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Orbitrap Tribrid Series

Getting Started Guide

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DOCUMENTATION SURVEY

ThermoFisher SCIENTIFIC

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Software version: (Thermo) Foundation 3.1 SP5 and later, Xcalibur 4.2 and later, Tune 3.1 and later

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Regulatory Compliance

Thermo Fisher Scientific performs complete testing and evaluation of its products to ensure full compliance with applicable North American and European regulations. Your system meets the applicable requirements in the electromagnetic compatibility (EMC) and product safety standards described in this section.

Unauthorized changes that you make to your system will void regulatory compliance and may defeat the built-in protections for your instrument. Some examples of unauthorized changes include using replacement parts or adding components, options, or peripherals that Thermo Fisher Scientific has not qualified and authorized. Unauthorized changes can also result in bodily injury and/or damage to your system and laboratory.

Ensure continued compliance with regulatory standards:

- Follow all installation instructions provided in the documentation that comes with your system.
- Order replacement parts (as specified in the instrument manual) and additional components, options, and peripherals directly from Thermo Fisher Scientific or an authorized representative.

Regulatory compliance results for the following Thermo Scientific™ mass spectrometers:

- Orbitrap Fusion Lumos
- Orbitrap Fusion and Orbitrap ID-X

Orbitrap Fusion Lumos

Low Voltage Directive 2014/35/EU
This device complies with Low Voltage Directive 2014/35/EU and the harmonized safety standard IEC/EN/CSA/UL 61010-1, 3rd Edition.

EMC Directive 2014/30/EU and other EMC test standards		
This device was tested by TÜV Rheinland of North America and complies with the following EMC standards:		
47 CFR 15, Subpart B, Class A: 2015	EN 61000-3-2: 2006 + A1 + A2	EN 61000-4-5: 2006
CISPR 11: 2009 + A1	EN 61000-3-3: 2008	EN 61000-4-6: 2009
ICES-003: 2014	EN 61000-4-2: 2009	EN 61000-4-8: 2010
EN 55011: 2009 + A1	EN 61000-4-3: 2006 + A1 + A2	EN 61000-4-11: 2004
EN 61326-1: 2013	EN 61000-4-4: 2004 + A1	

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Orbitrap Fusion and Orbitrap ID-X

Low Voltage Directive 2014/35/EU

This device complies with Low Voltage Directive 2014/35/EU and the harmonized safety standard IEC/EN/CSA/UL 61010-1, 3rd Edition.

EMC Directive 2014/30/EU and other EMC test standards

This device was tested by TÜV Rheinland of North America and complies with the following EMC standards:

47 CFR 15, Subpart B, Class A: 2012	EN 61326-1: 2013	EN 61000-4-4: 2004 + A1
CISPR 11: 2009 + A1	EN 61000-3-2: 2006 + A1 + A2	EN 61000-4-5: 2006
AS/NZS CISPR 22: 2009 + A1	EN 61000-3-3: 2008	EN 61000-4-6: 2009
ICES-003: 2012	EN 61000-4-2: 2009	EN 61000-4-8: 2010
EN 55011: 2009 + A1	EN 61000-4-3: 2006 + A1 + A2	EN 61000-4-11: 2004

FCC Compliance Statement

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

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

Notice on the Proper Use of Thermo Scientific Instruments

In compliance with international regulations: This instrument must be used in the manner specified by Thermo Fisher Scientific to ensure protections provided by the instrument are not impaired. Deviations from specified instructions on the proper use of the instrument include changes to the system and part replacement. Accordingly, order replacement parts from Thermo Fisher Scientific or one of its authorized representatives.

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WEEE Directive

2012/19/EU

Thermo Fisher Scientific is registered with B2B Compliance (B2Bcompliance.org.uk) in the UK and with the European Recycling Platform (ERP-recycling.org) in all other countries of the European Union and in Norway.

If this product is located in Europe and you want to participate in the Thermo Fisher Scientific Business-to-Business (B2B) Recycling Program, send an email request to weee.recycle@thermofisher.com with the following information:

- WEEE product class
- Name of the manufacturer or distributor (where you purchased the product)
- Number of product pieces, and the estimated total weight and volume
- Pick-up address and contact person (include contact information)
- Appropriate pick-up time
- Declaration of decontamination, stating that all hazardous fluids or material have been removed from the product

For additional information about the Restriction on Hazardous Substances (RoHS) Directive for the European Union, search for RoHS on the Thermo Fisher Scientific European language websites.

IMPORTANT This recycling program is **not** for biological hazard products or for products that have been medically contaminated. You must treat these types of products as biohazard waste and dispose of them in accordance with your local regulations.

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Preface

The *Orbitrap Tribrid Series Getting Started Guide* is intended for the following Thermo Scientific™ mass spectrometer (MS):

- Orbitrap Fusion™ (also known as Fusion™)
- Orbitrap Fusion Lumos™ (also known as Lumos™)
- Orbitrap ID-X™ (also know as ID-X™)

This guide describes how to set up and calibrate the MS system. Also, because this guide uses drawings of various connections and component parts to help illustrate procedures, be sure to start from 1, no matter where it appears.

Contents

Accessing Documentation

The Orbitrap Tribrid Series MS includes complete documentation.

For system requirements, refer to the release notes on the software DVD.

Viewing the Product Manuals

The Thermo Fisher Scientific service engineer installs the instrument control applications and the instrument manuals on the data system computer.

❖ **To view the product manuals**

From the Microsoft™ Windows™ taskbar, choose **Start > All Apps** (Windows 10) or **All Programs** (Windows 7) **> Thermo Instruments >** ***model x.x***, and then open the applicable PDF file.

Accessing the Help Menu Options

Follow this procedure to view the Help systems for the instrument-control applications.

❖ **To view the Help**

Do the following as applicable:

- Thermo Tune instrument-control application: Click the **Options** icon,  , and choose **Tune Help**.
- Thermo Xcalibur™ Method Editor application: Choose an option from the **Help** menu (or press the F1 key).

Viewing Online User Documentation

Visit the Thermo Fisher Scientific website for product manuals and more.

❖ **To view user documentation from the Thermo Fisher Scientific website**

1. Go to thermofisher.com.
2. Point to **Services & Support** and click **Manuals** on the left.
3. In the Refine Your Search box, search by the product name.
4. From the results list, click the title to open the document in your web browser, save it, or print it.

   To return to the document list, click the browser **Back** button.

Providing Documentation Feedback

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

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

License for the 1M Option

During the Orbitrap Fusion Lumos MS installation or upgrade process, the Thermo Fisher Scientific field service engineer activates the optional 1M license (with purchase of the 1M option).

Special Notices, Symbols, and Cautions

Make sure you understand the special notices, symbols, and caution labels in this guide. Most of the special notices and cautions appear in boxes; those pertaining to safety also have corresponding symbols. Some symbols are also marked on the instrument itself and can appear in color or in black and white. For complete definitions, see Table 1.

Table 1. Notices, symbols, labels, and their meanings (Sheet 1 of 2)

Notice, symbol, or label	Meaning
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 product.
Note	Highlights information of general interest.
Tip	Highlights helpful information that can make a task easier.

Table 1. Notices, symbols, labels, and their meanings (Sheet 2 of 2)

Notice, symbol, or label	Meaning
	**Caution:** Read the cautionary information associated with this task.
	**Chemical hazard:** Observe safe laboratory practices and procedures when handling chemicals. Only work with volatile chemicals under a fume or exhaust hood. Wear gloves and other protective equipment, as appropriate, when handling toxic, carcinogenic, mutagenic, corrosive, or irritant chemicals. Use approved containers and proper procedures to dispose of waste oil and when handling wetted parts of the instrument.
	**Heavy object:** The Orbitrap Tribrid Series MS, excluding its workbench, weighs over 227 kg (500 lb). Never try to detach and move the instrument from its workbench; you can suffer personal injury or damage the instrument.
	**Risk of electric shock:** This instrument uses voltages that can cause electric shock and personal injury. Before servicing the instrument, shut it down and disconnect it from line power. While operating the instrument, keep covers on.
	**Risk of eye injury:** Eye injury can occur from splattered chemicals, airborne particles, or sharp objects. Wear safety glasses when handling chemicals or servicing the instrument.
	**Risk of laser radiation—Orbitrap Fusion Lumos MS with the optional ultraviolet photodissociation (UVPD) module only:** Failure to understand and comply with laser cautions and operating instructions in the *UVPD Module User Guide* can result in property damage or serious injuries to the user.
	**Trip obstacle:** Be aware of cords, hoses, or other objects located on the floor.

Model Differences

This table lists the required number of forepumps and the available options for the Orbitrap Tribrid Series MSs.

Instrument	Number of forepumps	Available options				
		EASY-IC ion source	EASY-ETD ion source	Advanced peak determination	1M Orbitrap resolution	UVPD laser module
Orbitrap Fusion Lumos	2	✔	✔	✔	✔	✔
Orbitrap Fusion	1	✔	✔	✔		
Orbitrap ID-X	1	✔				

Contacting Us

Contact	Email	Telephone	QR Code[a]
U.S. Technical Support	us.techsupport.analyze@thermofisher.com	(U.S.) 1 (800) 532-4752	
U.S. Customer Service and Sales	us.customer-support.analyze@thermofisher.com	(U.S.) 1 (800) 532-4752	
Global Support	❖ **To find global contact information or customize your request** 1. Go to thermofisher.com. 2. Click **Contact Us**, select the country, and then select the type of support you need. 3. At the prompt, type the product name. 4. Use the phone number or complete the online form. ❖ **To find product support, knowledge bases, and resources** Go to thermofisher.com/us/en/home/technical-resources. ❖ **To find product information** Go to thermofisher.com/us/en/home/brands/thermo-scientific.		

Note To provide feedback for this document, go to surveymonkey.com/s/PQM6P62 or send an email message to Technical Publications (techpubs-lcms@thermofisher.com).

[a] You can use your smartphone to scan a QR Code, which opens your email application or browser.

1

Introduction

This chapter provides general information about the Orbitrap Tribrid Series MS. For information about using the Thermo Tune application, see Appendix A, "Using Basic Tune Functions." For information about daily operation, maintenance, and system startup and shutdown, refer to the Hardware Manual.

Note To ensure the proper operation of the MS, Thermo Fisher Scientific recommends that you perform the daily preventive maintenance described in the Hardware Manual.

Contents

Ionization Techniques

This section briefly describes the following ionization modes: heated-electrospray (H-ESI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI), and nanoelectrospray ionization (nanoESI or NSI). For additional information, refer to the ion source manuals.

- H-ESI Mode (Typically preferred for polar compounds)
- APCI Mode (Typically preferred for medium polar compounds)
- APPI Mode (Typically preferred for certain polar and nonpolar compounds)
- NSI Mode (Typically preferred for peptides and proteins)

Note For instruments with the optional EASY-ETD™ source, refer to the *EASY-ETD and EASY-IC Ion Sources User Guide*. For an Orbitrap Fusion Lumos MS with the optional UVPD device, refer to the *UVPD Module User Guide*.

H-ESI Mode

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 MS 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 H-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.

The upper limit for the system's flow rate is 3000 µL/min. However, for optimum H-ESI performance, vary the flow rate[1] into the MS over a range of 1–1000 µL/min, and configure the H-ESI spray insert for the high- or low-flow[2] range as applicable. This flow rate range is optimal for a wide range of separation techniques: capillary electrophoresis (CE), capillary electrochromatography (CEC), analytical LC, capillary LC, and microbore LC. For the lower range, make sure that the H-ESI spray insert contains the low-flow metal needle insert. See Table 2 for H-ESI 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.

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[1] The H-ESI spray insert can generate ions from liquid flows as low as 1 µL/min.

[2] For the range of 1–50 µL/min, install the low-flow needle insert (OPTON-30139) into the H-ESI spray insert.

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. 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, aim for a slight acidic mobile phase (pH 2–5). The acidic pH tends to keep positive ions in solution.

APCI Mode

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 upper limit for the system's flow rate is 3000 µL/min. However, for optimum APCI performance, vary the flow rate[3] into the MS over a range of 200–2000 µL/min. This flow range is optimal for the following separation techniques: analytical LC, microbore LC, and semi-preparative LC. See Table 3 APCI 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.

[3] For the APCI spray insert, flows below 200 µL/min require more care to maintain a stable spray.

APPI Mode

APPI is also a soft ionization technique. In APPI an ion is generated from a molecule when it interacts with a photon from a light source, such as the Syagen™ Technology PhotoMate™ APPI™ light source. APPI generates molecular ions for molecules that have an ionization potential below the photon energy of the light being emitted by the light source.

Molecules that include steroids, basic-drug entities, and pesticides have ionization potentials below the threshold. APPI reduces fragmentation because only a small amount of energy is deposited in the molecule. Molecules, such as the nitrogen sheath and auxiliary gas and the simple solvents used for LC/MS, are not ionized because their ionization potentials are greater than the photon energy. The result is selective ionization of an analyte versus the background.

NSI Mode

Conventional electrospray (ESI) employs flow rates from 1 µL/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. This produces significant increases in sensitivity as demonstrated by the signal response of the MS. See Table 4 for NSI guidelines.

LC Flow Rate Ranges

While changing the flow rate of solvents entering the MS, 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 general guidelines (default parameter values for the ion source) for system operation using H-ESI (Table 2), APCI (Table 3), and NSI (Table 4) for a range of LC solvent flow rates. For the Orbitrap ID-X MS, use the Orbitrap Fusion MS values.

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

LC flow rate (µL/min)	Spray voltage (V)[a]	Gas (arbitrary units)			Ion transfer tube temp (°C)	Vaporizer temp (°C)	Typical nitrogen gas consumption (L/min)
		Sheath	Auxiliary	Sweep			
Up to 15	Pos: 3500 Neg: –2500	5	2	0	Fusion MS: 275 Lumos MS: 325	20	3.5
16–99	Pos: 3500 Neg: –2500	25	5	0	Fusion MS: 275 Lumos MS: 325	75	8.0
100–199	Pos: 3500 Neg: –2500	35	7	0	Fusion MS: 300 Lumos MS: 325	200–275	10.5
200–500	Pos: 3500 Neg: –2500	50	10	1	325	275–350	15.0

[a] Positive and negative polarity modes

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

LC flow rate (µL/min)	Gas (arbitrary units)			Ion transfer tube temp (°C)	Vaporizer temp (°C)	Corona discharge current (µA)[a]
	Sheath	Auxiliary	Sweep			
Up to 5	15	5	0	Fusion MS: 250 Lumos MS: 300	275	Pos: 4 Neg: –10
5–199	25	5	0	Fusion MS: 250 Lumos MS: 300	325	Pos: 4 Neg: –10
200–500	45	5	0	Fusion MS: 275 Lumos MS: 325	350	Pos: 4 Neg: –10

[a] Positive and negative polarity modes

Table 4. Guidelines for setting operating parameters for LC/**NSI**/MS

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

Types of Buffers

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

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

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

For a list of recommended solvents, refer to the 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 MS's optional ion sweep cone.
- Reduce the concentration of buffers to an absolute minimum.

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

Templates in Thermo Xcalibur Instrument Setup (Method Editor)

Use the Method Editor that opens in the 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 name extension). For additional information, refer to the Help.

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

2

Setting Up the Ion Source

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

Shipment of the ion source includes the H-ESI spray insert. For APCI experiments, order the APCI Installation Kit (P/N 80000-62060), which includes the APCI spray insert. For APPI experiments, order the APPI Interface Kit (P/N OPTON-30185).

Contents

Preparing the Mass Spectrometer

Before you install the ion 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 ion source's spray insert or the MS's ion sweep cone.

❖ To prepare the mass spectrometer

1. Complete all data acquisition, as applicable.
2. In the Tune window, place the MS in **Off** mode (Table 9).

   The LC/MS system is now in off mode (see Chapter 6 in the Hardware Manual for a list of components that remain on). After the ion source housing and spray insert 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 ion source housing.

