



TSQ Series II

TSQ Endura and TSQ Quantiva

Getting Started Guide

80100-97228 Revision A • June 2017



© 2017 Thermo Fisher Scientific Inc. All rights reserved.

EASY-Max NG, Ion Max NG, nanoViper, and Optima are trademarks; Unity is a registered service mark; and Hypersil GOLD AQ, TSQ Endura, TSQ Quantiva, Thermo Scientific, and Xcalibur are registered trademarks of Thermo Fisher Scientific Inc. in the United States.

The following are registered trademarks in the United States and other countries: Excel, Microsoft, and Windows are registered trademarks of Microsoft Corporation. Teflon is a registered trademark of E.I. du Pont de Nemours & Co.

The following are registered trademarks in the United States and possibly other countries: APPI, PhotoMate, and Syagen are registered trademarks of Morpho Detection, Inc. Rheodyne is a registered trademark of IDEX Health & Science, LLC. Tygon is a registered trademark of the division of Saint-Gobain Performance Plastics Corporation.

Chemyx is a trademark of Chemyx Inc. MX Series II is a trademark of IDEX Health & Science, LLC.

All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

Thermo Fisher Scientific Inc. provides this document to its customers with a product purchase to use in the product operation. This document is copyright protected and any reproduction of the whole or any part of this document is strictly prohibited, except with the written authorization of Thermo Fisher Scientific Inc.

The contents of this document are subject to change without notice. All technical information in this document is for reference purposes only. System configurations and specifications in this document supersede all previous information received by the purchaser.

This document is not part of any sales contract between Thermo Fisher Scientific Inc. and a purchaser. This document shall in no way govern or modify any Terms and Conditions of Sale, which Terms and Conditions of Sale shall govern all conflicting information between the two documents.

Release history: Rev A, June 2017

Software version: (Thermo) Foundation 3.1 SP4 and later, Xcalibur 4.1 and later, Tune 3.0 and later

For Research Use Only. Not for use in diagnostic procedures.

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.

Changes that you make to your system might void compliance with one or more of these regulatory standards. Changes to your system include replacing a part or adding components, options, or peripherals not specifically authorized and qualified by Thermo Fisher Scientific. To ensure continued compliance with the regulatory standards, order replacement parts and additional components, options, and peripherals directly from Thermo Fisher Scientific or an authorized representative.

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, 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 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: 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
EN 61326-1: 2013	EN 61000-4-4: 2004 + A1	

FCC Compliance Statement

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



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

Notice on Lifting and Handling of Thermo Scientific Instruments

For your safety, and in compliance with international regulations, the physical handling of this Thermo Fisher Scientific instrument *requires a team effort* to lift and/or move the instrument. This instrument is too heavy and/or bulky for one person alone to handle safely.

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.

WEEE Directive 2012/19/EU



Thermo Fisher Scientific is registered with B2B Compliance ([B2Bcompliance.org.uk](https://www.b2bcompliance.org.uk)) in the UK and with the European Recycling Platform ([ERP-recycling.org](https://www.eur-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.

Contents

	Preface	xiii
	Accessing Documentation	xiv
	Special Notices, Symbols, and Cautions	xiv
	Contacting Us	xvi
Chapter 1	Introduction	1
	Ionization Techniques	1
	Using H-ESI	2
	Using APCI	3
	Using APPI	4
	Using NSI	4
	LC Flow Rates Ranges	4
	Types of Buffers	6
	Templates in Thermo Xcalibur Instrument Setup (Method Editor)	7
Chapter 2	Setting Up the API Source	9
	Preparing the Mass Spectrometer	9
	Installing or Removing the API Source	11
	Installing the API Source	11
	Removing the API Source	12
	Preparing the Spray Insert for the API Source	13
	Installing the Spray Insert	13
	Adjusting the Spray Direction	13
Chapter 3	Connecting the Inlet Plumbing	17
	Plumbing Connections	17
	Setting Up the Syringe Pump	18
	Setting Up the Inlet Plumbing for the Sample Introduction Techniques	19
	Setting Up the Inlet for Direct Infusion	21
	Setting Up the Inlet for HPLC with an Autosampler	22
	Setting Up the Inlet for High-Flow Infusion Without an Autosampler	23
	Setting Up the Inlet for Loop Injections (Flow-Injection Analysis)	24
	Connecting the Grounding Union to the H-ESI Spray Insert	26

Chapter 4	Using the Syringe Pump and Divert/Inject Valve	27
	Syringe Pump	27
	Divert/Inject Valve	28
	Configurations	29
	Controlling the Divert/Inject Valve.....	30
Chapter 5	Preparing the System for Calibration	31
	Setting Up the Syringe Pump for Direct Infusion	31
	Setting Up the Mass Spectrometer for Calibration.....	32
Chapter 6	Establishing a Stable Ionization Spray	35
	Determining the Initial API Source Settings	35
	Evaluating the Spray Stability	36
Chapter 7	Performing the Tune, Calibration, or Calibration Check	39
	Calibration Solution Peak Values	40
	System Tune and Calibration	41
	Spray Stability Evaluation	43
	System Tune and Check	43
	Check Mass Position and Resolution.....	43
	Calibrate Mass Position and Resolution.....	44
	Electron Multiplier Calibration	44
Chapter 8	Optimizing the System Parameters	45
	Setting Up the Plumbing for Compound Optimization	45
	Optimizing Parameters in the Optimization Pane	45
Chapter 9	Acquiring Sample Data	51
	Using Tune to Acquire Sample Data	51
	Using the Xcalibur Data System to Acquire Sample Data	53
Appendix A	Using Basic Tune Functions	55
	Opening the Tune Window	56
	Setting the Instrument System Controls	57
	Setting the Instrument Power Mode	58
	Checking the Instrument Readback Status	59
	Setting the Tune Preferences	60
	Using the Mass List Table in the Define Scan Pane.....	61
	Using the History Pane	63
	Using the Favorites Pane to Save System Settings	63
Appendix B	Flushing the Inlet Components	65
	Supplies.....	66
	Flushing the Inlet Components after Calibration	67

Appendix C Preparing the Polytyrosine Calibration Solution69

Appendix D Installation Kits71

 Chemical Kit 71

 MS Calibration Kit 72

 Performance Specification Kit 73

Glossary75

Index79

Contents

Figures

Figure 1.	Method Editor showing the experiment template categories	7
Figure 2.	MS API source mount assembly and ion sweep cone	10
Figure 3.	Proper connection for the PEEK tubing and fitting (syringe adapter assembly)	18
Figure 4.	Schematics of the sample introduction techniques (examples)	20
Figure 5.	Plumbing connections for the grounding union (H-ESI mode)	26
Figure 6.	Syringe pump setup (top view)	28
Figure 7.	Divert/inject valve positions	29
Figure 8.	Divert/inject valve plumbed as a loop injector and as a divert valve	30
Figure 9.	Divert/inject valve (front view)	30
Figure 10.	Syringe pump settings box	32
Figure 11.	Plot Chromatogram dialog box with the TIC option selected	37
Figure 12.	Mass spectrum of the calibration solution in positive ion polarity mode	40
Figure 13.	Mass spectrum of the calibration solution in negative ion polarity mode	41
Figure 14.	Calibration procedures and options	42
Figure 15.	Error range of the polytyrosine 1-3-6	44
Figure 16.	Optimizing the source parameters in the Optimization pane	47
Figure 17.	Example optimization of the RF lens (SRIG RF) tuning for m/z 997.55	48
Figure 18.	Examples of the optimization results tables	49
Figure 19.	Data Acquisition pane in the Tune window	52
Figure 20.	Run Sequence dialog box (partial) showing the selected start instrument	53
Figure 21.	Change Instruments In Use dialog box showing the MS as the start instrument	54
Figure 22.	Tune window showing the Define Scan pane	56
Figure 23.	Tune Preferences dialog box	60
Figure 24.	Q3 Resolution selected and added to the SRM Table	62
Figure 25.	Favorites pane	64
Figure 26.	State name box	64


Figures

Preface

The *TSQ Endura and TSQ Quantiva Getting Started Guide* describes how to set up and calibrate the Thermo Scientific™ TSQ Endura™ and TSQ Quantiva™ triple-stage quadrupole mass spectrometry systems.

