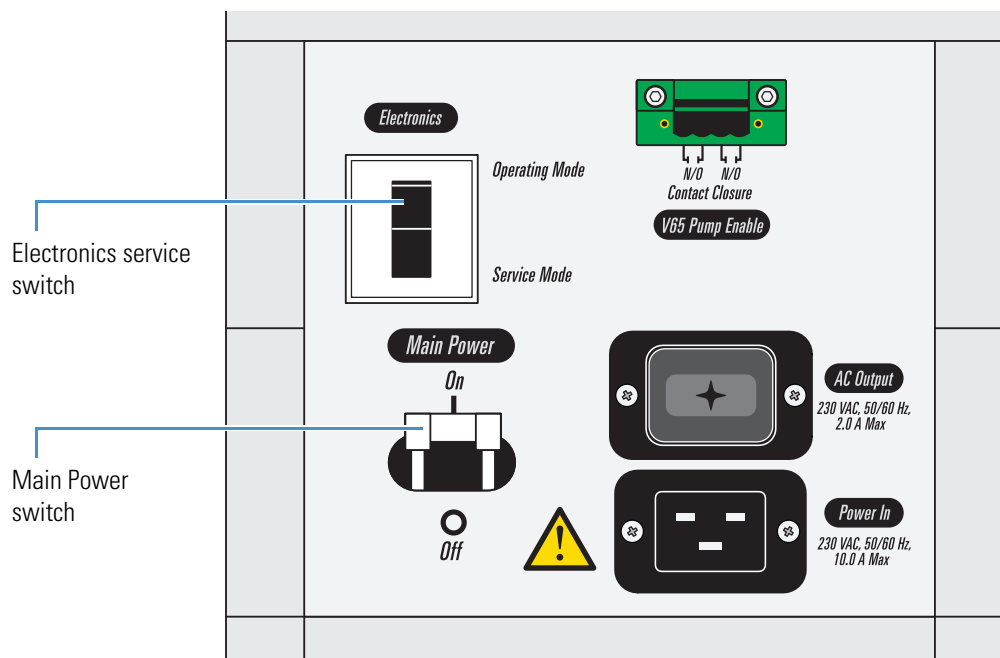
Pumping Down the Mass Spectrometer

To help optimize the performance of the mass spectrometers, pump down the vacuum system for at least 15 hours and bakeout the Orbitrap FT chamber for 12 hours during that period.

❖ To pump down the mass spectrometer

1. Check that the forepump's exhaust tubing connects to the exhaust system and that any valves in the exhaust path are open.
2. Turn on the forepump's power switch.
3. Place the electronics service switch in the Service Mode (down) position ([Figure 21](#)).

Figure 21. Power entry module (right side of the instrument)



4. Turn on the Main Power switch.

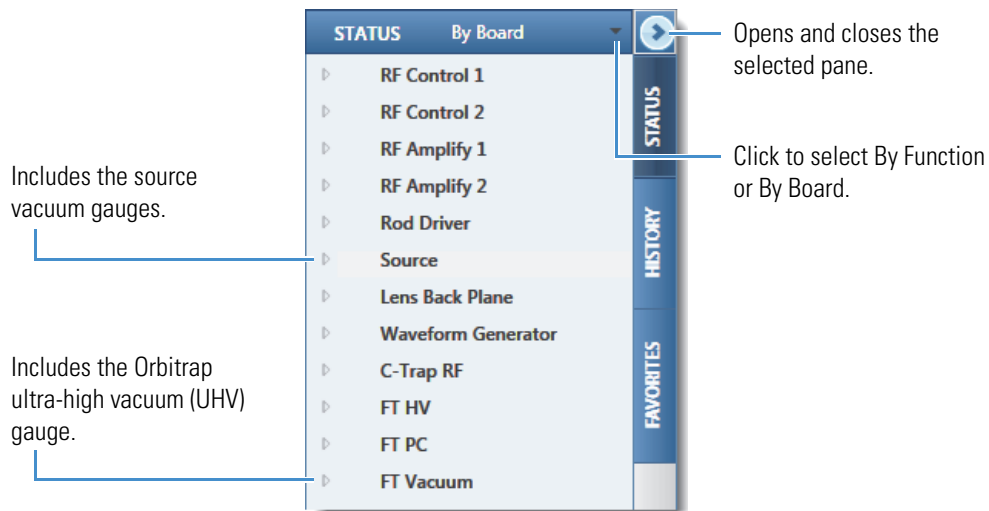
The LEDs on the front panel remain off.

5. Verify that the forepump is running and that there are no leaks in the connection between the forepump and the mass spectrometer.
6. Wait 1 hour.
7. Place the electronics service switch in the Operating Mode (up) position.

The mass spectrometer's restart sequence begins. The Power LED on the front panel turns green, and the Vacuum, Communication, and System LEDs remain off. When the instrument startup process is complete, the Vacuum and Communication LEDs are green and the System LED is yellow.

8. Open the Status pane in the Tune window (see [page 70](#)), click the downward arrow, and then choose **By Board** ([Figure 22](#)).

Figure 22. By Board page in the Status pane



9. Check the readback values for the source and Orbitrap (UHV) pressure gauges as follows:

- Double-click **Source**, and then verify that the Source Pressure and Ion Gauge Pressure readback values are normal (see [Table 10](#)).
- Double-click **FT Vacuum**, and then verify that the UHV Pressure readback value is normal (see [Table 10](#)).

Table 10. Normal readback values for the vacuum pressure gauges

Source pressure (Torr)	Ion gauge pressure (Torr)	UHV pressure (Torr)
< 3.0	1.5×10^{-4}	5×10^{-8}

Normal readback measurements show a green square (■). If the vacuum pressure values are normal, follow the next procedure “[To bakeout the Orbitrap FT chamber.](#)”

❖ **To bakeout the Orbitrap FT chamber**



1. When the vacuum pressures become normal, place the mass spectrometer in **Off** mode (see [page 71](#)).



2. Click the **Diagnostics** icon (lower left corner) to open the Diagnostics pane.

3. In the **System - Vacuum** list, select **Bake FT Chamber** ([Figure 23](#)), and then set the following values in the parameters table:

- In the Bake Time (hours) box, enter 12.
- In the Cool Time (hours) box, enter 2.

Figure 23. Diagnostics pane showing the Bake FT Chamber parameter table

Parameter Name	Parameter Value
Bake Time (hours)	12
Cool Time (hours)	2

4. Click **Start**.

After the 14-hour period, you can turn on the LC system, if applicable.

Setting Up the Syringe Pump for Direct Infusion

Use the syringe pump to infuse the calibration solution directly into the H-ESI source. For information about the syringe pump, see [Chapter 4, “Syringe Pump and Divert/Inject Valve.”](#)

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

❖ To set up the syringe pump for direct infusion of the calibration solution

1. Turn on the syringe pump's power switch.

The power switch is located on the back of the device.



2. In the Tune window, place the mass spectrometer in **Standby** mode.
3. Load a clean, 500 µL syringe with 500 µL of the ESI positive calibration solution.

For a list of provided solutions, see [“Orbitrap Fusion Chemicals Kit”](#) on [page xvi](#).

Note To minimize the possibility of cross-contamination of the assembly, be sure to wipe off the tip of the needle with a clean, lint-free tissue before reinserting it into the syringe adapter assembly ([Figure 8](#) on [page 21](#)).

4. Plumb the inlet for direct infusion as follows:
 - a. Follow steps 2–5 in [“To set up the syringe for infusion or high-flow infusion experiments”](#) on [page 22](#).
 - b. Follow steps 2 and 3 in [“To connect an infusion line between the LC union and the grounding union”](#) on [page 23](#).



CAUTION To prevent electric shock, verify that the grounding union is made of stainless steel and is completely inserted into the grounding union holder.

Go to the next section, [“Setting Up the Mass Spectrometer for Calibration.”](#)

Setting Up the Mass Spectrometer for Calibration

Before you calibrate the mass spectrometer, set up the operational parameters.



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

❖ To set up the mass spectrometer for calibration



1. In the Tune window, place the mass spectrometer in **On** mode.
2. Open the Ion Source page in the Ion Source pane, and then do the following:
 - a. In the Current LC Flow ($\mu\text{L}/\text{min}$) box, enter 3.
 - b. Click **Get Defaults**, and then click **Apply**.

The Tune application sets the default parameters for the H-ESI source.


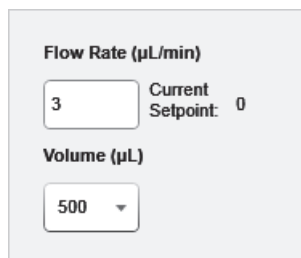
3. Set the syringe pump parameters as follows:
 - a. Click the dropdown arrow, , next to the Syringe On/Off button to open the syringe parameters box (Figure 24).

Figure 24. Syringe parameters box



Flow Rate ($\mu\text{L}/\text{min}$)

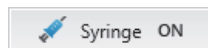
3 Current Setpoint: 0

Volume (μL)

500 ▾

- b. In the Flow Rate ($\mu\text{L}/\text{min}$) box, type 3.
- c. In the Volume (μL) list, select 500.

The Tune application automatically sets the internal diameter (ID) for the syringe volume.



- d. Click **Syringe On (Off)** to start the syringe pump.
4. Click **Positive (Negative)** to select the positive ion polarity mode.
 5. Verify that the system readback is normal (see [page 71](#)).

This completes the setup for calibrating the mass spectrometer. Go to [Chapter 6, “Ionization Spray Stability.”](#)

Ionization Spray Stability

Before you calibrate the mass spectrometer, make sure that you establish stable ionization spray conditions. The intensity and stability of the ionization spray largely depend on the performance of the API source.


IMPORTANT

- Failure to maintain a stable spray might compromise the data quality or result in a poor calibration or diagnostic result.
- If the spray becomes unstable with your analyte solution, return to this chapter to evaluate the spray stability.

Contents

- [Evaluating the Spray Stability](#)
- [Optimizing the API Source Parameters](#)

Evaluating the Spray Stability

Use the Plot Chromatogram tool () to evaluate the API source's ionization spray.

❖ To evaluate the spray stability

1. Open the Tune window (see [page 70](#)).
2. Set up the system to use the calibration solution as follows:
 - a. Verify that the syringe contains the appropriate calibration solution.
 - b. In the Tune window, verify the following syringe and instrument settings:
 - 3 μ L/min in the Current LC Flow box
 - 3 μ L/min flow rate and 500 μ L syringe volume (see [page 72](#))
 - Positive ion polarity mode (see [page 73](#))
 - Profile data type (see [page 73](#))
 - c. Go to [step 4](#).