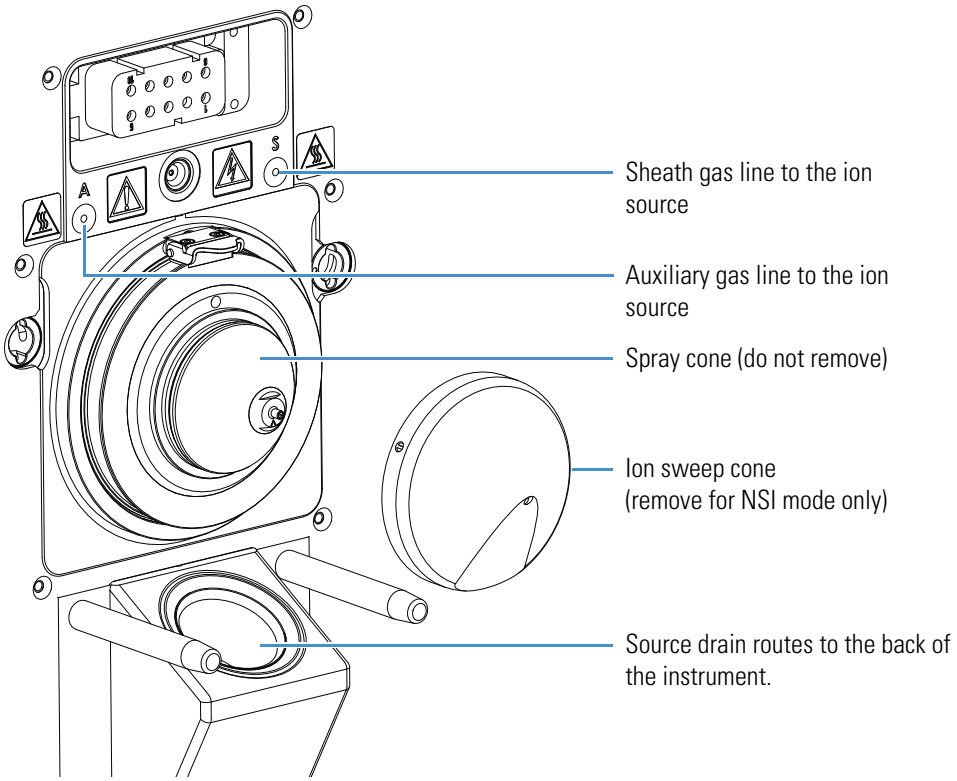
3. If you want to change the installed source, wait until it has cooled to room temperature.

   For instructions on how to remove the source, refer to its manual.

CAUTION Hot surface. Avoid touching the ion source housing when the MS is in operation. The external surface of the housing can become hot enough to burn your skin. Allow the housing to cool before you touch it.

4. Depending on the ionization mode, do the following (Figure 1):
   - For H-ESI, APCI, or APPI mode, install the ion sweep cone over the MS's spray cone (Figure 1).
   - For NSI mode, loosen the screws on the ion sweep cone, and then remove the cone by grasping its outer ridges and pulling it off.

Figure 1. MS ion source mount assembly and ion sweep cone



Installing the Ion Source

All the wiring and gas plumbing for the ion source are internal. This means you can install or remove the source or change the ionization mode[1] (H-ESI, APCI, or APPI) by changing the spray insert—all without the use of tools.

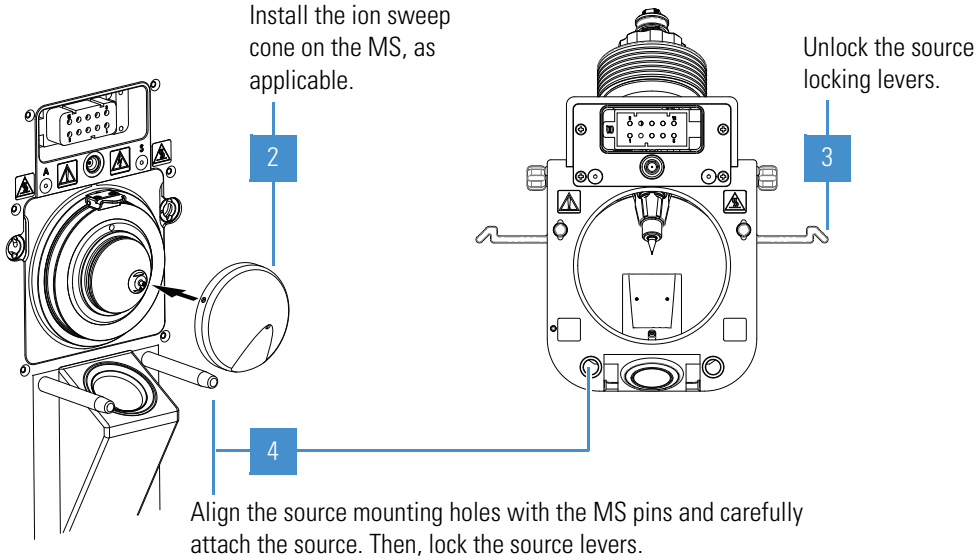
Note The MS internally routes the solvent waste from the bottom of the ion source to the back Drain/Waste port. Make sure that the solvent waste system is connected as described in the Getting Connected Guide.

[1] APCI and APPI modes require that you purchase and install the applicable kits.

❖ **To install the ion source for MS calibrations and experiments**

1

For APCI mode, check that the corona discharge needle assembly (not shown) is installed in the source housing. For instructions, refer to the ion source manual.



Preparing the Spray Insert for the Ion Source

See these topics:

- Installing the Spray Insert
- Spray Insert Positions

Installing the Spray Insert

For information about switching the between ionization modes or changing the needle insert, refer to the ion source manual.

IMPORTANT For calibrations in negative ion polarity mode and experiments with flow rates that are less than 50 µL/min, install the low-flow metal needle insert into the H-ESI spray insert. For part numbers, refer to “Replaceable Parts” in the Hardware Manual.

❖ **To install the spray insert into the ion source**

Do one of the following:

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

–or–

- For APPI mode, you can install either of the spray inserts.

Spray Insert Positions

Table 5 describes the positions of the spray insert within the ion source.

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

Table 5. Guidelines for adjusting the heater and spray insert position

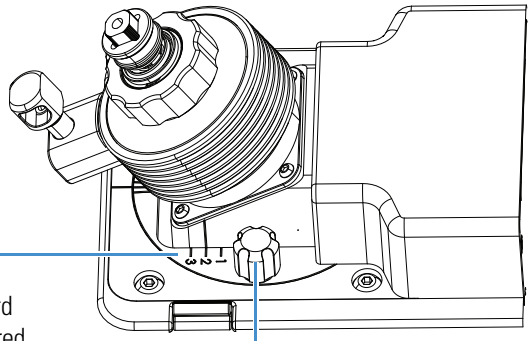
Adjustment control	Description
Front-to-back position	
1	For H-ESI mode, use this position for calibrating the MS and for low liquid flow rates (less than 50 µL/min). In position 1, the spray is closest to the entrance of the MS.
2	(Default) Use this position for liquid flow rates greater than 50 µL/min.
3	Use this position for enhanced robustness, for example, when you use a biological matrix. In position 3, the spray is farthest from the entrance of the MS.
Rotational position	
Left, center, right (marks)	Use the center mark to position the spray closest to the entrance of the MS.

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 ion source parameters for the analytes.

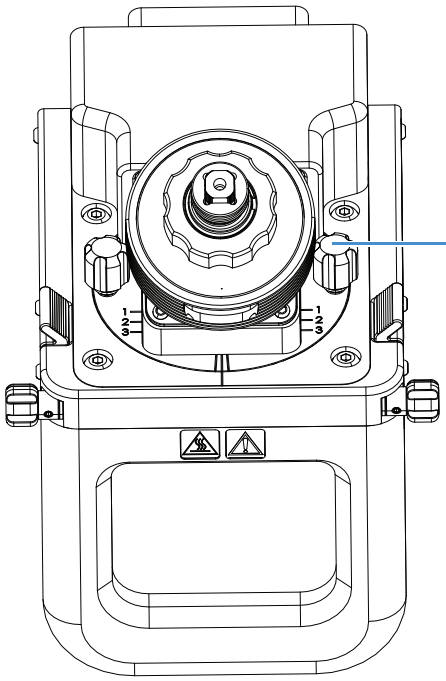
❖ **To adjust the spray direction**

CAUTION Hot surface. Avoid touching the ion source housing when the MS is in operation. The external surface of the housing can become hot enough to burn your skin. Allow the housing to cool before you touch it.



2 Move the heater forward or backward to the desired position (see Table 5).

1 Using your fingers, loosen the top two retainer knobs.



4 Using your fingers, tighten the retainer knobs.

3 Turn the side rotational knob to rotate the heater to the desired position.

Removing the Ion Source

To access the ion sweep cone, ion source interface, ion transfer tube, internal APCI corona needle (APCI-configured source), or internal APPI lamp (APPI-configured source), you must remove the source from the MS.

❖ **To remove the ion source**

1. Complete all data acquisition, as applicable.
2. Turn off the liquid flow from the LC (or other sample introduction device).
3. In the Tune window, place the MS in **Off** mode.

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.
6. Pull the source straight off of the MS.
7. Place the source in a safe location for temporary storage.

Installing the NSI Source

For NSI experiments, remove the MS ion sweep cone (page 10) before you install the NSI source.

❖ **To install the NSI source**

Refer to the NSI source manual.

3

Connecting the Inlet Plumbing

This chapter describes how to set up the inlet plumbing for the sample introduction techniques. Figure 3 shows schematic drawings of these sample introduction techniques. For operational information about the syringe pump and divert/inject valve, refer to the Hardware Manual.

IMPORTANT Compound optimization solutions, such as the reserpine sample solution, can contaminate your system at high concentrations.

The Calibration Kit and Performance Specification Kit contain the required components for the inlet plumbing connections (see page 85).

Contents

Guidelines for PEEK Fittings and Tubing

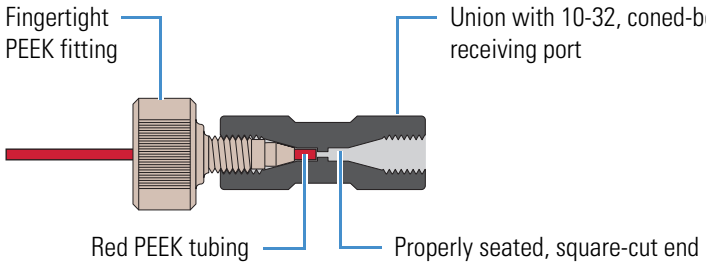
The modular divert/inject valve shipped with your order is a six-port, two-position, Rheodyne™ injection valve. The ports use standard 10-32 fittings for high-pressure and 1/16 in. OD tubing. To connect the high-pressure tubing to the valve, use the one-piece fingertight fittings provided in the Calibration Kit (see page 86).

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 2). 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 LC union's 10-32, coned-bottom receiving port. 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 2. Proper connection for the PEEK tubing and fitting (syringe adapter assembly)



Setting Up the Syringe and Syringe Pump

Use the syringe pump to directly infuse sample into the ion 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.

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

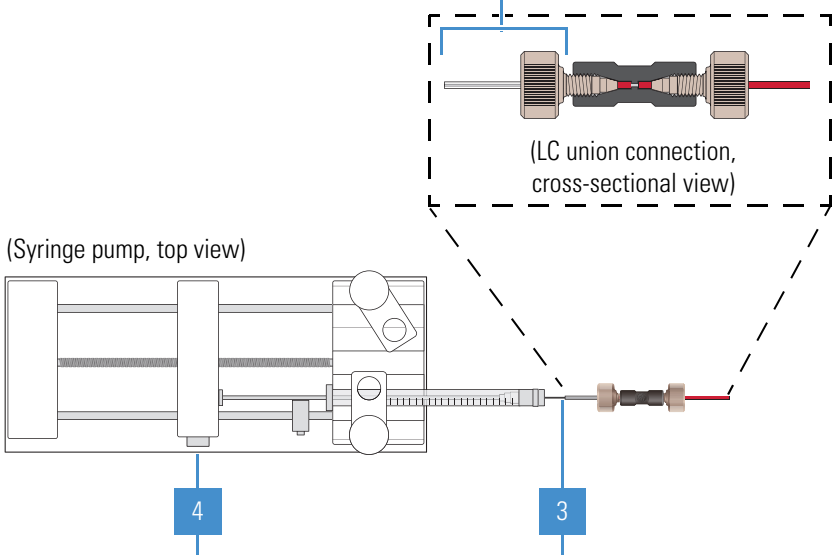
IMPORTANT To minimize the possibility of cross-contamination, do the following:

- Use a dedicated 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 and syringe pump

1 (Not shown) Load a clean, 500 µL syringe with the sample solution.

2 Using a two-piece, fingertight fitting, connect 4 cm (1.5 in.) of Teflon™ tubing to the (black) LC union.



4 Squeeze the syringe pump's release button and slowly move the pusher block until it contacts the syringe plunger.

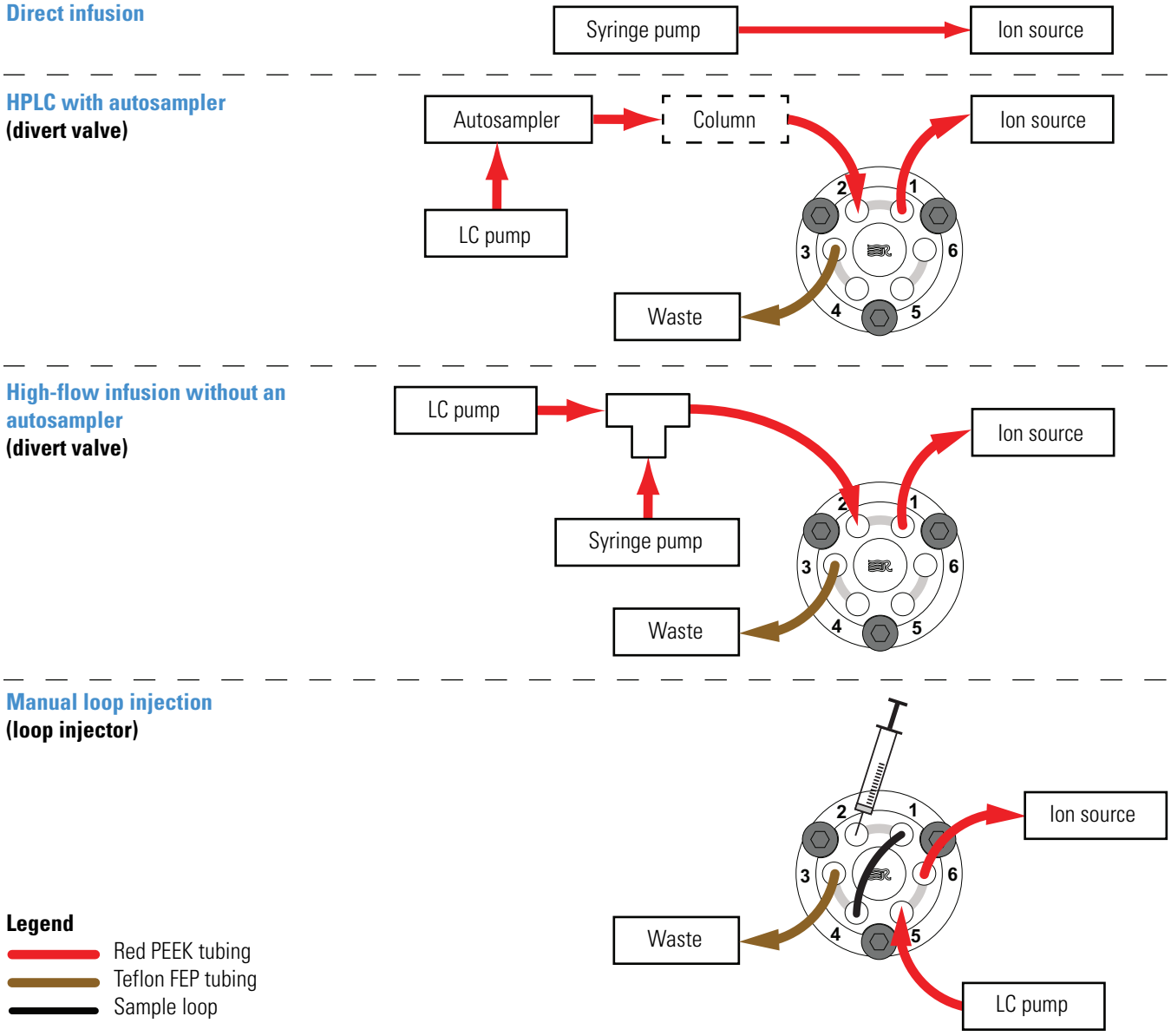
3 Hold the plunger of the syringe in place and carefully insert the tip of the syringe needle into the free end of the Teflon tubing. Then, place the syringe into the syringe pump with the end flange against the pump.

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

Plumbing Schematics for the Sample Introduction Techniques

Figure 3 shows the plumbing schematics for the sample introduction techniques.