This guide is intended for the following Thermo Scientific mass spectrometers (MSs):

- TSQ Endura (requires one forepump)
- TSQ Quantiva (requires two forepumps)

Also, because this guide uses drawings of various connections and component parts to help illustrate procedures, be sure to start from , no matter where it appears.

Contents

- [Accessing Documentation](#)
- [Special Notices, Symbols, and Cautions](#)
- [Contacting Us](#)

❖ 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.



Accessing Documentation


The TSQ Endura and TSQ Quantiva MSs include complete documentation. For system requirements, refer to the release notes on the software DVD.

❖ To view the product manuals

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

❖ To view the Help

Do the following as applicable:

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

❖ 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.







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.
	Hot surface: Before touching the API source assembly, allow heated components to cool.
	Risk of electric shock: This instrument uses voltages that can cause electric shock and/or personal injury. Before servicing, shut down the instrument and disconnect it from line power. While operating the instrument, keep covers on.
	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.
	Sharp object: Avoid handling the tip of the syringe needle.

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	<ul style="list-style-type: none"> ❖ To find global contact information or customize your request <ol style="list-style-type: none"> 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 <p>Go to thermofisher.com/us/en/home/technical-resources.</p> ❖ To find product information <p>Go to thermofisher.com/us/en/home/brands/thermo-scientific.</p> 		

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.

Introduction

This chapter provides general information about the TSQ Endura and TSQ Quantiva MSs. 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 *TSQ Endura and TSQ Quantiva 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 *TSQ Endura and TSQ Quantiva Hardware Manual*.

Contents

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

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 API source’s manual.

- [Using H-ESI](#) (Typically preferred for polar compounds)
- [Using APCI](#) (Typically preferred for medium polar compounds)
- [Using APPI](#) (Typically preferred for certain polar and nonpolar compounds)
- [Using NSI](#) (Typically preferred for peptides and proteins)

Using H-ESI

H-ESI is a soft gas phase ionization technique. The H-ESI source transfers ions in solution to the gas phase. H-ESI can analyze many samples that previously were not suitable for mass analysis (for example, heat-labile compounds or high molecular mass compounds). You can use H-ESI to analyze any polar compound that is an ion in solution, including adduct ions. Included in this class of compounds are biological polymers (such as proteins, peptides, glycoproteins, and nucleotides), pharmaceuticals and their metabolites, and industrial polymers. For example, you might analyze polyethylene glycols from a solution containing ammonium acetate because of adduct formation between NH_4^+ ions in the solution and oxygen atoms in the polymer. With H-ESI, the range of molecular masses that the 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 $\mu\text{L}/\text{min}$. However, for optimum H-ESI performance, vary the flow rate¹ into the MS over a range of 1–1000 $\mu\text{L}/\text{min}$, and configure the H-ESI spray insert for the high- or low-flow² 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.

¹ The H-ESI spray insert can generate ions from liquid flows as low as 1 $\mu\text{L}/\text{min}$.

² For the range of 1–50 $\mu\text{L}/\text{min}$, 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.

Using APCI

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

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

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

The upper limit for the system's flow rate is 3000 $\mu\text{L}/\text{min}$. However, for optimum APCI performance, vary the flow rate³ into the MS over a range of 200–2000 $\mu\text{L}/\text{min}$. This flow range is optimal for the following separation techniques: analytical LC, microbore LC, and semi-preparative LC. See [Table 3](#) for APCI 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 $\mu\text{L}/\text{min}$ require more care to maintain a stable spray.

Using APPI

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 (as in the nitrogen, sheath, and auxiliary gases, 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.

Using NSI

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 Rates Ranges

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

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

The following tables list the general guidelines (default parameter values for the API source) for system operation using H-ESI ([Table 2](#)), APCI ([Table 3](#)), and NSI ([Table 4](#)) for a range of LC solvent flow rates.

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	TSQ Endura MS: 275 TSQ Quantiva MS: 325	20	3.5
16–99	Pos: 3500 Neg: -2500	25	5	0	TSQ Endura MS: 275 TSQ Quantiva MS: 325	75	8.0
100–199	Pos: 3500 Neg: -2500	35	7	0	TSQ Endura MS: 300 TSQ Quantiva MS: 325	275	10.5
200–500	Pos: 3500 Neg: -2500	50	10	1	325	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	TSQ Endura MS: 250 TSQ Quantiva MS: 300	275	Pos: 4 Neg: -10
5–199	25	5	0	TSQ Endura MS: 250 TSQ Quantiva MS: 300	325	Pos: 4 Neg: -10
200–500	45	5	0	TSQ Endura MS: 275 TSQ Quantiva 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	0	325

Note Use the Ion Source pane to determine the initial spray voltage, API gas pressures, ion transfer tube temperature, and vaporizer temperature for the LC flow rate of your experiment. See [Determining the Initial API Source Settings](#).

Use the Optimization pane to optimize the spray voltage and API gas pressures for your experiment. See [Chapter 8, “Optimizing the System Parameters.”](#)

Types of Buffers

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

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

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

For a list of recommended solvents, refer to the *TSQ Endura and TSQ Quantiva 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.