3. (Optional) Set up the system to use an analyte solution as follows:

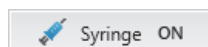
- Verify that the LC device or the syringe contains a sufficient amount of the analyte.
- Open the Ion Source page in the Ion Source pane, and verify the value in the Current LC Flow ($\mu\text{L}/\text{min}$) box.



4. Place the mass spectrometer in **On** mode (see [page 71](#)).

The mass spectrometer begins scanning and applies high voltage to the spray insert. A real-time mass spectrum appears in the Tune window.

5. Turn on the flow for the solution as follows:



- For the calibration solution, click **Syringe On (Off)** to start the syringe pump.

—or—

- For an analyte solution, turn on the flow from the LC device or the syringe pump.

A real-time plot of the solution's mass spectrum appears.

6. Plot the full **total ion current (TIC)** and RSD graphs as follows:


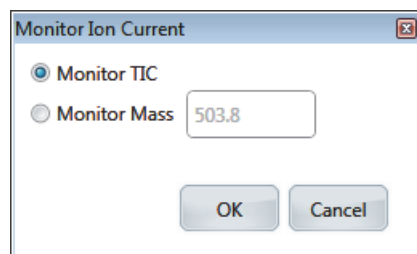
- a. Click the **Plot Chromatogram** icon, , to open the Monitor Ion Current dialog box ([Figure 25](#)).

Figure 25. Monitor Ion Current dialog box with the Monitor TIC option selected



- b. Select the **Monitor TIC** option.
- c. Click **OK** to plot the full TIC chromatogram.

The Plot Chromatogram tool generates a real-time graph (plot) of the full TIC where you can observe the signal stability and the effects of changes to various parameters. The tool also generates a real-time graph of the **relative standard deviation (RSD)** of the TIC for a 10 Da selected ion monitoring (SIM) scan that is centered around the most abundant mass-to-charge ratio in the current spectrum.

7. Observe the RSD graph, and review the signal stability rating and maximum %RSD value.

Table 11 lists the criteria for a stable spray in either positive or negative ion polarity mode.

Table 11. Recommended %RSD values and ratings for the calibration solutions

Ion polarity mode	Acceptable signal stability rating	Maximum %RSD (threshold)
Positive	Excellent or Good	15
Negative	Excellent or Good	15

8. If the signal stability rating is poor or the %RSD value is above the threshold, follow the procedure in the next section, “[Optimizing the API Source Parameters](#).”

This completes the spray stability evaluation.

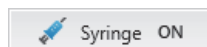
Optimizing the API Source Parameters

If the ionization spray is unstable, follow the procedure in this section to optimize the API source parameters.

IMPORTANT Thermo Fisher Scientific recommends that you optimize the API source parameters only if the preceding spray evaluation determines that the ionization spray is unstable.

❖ To optimize the API source parameters

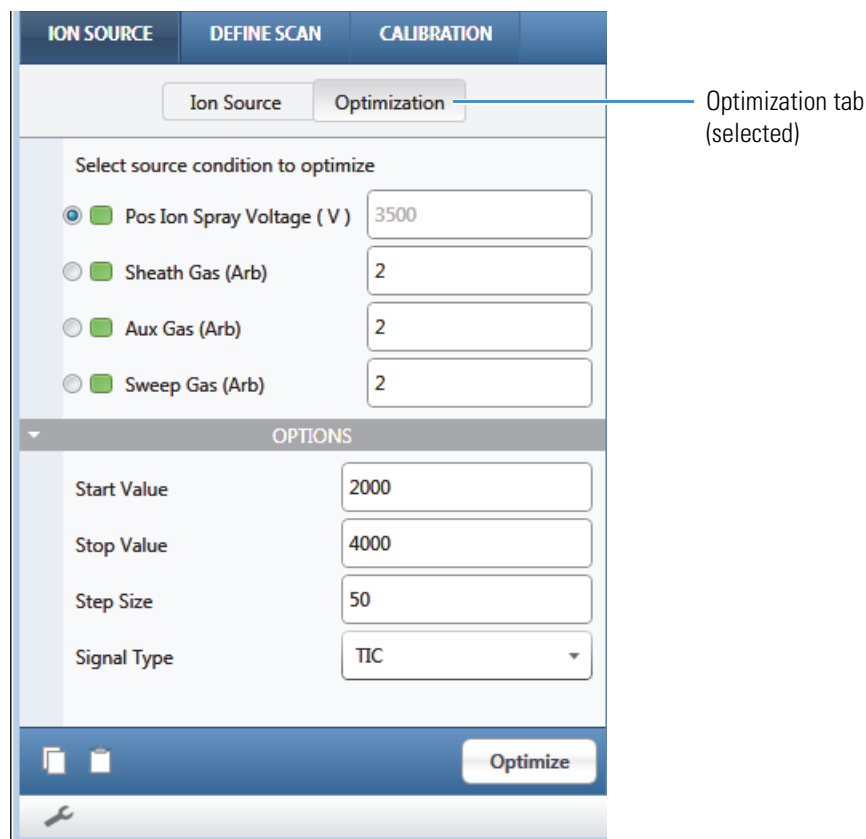
1. Verify that the syringe has a sufficient amount of the calibration solution.
2. In the Tune window, click **Syringe On (Off)** to start the syringe pump.



For an analyte solution, turn on the flow from the syringe pump or the LC device.

3. Open the Optimization page of the Ion Source pane, and then do the following:
 - a. Select the **Polarity Ion Spray Voltage (V)** option (Figure 26).

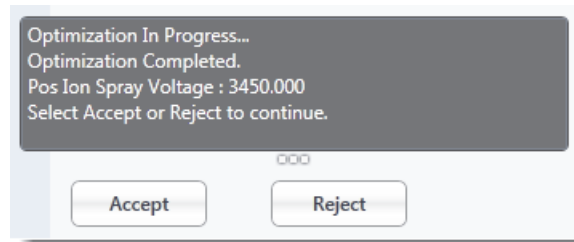
Figure 26. Optimization page of the Ion Source pane



- b. In the Signal Type list, select **TIC**.
 - c. Click **Optimize**.

The status area displays the message “Optimization In Progress.” After Tune completes the optimization, the optimized value and the Accept and Reject buttons appear (Figure 27).

Figure 27. Completed optimization message



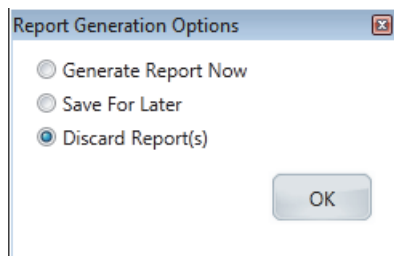
- d. Click **Accept**.

The Report Generation Options dialog box opens (Figure 28).

- e. Select an option, and then click **OK**.

Tip To turn off the Report Generation Options dialog box, see [“Setting the Tune Preferences” on page 73](#).

Figure 28. Report Generation Options dialog box



4. Optimize the remaining source parameters.
5. (Optional) Save the parameters' state in the Favorites pane (see [page 76](#)). For additional information about the Favorites pane, refer to the Tune Help.

Calibrating the Mass Spectrometer in H-ESI Mode

This chapter describes how to calibrate the Orbitrap Fusion mass spectrometer in H-ESI mode by using the ESI calibration solution that the syringe pump introduces into the instrument at a steady flow rate.

Calibration parameters are mass spectrometer parameters whose values do not vary with the type of experiment. In positive mode, you can calibrate the ion optics, linear ion trap, quadrupole, Orbitrap, and ETD source, if your instrument includes this option. In negative mode, you can calibrate the ion optics, linear ion trap, and Orbitrap.

Note

- Calibrate the Orbitrap Fusion mass spectrometer in H-ESI mode before acquiring data in H-ESI or APCI modes. Generally, you must calibrate the mass spectrometer every one to three months of operation for optimum performance over the entire mass range of the mass detector.
- The figures shown in this chapter exclude the features for the ETD and Internal Calibration (IC) options. If the optional EASY-ETD or EASY-IC ion source is installed in your mass spectrometer, refer to the *EASY-ETD and EASY-IC Ion Sources User Guide* for the applicable figures.

Contents

- [Running the Positive Ion Polarity Calibrations](#)
- [Running the Negative Ion Polarity Calibrations](#)

Running the Positive Ion Polarity Calibrations

Always run the positive ion polarity calibrations before the negative ion polarity calibrations.

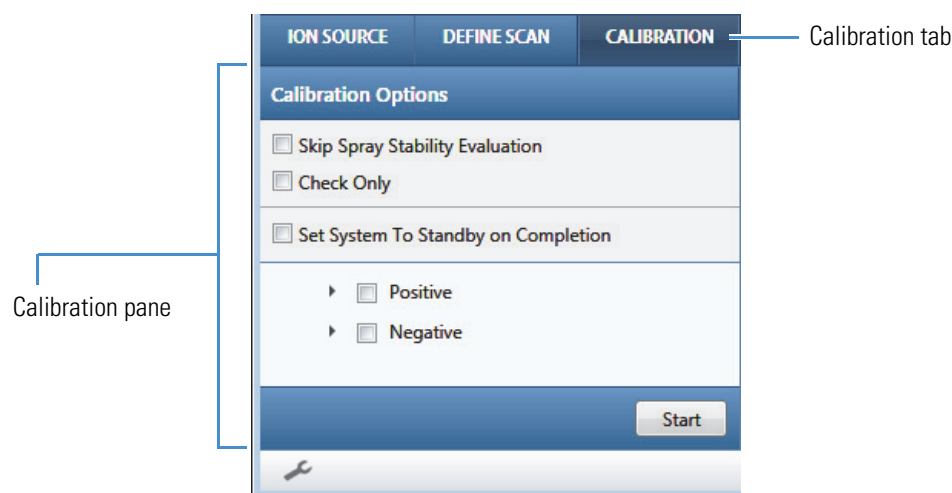
IMPORTANT

- Before you proceed, verify that you have set up the API source ([Chapter 2](#)), prepared the system for calibrating in positive H-ESI mode ([Chapter 5](#)), and verified that the infused calibration solution produces a stable ionization spray ([Chapter 6](#)).
- To minimize the possibility of cross-contamination, use a different syringe and length of PEEK tubing for each type of calibration solution.