Figure 3. Schematics of the sample introduction techniques (examples)



Setting Up the Inlet Plumbing for the Sample Introduction Techniques

The Orbitrap Tribrid Series MS has an external syringe pump and divert/inject valve. The following techniques are available to introduce samples into the ion source:

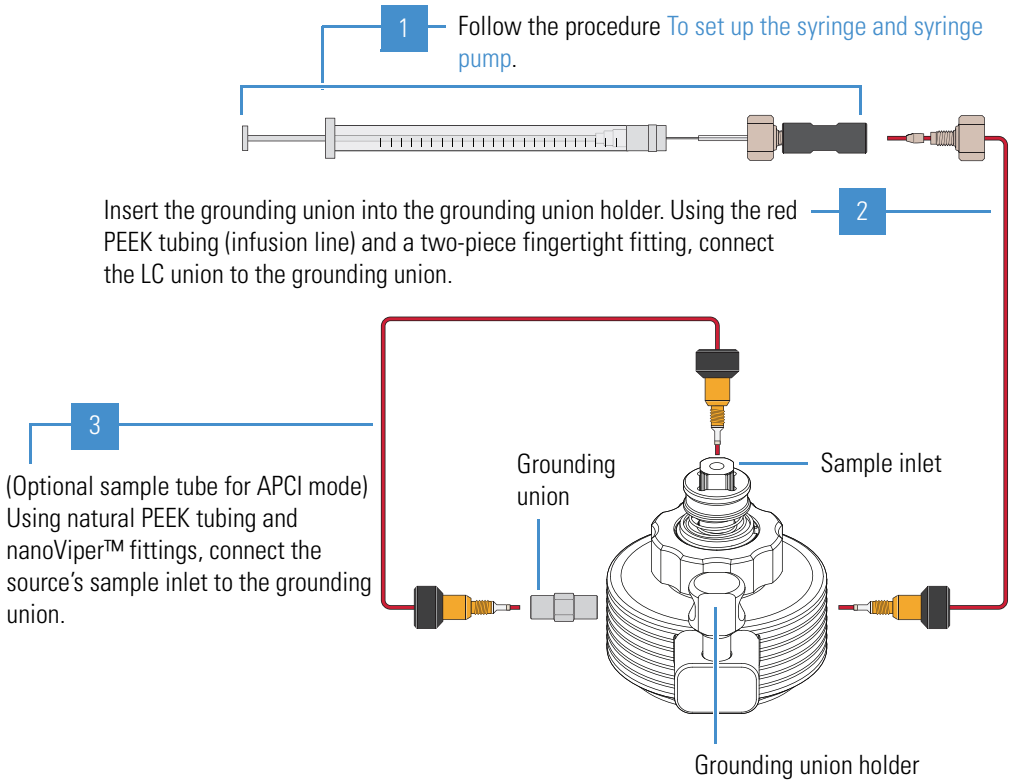
- Setting Up the Inlet for Direct Infusion
- Setting Up the Inlet for HPLC with an Autosampler
- Setting Up the Inlet for High-Flow Infusion Without an Autosampler
- Setting Up the Inlet for Loop Injections (Flow-Injection Analysis)

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

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

❖ **To set up the ion source for direct infusion (H-ESI mode)**



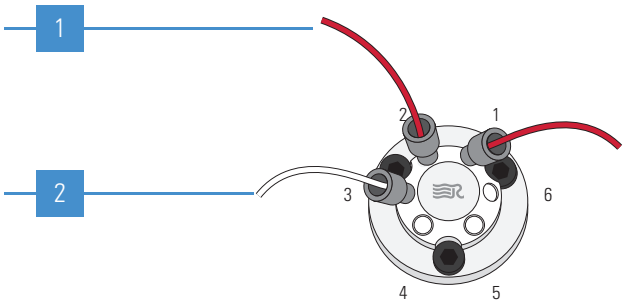
Setting Up the Inlet for HPLC with an Autosampler

To automatically inject a set of samples, connect an LC system with an autosampler to the divert/inject valve and connect the valve to the ion 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 ion source.

❖ To set up the inlet for HPLC infusion with an autosampler (H-ESI or APCI mode)

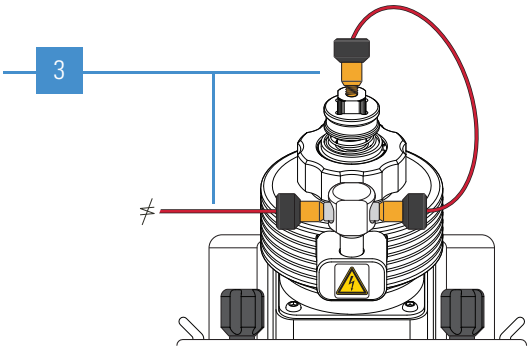
Using red PEEK tubing, connect **port 2** (with a one-piece fingertight fitting) to the outlet of the autosampler or LC pump (with an appropriate fitting and ferrule). (Optional) Install an LC column between the LC device and the divert valve.

Using a Rheodyne fitting, connect one end of the Teflon tubing to **port 3**. Place the other end into an appropriate waste container.



Divert valve

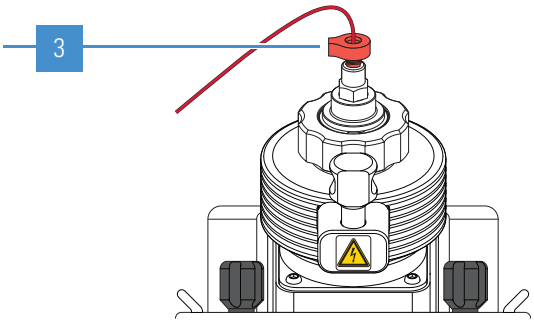
(H-ESI mode) Using two pieces of red PEEK tubing, make these connections:
- Connect **port 1** (with a one-piece fingertight fitting) to the installed grounding union (with a two-piece fingertight fitting).
- Connect the other end of the grounding union (with a two-piece fingertight fitting) to the H-ESI spray insert's sample inlet (with a one-piece fingertight fitting).



H-ESI mode

(APCI mode) Using red PEEK tubing, connect **port 1** (with a one-piece fingertight fitting) to the APCI spray insert's sample inlet (with a two-piece fingertight fitting).
(Optional, not shown) If you installed the H-ESI plumbing path, you can connect the tubing to the installed grounding union.

Note For APCI mode, you do not need to remove the grounding bar if you choose not to use the H-ESI plumbing path.



APCI mode

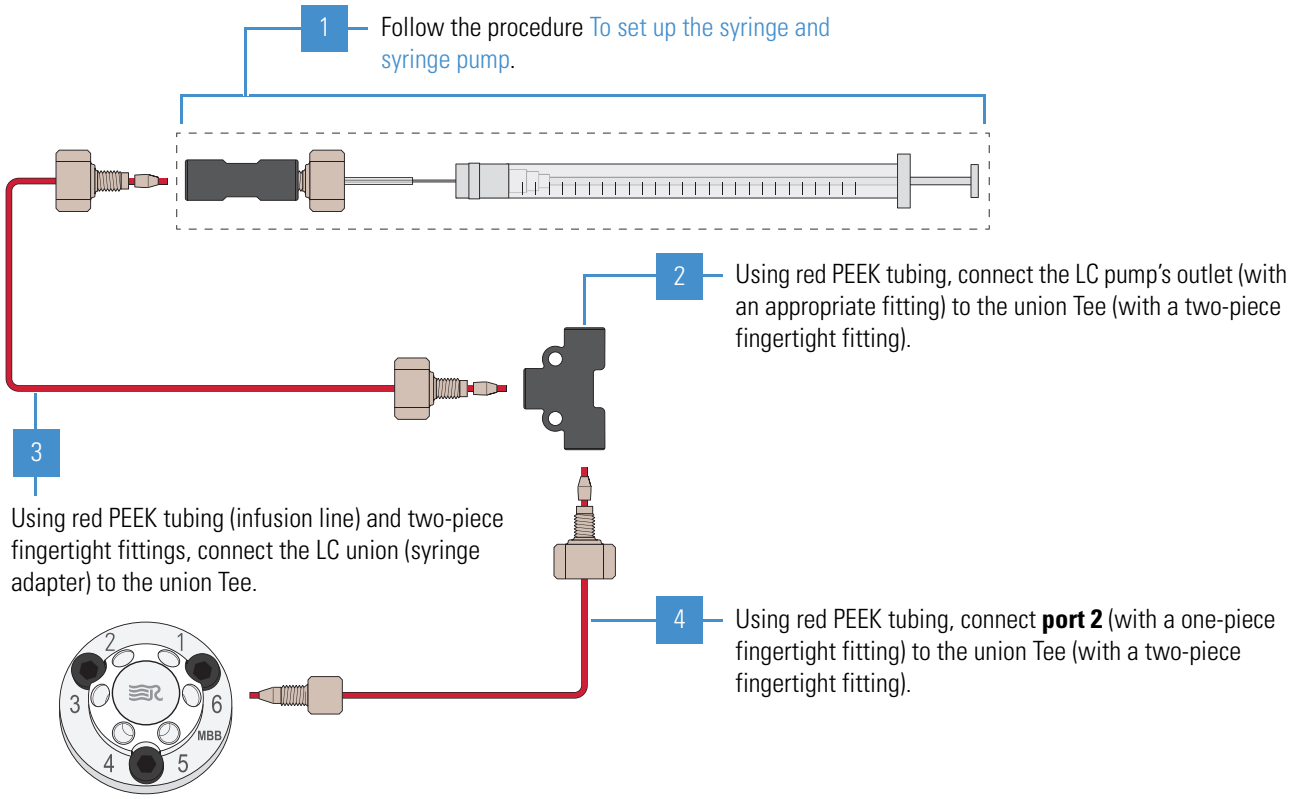
Setting Up the Inlet for High-Flow Infusion Without an Autosampler

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 ion source. Use this infusion method to perform experiments at a higher flow rate with an LC system. The high-flow infusion method allows you to optimize the source parameters (such as sheath gas and vaporizer temperature) at the flow rate and mobile phase composition of the assay.

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 ion 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 information about the valve configurations, refer to the Hardware Manual.

❖ **To set up the ion source for high-flow infusion without an autosampler**



4 (Not shown) Make the following connections:
- Using red PEEK tubing, connect **port 1** (with a one-piece fingertight fitting) to the ion source (with a two-piece fingertight fitting).
- Using a Rheodyne fitting, connect one end of the Teflon tubing to **port 3**. Place the other end into an appropriate waste container.

Setting Up the Inlet for Loop Injections (Flow-Injection Analysis)

Use the loop injection technique when there is a limited amount of sample. To use this technique, attach a sample loop, an injection port fitting (needle port), and an LC pump to the divert/inject valve, and then connect the valve to the ion 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 ion 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 ion source.

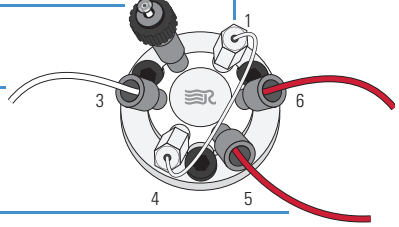
❖ To set up the inlet for manual-loop injection (H-ESI or APCI mode)

1 Connect a sample loop across **ports 1** and **4**.

2 Connect a loop filler (needle port) to **port 2**.

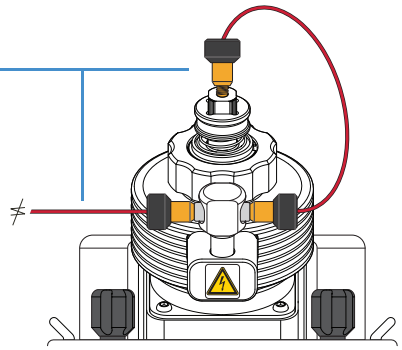
3 Using a Rheodyne fitting, connect one end of the Teflon tubing to **port 3**. Place the other end into an appropriate waste container.

4 Using red PEEK tubing, connect **port 5** (with a one-piece fingertight fitting) to the LC pump outlet (with an appropriate fitting and ferrule).



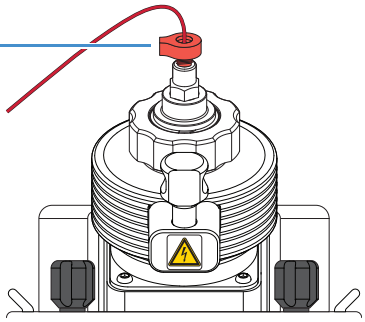
Loop injector valve

5 (H-ESI mode) Using two pieces of red PEEK tubing, make these connections:
- Connect **port 6** (with a one-piece fingertight fitting) to the installed grounding union (with a two-piece fingertight fitting).
- Connect the other end of the grounding union (with a two-piece fingertight fitting) to the H-ESI spray insert's sample inlet (with a one-piece fingertight fitting).


H-ESI mode

5 (APCI mode) Using red PEEK tubing, connect **port 6** (with a one-piece fingertight fitting) to the APCI spray insert's sample inlet (with a two-piece fingertight fitting).
(Optional, not shown) If you installed the H-ESI plumbing path, you can connect the tubing to the installed grounding union.

Note For APCI mode, you do not need to remove the grounding bar if you choose not to use the H-ESI plumbing path.


APCI mode

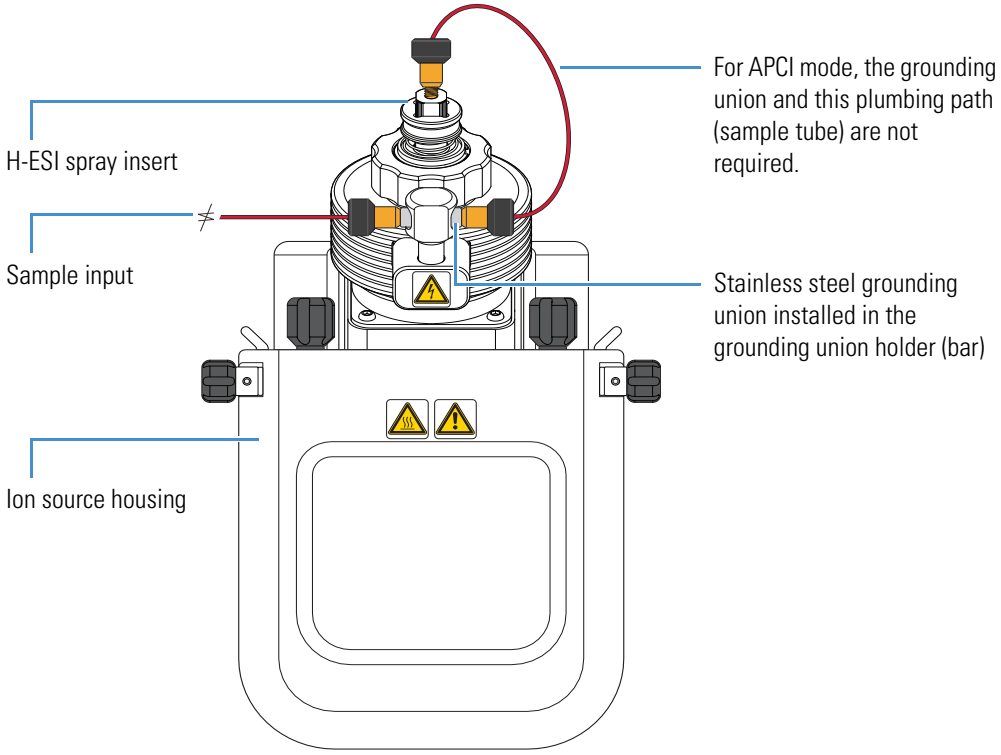
6 (Not shown) Load a clean syringe with the sample solution, carefully insert it into the needle port that is installed in **port 2**, and then slowly press the syringe plunger.

Connecting the Grounding Union to the H-ESI Spray Insert

Figure 4 shows the connection between the grounding union and the H-ESI spray insert's sample inlet.

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



4

Preparing the System for Calibration

Prepare the Orbitrap Tribrid Series system before you calibrate the instrument.

IMPORTANT

- For calibrations in negative ion polarity mode and experiments with flow rates that are less than 50 µL/min, install the low-flow metal needle insert into the H-ESI spray insert. For part numbers, refer to "Replaceable Parts" in the Hardware Manual.
- If the MS was turned off for an extended period of time, pump down the vacuum system for at least 15 hours and complete the bakeout period before you start the instrument calibration process. For instructions, refer to Chapter 8, "Maintenance," in the Hardware Manual.
- 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 MS, refer to the *EASY-ETD and EASY-IC Ion Sources User Guide* for the applicable figures.

Contents

Setting Up the Syringe Pump for Direct Infusion

Use the syringe pump to infuse the calibration solution directly into the H-ESI source.

IMPORTANT To minimize the possibility of cross-contamination, use a dedicated 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. Load a clean, 500 µL syringe with 500 µL of the ESI positive calibration solution.

   For a list of provided solutions, see Orbitrap Tribrid Series Chemicals Kit.

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 2).

2. Follow the procedure Setting Up the Inlet for Direct Infusion.
3. Turn on the syringe pump's power switch (on the back of the device).
4. In the Tune window, place the MS in **Standby** mode.

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.

Go to the next section.