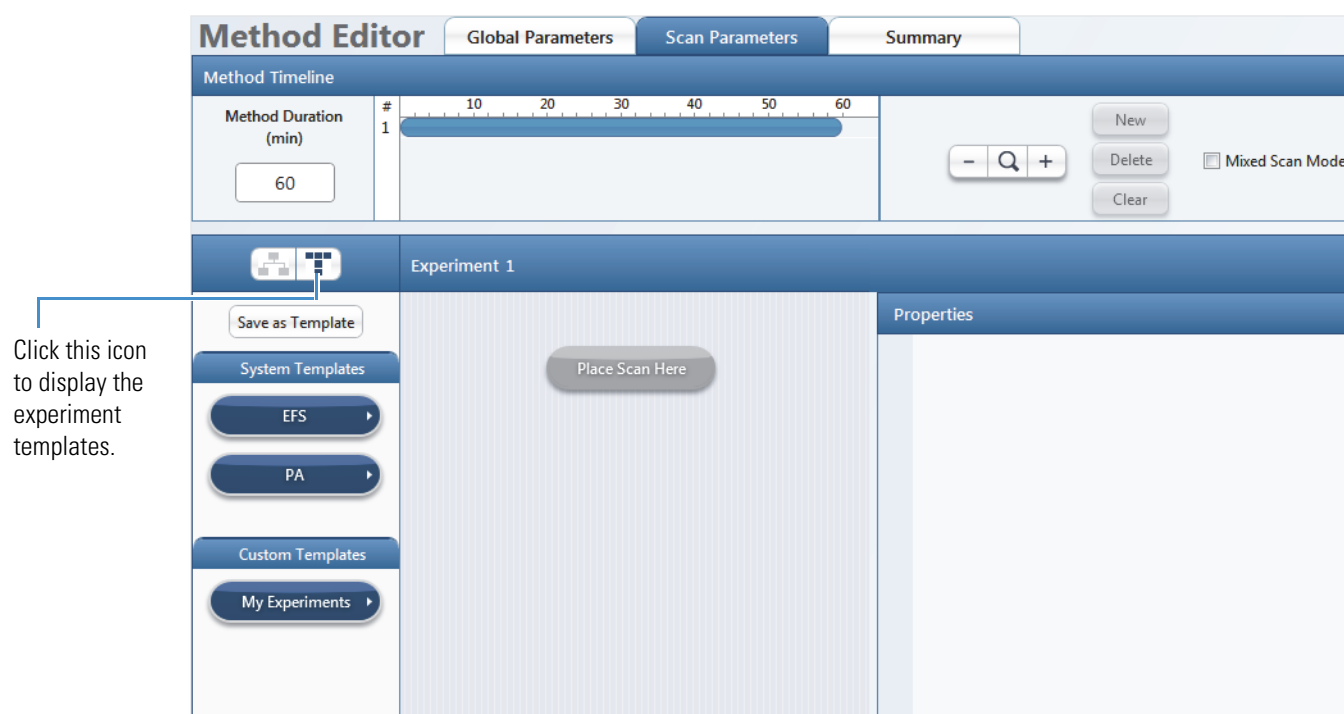
Note You might need to increase the frequency of API source maintenance when you use nonvolatile buffers.

Templates in Thermo Xcalibur Instrument Setup (Method Editor)

Use the Method Editor (Figure 1) that opens in the Xcalibur Instrument Setup window to create an instrument method 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.

The Method Editor provides default system templates for several types of experiments, including environmental and food safety (EFS) and peptide analysis (PA).

Figure 1. Method Editor showing the experiment template categories



1 Introduction

Templates in Thermo Xcalibur Instrument Setup (Method Editor)

Setting Up the API Source

This chapter provides information about setting up the Thermo Scientific EASY-Max NG™ API source (for the TSQ Endura MS) or Ion Max NG™ API source (for the TSQ Quantiva MS) for H-ESI, APCI, and APPI experiments. For NSI experiments, use one of the compatible Thermo Scientific nanospray sources.

Shipment of the API source includes the H-ESI spray insert. For APCI experiments, order the APCI Installation Kit (P/N OPTON-30159), which includes the APCI spray insert. For APPI experiments, order the APPI Interface Kit (P/N OPTON-30185).

Contents

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

Preparing the Mass Spectrometer

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

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

❖ To prepare the mass spectrometer

1. Complete all data acquisition, as applicable.
2. Open the Tune window (see [page 56](#)).
3. Place the MS in **Off** mode (see [page 58](#)).

The LC/MS system is now in off mode (see Chapter 6 in the *TSQ Endura and TSQ Quantiva Hardware Manual* for a list of components that remain on). After the API 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 API source housing.

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

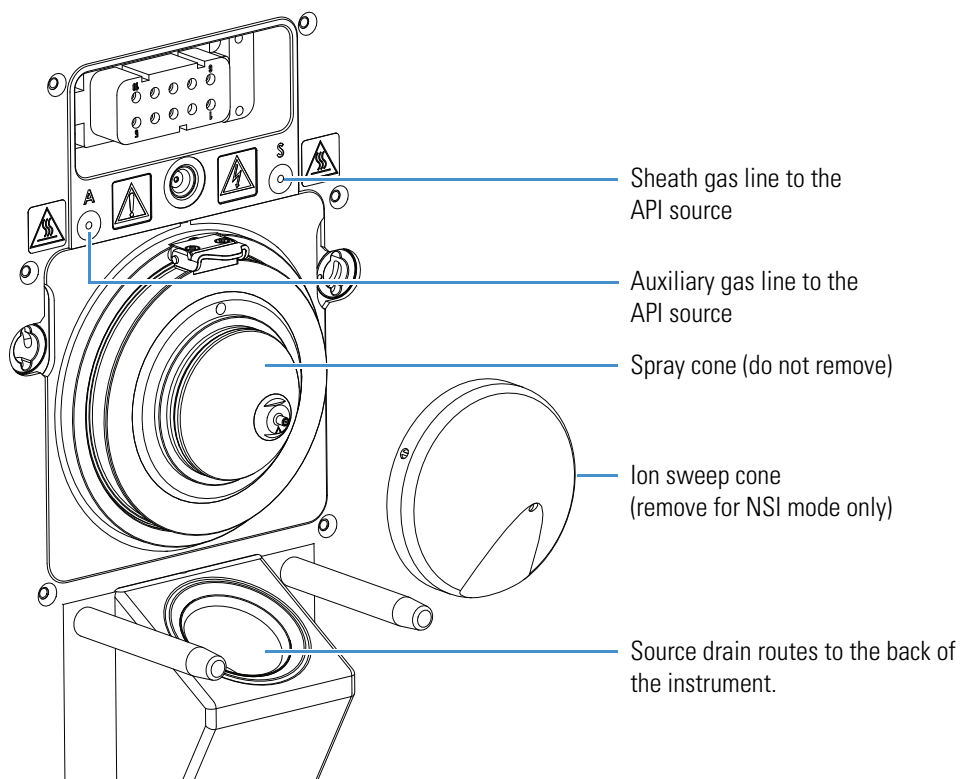
For instructions on how to remove the API source, refer to the *Ion Max NG and EASY-Max NG Ion Sources User Guide*.



CAUTION Hot surface. Avoid touching the API source housing when the MS is in operation. The external surface of the API source housing can become hot enough to burn your skin.

- Depending on the ionization mode, do the following (Figure 2):
 - For H-ESI, APCI, or APPI mode, install the ion sweep cone over the MS's spray cone (Figure 2).
 - 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 2. MS API source mount assembly and ion sweep cone



Installing or Removing the API Source

All the wiring and gas plumbing for the API source are internal. This means you can install or remove the API source or change the ionization mode¹ (H-ESI, APCI, or APPI) by changing the spray insert—all without the use of tools.

Installing the API Source

The TSQ Endura or TSQ Quantiva MS internally routes the solvent waste from the bottom of the API source to the left side Drain/Waste port. Make sure that the solvent waste system is connected as described in Chapter 3 of the *TSQ Endura and TSQ Quantiva Getting Connected Guide*.