❖ To calibrate the mass spectrometer in positive polarity mode

1. Open the Tune window (see [page 70](#)).
2. Click **Calibration** to open the Calibration pane ([Figure 29](#)).

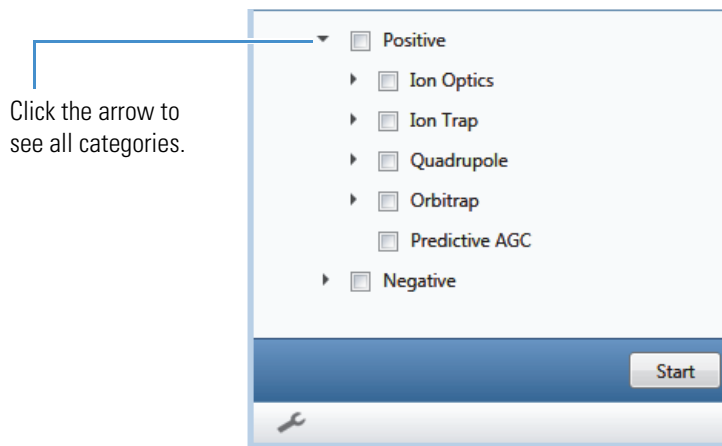
Figure 29. Calibration pane



3. Verify that the **Skip Spray Stability Evaluation** check box is clear so that this test runs.
4. (Optional) Select the **Set System to Standby on Completion** check box.

- Click the arrow next to the Positive check box to display the calibration categories (Figure 30).

Figure 30. Calibration categories for positive polarity



- Select the **Ion Optics** check box.
- Click **Start**, and then review the real-time plot of the mass spectrum.

After the Tune parameters reach their specified settings, the calibration process begins and the status area appears in the Calibration pane. After completing the calibration, the Tune application adds a change record to the History pane under History Logs.

- Run the calibration for each of the remaining categories—one at a time and in the order specified in the Tune window (Figure 30 on page 53).

IMPORTANT The Predictive AGC calibration depends on the other calibrations. Therefore, you must run this as the last positive calibration.

Tip After performing the calibration, you see either a green checkmark ✓ adjacent to the calibration name to indicate a successful calibration or a red X mark ✗ to indicate a failed calibration.

This completes the positive polarity calibration process.

IMPORTANT Before you run the negative polarity calibrations, follow the procedure “To flush the inlet components” on page 79.

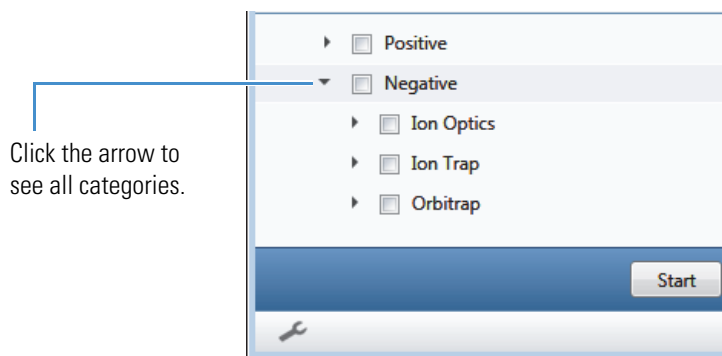
Running the Negative Ion Polarity Calibrations

Make sure that you have completed the positive ion polarity calibrations and flushed the inlet components before you start the negative ion polarity calibrations.

❖ To calibrate the mass spectrometer in negative polarity mode

1. Load another clean, 500 μ L syringe with 500 μ L of the ESI negative calibration solution.
2. In the Calibration pane, verify that the **Skip Spray Stability Evaluation** check box is clear so that this test runs.
3. (Optional) Select the **Set System to Standby on Completion** check box.
4. Click the arrow next to the Negative check box to display the calibration categories (Figure 31).

Figure 31. Calibration categories for negative polarity



5. Select the **Ion Optics** check box.
6. Click **Start**, and then review the real-time plot of the mass spectrum.

The status area appears in the pane. The Tune application adds change records to the History pane under History Logs.

7. Run the calibration for each of the remaining categories—one at a time and in the order specified in the Tune window (Figure 31 on page 54).

This completes the negative polarity calibration process. You can now start using your analyte solution for data acquisition.

IMPORTANT Before you start using your analyte, follow the procedure “To flush the inlet components” on page 79.

Acquiring Sample Data

This chapter describes how to use the Tune application to manually acquire sample data and how to use the Xcalibur data system to set the start (trigger) instrument before you run an instrument method to acquire sample data.

You can follow these procedures with any suitable analyte. For demonstration purposes only, the Thermo Fisher Scientific field service engineer infuses a 50 fg/μL reserpine sample solution. See [Appendix C, “Preparing the Reserpine Sample Solution.”](#)

Note

- Before you begin the analysis of the sample solution, make sure that you have calibrated the mass spectrometer in H-ESI mode within the last three months.
- The data system computer automatically saves the acquired data to its hard drive.

Contents

- [Using the Tune Application to Acquire Sample Data](#)
- [Using the Xcalibur Data System to Acquire Sample Data](#)

Using the Tune Application to Acquire Sample Data

Follow these procedures:

1. [Setting Up the LC/MS System for Analyte Optimization](#)
2. [Defining the Scan Parameters for Precursor Optimization](#), on page 58
3. [Optimizing the Fragmentation Parameters](#), on page 63
4. (Optional) [Defining the Scan Parameters for the SIM Scan Type](#), on page 63
5. [Acquiring a Data File By Using the Tune Application](#), on page 64

Setting Up the LC/MS System for Analyte Optimization

Follow these procedures:

1. [To set up the inlet](#)
2. [To configure the syringe pump](#)
3. [To configure the LC pump](#)

❖ To set up the inlet

Do one of the following:

- If infusing an analyte, set up the inlet for high-flow infusion (see [“Setting Up the Inlet for High-Flow Infusion”](#) on page 24).
- If infusing the reserpine sample solution, set up the inlet for manual-loop injection (see [“Setting Up the Inlet for Manual or Auto-Loop Injections”](#) on page 28).

❖ To configure the syringe pump

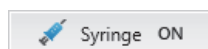


1. In the Tune window, place the mass spectrometer in **On** mode.

The mass spectrometer begins scanning and applies high voltage to the spray insert. A real-time mass spectrum appears in the Tune window.

2. Open the syringe parameters box ([Figure 24](#) on page 44), and then enter the following:
 - **3** for the flow rate (μL/min)
 - **500** for the Volume (μL)

The Tune application automatically saves the parameter values.

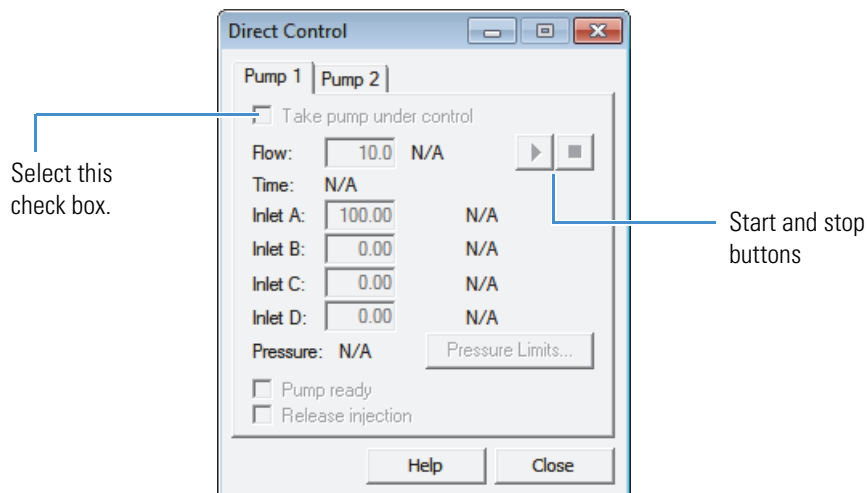


3. Click **Syringe On (Off)** to start the syringe pump.
4. Verify that the inlet plumbing connections do not leak.

❖ **To configure the LC pump**

1. In the Xcalibur Instrument Setup window, click the icon for the LC pump.
2. In the menu bar, choose **pump model > Direct Control** to open the Direct Control dialog box (Figure 32).

Figure 32. Direct Control dialog box (Instrument Setup window)



3. Click the tab for the LC pump, and then select the **Take Pump Under Control** check box.
4. In the Flow box, type **0.4** (in mL/min).
5. Click the **Start** button.
6. Verify that the inlet plumbing connections do not leak.

Defining the Scan Parameters for Precursor Optimization

Before you perform the experiment, define the scan parameters, and then check the [isolation width](#) for the analyte to ensure the effective isolation of the target ion.

Follow these procedures:

1. [To define the MS/MS scan parameters for precursor optimization](#)
2. [To optimize the isolation width](#), on [page 60](#)
3. [To optimize the API source parameters on an analyte](#), on [page 62](#)

❖ To define the MS/MS scan parameters for precursor optimization

1. In the Tune window, click **Define Scan** to open the Define Scan pane.
2. Set the MS/MS scan parameters that are appropriate for your analyte, starting with Scan Type.

See [Table 12](#) and [Figure 33](#) for an example.

Table 12. MS/MS scan parameters (lidocaine example)

Parameter	Value	Parameter	Value
Scan Type	MSn Scan	S-Lens RF Level	70
Detector Type	Ion Trap	Isolation Mode	Ion Trap
Ion Trap Scan Rate	Normal	Isolation Width	2 ^a
Mass Range	Normal	Activation Type	CID
Scan Range (<i>m/z</i>)	50–300	<i>Type</i> ^b Collision Energy (%)	0 ^c
AGC Target	1e4		

^a Start with the default value.

^b *Type* is the selected activation type.

^c No ion fragmentation

Figure 33. Scan parameters to acquire MS/MS data (lidocaine example)

ION SOURCE DEFINE SCAN CALIBRATION

Scan Type: MSn Scan (Select the MSⁿ scan type.)

Detector Type: Ion Trap

Ion Trap Scan Rate: Normal

Mass Range: Normal

Scan Range (m/z): 50-300

AGC Target: 1e4

Maximum Injection Time (ms): 100

Microscans: 1

S-Lens RF Level: 70

Isolation Mode: Ion Trap

Isolation Width (m/z): 2 (Isolation width (default))

Activation Type: CID (CID activation type)

CID Collision Energy (%): 0

Source Fragmentation: ☐ (Turns fragmentation on and off.)