Related Topics

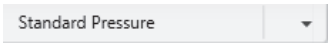
- Guidelines for PEEK Fittings and Tubing
- Syringe pump (refer to the Hardware Manual)

Setting Up the Mass Spectrometer for Calibration

Before you calibrate the MS, set up the operational parameters.

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

❖ To set up the MS for calibration

1. In the Tune window, place the MS in **On** mode,  .
2. Click **Profile (Centroid)** and select the profile data type.
3. Click **Positive (Negative)** and select the positive ion polarity mode.
4. (Orbitrap Fusion and Orbitrap Fusion Lumos MSs) Select **Standard Pressure** mode, 

.
5. Set the syringe pump parameters as follows:
   a. Click **Syringe Off** to turn on the syringe pump.

      The button name changes to Syringe On.

b. Click the arrow next to the Syringe On/Off button to open the syringe pump settings box (Figure 5), and then enter the following:

Flow Rate (µL/min): **3**

Volume (µL): **500**

Figure 5. Syringe pump settings box

c. Click **Apply**.

6. Verify that the inlet plumbing connections do not leak.
7. Open the syringe pump settings box again, and press and hold **Prime** to prime the syringe.

   The preset priming flow rate is 100 µL/min.

8. Verify that the system readback is normal (see Checking the Instrument Readback Status).

This completes the setup for calibrating the MS. Go to Chapter 5, "Establishing a Stable Ionization Spray."

Establishing a Stable Ionization Spray

Before you calibrate the MS, make sure that you establish stable ionization spray conditions. The intensity and stability of the ionization spray largely depend on the performance of the ion 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

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

❖ **To evaluate the spray stability**

1. Open the Tune window.
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:

      Current LC Flow (µL/min): **3**

      Syringe pump settings: **3** µL/min flow rate and **500** µL syringe volume

      Ion polarity mode: **Positive**

      Data type: **Profile**

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.
   - In the Ion Source - Ion Source pane, verify the value in the Current LC Flow (µL/min) box.
4. In the Define Scan pane, define the scan on which to evaluate the spray stability.
5. Place the MS in **On** mode.

   The MS begins scanning and applies high voltage to the spray insert. A real-time mass spectrum appears in the Tune window.
6. Turn on the flow for the solution as follows:
   - For the calibration solution, click **Syringe Off** to turn on the syringe pump.

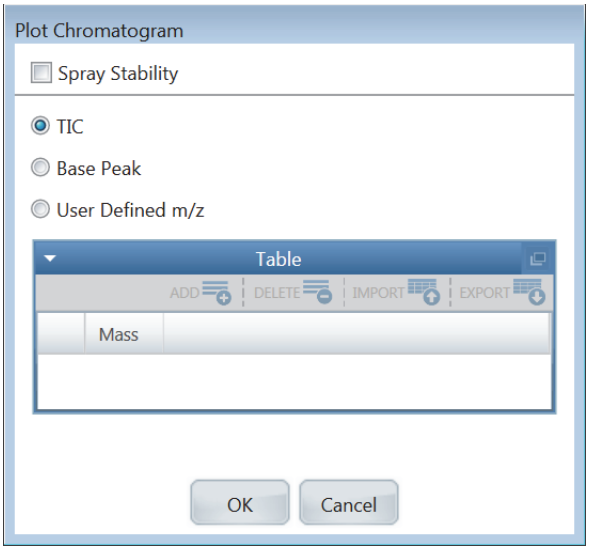
     The button name changes to Syringe On.

   –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.
7. Plot the full total ion current (TIC) and relative standard deviation (RSD) graphs as follows:
   a. Click the **Plot Chromatogram** icon,  , to open the Plot Chromatogram dialog box (Figure 6).

      Figure 6. Plot Chromatogram dialog box with the TIC option selected

   b. Select the **Spray Stability** check box to monitor the relative standard deviation (RSD) of the target ion current.

c. Select the **TIC** option.

d. To plot the full TIC chromatogram, click **OK**.

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 RSD of the TIC for a 10 Da-selected ion monitoring (SIM) scan that is centered around the most abundant mass-to-charge ratio (m/z) in the current spectrum.

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

Table 6 lists the criteria for a stable spray in either positive or negative ion polarity mode.

Table 6. 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

9. If the signal stability rating is poor or the %RSD value is above the threshold, follow the procedure in the next section, Optimizing the Ion Source Parameters.

This completes the spray stability evaluation.

Optimizing the Ion Source Parameters

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

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

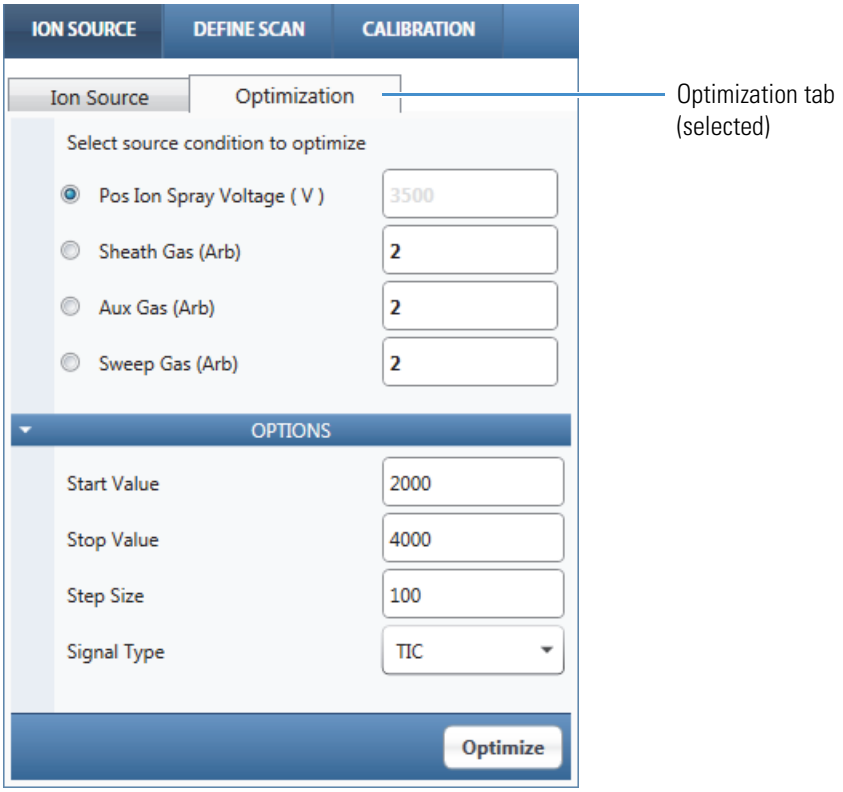
❖ To optimize the ion source parameters

1. Verify that the syringe has a sufficient amount of the calibration solution.
2. In the Tune window, click **Syringe Off** to turn on 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 7).

Figure 7. Optimization page of the Ion Source pane

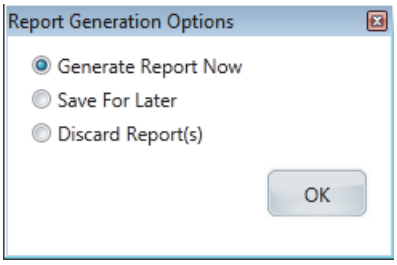


   b. In the Signal Type list, select **TIC**.
   c. Click **Optimize**.

      The status area displays the message "Optimization In Progress." After Tune completes the optimization, the optimized value and the Accept and Reject buttons appear.
   d. Click **Accept**.

      The Report Generation Options dialog box opens (Figure 8).

Figure 8. Report Generation Options dialog box



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

> **Tip** To turn off the Report Generation Options dialog box, see "Setting the Tune Preferences" in Appendix A.

4. Optimize the remaining source parameters.
5. (Optional) Save the parameters' state in the Favorites pane (see page 70). For additional information about the Favorites pane, refer to the Tune Help.

6

Calibrating the Mass Spectrometer in H-ESI Mode

Calibrate the Orbitrap Tribrid Series MS in H-ESI mode by having the syringe pump introduce the ESI calibration solution into the instrument at a steady flow rate.

Calibration parameters are MS 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 MS in H-ESI mode before acquiring data in H-ESI, NSI, APCI, or APPI mode. Generally, you must calibrate the MS 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 MS, refer to the *EASY-ETD and EASY-IC Ion Sources User Guide* for the applicable figures.

Contents

Calibration Solution Peak Values

IMPORTANT If you observe interfering peaks in the spectrum that are within ±10 Da of any of these calibration masses, follow the procedure To flush the inlet components until the interfering masses show less than 25 percent of the intensity of the calibrant ions.

See these topics:

- Peak Values for the Traditional Calibration Solutions
- Peak Values for the Pierce FlexMix Calibration Solution

Peak Values for the Traditional Calibration Solutions

The mass spectra of the calibration solution in positive (Figure 9) and negative (Figure 10) ion polarity modes have peaks at *m/z* values close to the following theoretical values:

- Positive mode peaks

m/z 195.0874	*m/z* 1022.0028	*m/z* 1321.9838	*m/z* 1621.9639
m/z 262.6359	*m/z* 1122.9963	*m/z* 1421.9774	*m/z* 1721.9579
m/z 393.2242	*m/z* 1221.9902	*m/z* 1521.9707	*m/z* 1821.9506
m/z 524.2646			

- Negative mode peaks

m/z 265.1479	*m/z* 1333.9694	*m/z* 1533.9569	*m/z* 1733.9438
m/z 514.2849	*m/z* 1379.9916	*m/z* 1579.9787	*m/z* 1786.1122
m/z 1233.9760	*m/z* 1433.9635	*m/z* 1633.9498	*m/z* 1886.1056
m/z 1279.9980	*m/z* 1479.9853	*m/z* 1679.9726	

Figure 9. Traditional n-Butylamine calibration solution's normal mass-range spectrum in positive ion polarity mode

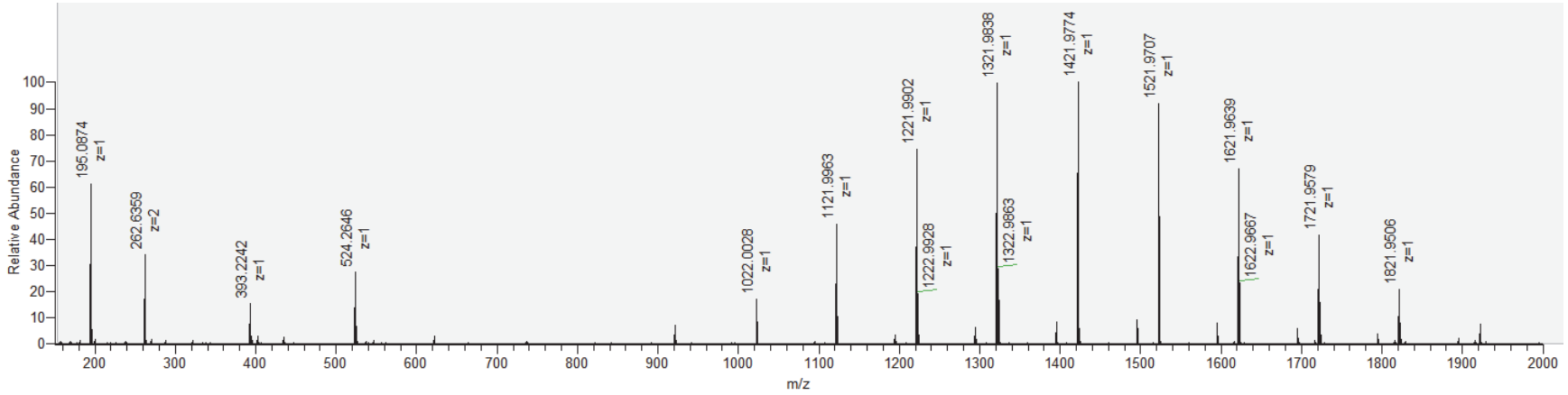
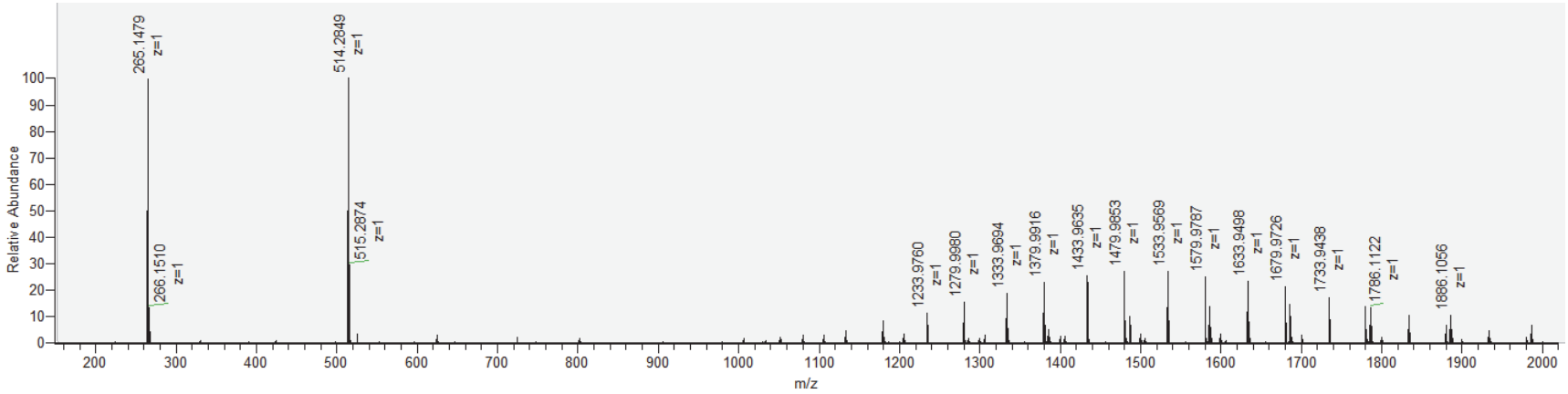


Figure 10. Traditional Ultramark 1621 calibration solution's normal mass-range spectrum in negative ion polarity mode



Peak Values for the Pierce FlexMix Calibration Solution

The mass spectra of the Pierce™ FlexMix™ calibration solution (P/N A39239) in positive (Figure 11, Figure 13, and Figure 15) and negative (Figure 12, Figure 14, and Figure 16) ion polarity modes have peaks at *m/z* values close to the following theoretical values:

- Positive mode peaks

m/z 69.0447	*m/z* 524.2649	*m/z* 1221.9905	*m/z* 1721.9585
m/z 102.1277	*m/z* 622.0289	*m/z* 1321.9841	*m/z* 1821.9521
m/z 142.1590	*m/z* 922.0097	*m/z* 1421.9777	*m/z* 1921.9457
m/z 195.0876	*m/z* 1022.0033	*m/z* 1521.9713	*m/z* 2121.9329
m/z 262.6361	*m/z* 1121.9969	*m/z* 1621.9649	*m/z* 2721.8945
m/z 322.0481			

- Negative mode peaks

m/z 59.0138	*m/z* 1033.9880	*m/z* 1433.9624	*m/z* 1833.9368
m/z 112.9856	*m/z* 1133.9816	*m/z* 1533.9560	*m/z* 1933.9304
m/z 162.9824	*m/z* 1233.9752	*m/z* 1633.9496	*m/z* 2233.9111
m/z 362.9696	*m/z* 1333.9688	*m/z* 1733.9432	*m/z* 2833.8728
m/z 601.9789			

Note The Pierce FlexMix calibration solution (P/N A39239) is available for purchase Summer 2018. To check the availability status, visit this web page:

www.thermofisher.com/search/browse/category/us/en/600654/Instrument+Calibration+Kits%2C+Standards+%26+Reagents%2FMass+Spectrometry+Controls+%26+Standards

Figure 11. FlexMix calibration solution's low-mass range spectrum in positive ion polarity mode