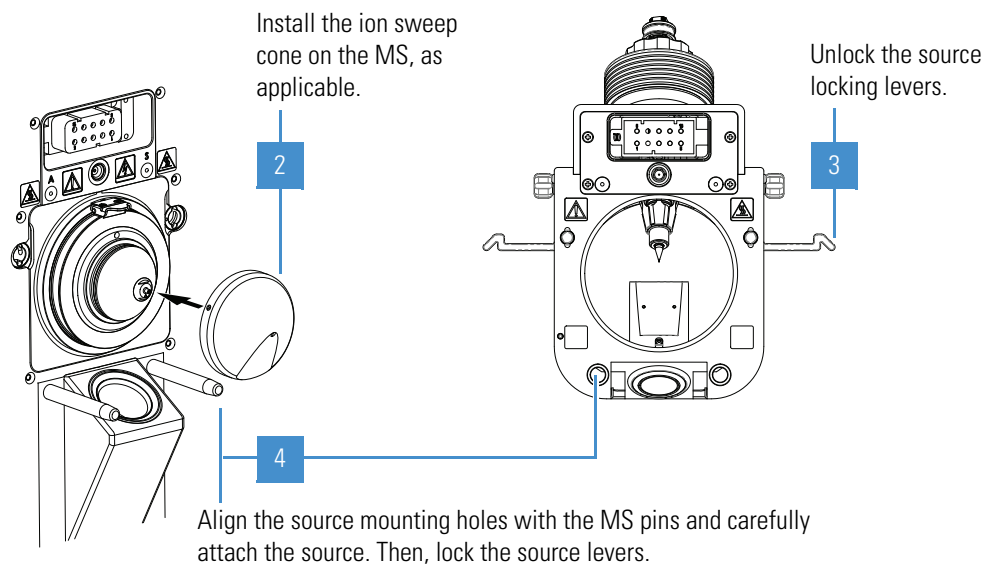
Complete the appropriate procedure:

- To install the API source onto the MS (instrument calibration and experiments)
- To install the NSI source (experiments)

❖ To install the API source onto the MS

1

For APCI mode, check that the corona discharge needle assembly (not shown) is installed in the API source housing. For instructions, refer to the *Ion Max NG and EASY-Max NG Ion Sources User Guide*.



¹ APCI and APPI modes require that you purchase and install the applicable kits. Refer to the *TSQ Endura and TSQ Quantiva Hardware Manual*.

❖ **To install the NSI source**

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

Removing the API Source

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

❖ **To remove the API 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 (see [page 58](#)).



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.

Preparing the Spray Insert for the API Source

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

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

Installing the Spray Insert

For H-ESI mode, install the H-ESI spray insert and turn on the source heater. For APCI mode, install the APCI spray insert. For APPI mode, you can use either of the spray inserts; refer to “Spray Insert Selection” in Chapter 1 of the *Ion Max NG and EASY-Max NG Ion Sources User Guide*.

IMPORTANT For experiments with flow rates that are less than 50 $\mu\text{L}/\text{min}$, install the low-flow metal needle insert into the H-ESI spray insert. For part numbers, refer to “Replaceable Parts” in the *TSQ Endura and TSQ Quantiva Hardware Manual*.

❖ **To install the spray insert**

1. Follow the procedure [Installing the API Source](#).
2. To switch between ionization modes or change the needle insert, refer to the *Ion Max NG and EASY-Max NG Ion Sources User Guide*.

Adjusting the Spray Direction

To maximize sensitivity or robustness, you can adjust the spray direction by a few millimeters. Typically, you adjust the spray direction while optimizing the API source parameters for the analytes. Make the adjustment according to the guidelines in [Table 5](#).

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 (Sheet 1 of 2)

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 $\mu\text{L}/\text{min}$). 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 $\mu\text{L}/\text{min}$.
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.

2 Setting Up the API Source

Preparing the Spray Insert for the API Source

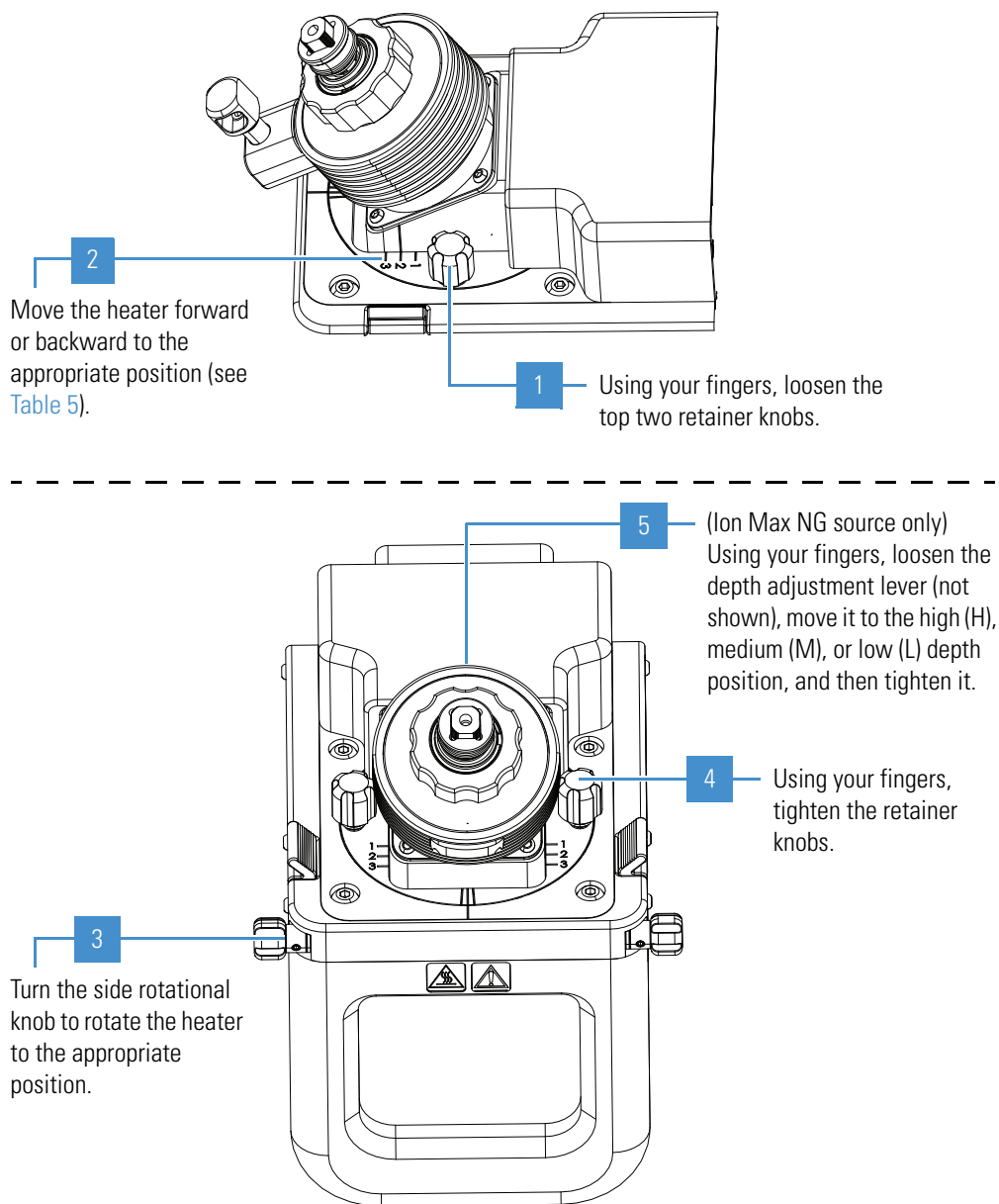
Table 5. Guidelines for adjusting the heater and spray insert position (Sheet 2 of 2)

Adjustment control	Description
Rotational position	
Left, center, right (marks)	Use the center mark to position the spray closest to the entrance of the MS.
Spray insert depth (Ion Max NG ion source only)	
Move the lever to the left.	Raises the spray insert (H position).
Move the lever to the right.	Lowers the spray insert (L position).