MSn Setting Table	
	Precursor (m/z)
1	235.2 (m/z for lidocaine solution)

Apply

3. In the MSⁿ Setting Table, enter the analyte's precursor ion (for example, *m/z* 235.2).

For information about the MSⁿ Setting Table, see [“Using the MSⁿ Setting Table in the Define Scan Pane”](#) on page 74.

4. Click **Apply**.

The MS/MS scan starts and the mass spectrum appears.

❖ To optimize the isolation width


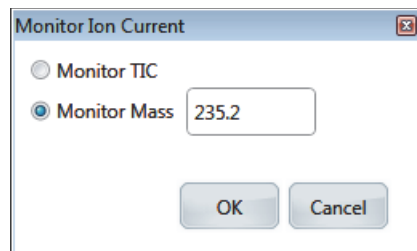
1. Display the chromatogram for the analyte's precursor ion as follows:
 - a. Click the **Plot Chromatogram** icon, , to open the Monitor Ion Current dialog box (Figure 34).

Figure 34. Monitor Ion Current dialog box with the Monitor Mass option selected

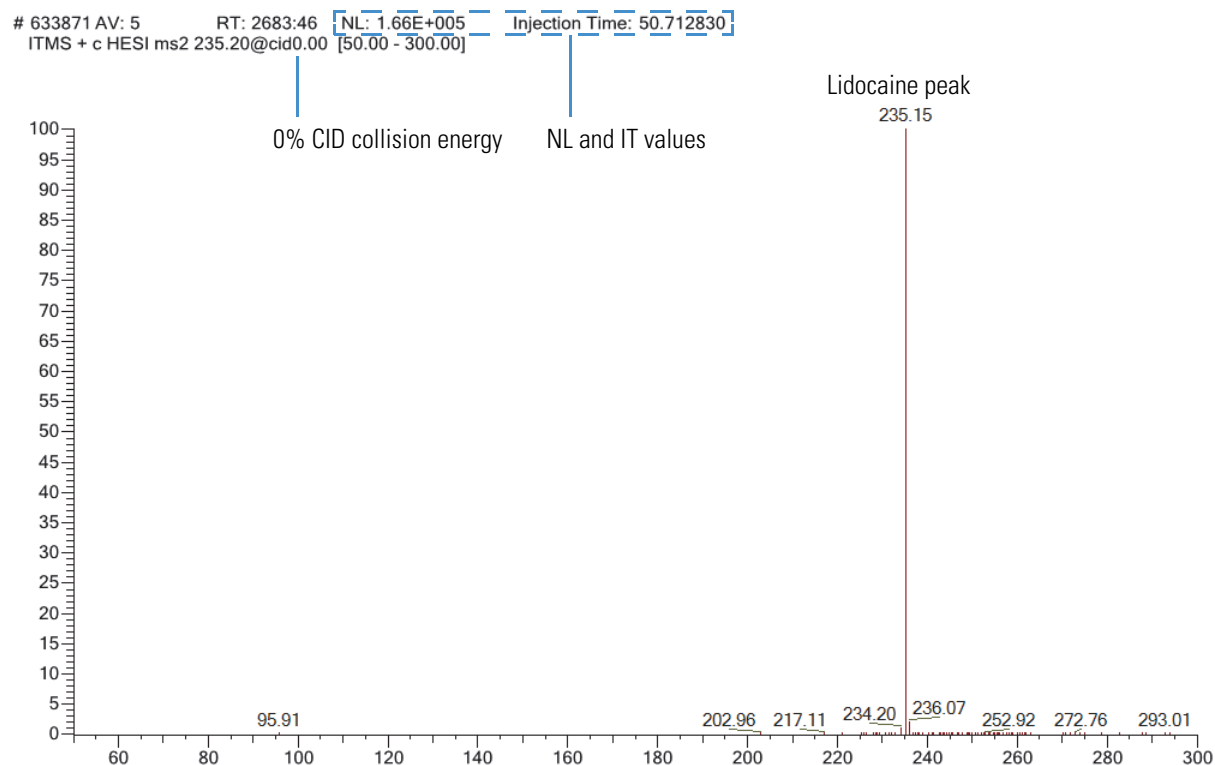
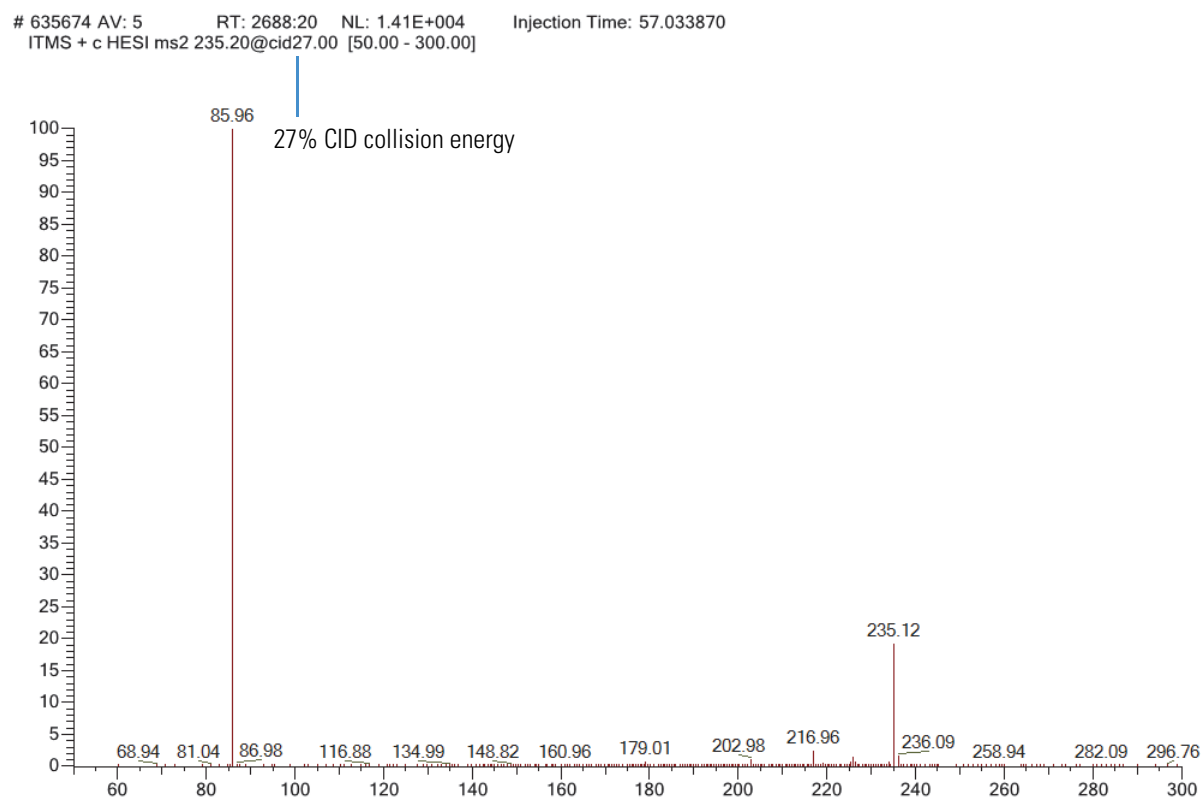
- b. Select the **Monitor Mass** option, and then enter the analyte's m/z value (for example, m/z 235.2).
 - c. Click **OK** to plot the chromatogram.
2. In the Define Scan pane, in the Isolation Width (m/z) box, enter a slightly lower value.

Note The isolation width setting is typically m/z 1–3. The optimum value for the isolation width is the smallest m/z width (instrument minimum width = m/z 0.1) that gives a mass spectrum of maximum intensity for only the target ions. A narrow isolation width increases the specificity of the scan results while a wider width increases the signal at the expense of specificity.

When you obtain the optimum isolation width, the normalization level (NL) and ionization time (IT) values are stable and the mass peak for the precursor ion is at its maximum intensity and appears symmetrical. An isolation width value that is less than optimum causes a substantial drop in the NL reading. A significant drop in sensitivity indicates that the ions are not effectively isolated.

3. Enter successively smaller values for the isolation width, until the intensity of the chromatogram is acceptable for your needs.
 4. After you optimize the isolation width, compensate for minor changes in stability by increasing the isolation width by an amount not to exceed m/z = 1.
 5. In the Collision Energy (%) box, enter an appropriate value for the analyte (for example, 27).
 6. Click **Apply** to start the fragmentation process.

Figure 35 and Figure 36 show spectra examples of CID-MS/MS scans without and with fragmentation, respectively. For descriptions of the spectrum header information and controls, refer to the Spectrum View topic in the Tune Help.

Figure 35. CID-MS/MS scan spectrum without fragmentation (lidocaine example)**Figure 36.** CID-MS/MS scan spectrum with fragmentation (lidocaine example)

❖ To optimize the API source parameters on an analyte

1. Follow the procedure “To optimize the API source parameters” on page 47, except use your analyte solution, select *m/z* in the Signal Type list, and then type the *m/z* value for the analyte in the *m/z* box (Figure 37).

Figure 37. Optimization page in the Ion Source pane (*m/z* signal type)

ION SOURCE DEFINE SCAN CALIBRATION

Ion Source Optimization

Select source condition to optimize

☒ Pos Ion Spray Voltage (V) 3500

☐ Sheath Gas (Arb) 2

☐ Aux Gas (Arb) 2

☐ Sweep Gas (Arb) 2

OPTIONS

Start Value 2000

Stop Value 4000

Step Size 50

Signal Type *m/z*

m/z 235.2

Optimize

2. (Optional) If you need to increase the sensitivity, optimize the following:
 - Vaporizer Temperature (Ion Source page of the Ion Source pane)
 - S-Lens RF Level (Define Scan pane)
 - Spray direction (see “Adjusting the Spray Direction” on page 15).
3. (Optional) Save the parameters’ state in the Favorites pane (see page 76). For additional information about the Favorites pane, refer to the Tune Help.

Optimizing the Fragmentation Parameters

After you optimize the isolation width and the API source parameters for an MS/MS scan, optimize the collision energy for optimum fragmentation.

❖ To optimize the collision energy

1. Open the Define Scan pane.
2. In the Collision Energy (%) box, enter an appropriate value (for example, 27).
3. Click **Apply**.
4. Observe the mass spectrum from the specified fragmentation method, which is CID for this chapter's example.
5. Repeat steps 2 through 4, entering new values in 5% increments until you are satisfied with the spectrum.

The normal range for CID collision energy is 20–40 percent. The normal range for HCD collision energy is 10–50 percent.