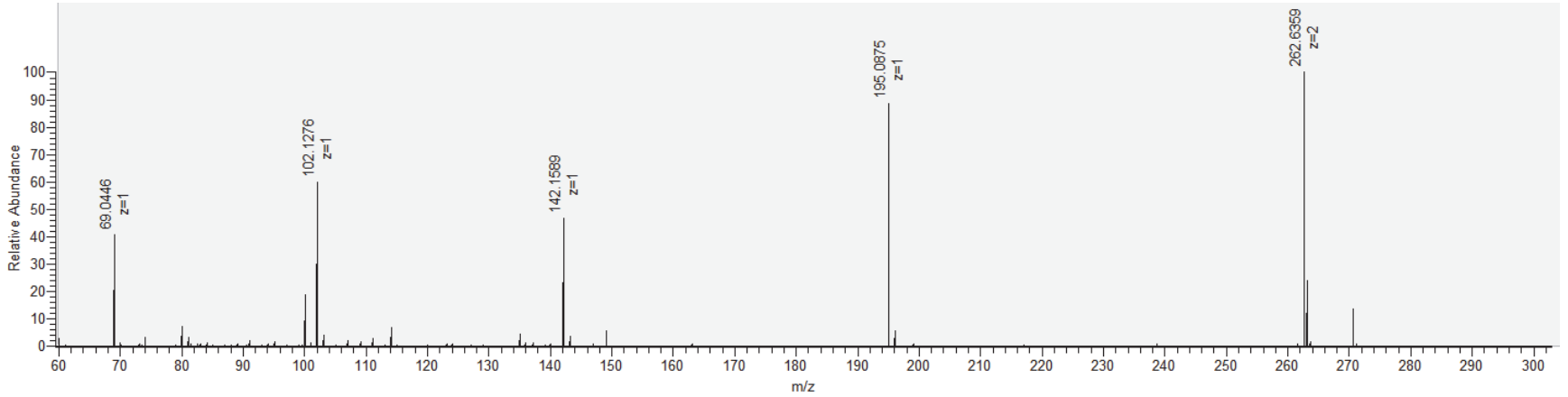


Figure 12. FlexMix calibration solution's low-mass range spectrum in negative ion polarity mode

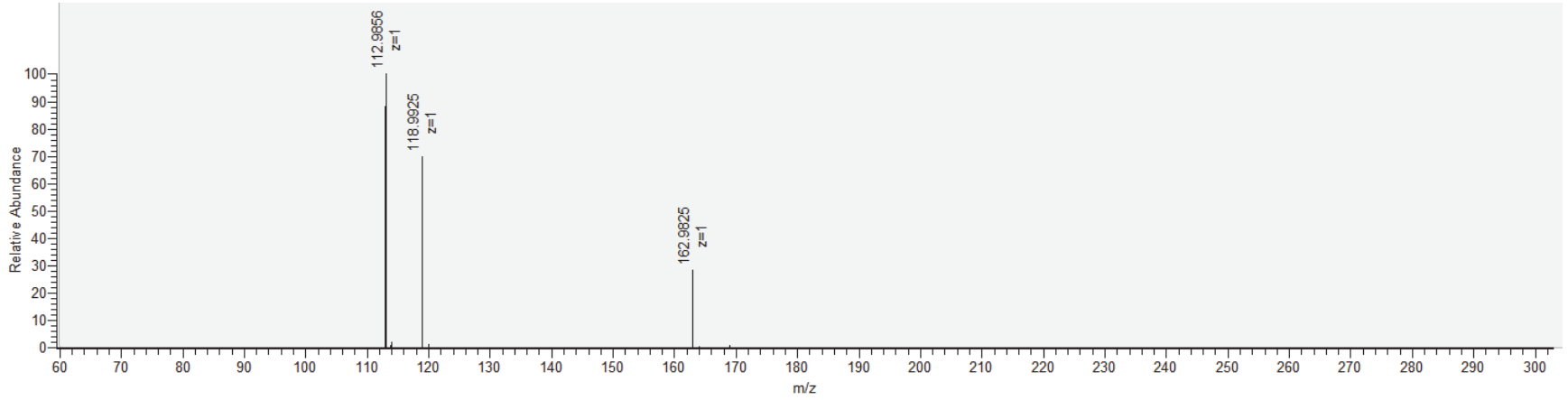


Figure 13. FlexMix solution's normal-mass range spectrum in positive ion polarity mode

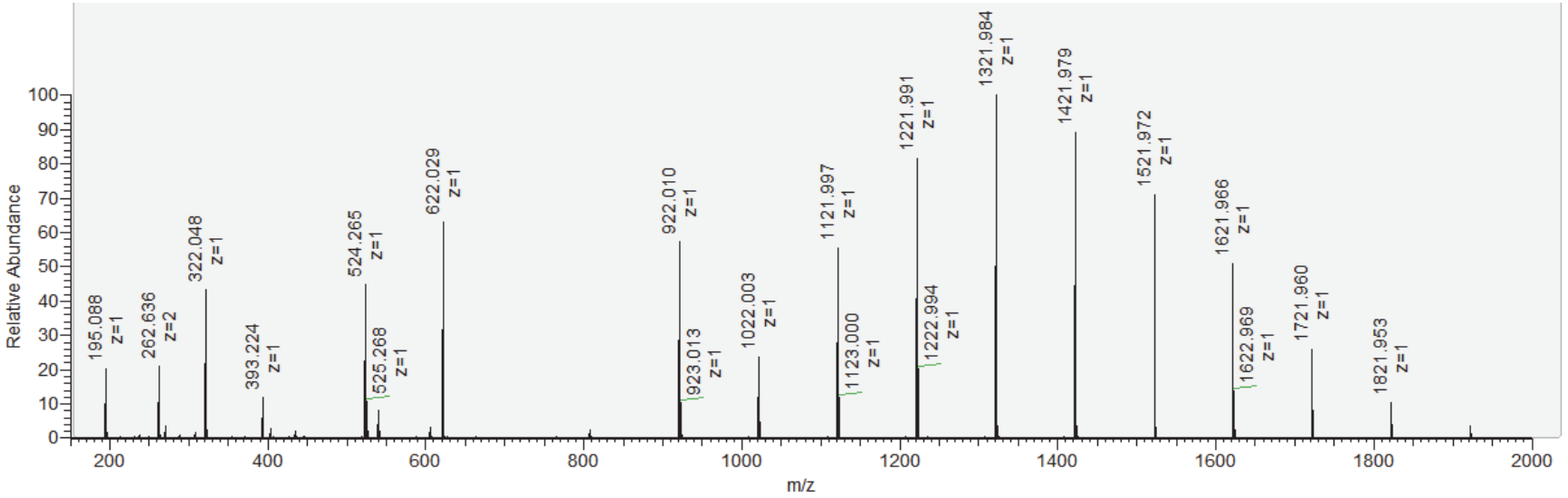


Figure 14. FlexMix solution's normal-mass range spectrum in negative ion polarity mode

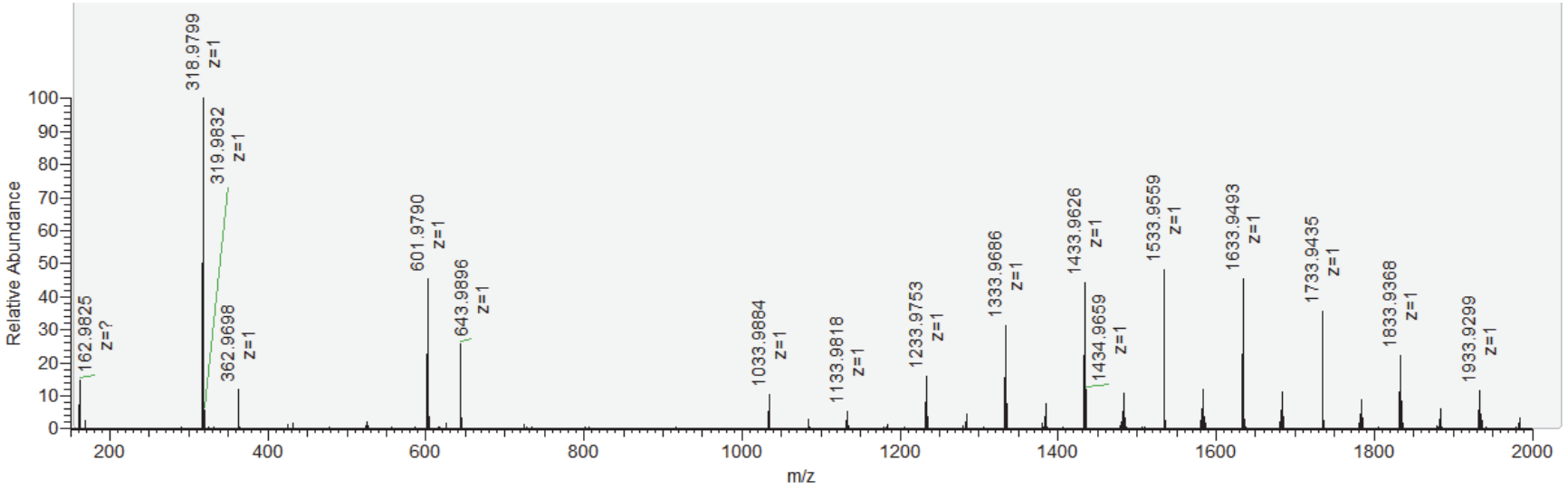


Figure 15. FlexMix solution's high-mass range spectrum in positive ion polarity mode

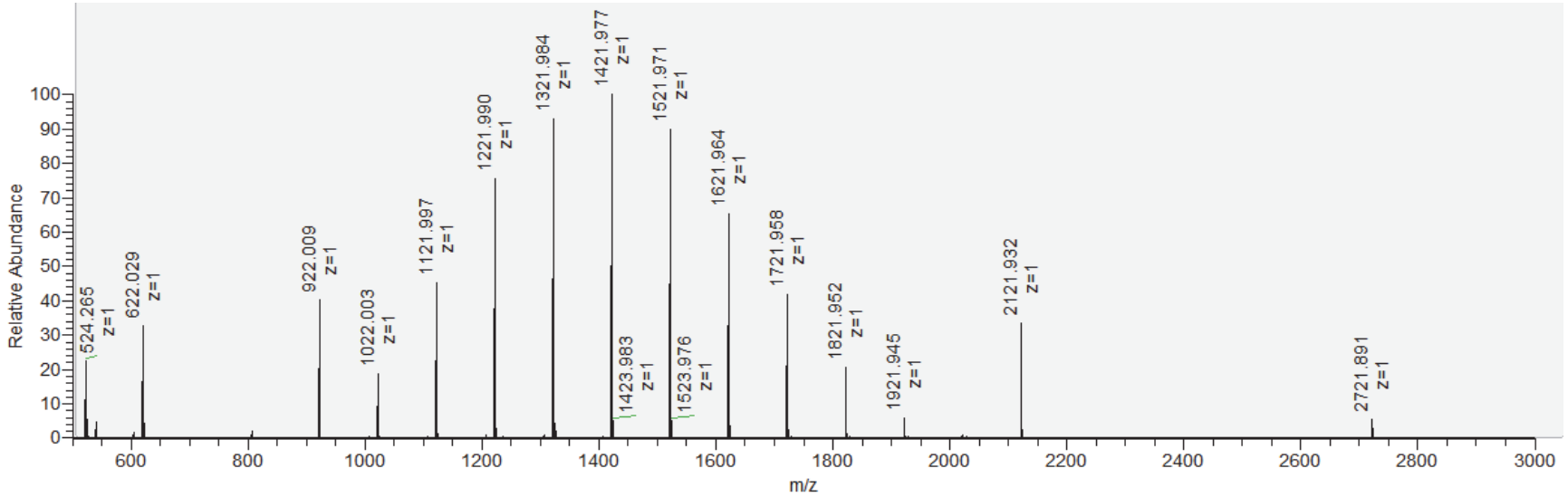
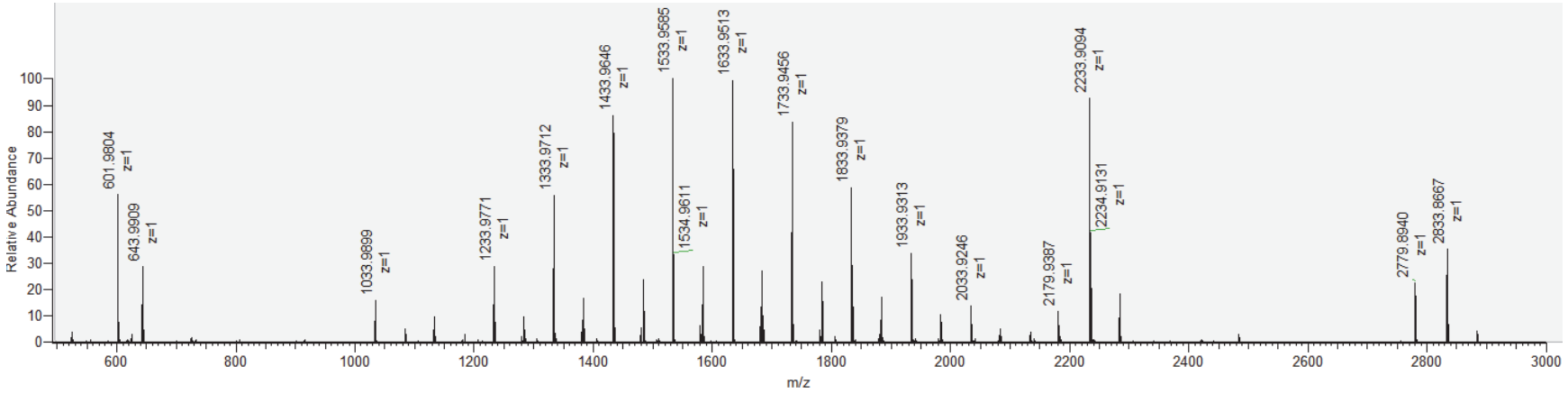


Figure 16. FlexMix solution's high-mass range spectrum in negative ion polarity mode



Running the Calibration Procedures

Before you begin, you must specify the type of calibration solution in the Tune Preferences dialog box. If you use the traditional calibration solution, always run the positive ion polarity calibrations before the negative ion polarity calibrations. You can use the FlexMix calibration solution for both the positive and negative calibration procedures.

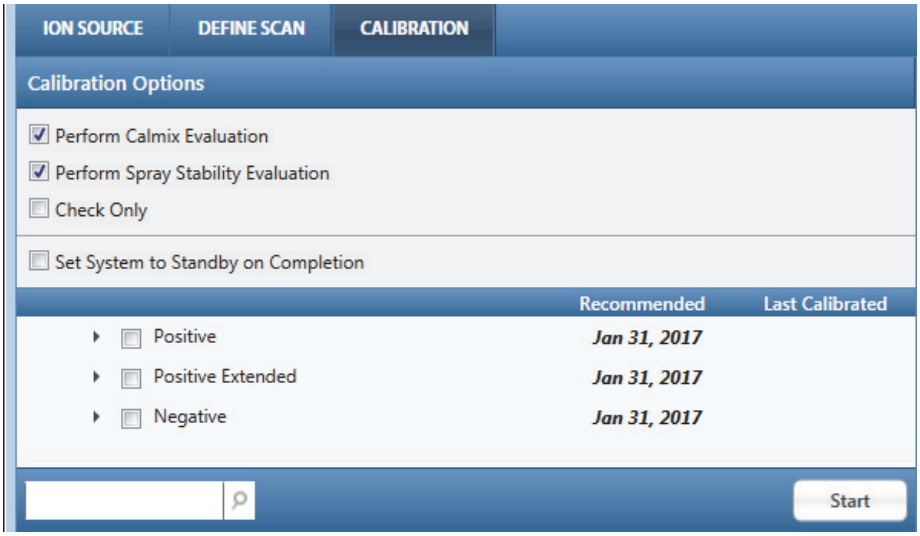
IMPORTANT

- Before you continue, verify that you have set up the ion source (Chapter 2), prepared the system for calibrating in positive H-ESI mode (Chapter 4), and verified that the infused calibration solution produces a stable ionization spray (Chapter 5).
- To minimize the possibility of cross-contamination, use a dedicated syringe and length of PEEK tubing for each type of calibration solution.

❖ To calibrate the MS

1. Load separate, clean, 500 µL syringes with 500 µL of the applicable calibration solution.
2. In the Tune window, specify the calibration solution as follows:
   a. Click the **Options** icon,  , and choose **Preferences**.
   b. Under Current Calibration Mix (Figure 25), select the **Traditional** or **FlexMix** option, and then click **OK**.
3. In the Calibration pane, select the **Perform Calmix Evaluation** and **Perform Spray Stability Evaluation** check boxes (Figure 17).

   Figure 17. Calibration pane (example for Orbitrap Fusion and Orbitrap Fusion Lumos MSs)



4. (Optional) Select the **Set System to Standby on Completion** check box.

5. Click the arrow next to the Positive check box, and then select the **Ion Optics** check box.
6. Click **Start** and 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.
7. Run the calibration for each of the remaining Positive and Positive Extended categories (as applicable for your MS)—one at a time and in the order specified in the Tune window.

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

   Tip After the calibration, you see either a green check (✔) adjacent to the calibration name to indicate a successful calibration or a red X mark (✖) to indicate a failed calibration.

   A date appears in the Last Calibrated column for each successful calibration test. A date does not appear for failed calibrations.
8. If using the traditional calibration solution, do the following:
   a. Follow the procedure in Flushing the Inlet Components After Calibration.
   b. Load the syringe with the negative calibration solution.
   c. Run the Negative calibrations one at a time and in the order specified in the Tune window.
9. If using the FlexMix calibration solution, run the Negative calibrations one at a time and in the order specified in the Tune window.