❖ To adjust the spray direction



CAUTION Hot surface. Avoid touching the API 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 Setting Up the API Source

Preparing the Spray Insert for the API Source

Connecting the Inlet Plumbing

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

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 71](#)).

Contents

- [Plumbing Connections](#)
- [Setting Up the Syringe Pump](#)
- [Setting Up the Inlet Plumbing for the Sample Introduction Techniques](#)
- [Connecting the Grounding Union to the H-ESI Spray Insert](#)

Plumbing Connections

The modular divert/inject valve shipped with your order is a six-port, two-position, Rheodyne™ injection valve. The ports use standard 10-32 fittings for high-pressure and 1/16 in. OD tubing. To connect the high-pressure tubing to the valve, use the one-piece fingertight fittings provided in the MS Calibration Kit (see [page 72](#)).

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

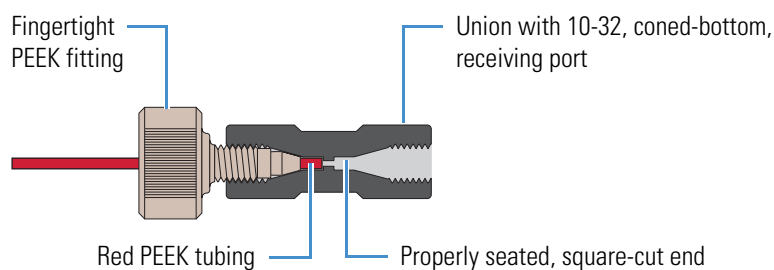
3 Connecting the Inlet Plumbing

Setting Up the Syringe Pump

Ensure the following when you make the plumbing connections:

- The ends of the PEEK tubing are squarely cut (Figure 3). 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 3. Proper connection for the PEEK tubing and fitting (syringe adapter assembly)



Setting Up the Syringe Pump

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



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

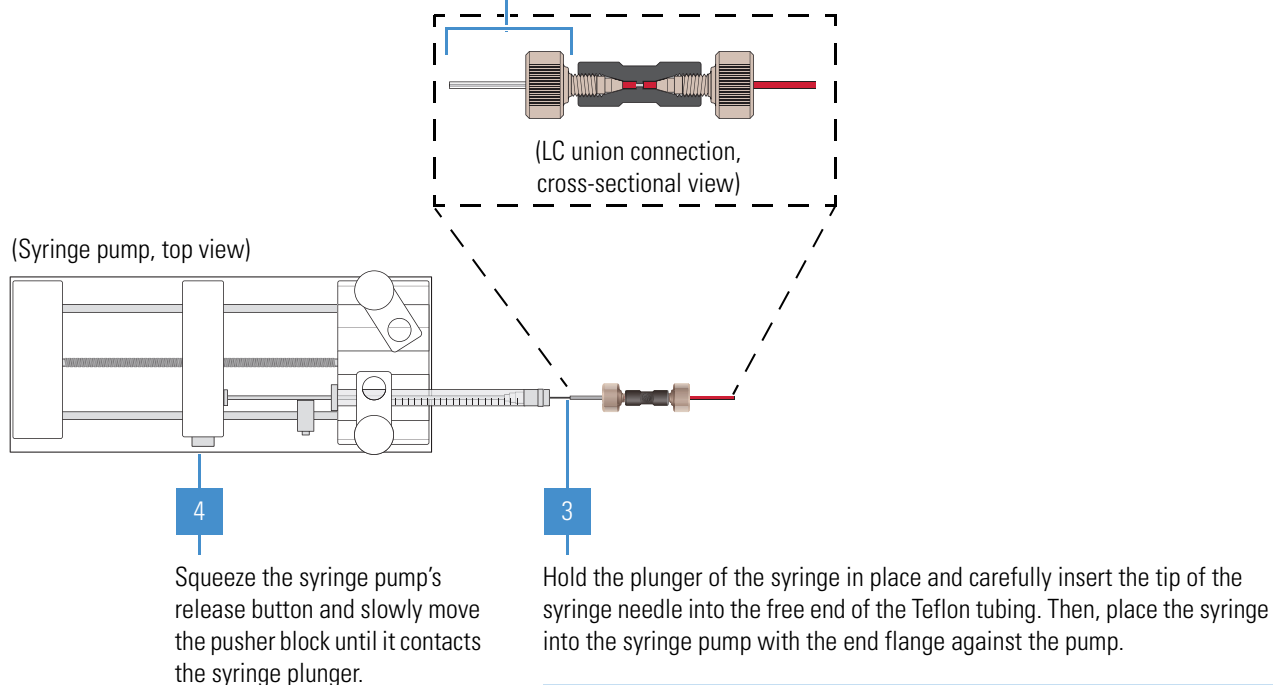
IMPORTANT To minimize the possibility of cross-contamination, do the following:

- 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 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.



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

Setting Up the Inlet Plumbing for the Sample Introduction Techniques

The TSQ Endura or TSQ Quantiva MS has an external [syringe pump](#) and [divert/inject valve](#). The following techniques are available to introduce samples into the API 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 \(Flow-Injection Analysis\)](#)

Figure 4 summarizes these configurations.

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

3 Connecting the Inlet Plumbing

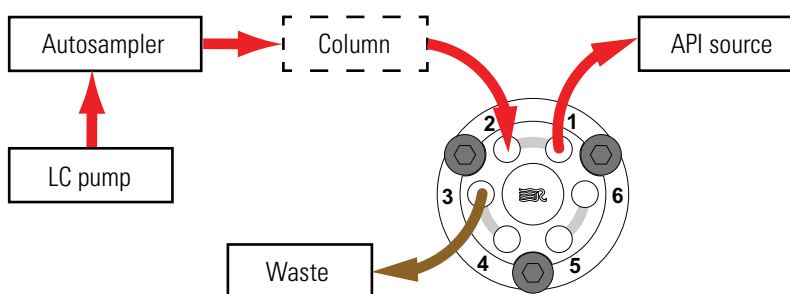
Setting Up the Inlet Plumbing for the Sample Introduction Techniques

Figure 4. Schematics of the sample introduction techniques (examples)

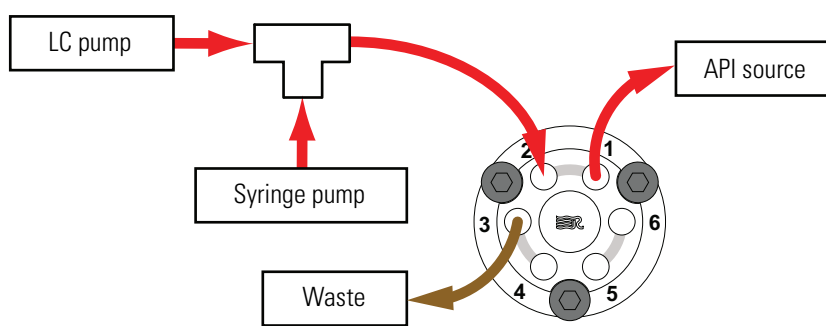
Direct infusion



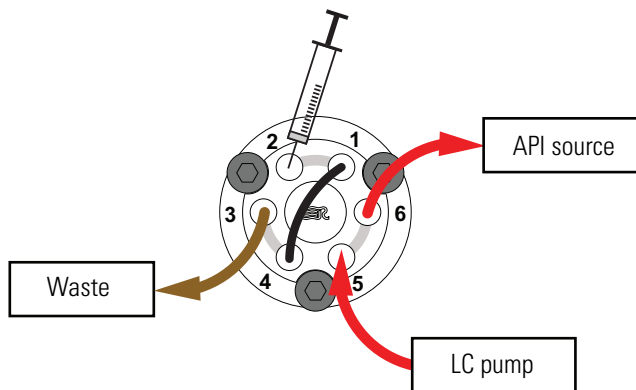
HPLC with autosampler (divert valve)