Defining the Scan Parameters for the SIM Scan Type

This section is optional. If you want to further improve the sensitivity of the data acquisition, use the SIM scan type.

❖ To define the SIM scan parameters

1. In the Define Scan pane, in the Scan Type list, select **SIM Scan**.
2. Set the SIM scan parameters that are appropriate for your analyte, starting with Scan Type.

See [Table 13](#) and [Figure 38](#) for an example.

Table 13. SIM scan parameters (lidocaine example)

Parameter	Value	Parameter	Value
Scan Type	SIM Scan	AGC Target	1e4
Detector Type	Ion Trap	S-Lens RF Level	70
Ion Trap Scan Rate	Normal	Isolation Mode	Ion Trap
Mass Range	Normal	Isolation Width (<i>m/z</i>)	2
Precursor (<i>m/z</i>)	235.2		

8 Acquiring Sample Data

Using the Tune Application to Acquire Sample Data

Figure 38. Scan parameters to acquire SIM data (lidocaine example)

ION SOURCE	DEFINE SCAN	CALIBRATION
Scan Type	SIM Scan	
Detector Type	Ion Trap	
Ion Trap Scan Rate	Normal	
Mass Range	Normal	
Precursor (m/z)	235.2	
AGC Target	1e4	
Maximum Injection Time (ms)	100	
Microscans	1	
S-Lens RF Level	70	
Isolation Mode	Ion Trap	
Isolation Width (m/z)	2	
Source Fragmentation	<input type="checkbox"/>	

Apply

3. Click **Apply**.

Acquiring a Data File By Using the Tune Application

❖ To acquire a sample data file

1. Open the Data Acquisition pane (Figure 39), and then do the following:



- a. If you want to change the destination folder for the RAW file, click the **Browse** icon.
The default folder location is in *drive:\Thermo\Data*.
- b. In the File Name box, type **reserpine** (or the name of the analyte).
The RAW file name is a combination of a base name, which you enter, and an automatic time stamp that consists of the year (YY), month (MM), day (DD), and time (HHMMSS).
- c. In the Sample Name box, type the name of the analyte (or other suitable label).

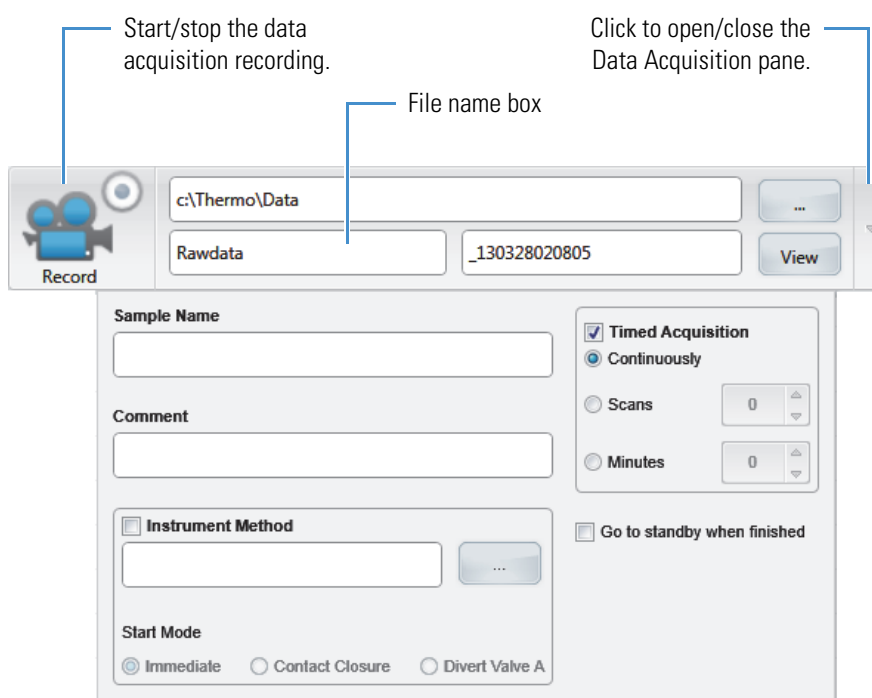
- d. In the Comment box, type a comment about the experiment.

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

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

- e. Under Timed Acquisition, select the **Continuously** option (acquires data until you stop the acquisition).

Figure 39. Data Acquisition pane in Tune



2. Click **Record** (Figure 39) to start data acquisition.

After the Tune parameters reach their specified settings, the data acquisition process begins and the small circle on the Record button turns red (●).

3. When you are ready, click **Record** again to stop the acquisition.

The small circle on the Record button turns gray (not recording).

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

8 Acquiring Sample Data

Using the Xcalibur Data System to Acquire Sample Data

Using the Xcalibur Data System to Acquire Sample Data

Thermo mass spectrometry applications, such as the Xcalibur data system, can control the connected external device. If the Xcalibur application can control the external device, it selects the autosampler as the default start (trigger) instrument for a sequence run. If the Xcalibur application cannot control the external device, it selects the mass spectrometer as the start instrument, which means that you must change the start instrument as part of the Xcalibur sequence run setting.

Follow these procedures:

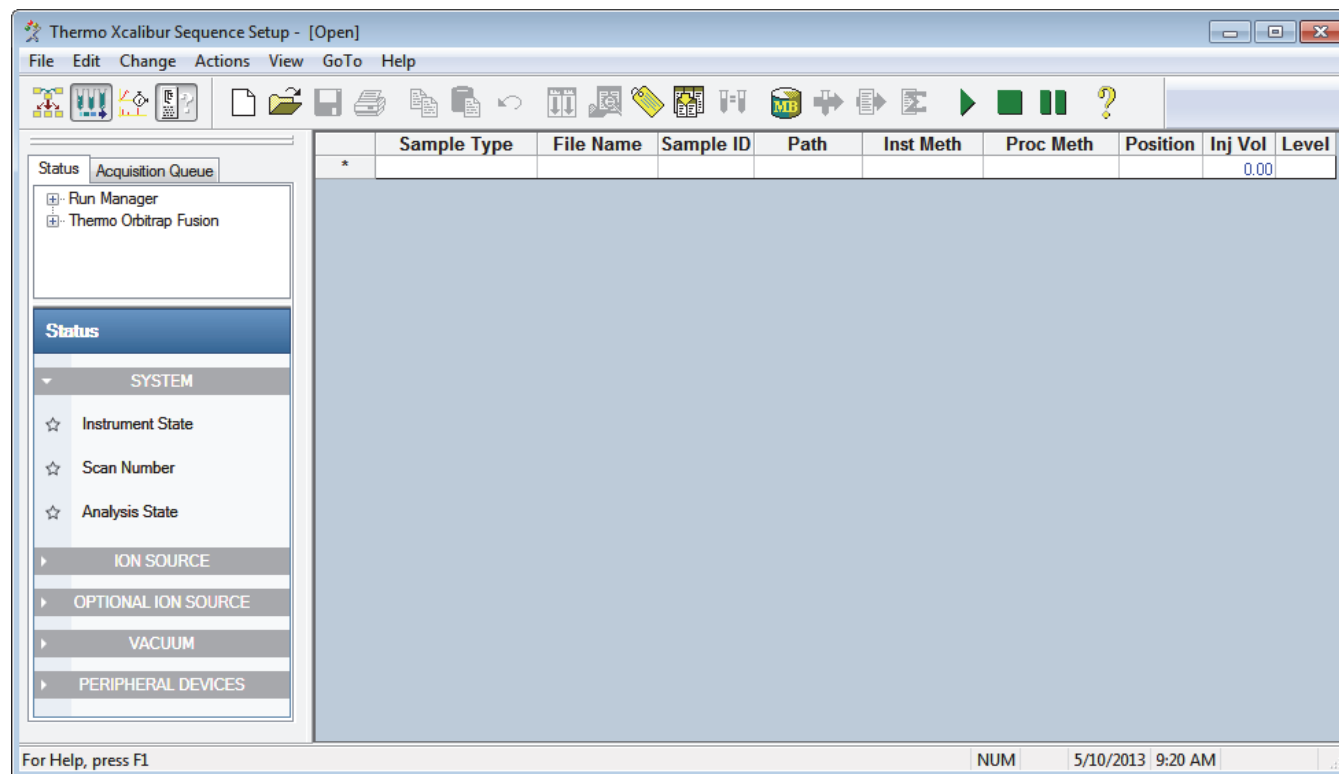
1. To select the external start instrument
2. To acquire a data file by using the Xcalibur data system, on page 67

❖ To select the external start instrument



1. Open the Xcalibur data system, and then click the **Sequence Setup** icon to open the Sequence Setup window (Figure 40).

Figure 40. Thermo Xcalibur Sequence Setup window



2. Open the sequence that you want to run as follows:

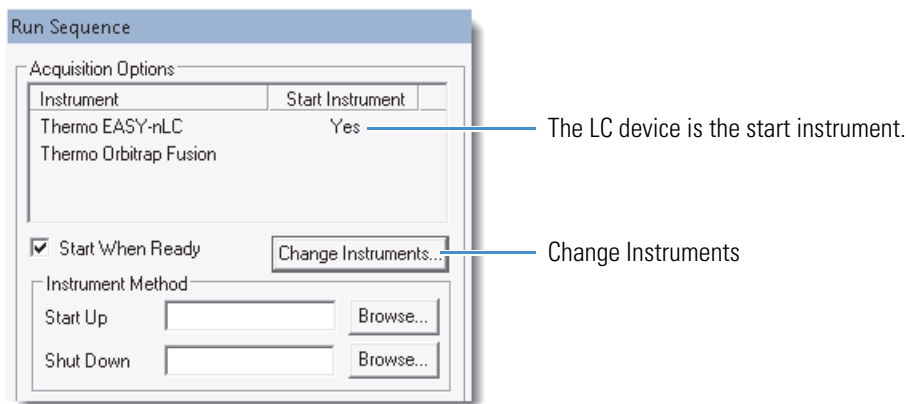


- a. Click the **Open** button and browse to the appropriate folder.
- b. Select the sequence (.sld) file and click **Open**.

3. Choose **Actions > Run Sequence** or **Actions > Run This Sample** to open the Run Sequence dialog box (Figure 41).

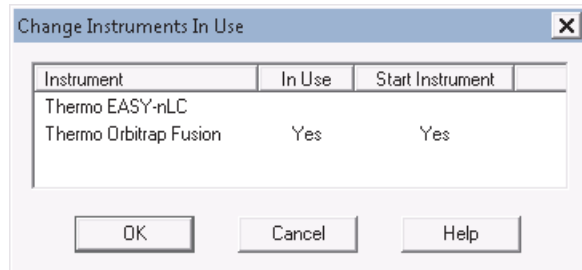
The Yes in the Start Instrument column indicates the default start instrument for the sequence run.