IMPORTANT Before you start using your analyte, follow the procedure in Flushing the Inlet Components After Calibration.

7

Using Tune to Acquire Sample Data

To manually acquire sample data, use the Tune application. The data system computer automatically saves the acquired data to its hard drive. The data file size depends on several instrument method parameters, such as the data type, detector type, scan types, mass range, and more. For example, a proteomics data file is approximately 2 GB.

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

IMPORTANT Before you begin, check the following:

- In the Calibration pane, ensure that all calibrations are up to date.
- Connect the Ready Out cable (not provided) and the contact closure cable to help prevent sample loss. Refer to the Getting Connected Guide.

Contents

Setting Up the LC/MS System for Analyte Optimization

See these topics:

Setting Up the Inlet

❖ To set up the inlet

Do one of the following:

- To infuse an analyte, see Setting Up the Inlet for High-Flow Infusion Without an Autosampler.
- To infuse the reserpine sample solution, see Setting Up the Inlet for Loop Injections (Flow-Injection Analysis).

Configuring the Syringe Pump

❖ To configure the syringe pump

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

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

2. Set the syringe pump parameters as follows:

   a. Click the down arrow next to the Syringe On/Off button to open the syringe pump settings box (Figure 18), and then enter the following:

   Flow rate (µL/min): **3**

   Volume (µL): **500**

   Figure 18. Syringe pump settings box

   b. Click **Apply**.

   c. Click **Syringe Off** to turn on the syringe pump.

3. Verify that the inlet plumbing connections do not leak.

Configuring the LC Pump

When controlling the LC pump through the Xcalibur data system, use the Direct Control dialog box to turn off the solvent flow.

❖ **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.
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.

Configuring the Ion Source for Reserpine (Example)

❖ **To configure the ion source for the reserpine example**

On the Ion Source - Ion Source page, set the parameters as listed in the following table.

Parameter	Setting
Positive Ion Spray Voltage (V)	3500
Sheath Gas (Arb)	50
Aux Gas (Arb)	20
Sweep Gas (Arb)	2
Ion Transfer Tube Temp (°C)	350
Vaporizer Temp (°C)	500

Setting the Instrument's Optimal Pressure

For the Orbitrap Fusion and Orbitrap Fusion Lumos MSs only, use Standard Pressure mode for the reserpine example. (The Orbitrap ID-X MS does not have this button because it is always set to standard pressure mode.)

❖ **To set the instrument's optimal pressure**

Do one of the following:

- For small molecules, bottom-up, and top-down protein experiments, select **Standard Pressure** mode, Standard Pressure.
- For intact protein experiments and top-down experiments with large fragment ions, select **Low Pressure (Intact Protein)** mode, Low Pressure (Intact Protein).

Defining the Scan Parameters for Precursor Optimization

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

See these topics:

1. Defining the MS/MS Scan Parameters for Precursor Optimization
2. Optimizing the Isolation Window
3. Optimizing the Ion Source Parameters on an Analyte
4. Optimizing the Fragmentation Parameters

Defining the MS/MS Scan Parameters for Precursor Optimization

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

1. In the Tune window, click the **Define Scan** tab, and then select the **MS^2 Scan** type.
2. Set the scan parameters that are appropriate for your analyte.

   See Table 7 for an example using the 50 fg/µL reserpine.

Table 7. MS/MS scan parameters (reserpine example) (Sheet 1 of 2)

Parameter	Value	Parameter	Value
Scan Type	MS^2 Scan	Detector Type	Ion Trap
Isolation Mode	Ion Trap	Ion Trap Scan Rate	Normal
Isolation Width (m/z)	12	Mass Range	Normal

Table 7. MS/MS scan parameters (reserpine example) (Sheet 2 of 2)

Parameter	Value	Parameter	Value
Activation Type	CID	Scan Range (m/z)	165–615
Type[a] Collision Energy (%)	33	RF Lens (%)	70
		AGC Target	1.0e4

[a] *Type* is the selected activation type.

3. In the MS^n Setting Table, enter the analyte's precursor ion (for example, *m/z* **609**).

   For information about the MS^n Setting Table, see "Using the MS^n Setting Table in the Define Scan Pane" in Appendix A.

4. Click **Apply** (or press ENTER).

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

Optimizing the Isolation Window

❖ To optimize the isolation window

1. Display the chromatogram for the analyte's precursor ion as follows:
   a. Click the **Plot Chromatogram** icon,  , to open the Plot Chromatogram dialog box.
   b. Clear the **Spray Stability** check box.
   c. Select the **User Defined m/z** option, and then enter the analyte's *m/z* value (for example, *m/z* **609**).
   d. Click **OK** to plot the chromatogram.
2. In the Define Scan pane, in the Isolation Window (*m/z*) box, enter a slightly lower value.

   Note The isolation window setting is typically *m/z* 1–3. The optimum value for the isolation window 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 window increases the specificity of the scan results while a wider width increases the signal at the expense of specificity.

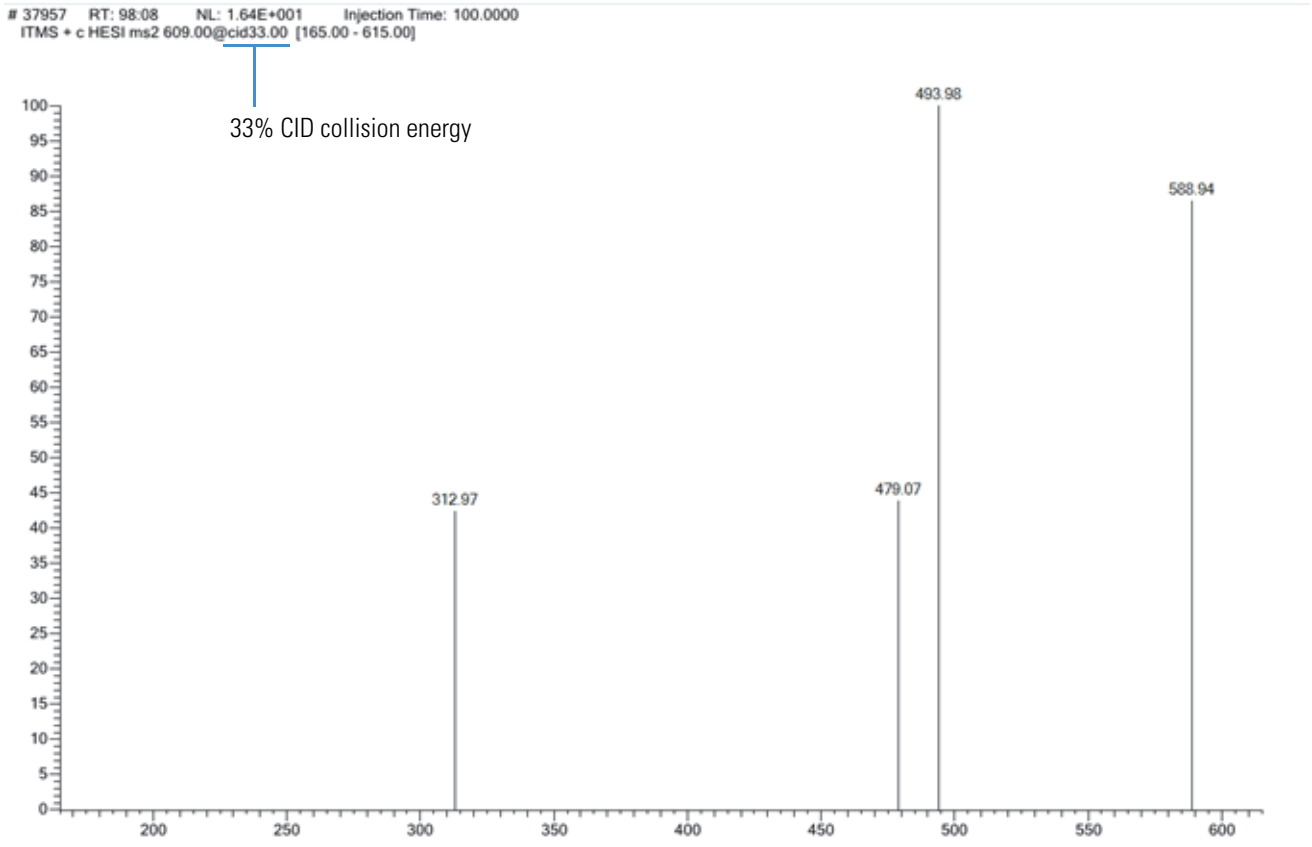
   When you obtain the optimum isolation window, the normalization level (NL) and ionization time (IT) values are stable and the mass peak for the precursor ion is at its maximum intensity and appears symmetrical. An isolation window value that is less than optimum causes a substantial drop in the NL reading.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

3. Enter successively smaller values for the isolation window, until the intensity of the chromatogram is acceptable for your needs.

4. After you optimize the isolation window, compensate for minor changes in stability by increasing the isolation window by an amount not to exceed *m/z* = 1.
5. In the Collision Energy (%) box, enter an appropriate value for the analyte (for example, **33**).
6. Click **Apply** (or press ENTER) to start the fragmentation process.

   Figure 19 shows a spectrum example of a CID-MS/MS scan with fragmentation. For descriptions of the spectrum header information and controls, refer to the Spectrum View topic in the Tune Help.

Figure 19. CID-MS/MS scan spectrum with fragmentation (reserpine example)



Optimizing the Ion Source Parameters on an Analyte

❖ **To optimize the ion source parameters on an analyte**

1. Follow the procedure To optimize the ion source parameters, 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.
2. (Optional) If you need to increase the sensitivity, optimize the following:
   - Vaporizer Temperature (Ion Source - Ion Source pane)
   - RF Lens (Define Scan pane)
   - Spray direction (see page 14)
3. (Optional) Save the parameters' state in the Favorites pane. For additional information about the Favorites pane, refer to the Tune Help.

Optimizing the Fragmentation Parameters

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

❖ **To optimize the collision energy**

1. In the Define Scan pane, select the **MS^2 Scan** type.
2. In the Collision Energy (%) box, enter an appropriate value (for example, **33**).
3. Click **Apply** (or press ENTER).
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 an MS^3 Scan

This section is optional. If you want to further improve the sensitivity of the data acquisition example, use the MS^n scan type (n = 3).

❖ To define the MS^3 scan parameters

1. In the Define Scan pane, select the **MS^n Scan** type.
2. Set the scan parameters that are appropriate for your analyte.

   See Table 8 for an example using the 50 fg/µL reserpine.

   Table 8. MS^3 scan parameters (reserpine example)

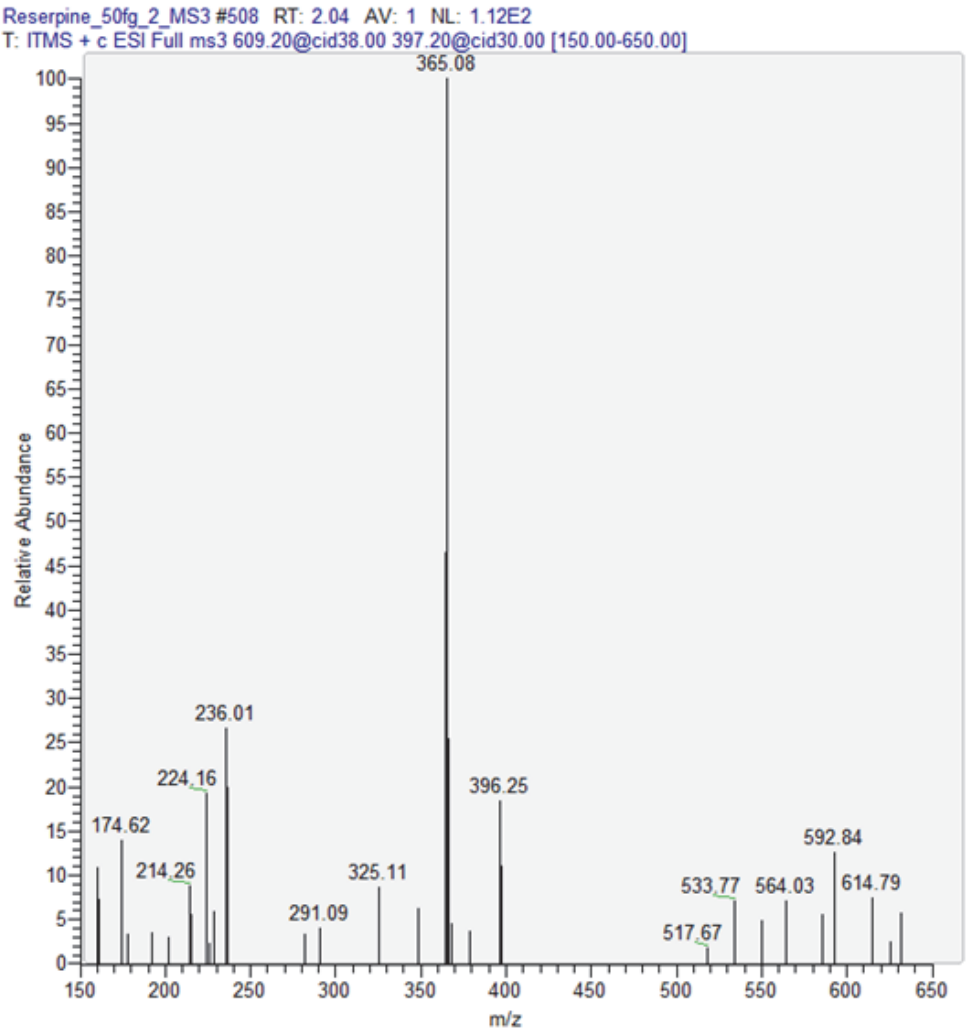
Parameter	Value	Parameter	Value
Scan Type	MS^n Scan	Detector Type	Ion Trap
Isolation Mode	Ion Trap	Ion Trap Scan Rate	Normal
Isolation Width (m/z)	1.2	Mass Range	Normal
Activation Type	CID	RF Lens (%)	70
Type[a] Collision Energy (%)	33	AGC Target	1.0e4
		m/z (setting table)	397

   [a] *Type* is the selected activation type.

3. Click **Apply** (or press ENTER).

   Figure 20 shows a spectrum example of a CID-MS^3 scan with fragmentation.

Figure 20. CID-MS^3 scan spectrum with fragmentation (reserpine example)



Acquiring a Data File by Using the Tune Application

❖ **To acquire a sample data file**

1. Open the Data Acquisition pane (Figure 21), and then do the following:

   a. (Optional) To change the destination folder for the raw data, 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).

      If the base file name already exists in the save location, the Tune application adds a time-stamp suffix that consists of the year (*YYYY*), month (*MM*), day (*DD*), and time (*HHMMSS*).

   c. In the Sample Name box, type the name of the analyte (or other suitable label).

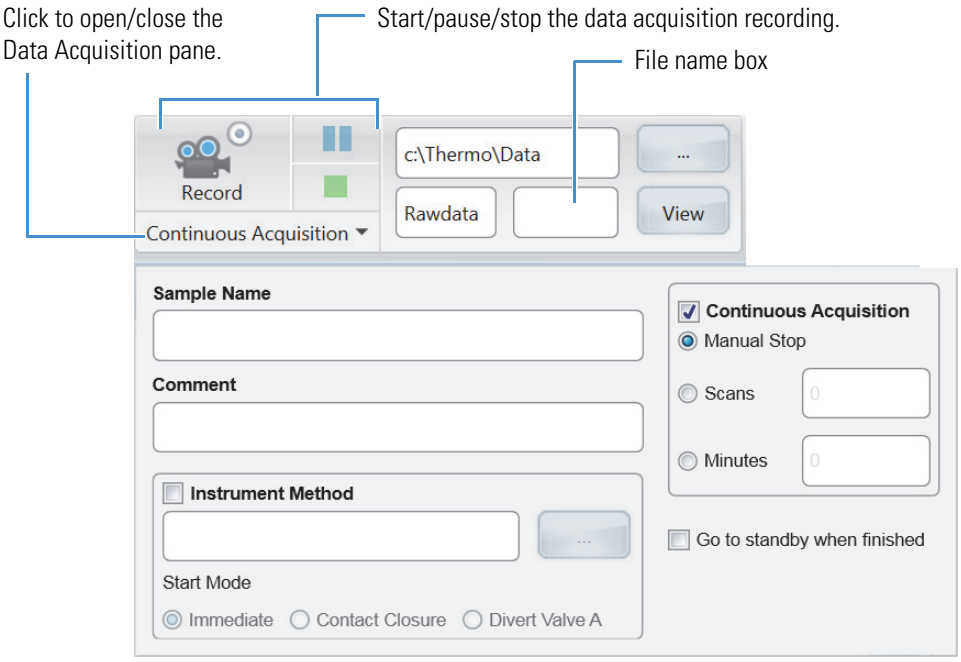
d. In the Comment box, type a comment about the experiment.