High-flow infusion without an autosampler (divert valve)



Manual loop injection (loop injector)



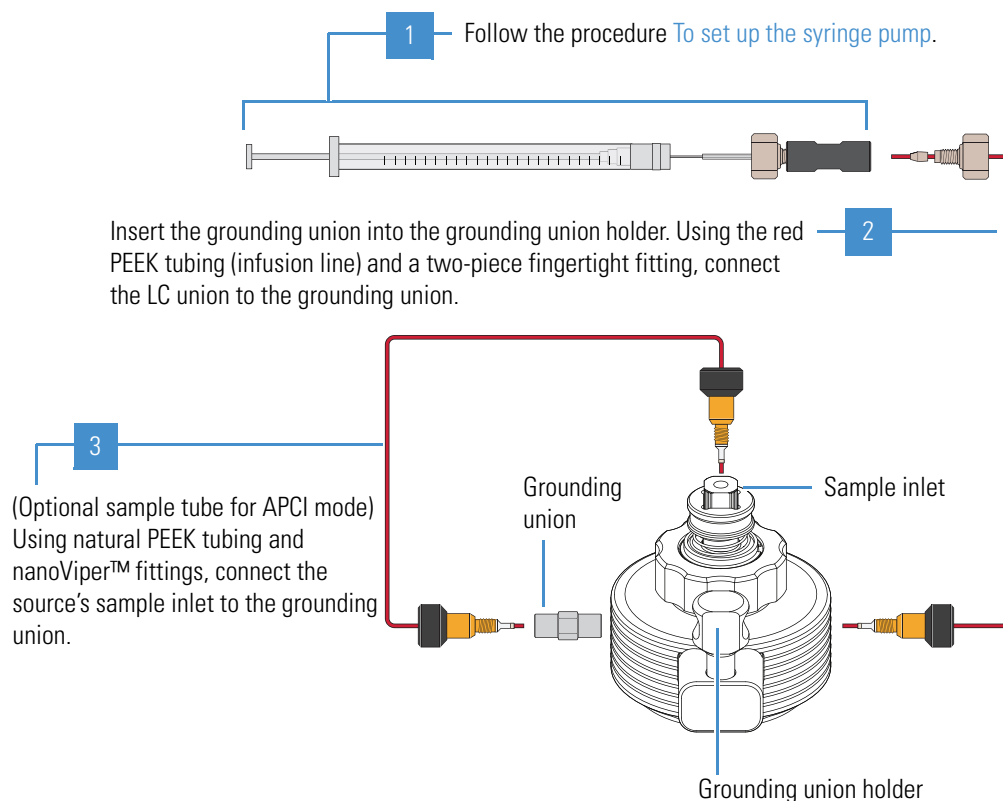
Legend

- Red PEEK tubing
- Teflon FEP tubing
- Sample loop

Setting Up the Inlet for Direct Infusion

The direct infusion technique uses the syringe pump to infuse sample directly into the API source. Use this technique to introduce the calibration solution for calibrating in H-ESI mode—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 API source for direct infusion (H-ESI mode)



3 Connecting the Inlet Plumbing

Setting Up the Inlet Plumbing for the Sample Introduction Techniques

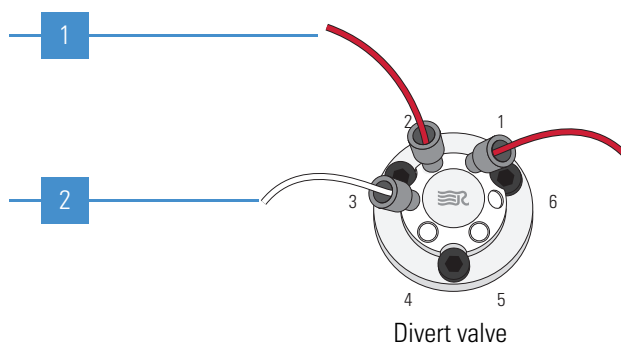
Setting Up the Inlet for HPLC with an Autosampler

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 API source. Use the autosampler to inject sample solution into the flow from an LC pump. In a typical LC/MS experiment, direct the solvent flow through an LC column to separate the compounds of a mixture before they are directed into the API source.

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

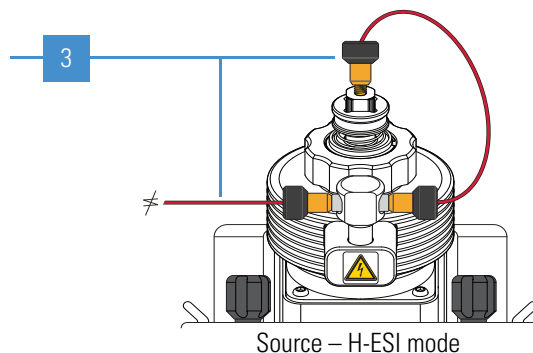
Using red PEEK tubing, connect **port 2** (with a one-piece fingertight fitting) to the outlet of the autosampler or LC pump (with an appropriate fitting and ferrule). (Optional) Install an LC column between the LC device and the divert valve.

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



(H-ESI mode) Using two pieces of red PEEK tubing, make these connections:

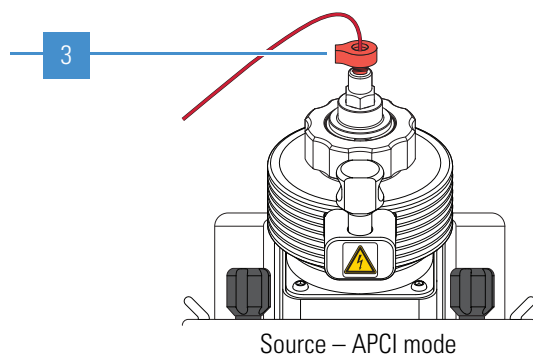
- Connect **port 1** (with a one-piece fingertight fitting) to the installed grounding union (with a two-piece fingertight fitting).
- Connect the other end of the grounding union (with a two-piece fingertight fitting) to the H-ESI spray insert's sample inlet (with a one-piece fingertight fitting).



(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.