Figure 41. Run Sequence dialog box (partial) showing the selected start instrument



4. If Yes appears in the Start Instrument column for the mass spectrometer or if you need to change the start instrument to another device, click **Change Instruments** to open the Change Instruments In Use dialog box (Figure 42).

Figure 42. Change Instruments In Use dialog box showing the MS as the start instrument



- a. In the Start Instrument column, click the blank field to the right of the appropriate triggering device (typically an autosampler) to move “Yes” to that field.
 - b. Click **OK**.
5. In the Run Sequence dialog box, complete the remaining selections.
 6. Click **OK**.

This completes the start instrument setup.

❖ **To acquire a data file by using the Xcalibur data system**

For instructions, refer to the Instrument Setup and Sequence Setup topics in the Xcalibur Help.

Basic Tune Functions

This appendix describes some of the basic Tune functions that are used throughout this guide. For additional information, refer to the Tune Help.

Contents

- [Opening the Tune Window](#)
- [Setting the Instrument Power Mode](#)
- [Checking the Instrument Readback Status](#)
- [Controlling the Syringe Pump](#)
- [Setting the Data Type](#)
- [Setting the Ion Polarity Mode](#)
- [Setting the Tune Preferences](#)
- [Using the MSⁿ Setting Table in the Define Scan Pane](#)
- [Using the Favorites Pane to Save System Settings](#)

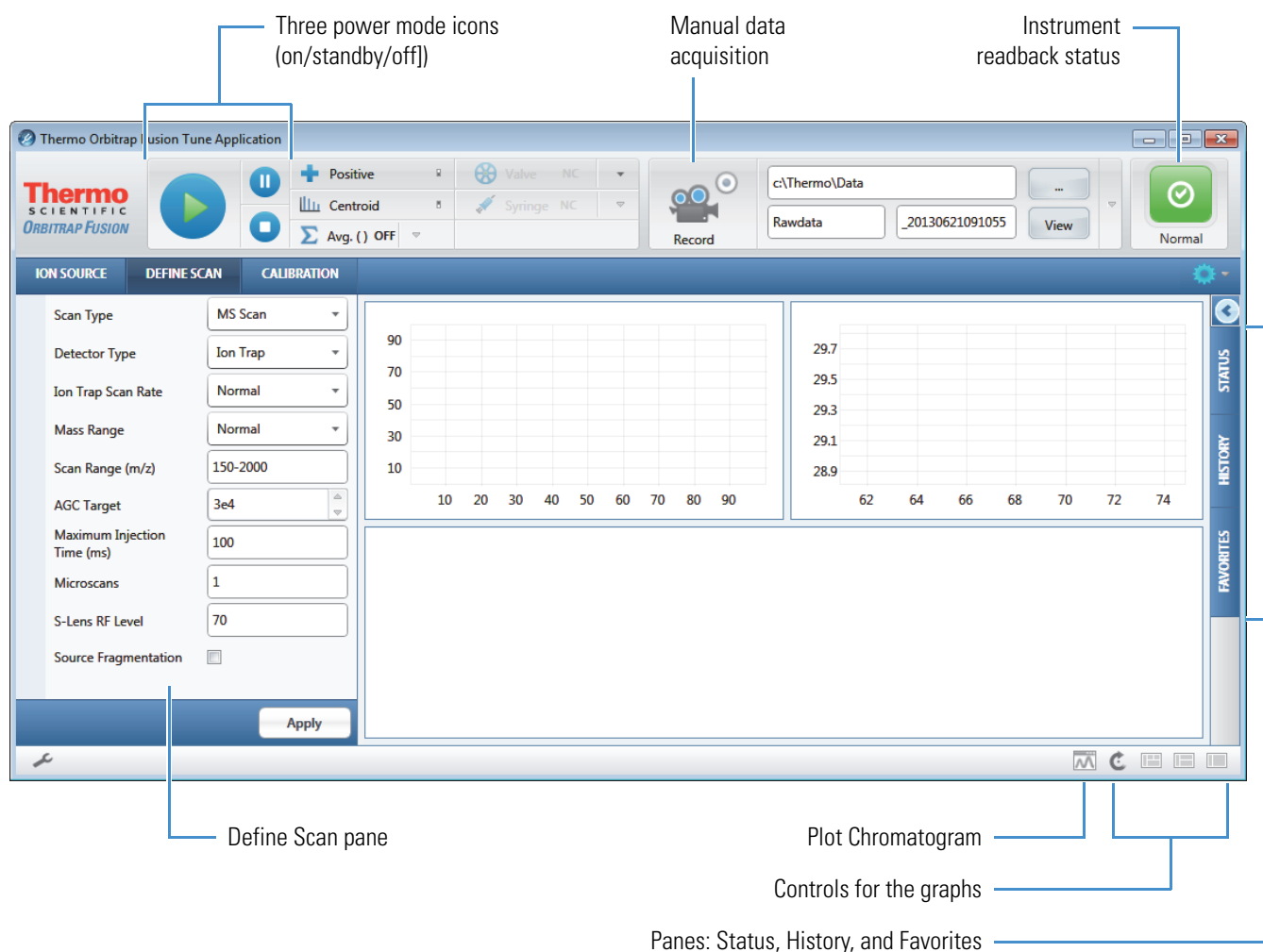
Opening the Tune Window

❖ To open the Tune window

On the Windows taskbar, choose **Start > All Programs > Thermo Instruments > Thermo Orbitrap Fusion > Thermo Orbitrap Fusion Tune** to open the Tune window (Figure 43).

For information about the buttons and controls, refer to the Tune Help.

Figure 43. Tune window showing the Define Scan pane



Setting the Instrument Power Mode

Use the three power mode icons in the Tune window (Figure 43 on page 70) to set the mass spectrometer's power mode (on, standby, and off).

When you remove the API source housing or the spray insert, the mass spectrometer automatically switches to off mode.

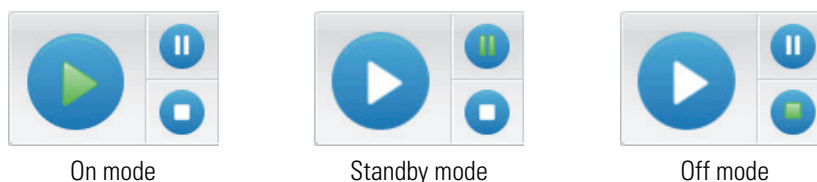
In standby mode, the System LED on the front panel turns yellow and the mass spectrometer turns off the [electron multipliers](#), [conversion dynodes](#), 8 kV power to the API source, main rf voltage, and ion optic rf voltages. The auxiliary, sheath, and sweep gas flows remain on and return to their standby default settings (2 arbitrary). For a list of the on/off status of the mass spectrometer components under varying power conditions, refer to Chapter 6 in the *Orbitrap Fusion Hardware Manual*.

❖ To set the instrument power mode

Click the icon for the power mode that you want (Figure 44).

The center of the selected icon changes from white to green.

Figure 44. Power mode icons showing the selected icon (mode)






Checking the Instrument Readback Status

The system readback icon is located in the top, right corner of the Tune window. Table 14 lists the various readback states.

Table 14. Instrument readback icons and their meanings (Sheet 1 of 2)

Icon	Background color	Meaning
	Green	Normal
	Green	Busy
	Amber	Waiting for the settings to change
	Yellow	Warning

Table 14. Instrument readback icons and their meanings (Sheet 2 of 2)

Icon	Background color	Meaning
	Red	Error
	Gray	Power off
	Dark gray	Disconnected data system communication

Controlling the Syringe Pump

Follow these procedures, as applicable:

- [Using the Favorites Pane to Save System Settings](#)
- [To set the syringe pump parameters](#)

❖ To turn the syringe pump on or off

Click **Syringe On (Off)** to switch between on and off ([Figure 45](#)).

Figure 45. Buttons for the syringe modes



❖ To set the syringe pump parameters


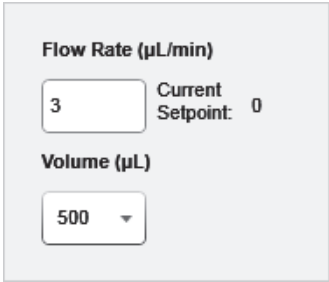
1. Click the dropdown arrow, , next to the Syringe On/Off button to open the syringe parameter box ([Figure 46](#)).

Figure 46. Syringe parameter box



Flow Rate (µL/min)

3 Current Setpoint: 0

Volume (µL)

500 ▼

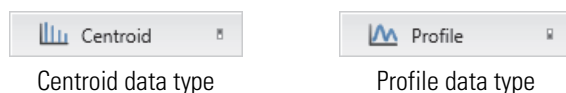
2. Type the parameter values that you want.
The Tune application automatically saves the values.
3. Click the dropdown arrow again or click elsewhere in the Tune window to close the syringe parameter box.

Setting the Data Type

❖ To set the data type

Click **Centroid (Profile)** to select the data type that you want (Figure 47).

Figure 47. Buttons for the data types

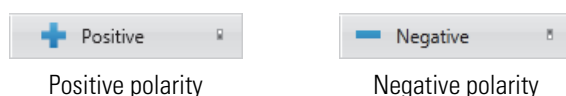


Setting the Ion Polarity Mode

❖ To set the ion polarity mode

Click **Positive (Negative)** to select the polarity mode that you want (Figure 48).

Figure 48. Buttons for the instrument polarity modes



Setting the Tune Preferences

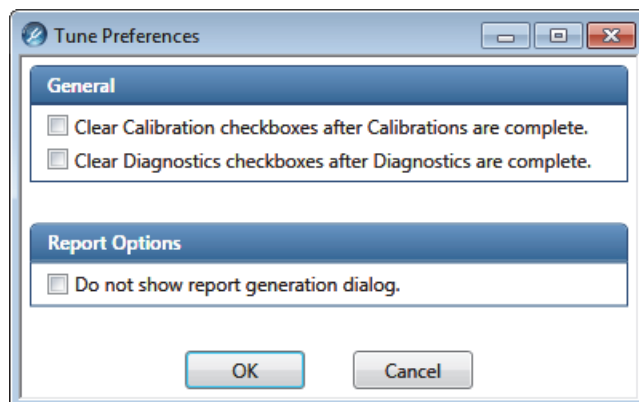
You can set a few preferences for how the Tune application works.