   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 application. To open this application, choose **Start > All Programs > Thermo Xcalibur > XReport**.

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

Figure 21. Data Acquisition pane in the Tune window



2. Click **Record** 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 Qual Browser manual or Help.

8

Using the Xcalibur Data System to Acquire Data

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

The data system computer automatically saves the acquired data to its hard drive. The data file size depends on several instrument method parameters, such as the data type, detector type, scan types, mass range, and more. For example, a proteomics data file is approximately 2 GB.

IMPORTANT Before you begin, check the following:

- In the Calibration pane, ensure that all calibrations are up to date.
- Connect the Ready Out cable (not provided) and the contact closure cable to help prevent sample loss. Refer to the Getting Connected Guide.

Contents

Selecting the External Start Instrument

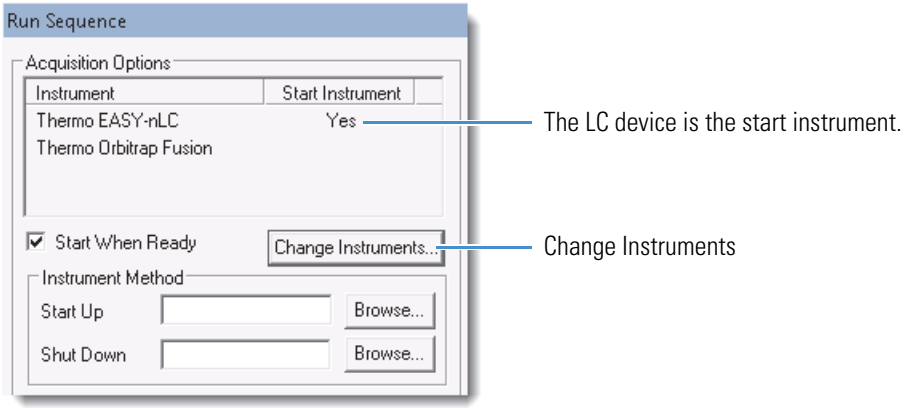
Set the start (trigger) instrument in the Xcalibur data system before running an instrument method to acquire data.

❖ To select the external start instrument

1. Open the Xcalibur data system, and then choose **View > Sequence Setup View** to open the 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 22).

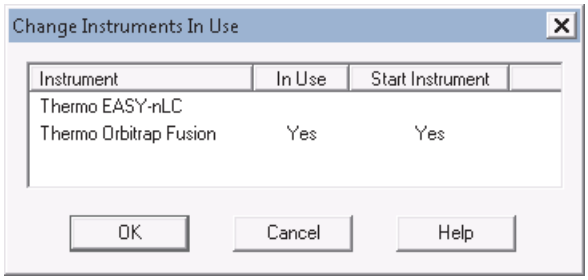
   The Yes in the Start Instrument column indicates the default start instrument for the sequence run.

   Figure 22. Run Sequence dialog box (partial) showing the selected start instrument



4. If Yes appears in the Start Instrument column for the MS 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 23).
   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**.

Figure 23. Change Instruments In Use dialog box showing the MS as the start instrument



5. In the Run Sequence dialog box, complete the remaining selections.
6. Click **OK**.

Acquiring a Data File by Using the Xcalibur Data System

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

For instructions, refer to the Method Editor Help and the Xcalibur Help (Instrument Setup and Sequence Setup topics).

A

Using Basic Tune Functions

This appendix describes some of the basic Tune functions that are referenced throughout this guide. You activate several of the functions by pressing different toggle buttons. For additional information about the Tune window, refer to the Tune Help.

Note In the Tune window, point to a parameter name to display its description. For additional information, open the Tune Help (press the F1 key).

Contents

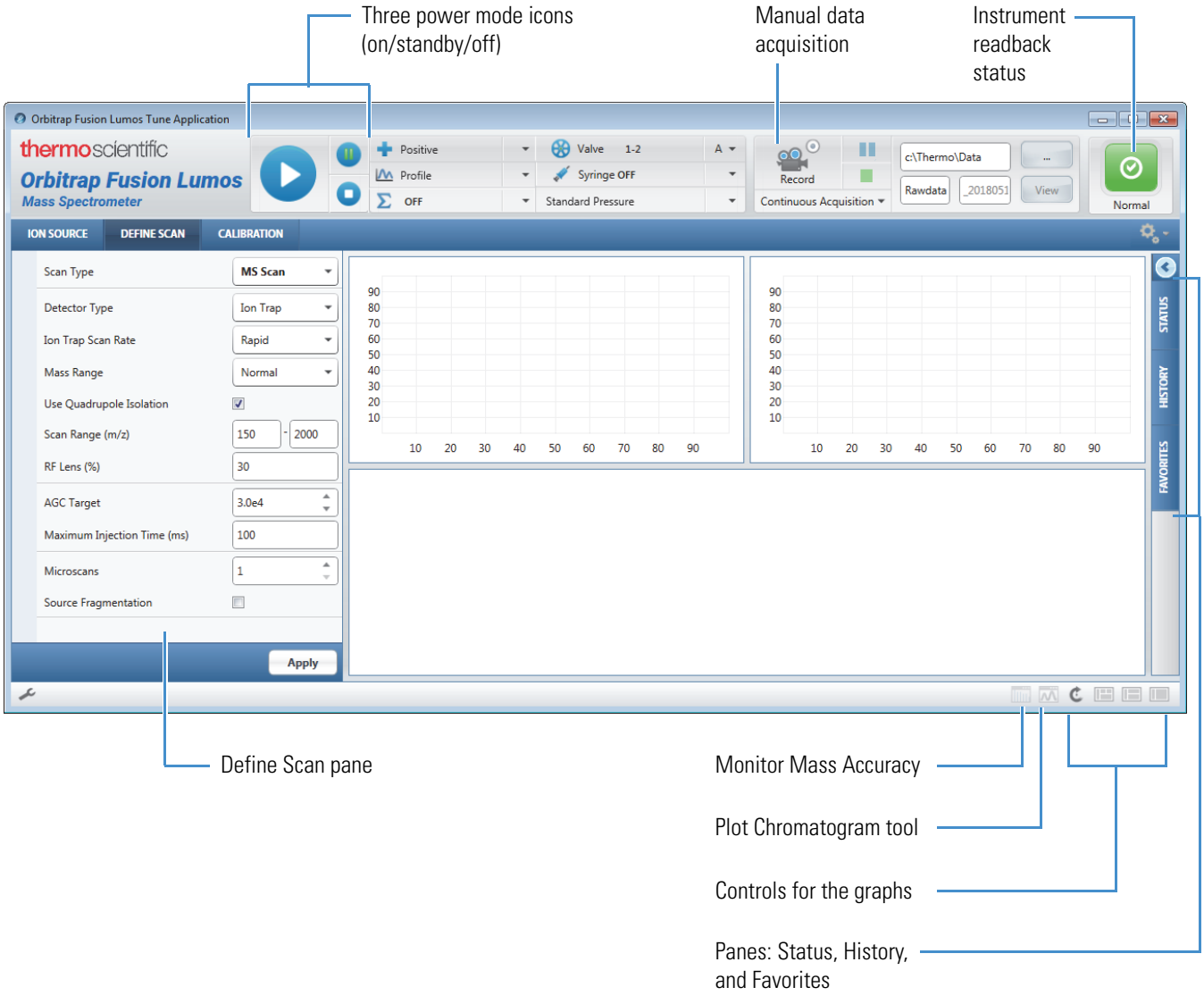
Opening the Tune Window

❖ To open the Tune window

From the Windows taskbar, choose **Start > All Apps** (Windows 10) or **All Programs** (Windows 7) **> Thermo Instruments >** ***model x.x*** **>** ***model x.x*** **Tune** (Figure 24).

For information about the buttons and icons in the Tune application and what they control, refer to the Tune Help.

Figure 24. Orbitrap Fusion Lumos Tune window showing the Define Scan pane



Setting the Instrument System Controls

Table 9 shows the options for each of the system control buttons at the top of the Tune window.

Table 9. Procedures for using the instrument control buttons (Sheet 1 of 2)

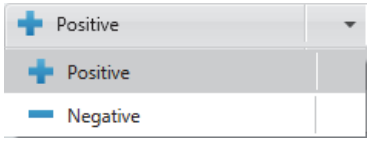
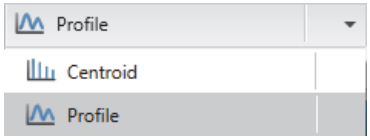
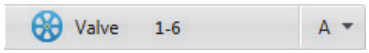
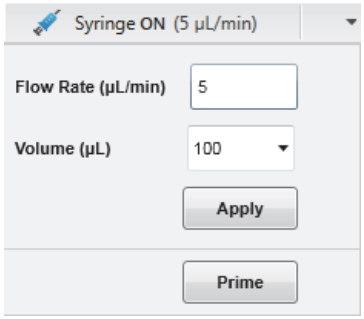
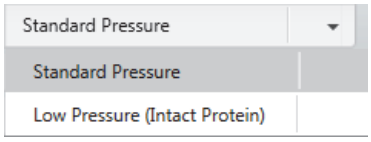
Button	Function	Procedure
	Power mode	❖ **To set the instrument power mode** Click the icon for the applicable power mode. The center of the selected icon changes from white to green. See Setting the Instrument Power Mode.
	Ion polarity mode	❖ **To set the ion polarity mode** Click **Positive (Negative)** and select the ion polarity mode.
	Data type	❖ **To set the data type** Click **Centroid (Profile)** and select the data type.
	Spectrum scan averaging	❖ **To turn the scan averaging on or off** Click the button to switch between on and off. ❖ **To set the scan averaging value** Click the down arrow to enter the value, and then click **Apply**.
	Divert/inject valve	❖ **To set the divert/inject valve module and position** Click the down arrow to select the valve, and then click **Valve** to set the position.

Table 9. Procedures for using the instrument control buttons (Sheet 2 of 2)

Button	Function	Procedure
	Syringe pump	❖ **To turn the syringe pump on or off** Click **Syringe On (Off)** to switch between on and off. ❖ **To set the syringe pump parameters** 1. Click the down arrow to open the syringe pump settings box, and then type the parameter values. The Tune application automatically saves the values. 2. (Optional) Press and hold **Prime** to prime the syringe at 100 µL/min. 3. Click the down arrow again or click elsewhere to close the box.
	Pressure mode	(Orbitrap Fusion and Orbitrap Fusion Lumos MSs only) ❖ **To set the instrument pressure mode** Click **Standard Pressure (Low Pressure [Intact Protein])** and select the instrument pressure mode.

Setting the Instrument Power Mode

Use the three power mode icons in the Tune window (Table 9) to set the MS's power mode (on, standby, and off).

When you remove the ion source housing or the spray insert, the MS automatically switches to off mode.

In standby mode, the System LED on the front panel turns yellow and the MS turns off the electron multipliers, conversion dynodes, 8 kV power to the ion 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 MS components under varying power conditions, refer to Chapter 6 in the Hardware Manual.

Checking the Instrument Readback Status

The system readback icon is located in the top right of the Tune window. Table 10 lists the various readback states.

Table 10. Instrument readback icons and their meanings

Icon	Background color	Meaning
	Green	The system parameters are within tolerance.
	Green	The system is initializing.
	Amber	One or more settings are changing.
	Red	An error has occurred.
	Gray	The ion source is off.
	Gray	There is no communication between the MS and the data system.

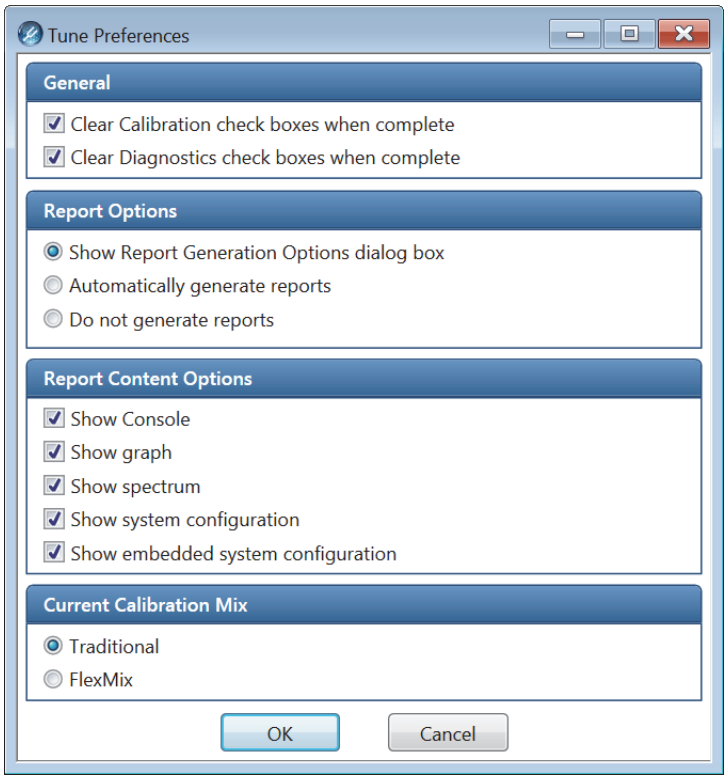
Setting the Tune Preferences

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 **Preferences** to open the Tune Preferences dialog box (Figure 25).

   Figure 25. Tune Preferences dialog box

2. Select all check boxes that apply.
3. Under both Report Options and Current Calibration Mix, select one of the options, and then click **OK**.

Using the MS^n Setting Table in the Define Scan Pane

The MS^n Setting Table appears when you select the MS^n Scan type in the Define Scan pane. Use this table to specify one or more precursor ions.

Importing a Mass List from a File

❖ To import a mass list from a file

1. Click **Import** to open the Open dialog box.
2. Browse to a CSV (Microsoft Excel™), TXT, or XML file, and then click **Open**.

   The list of *m/z* values appears in the table.

Exporting a Mass List to a File

❖ To export a mass list to a file

1. Complete the list of *m/z* values.
2. Click **Export** to open the Save As dialog box.
3. Browse to a location, enter a file name, and then select a file type (**CSV**, **TXT Only**, or **XML Data**).
4. Click **Save**.

Modifying Rows in the MS^n Setting Table

This table describes how to modify the number of rows in the MS^n Setting Table.

Task	Steps
Delete multiple rows	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**. • Press the DELETE key on your keyboard.
Delete one row	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**. • Press the DELETE key on your keyboard.
Add one row	Do one of the following: • Click the **Add Row** icon, . • Right-click the table, and then choose **Add Row**.

Adding or Removing Scan Parameters from the MS^n Setting Table

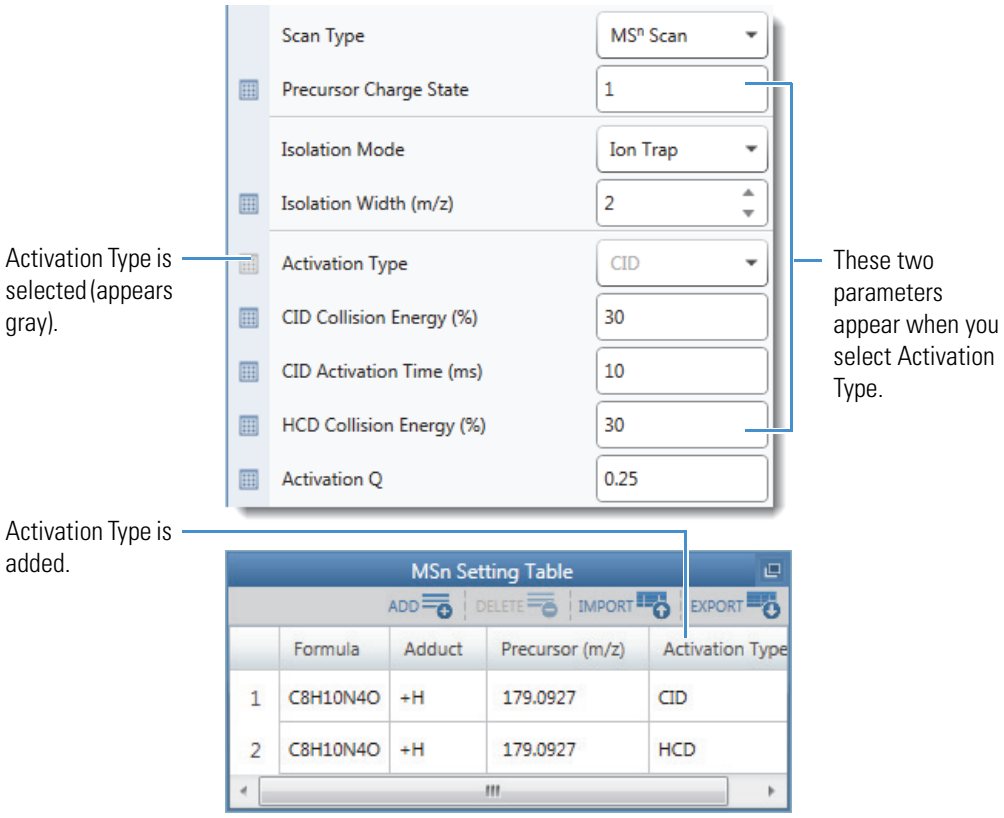
To set different scan parameters for the precursor ions, add the parameters to the MS^n Setting Table.