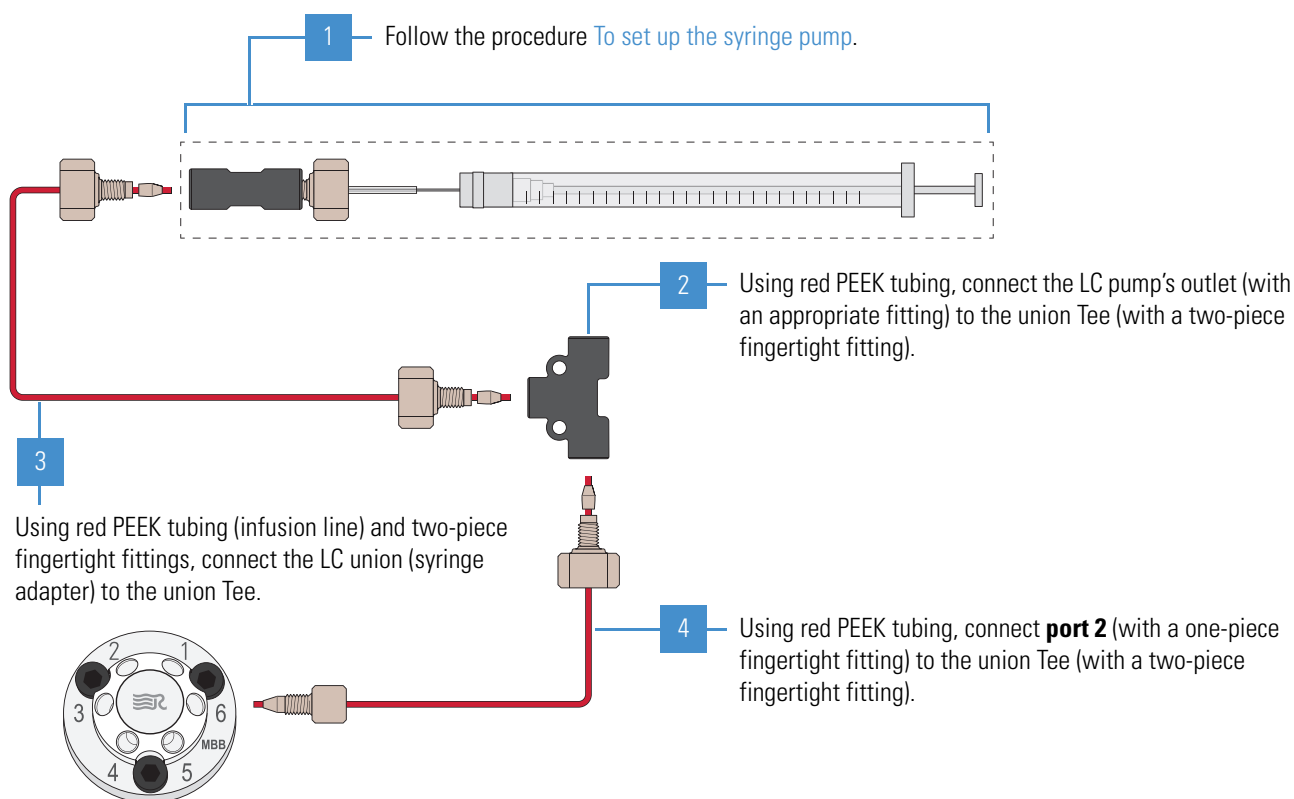
Setting Up the Inlet for High-Flow Infusion Without an Autosampler

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

For information about the valve configurations, see [Configurations](#).

❖ To set up the API 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 API 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.

3 Connecting the Inlet Plumbing

Setting Up the Inlet Plumbing for the Sample Introduction Techniques

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 API source. With the valve in the Load position, use a syringe to load sample through the injection port fitting into the sample loop, and then switch the position of the inject valve to the Inject position. Switching the valve to the Inject position allows the solvent flow from the LC pump to backflush the sample out of the loop and into the API source.

Additionally, follow these guidelines:

- Use a manual loop injection without chromatographic separation for qualitative or quantitative analysis when there is a limited amount of a pure sample.
- Use a manual loop injection with chromatographic separation for qualitative or quantitative analysis when there is a limited amount of a sample mixture. Requires an LC column between the injection valve and the API source.

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

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

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

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

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

(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).

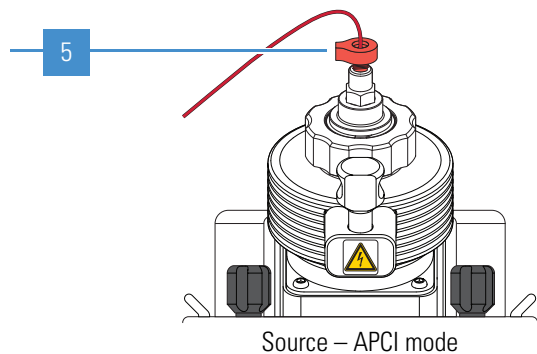
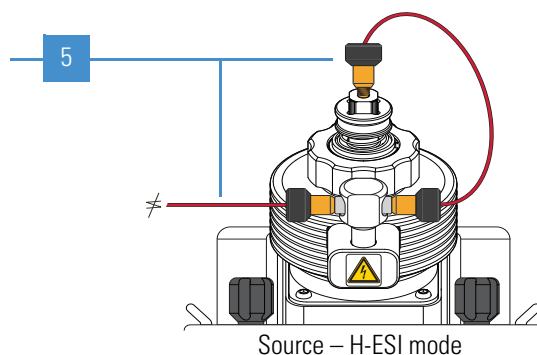
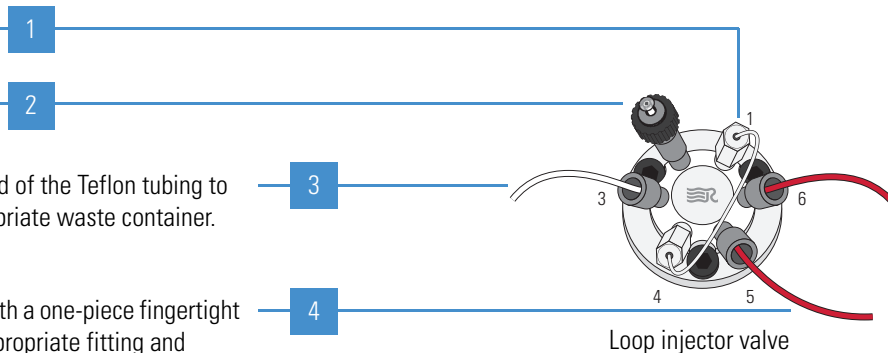
(APCI mode) Using red PEEK tubing, connect **port 6** (with a one-piece fingertight fitting) to the APCI spray insert's sample inlet (with a two-piece fingertight fitting).

(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.

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.