❖ To set the Tune preferences



1. Click the **Options** icon, and then choose **Tune Preferences** to open the Tune Preferences dialog box (Figure 49).

Figure 49. Tune Preferences dialog box



2. Select the check box for each preference that you want, and then click **OK**.


Using the MSⁿ Setting Table in the Define Scan Pane

The MSⁿ Setting Table appears when you select the MSⁿ Scan type in the Define Scan pane. Use this table to specify one or more precursor ions. To set different scan parameters for the precursor ions, add the parameters to the MSⁿ Setting Table.


- [To add a row to the table](#)
- [To delete a row from the table](#)
- [To delete multiple rows from the table](#)
- [To add or remove scan parameters from the table](#)

❖ To add a row to the table


Do one of the following:

- Click the **Add Row** icon, .
- Right-click the table, and then choose **Add Row** from the shortcut menu.

❖ To delete a row from the table

1. Select the row number to highlight the entire row.
2. Do one of the following:
 - Click the **Delete Selected Rows** icon, .
 - Right-click the selected row, and then choose **Delete Selected Rows** from the shortcut menu.
 - Press the DELETE key on your keyboard.

❖ To delete multiple rows from the table

1. Select the first row's number to highlight the entire row.
2. Do one of the following:
 - For an adjacent row or group of sequential rows, hold down the SHIFT key and select another row number.
 - For an adjacent row or nonsequential rows, hold down the CTRL key and select each additional row number.
3. Do one of the following:
 - Click the **Delete Selected Rows** icon, .
 - Right-click the selected row, and then choose **Delete Selected Rows** from the shortcut menu.
 - Press the DELETE key on your keyboard.

❖ **To add or remove scan parameters from the table**



Click the **Table** icon once to add the adjacent scan parameter to the table. Click it again to remove the parameter from the table.

Figure 50 shows an example with Activation Type added to the MSⁿ Settings Table.

Figure 50. Activation Type added to the MSⁿ Setting Table

The screenshot shows the MSⁿ Setting Table interface. The top section contains various parameters with their values: Isolation Mode (Quadrupole), Isolation Width (m/z) (2), Activation Type (CID), CID Collision Energy (%) (30), HCD Collision Energy (%) (30), Supplemental Activation (unchecked), Precursor Charge State (1), and Source Fragmentation (unchecked). The bottom section is the MSⁿ Setting Table, which has columns for Precursor (m/z) and Activation Type. The table contains two rows: Row 1 with Precursor (m/z) 609 and Activation Type CID; Row 2 with Precursor (m/z) 609.2 and Activation Type HCD. A dropdown menu is open for the Activation Type of Row 1, showing options: CID, HCD, and ETD. An 'Apply' button is at the bottom right.

This parameter is selected.

Selecting Activation Type activates these three parameters.

The Edit icon appears when you select a cell.

	Precursor (m/z)	Activation Type
1	609	CID
2	609.2	HCD

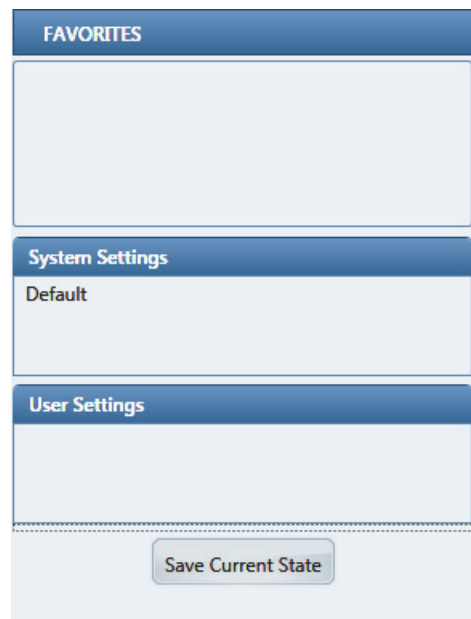
Using the Favorites Pane to Save System Settings

You can manually save the current settings for the ion source and scan parameters in the Favorites pane.

❖ To save the settings as a favorite state

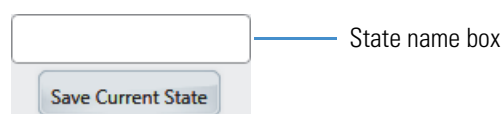
1. In the Tune window, open the Favorites pane (Figure 51).

Figure 51. Favorites pane



2. Click **Save Current State**, and then type a unique name in the box (Figure 52).

Figure 52. State name box



3. Click **Save Current State** again to save the state.

The most recent state appears at the top of the User Settings list.

❖ To apply, load, delete, or rename a favorite state

Under User Settings, right-click the state name, and then choose **Apply**, **Load**, **Delete**, or **Rename**, as applicable, from the shortcut menu.

Flushing the Inlet Components

This appendix describes how to flush the inlet components (sample transfer line, sample tube, and spray insert) after both the positive and negative calibration processes, and also before you change from one analyte solution to another.

In addition, Thermo Fisher Scientific recommends that you clean the ion sweep cone, the spray cone, and the ion transfer tube, on a regular basis to prevent corrosion and to maintain optimum performance of the API source. A good practice is to wash or flush the ion sweep cone and ion transfer tube at the end of each operating day after you pump a solution of 50:50 methanol/water from the LC system through the inlet components. If you use a mobile phase that contains a nonvolatile buffer or inject high concentrations of sample, you might need to clean these parts more often. Be aware that it is not necessary to vent the system to flush the ion sweep cone and ion transfer tube.

For instructions on how to clean the ion sweep cone, spray cone, and ion transfer tube, refer to the section “API Source Interface Maintenance,” in Chapter 8 of the *Orbitrap Fusion Hardware Manual*.



CAUTION When the ion transfer tube is installed, do not flush it with cleaning solution, which flushes the residue into the mass spectrometer.

Contents

- [Supplies](#)
- [Flushing the Inlet Components after Calibration](#)

Supplies



Table 15 lists the necessary supplies for flushing and cleaning specific components.

CAUTION Avoid exposure to potentially harmful materials.

By law, producers and suppliers of chemical compounds are required to provide their customers with the most current health and safety information in the form of Material Safety Data Sheets (MSDSs) or Safety Data Sheets (SDSs). The MSDSs and SDSs must be freely available to lab personnel to examine at any time. These data sheets describe the chemicals and summarize information on the hazard and toxicity of specific chemical compounds. They also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures to remedy spills or leaks.

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

Table 15. Supplies

Description	Part number
Gloves, lint-free and powder-free	Fisher Scientific: <ul style="list-style-type: none">• 19-120-2947A (size small)• 19-120-2947B (size medium)• 19-120-2947C (size large)• 19-120-2947D (size X-large) Thermo Scientific: <ul style="list-style-type: none">• 23827-0008 (size medium)• 23827-0009 (size large)
Methanol, LC/MS-grade	Fisher Scientific: A456-1
Water, LC/MS-grade	Fisher Scientific: W6-1

Flushing the Inlet Components after Calibration

This section describes how to flush the inlet components (sample transfer line, sample tube, and spray insert) after calibration. For best results, follow this procedure before you acquire data on an analyte.

❖ To flush the inlet components



1. Turn off the flow from the syringe pump (see [page 76](#)).
2. Place the mass spectrometer in **Standby** mode (see [page 71](#)).
3. Remove the syringe from the syringe pump as follows:
 - a. Lift the syringe holder off of the syringe.
 - b. Press the pusher block's release knob and slide the block to the left.
 - c. Remove the syringe from the holder.
 - d. Carefully remove the syringe needle from the Teflon tube on the syringe adapter assembly ([Figure 9](#) on [page 22](#)).
4. Clean the syringe as follows:
 - a. Rinse the syringe with a solution of 50:50 methanol/water.
 - b. Rinse the syringe with acetone several times.
5. Flush the sample transfer line, sample tube, and spray insert as follows:
 - a. Load the cleaned syringe with a solution of 50:50 methanol/water (or another appropriate solvent).
 - b. Carefully reinsert the syringe needle into the Teflon tube on the syringe adapter assembly.
 - c. Slowly depress the syringe plunger to flush the sample transfer line, sample tube, and spray insert with the solution.
 - d. Remove the syringe needle from the syringe adapter assembly.

This completes the procedure to flush the inlet components. Repeat this procedure after you complete the negative polarity calibrations.

Preparing the Reserpine Sample Solution

This appendix, for use by the Thermo Fisher Scientific field service engineer, describes how to prepare the 50 fg/ μ L reserpine sample solution for H-ESI and APCI modes. The procedure calls for potentially hazardous chemicals, including methanol and reserpine.

For a list of solvent recommendations, refer to the *Orbitrap Fusion Preinstallation Requirements Guide*. For a complete selection of LC/MS-grade consumables from Thermo Fisher Scientific, visit www.FisherLCMS.com.

IMPORTANT

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



CAUTION Avoid exposure to potentially harmful materials.

By law, producers and suppliers of chemical compounds are required to provide their customers with the most current health and safety information in the form of Material Safety Data Sheets (MSDSs) or Safety Data Sheets (SDSs). The MSDSs and SDSs must be freely available to lab personnel to examine at any time. These data sheets describe the chemicals and summarize information on the hazard and toxicity of specific chemical compounds. They also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures to remedy spills or leaks.

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

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

❖ **To prepare the reserpine sample solution**

1. Transfer 100 μL of the 100 $\text{pg}/\mu\text{L}$ reserpine standard solution into a clean, minimum 1.5 mL polypropylene tube.

The [Orbitrap Fusion Chemicals Kit](#) contains the reserpine standard solution.

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

Glossary

A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

A

activation time The time in milliseconds that the rf used for fragmentation is applied in an ion trap. The activation time value is 10 ms (not a user variable). In general, shorter activation time results in less fragmentation and a longer activation time results in more fragmentation.

APCI spray current The ion current carried by the charged particles in the APCI source. The APCI corona discharge voltage varies, as required, to maintain the set spray current.

API source The sample interface between the liquid chromatograph (LC) and the mass spectrometer (MS).

atmospheric pressure chemical ionization (APCI) A soft ionization technique done in an ion source operating at atmospheric pressure. Electrons from a corona discharge initiate the process by ionizing the mobile phase vapor molecules, forming a reagent gas.

atmospheric pressure ionization (API) Ionization performed at atmospheric pressure by using atmospheric pressure chemical ionization (APCI), heated-electrospray (H-ESI), or nanospray ionization (NSI).

auxiliary gas The outer-coaxial gas (nitrogen) that assists the sheath (inner-coaxial) gas in dispersing and/or evaporating sample solution as the sample solution exits the ESI or APCI (optional) spray insert.