❖ 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 26 shows an example with the Activation Type column added to the MS^n Settings Table.

Figure 26. Activation Type selected and added to the MS^n Setting Table (CID example)



Using the History Pane

The History pane records all parameter changes made in the Ion Source and Define Scan panes as "change records," which appear as sub-items under the date that they were created. A change record is inactive if the ion source type of the change record differs from the currently installed ion source type. The maximum number of change records is 100.

❖ To view or modify a change record

Choose from the following:

- Click a change record to display its parameters, or right-click it and choose **Load**. Parameters shown in bold differ from their default values.
- Double-click a change record, and then click **Apply** in the Ion Source or Define Scan pane to submit its parameters to the MS. You can also right-click the change record and choose Apply.

Related Topics

- "Instrument Status and History" (in the Tune Help)

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.

- Saving a Favorite State
- Loading a Favorite State
- Applying a Favorite State
- Deleting a Favorite State
- Renaming a Favorite State

Saving a Favorite State

You can save the current key parameters of the MS by using the Favorites pane.

❖ To save a favorite state

1. Modify parameters in one of the Ion Source or Define Scan panes.
2. Click **Apply**.
3. Click the **Favorites** tab to display the Favorites pane (Figure 4).

4. In the Favorites pane, click **Save Current State**.
5. Type a unique name in the box that appears, and then click **Save Current State**.

   The newest favorite state appears first in the Favorites list. You may enter up to 100 states.

Loading a Favorite State

You can load key MS parameters—current or previously saved—by using the Favorites pane. When you load a favorite state, its key parameters appear in the Ion Source and Define Scan panes, but the Tune application does not automatically submit them to the MS.

❖ **To load a favorite state**

Click the name in the Favorites list, or right-click it and choose **Load**.

The Tune application displays the key parameters in the Parameters box.

Applying a Favorite State

You can apply key MS parameters that you previously saved by using the Favorites pane. When you apply a favorite state, the Tune application submits the parameters to the MS and also displays them in the (upper) Parameters box.

❖ **To apply a favorite state**

Double-click the name in the Favorites list, and then click **Apply** in the Ion Source or Define Scan pane. Or, right-click the name and choose **Apply**.

Deleting a Favorite State

You can delete a saved favorites entry by using the Favorites pane.

❖ **To delete a favorite state**

Right-click the name in the Favorites list and choose **Delete**.

Renaming a Favorite State

You can rename a saved favorites entry by using the Favorites pane.

❖ **To rename a favorite state**

1. Right-click the name in the Favorites list and choose **Rename**.
2. Type a different name in the box that appears.

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, spray cone, and ion transfer tube, on a regular basis to prevent corrosion and to maintain optimum performance of the ion 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 Chapter 8 in the Hardware Manual.

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

Contents

Supplies

Table 11 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 11. Flushing and cleaning supplies

Description	Part number
Gloves, nitrile	Fisher Scientific™ 19-120-2947[a] Unity Lab Services: • 23827-0008 (medium size) • 23827-0009 (large size)
Methanol, Optima™ LC/MS-grade	Fisher Scientific: A456-1
Water, Optima LC/MS-grade	Fisher Scientific: W6-1

[a] Multiple sizes are available.

Flushing the Inlet Components After Calibration

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

Tip You can also use an LC pump to flush the 50:50 methanol/water solution through the inlet components to the ion source at a flow rate of 200–400 µL/min for approximately 15 minutes.

❖ To flush the inlet components

1. Turn off the flow from the syringe pump.
2. Place the MS in **Standby** mode.
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 button 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.
4. Rinse the syringe with a solution of 50:50 methanol/water.
5. Flush the sample transfer line, sample tube, and spray insert as follows:
   a. Load the cleaned syringe with a solution of 0.1% formic acid in 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 service engineer, describes how to prepare the 50 fg/µL reserpine sample solution for H-ESI, APCI, and APPI modes. The procedure calls for potentially hazardous chemicals, including methanol and reserpine.

For a list of solvent recommendations, refer to the Preinstallation Requirements Guide. For a complete selection of LC/MS-grade consumables from Thermo Fisher Scientific, visit www.fishersci.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 900 µL of 1% acetic acid in 50:50 methanol/water into a clean, minimum 1.5 mL polypropylene tube.
2. Add 100 µL of the 100 pg/µL reserpine standard solution to the tube.

   The Orbitrap Tribrid Series Chemicals Kit contains the reserpine standard solution.
3. Mix the solution (10 pg/µL) thoroughly.
4. Transfer 100 µL of the 10 pg/µL 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 pg/µL) thoroughly.
7. Transfer 50 µL of the 1 pg/µL 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 fg/µL)**.

D

Preparing the High Mass Range Calibration Solution

If using the traditional calibration solutions for the Orbitrap Fusion and Orbitrap Fusion Lumos MSs, follow these procedures to prepare the high mass range calibration solution for H-ESI mode. The procedures call for potentially hazardous chemicals, including enfuvirtide, which is supplied in the Orbitrap Tribrid Series Chemicals Kit (P/N 80000-62049).

Contents

Supplies

To prepare the high mass range calibration solution, see the list of required chemicals and equipment in Table 12 and Table 13, respectively. For a complete selection of LC/MS-grade consumables from Thermo Fisher Scientific, visit www.fishersci.com.

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 12. Required chemicals

Description	Grade	Part number
Acetic acid, glacial	Optima LC/MS	A113
Acetonitrile (ACN)	Optima UHPLC/MS	A956
Ammonium hydroxide, 28–30%	Certified ACS[a] Plus	A669
Enfuvirtide, 100 mg	–	HAZMAT-01-00083
Methanol	Optima UHPLC/MS	A458
Water	Optima UHPLC/MS	W8-1

[a] American Chemical Society

IMPORTANT

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

Table 13. Required equipment

Description	Quantity
Bottle, glass, 100 mL	1
Bottle, glass, 500 mL	1
Cylinder, glass, 100 mL	1
Cylinder, glass, 250 mL	1
Gloves, nitrile[a] (Fisher Scientific 19-120-2947 or Unity Lab Services 23827)	–
Pipet tips, 100 µL	3
Pipetter, 100–1000 µL	1
Sonicator	1
Spatula, stainless steel	1
Syringes, glass, 500 µL	3
Vial, scintillation, glass, 20 mL	1
Vortex mixer	1
Weighing container	1
Weight scale	1

[a] Multiple sizes are available.

Guidelines

For optimal results, follow these guidelines when performing the procedures in this appendix:

- Make sure that the surrounding area is neat, clean, and well ventilated.
- Have nearby the necessary chemicals and equipment.
- Always wear protective safety glasses and a new pair of nitrile gloves when handling solvents and samples—never reuse gloves after you remove them.
- Clean all bottles and vials with methanol and let dry before use.
- Proceed methodically.

Preparing the Enfuvirtide Calibration Solution

See these topics to prepare the enfuvirtide calibration solution:

1. Preparing the 0.15% Ammonium Hydroxide Solution
2. Preparing the 0.15% Ammonium Hydroxide/ACN-50:50 Solution
3. Preparing the Enfuvirtide Stock Solution
4. Preparing the 0.1% Acetic Acid/ACN-50:50 Solution
5. Preparing the Enfuvirtide Final Solution

CAUTION When using solvents or corrosives, always wear protective gloves and safety glasses, and use the appropriate vapor respiratory equipment. Prepare the solution under a fume hood.

Preparing the 0.15% Ammonium Hydroxide Solution

❖ **To prepare the 0.15% ammonium hydroxide solution**

1. Transfer 99.5 mL of the LC/MS-grade water into a clean 100 mL glass bottle.
2. Use a syringe to transfer 500 µL of the ammonium hydroxide to the bottle.
3. Put on the lid and vortex the bottle of the ammonium hydroxide (28–30%) solution for 2 minutes.
4. Label the bottle **0.15% Ammonium Hydroxide Solution**.

Preparing the 0.15% Ammonium Hydroxide/ACN-50:50 Solution

❖ **To prepare the 0.15% ammonium hydroxide/ACN-50:50 solution**

1. Pipet 60 mL of the acetonitrile into a clean 500 mL glass bottle.
2. Pipet 60 mL of the "0.15% Ammonium Hydroxide Solution" into the bottle.
3. Put on the lid and vortex the bottle of solution for 2 minutes.
4. Label the bottle **0.15% Ammonium Hydroxide/ACN-50:50 Solution**.

Preparing the Enfuvirtide Stock Solution

❖ **To prepare the enfuvirtide stock solution**

1. Obtain the enfuvirtide from the lab freezer, and verify the chemical name and expiration date before you continue.
2. Weigh out 22 ±0.5 mg of the enfuvirtide.

   IMPORTANT Refrigerate the opened enfuvirtide container. For long-term storage, keep frozen at –25 to –15 °C (–13 to 5 °F).

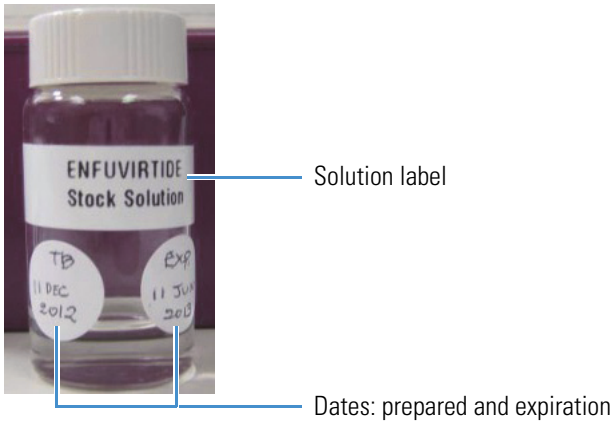
3. Carefully pour the measured enfuvirtide into a clean 20 mL scintillation vial.

   Discard the used weighing container according to established lab practices.
4. Pipet 5 mL of the "0.15% Ammonium Hydroxide/ACN-50:50 Solution" into the vial.
5. Put on the lid and vortex the bottle for 2 minutes.
6. Label the bottle **Enfuvirtide Stock Solution** and include the prepared and expiration dates (Figure 27).

   IMPORTANT

   - Freeze the stock solution at –20 °C (–4 °F) or colder for up to 6 months.
   - (Optional) To store the stock solution up to 1 year, make 1 mL aliquots.

Figure 27. Example labeling for the Enfuvirtide stock solution



Preparing the 0.1% Acetic Acid/ACN-50:50 Solution

❖ **To prepare the 0.1% acetic acid/ACN-50:50 solution**

1. Transfer 100 mL of the water/ACN-50:50 Solution into a clean 100 mL glass bottle.
2. Use a syringe to transfer 100 µL of the glacial acetic acid to the bottle.
3. Put on the lid and vortex the bottle of solution for 2 minutes.
4. Label the bottle **0.1% Acetic Acid/ACN-50:50 Solution**.

Preparing the Enfuvirtide Final Solution

❖ **To prepare the enfuvirtide final solution**

1. Transfer 10 mL of the "0.1% Acetic Acid/ACN-50:50 Solution" to a clean 20 mL glass scintillation vial.
2. Use a syringe to transfer 100 µL of the "Enfuvirtide Stock Solution" into the vial.
3. Put on the lid and vortex the bottle of solution for 2 minutes.
4. Loosen the lid slightly and sonicate the bottle for 5 minutes.
5. Label the bottle **Enfuvirtide Final Solution** and include the prepared and expiration dates.

Note Refrigerate the final solution at 4 °C (39 °F) for up to 4 weeks.

Installation Kits

The Orbitrap Tribrid Series MS ships with several kits. This appendix lists the necessary components to complete the procedures in this guide. For a full list of the kits and their contents, refer to the Hardware Manual.

Contents

Orbitrap Tribrid Series Chemicals Kit

IMPORTANT Be aware of the following storage precautions.

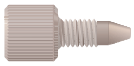
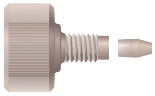
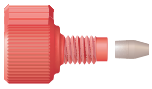
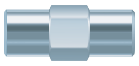
- Calibration and reserpine solutions—Refrigerate the containers after opening. For long-term storage, keep refrigerated at 2–8 °C (36–46 °F).
- Enfuvirtide—Refrigerate the container after opening. For long-term storage, keep refrigerated at –25 to –15 °C (–13 to 5 °F).

Table 14. Orbitrap Tribrid Series Chemicals Kit (P/N 80000-62049)

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

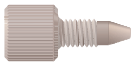
Calibration Kit

Table 15. Calibration Kit (P/N 80000-62078)

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
	HPLC union, black PEEK, 10-32, 0.01 in. thru-hole	1	00101-18202
—	Syringe, gas tight, 500 µL	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 fluorinated ethylene propylene (FEP), 1/16 in. OD, 0.03 in. ID, 3 cm (1.2 in.) long	1	00301-22915

Performance Specification Kit

Table 16. Performance Specification Kit (P/N 80100-62008)

Image	Item	Quantity	Part number
—	Column, HPLC, 20 × 2.1 mm ID, Hypersil GOLD™ AQ C18, 1.9 µm particles	1	00109-01-00013
	Fitting, fingertight, one-piece natural PEEK, 10-32	10	00109-99-00016
	Needle port, PEEK	1	00110-22030
	Sample loop, 2 µL, PEEK	1	00110-16012
—	Syringe, gas tight, 500 µL	1	00301-19016
—	Tubing, red PEEK, 1/16 in. OD, 0.005 in. ID, 3 m (10 ft) long	1	00301-22912
	Union Tee, HPLC, PEEK, 1/16 in. orifice, 0.020 in. (0.5 mm) thru-hole, 10-32 (provided with fingertight fittings)	1	00101-18204

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 operating at atmospheric pressure. Electrons from a corona discharge initiate the process by ionizing the mobile phase vapor molecules, forming a reagent gas. Charged species are generated in the gas phase.

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

atmospheric pressure photoionization (APPI) A soft ionization technique that shows an ion generated from a molecule when it interacts with a photon from a light source.

auxiliary gas The outer-coaxial gas (nitrogen) that assists in evaporating the sample solution as it exits the ESI, APCI (optional), or APPI (optional) spray insert. The mass spectrometer heats this gas to the user-specified vaporizer temperature.

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 in the collision cell 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. The collisions break the bonds and fragment the ions into smaller charged product ions and neutral fragments.

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 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 soft ionization technique operating at atmospheric pressure. A solution of the analyte passes through a small capillary such that the fluid sprays through an electric field, generating very fine droplets. The droplets evaporate until all ions are in the gas phase.

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

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

I

ion detection system A high sensitivity, off-axis system for detecting ions. It produces a high signal-to-noise ratio (S/N) and allows for switching of the voltage polarity between positive ion and negative ion modes of operation. The ion detection system includes 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 window The baseline width of a window for a mass peak (or peak cluster) of interest for an MS/MS or MS^n scan.

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

ion source See API source.

L

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 measures and analyses the ions according to their mass-to-charge ratio (*m/z*). The resulting mass spectrum is a characteristic pattern for identifying 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 $C7H7^{2+}$, $m/z = 45.5$.

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.

MS^n 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 MS^n. MS^n 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

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 (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.

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:

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

where S is the standard deviation and $\overline{X}$ 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 column.

S

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, storage, or both. 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 scan power n in MS^n, which expresses the number of stages of mass analysis. For example, a scan power of $n = 1$ corresponds to an MS^1 (or MS) scan with one stage of mass analysis. A scan power of

n = 2 corresponds to an MS^2 (or MS/MS) scan with two stages of mass analysis. A scan power of n = 3 corresponds to an MS^3 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 following the isolation of a range of 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.

sweep gas Nitrogen gas that flows out from the gap between the sweep cone and the ion transfer tube into 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.

U

ultraviolet photodissociation (UVPD) A method of fragmentation based on photon absorption by a precursor molecule. Photon absorption causes the molecule to undergo an electronic transition to an excited state followed by subsequent dissociation to fragment species. UVPD produces abundant fragments and is applicable to peptides, proteins, and many other compound classes.

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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