C

centroid data Data used to represent mass spectral peaks in terms of two parameters: the centroid (the weighted center of mass) and the intensity. The data is displayed as a bar graph. The normalized area of the peak provides the mass intensity data.

charge state The imbalance between the number of protons (in the nuclei of the atoms) and the number of electrons that a molecular species (or adduct ion) possesses. If the species possesses more protons than electrons, its charge state is positive. If it possesses more electrons than protons, its charge state is negative.

collision energy The energy used when ions collide with the collision gas.

collision gas A neutral gas used to undergo collisions with ions.

collision-induced dissociation (CID) A method of fragmentation where ions are accelerated to high-kinetic energy and then allowed to collide with neutral gas molecules such as helium for the Orbitrap Fusion MS. The collisions break the bonds and fragment the ions into smaller pieces.

conversion dynode A highly polished metal surface that converts ions from the mass analyzer into secondary particles, which enter the electron multiplier.

D

damping gas Helium gas introduced into the ion trap mass analyzer that slows the motion of ions entering the mass analyzer so that the ions can be trapped by the rf voltage fields in the mass analyzer.

divert/inject valve A valve on the mass spectrometer that can be plumbed as a loop injector or as a divert valve.

E

electron multiplier A device used for current amplification through the secondary emission of electrons. Electron multipliers can have a discrete dynode or a continuous dynode.

electron transfer dissociation (ETD) A method of fragmenting peptides and proteins. In ETD, singly charged reagent anions transfer an electron to multiply protonated peptides within the linear ion trap mass analyzer. This leads to a rich ladder of sequence ions derived from cleavage at the amide groups along the peptide backbone. Amino acid side chains and important modifications such as phosphorylation are left intact.

electrospray (ESI) A type of atmospheric pressure ionization that is currently the softest ionization technique available to transform ions in solution into ions in the gas phase.

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

F

flow rate, syringe pump status The syringe pump injection flow rate in milliliters per minute (mL/min) or microliters per minute (μL/min) for the current sample, as defined in the current experiment method.

forepump The pump that evacuates the foreline. A rotary-vane pump is a type of forepump. It might also be referred to as a backing, mechanical, rotary-vane, roughing, or vacuum pump.

fragment ion A charged dissociation product of an ionic fragmentation. Such an ion can dissociate further to form other charged molecular or atomic species of successively lower formula weights.

full-scan type Provides a full mass spectrum as opposed to the selected ion monitoring (SIM) scan type, which produces only one mass. With the full-scan type, the mass analyzer is scanned from the first mass to the last mass without interruption. Also known as single-stage full-scan type.

H

heated-electrospray (H-ESI) A type of atmospheric pressure ionization that converts ions in solution into ions in the gas phase by using electrospray (ESI) in combination with heated auxiliary gas.

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

high performance liquid chromatography (HPLC) Liquid chromatography where the liquid is driven through the column at high pressure. Also known as high pressure liquid chromatography.

higher energy collision-induced dissociation (HCD) Collision-induced dissociation that occurs in the ion-routing multipole. The ion-routing multipole consists of a straight multipole mounted inside a collision gas-filled tube. A voltage offset between the C-trap and multipole accelerates precursor ions into the collision gas inside the multipole, which causes the ions to fragment into product ions. The product ions are then returned to the Orbitrap mass analyzer for mass analysis. HCD produces triple quadrupole-like product ion mass spectra.

I

ion detection system The ion detection system is a high sensitivity, off-axis system for detecting ions. It produces a high signal-to-noise ratio (S/N) and allows for switching of the voltage polarity between

positive ion and negative ion modes of operation. The ion detection system includes two ± 12 kVdc conversion dynodes and a discrete dynode electron multiplier.

ion optics Focuses and transmits ions from the API source to the mass analyzer.

ion polarity mode The mass spectrometer can operate in either of two ion polarity modes: positive or negative.

ion sweep cone A removable cone-shaped metal cover that fits on top of the API ion transfer tube and acts as a physical barrier to protect the entrance of the tube.

isolation width The baseline width of a window for a mass peak (or peak cluster) of interest for an MS/MS or MSⁿ scan.

ion-routing multipole The collision cell where higher energy collision-induced dissociation (HCD) takes place.

L

LC pump A high pressure solvent pump in the LC that provides the pressure on the input side of a column to drive the eluent and sample through the column.

lens An element that provides focusing of the ion beam.

M

mass analysis A process that produces a mixture of ionic species that is then separated according to the mass-to-charge ratios (m/z) of the ions to produce a mass spectrum.

mass analyzer A device that determines the mass-to-charge ratios (m/z) of ions by one of a variety of techniques.

mass spectrometer An instrument that ionizes sample molecules and then separates the ions according to their mass-to-charge ratio (m/z). The resulting mass spectrum is a characteristic pattern for the identification of a molecule.

mass spectrum A graphical representation (plot) of measured ion abundance versus mass-to-charge ratio. The mass spectrum is a characteristic pattern for the identification of a molecule and is helpful in determining the chemical composition of a sample.

mass-to-charge ratio (m/z) An abbreviation used to denote the quantity formed by dividing the mass of an ion (in Da) by the number of charges carried by the ion. For example, for the ion C₇H₇²⁺, $m/z = 45.5$.

molecular ion An ion formed by the removal (positive ion) or addition (negative ion) of one or more electrons to/from a molecule without fragmentation of the molecular structure.

MS scan modes Scan modes where only one stage of mass analysis is performed. The scan types used with the MS scan modes are full-scan type and selected ion monitoring (SIM) scan type.

MSⁿ scan mode Scan modes where 2 to 10 stages of mass analysis are performed. The scan power equals 2 to 10, where the scan power is the power n in the expression MSⁿ. MSⁿ is the most general expression for the scan mode, which can include the following:

- The scan mode corresponding to the two or more stages of mass analysis in a two-stage full- or narrow-scan experiment.
- The scan mode corresponding to the 3 to 10 stages of mass analysis ($n = 3$ to $n = 10$) in a multistage full-scan experiment.

N

nano liquid chromatography (nanoLC) Liquid chromatography with typical flow rates of 10–1000 nL/min and 10–150 μ m diameter columns.

nanoelectrospray ionization (nanoESI or NSI) A type of electrospray (ESI) that accommodates very low flow rates of sample and solvent at 1–20 nL/min (for static nanoelectrospray) or 100–1000 nL/min (for dynamic nanoelectrospray, which is also called nanoESI nanoLC gradient separation).

nanoESI nanoLC gradient separation Employs microscale capillary columns to separate the analytes in complex mixtures. The sample is loaded onto a column using an injection valve or a gas pressure vessel. The mixture components are then eluted by a solvent gradient and pumped through the emitter.

nanoESI (NSI) spray current The flow of charged particles in the nanoESI (NSI) source. The voltage on the NSI spray needle supplies the potential required to ionize the particles.

nanoESI (NSI) spray voltage The high voltage that is applied to the spray needle in the nanoESI (NSI) source to produce the NSI spray current as liquid emerges from the nozzle. The NSI spray voltage is selected and set; the NSI spray current varies.

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

P

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

precursor mass The mass-to-charge ratio of a precursor ion. The location of the center of a target precursor-ion peak in mass-to-charge ratio (m/z) units.

product ion An electrically charged fragment of an isolated precursor ion.

product mass The mass-to-charge ratio of a product ion. The location of the center of a target production peak in mass-to-charge ratio (m/z) units.

profile data Data representing mass spectral peaks as point-to-point plots, with each point having an associated intensity value.

Q

qualitative analysis Chemical analysis designed to determine the identity of the components of a substance.

quantitative analysis Chemical analysis designed to determine the quantity or concentration of a specific substance in a sample.

R

reagent carrier gas Ultra-high-purity nitrogen gas used to transfer the reagent to the reagent ion source that is regulated by the backpressure regulator.

relative standard deviation (RSD) A measure of the dispersion of a group of measurements relative to the mean of the group. Relative standard deviation is expressed as a percentage of the average value. The percent relative standard deviation is calculated as:

$$\%RSD = 100 \times (S/\bar{X})$$

where S is the standard deviation and \bar{X} is the sample mean.

retention time (RT) The time after injection at which a compound elutes. The total time that the compound is retained on the chromatograph.

S

sample loop A loop of calibrated volume that is used to perform flow injection analysis.

scan Comprised of one or more microscans. Each microscan is one mass analysis (ion injection and storage/scan-out of ions) followed by ion detection. After the microscans are summed, the scan data is sent to the data system for display and/or storage. The process of ramping the amplitude of the rf and dc voltages on the multipole rods in the mass analyzer to transmit ions from the lowest mass to the highest mass of a specified scan range.

scan mode and scan type combinations A function that coordinates the three processes in the MS detector: ionization, mass analysis, and ion detection. You can combine the various scan modes and scan types to perform a wide variety of experiments.

scan power The power n in the expression MS^n . The number of stages of mass analysis, expressed as MS^n , where n is the scan power. For example, a scan power of $n = 1$ corresponds to an $MS1$ (or MS) scan with one stage of mass analysis. A scan power of $n = 2$ corresponds to an $MS2$ (or MS/MS) scan with two stages of mass analysis. A scan power of $n = 3$ corresponds to an $MS3$ scan with three stages of mass analysis, and so on.

selected ion monitoring (SIM) scan type A scan type where the mass spectrometer acquires and records ion current at only one or a few selected mass-to-charge ratio values.

sheath gas The inner coaxial gas (nitrogen), which is used in the API source to help nebulize the sample solution into a fine mist as the sample solution exits the H-ESI or APCI nozzle.

signal-to-noise ratio (S/N) The ratio of the signal height (S) to the noise height (N). The signal height is the baseline corrected peak height. The noise height is the peak-to-peak height of the baseline noise.

source See [API source](#).

static nanoelectrospray A device that performs continuous analysis of small analyte solution volumes over an extended period of time.

sweep gas Nitrogen gas that flows out from behind the sweep cone in the API source. Sweep gas aids in solvent declustering and adduct reduction.

syringe pump A device that delivers a solution from a syringe at a specified rate.

T

total ion current (TIC) The sum of the ion current intensities across the scan range in a mass spectrum.

Z

Zoom scan type A scan type that provides information about the charge state of one or more ions of interest. Zoom scans are slower scans with higher resolution than normal scans.

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