3 Connecting the Inlet Plumbing

Connecting the Grounding Union to the H-ESI Spray Insert

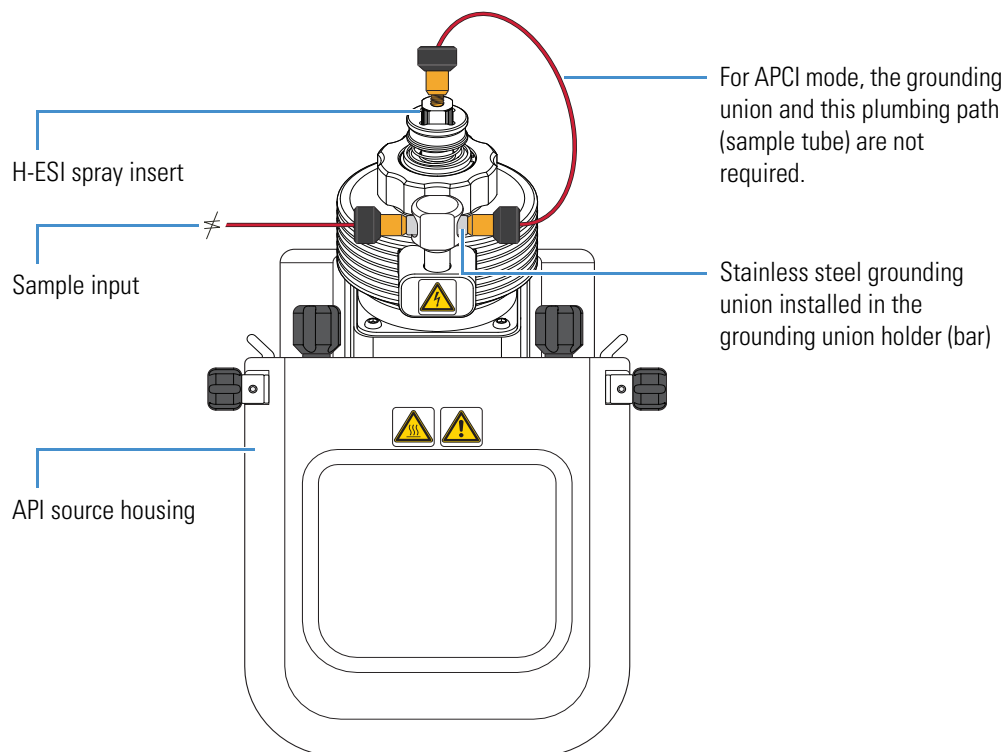
Connecting the Grounding Union to the H-ESI Spray Insert

Figure 5 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 5. Plumbing connections for the grounding union (H-ESI mode)



Using the Syringe Pump and Divert/Inject Valve

This chapter describes the external syringe pump and module divert/inject valve that ship with the TSQ Endura or TSQ Quantiva MS. For information about installing these components, refer to the *TSQ Endura and TSQ Quantiva Getting Connected Guide*.

Contents

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

Syringe Pump

The external Chemyx™ Fusion 100T syringe pump delivers sample solution from an installed syringe, through the sample transfer line (red PEEK), and into the API source. The motorized pusher block ([Figure 6](#)) depresses the syringe plunger at the flow rate specified in the data system. (The default flow rate for calibration is 5 $\mu\text{L}/\text{min}$.)

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

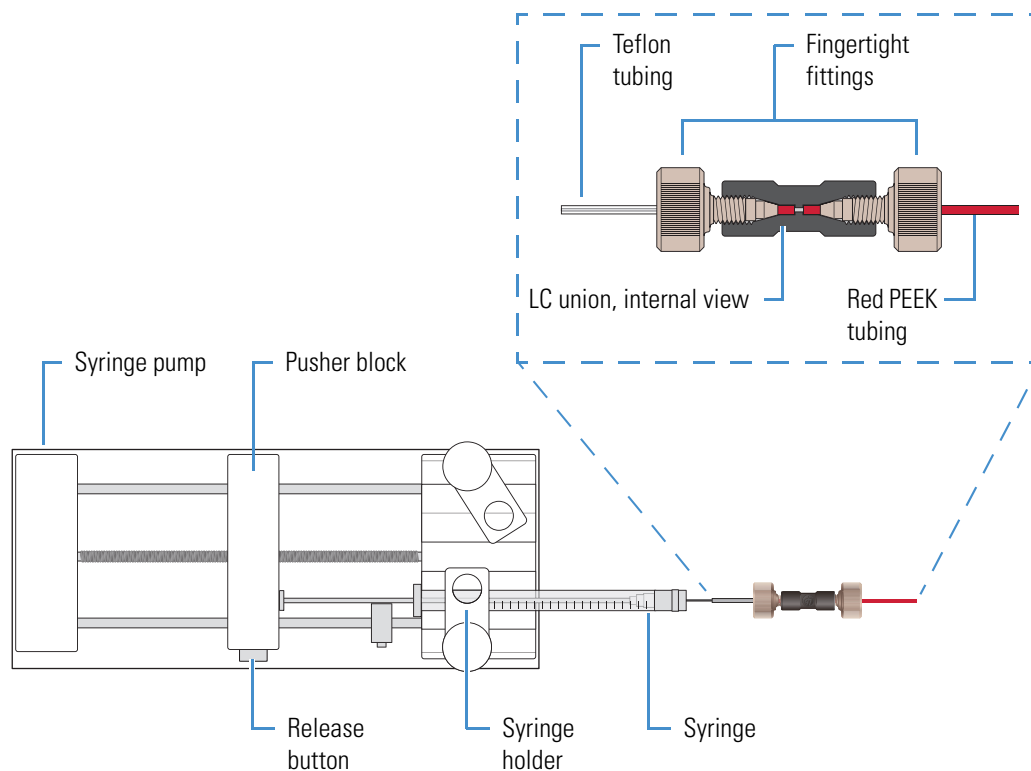
Note If you choose to provide a syringe pump other than the Fusion 100T, ensure that it can provide a steady, continuous flow of 1–5 $\mu\text{L}/\text{min}$.

In the Tune application version 3.0 and later, you can prime the syringe while it is in the provided syringe pump (see [page 58](#)).

4 Using the Syringe Pump and Divert/Inject Valve

Divert/Inject Valve

Figure 6. Syringe pump setup (top view)

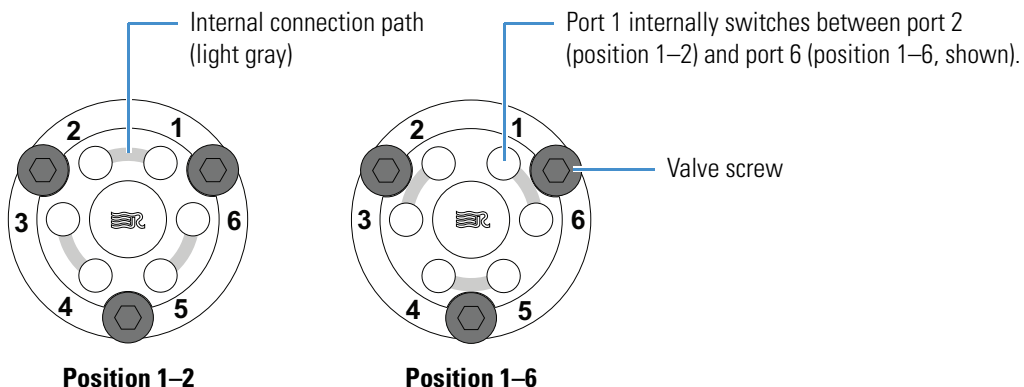


Divert/Inject Valve

The external Rheodyne MX Series II™ divert/inject valve is a 6-port motorized valve that switches between two positions. In the first position, port 1 connects internally to port 2, port 3 connects to port 4, and port 5 connects to port 6. In the second position, the valve rotates clockwise one position so that port 1 connects internally to port 6, port 2 connects to port 3, and port 4 connects to port 5. [Figure 7](#) shows the valve's internal flow paths for both positions.

The Method Editor application identifies the valve's two positions as "1-2" (port 1 to 2) and "1-6" (port 1 to 6). Therefore, ignore the number shown on the valve position indicator ([Figure 9](#)).

Figure 7. Divert/inject valve positions



Configurations

You can configure (plumb) the divert/inject valve as a loop injector (for flow injection analysis) or as a divert valve. The divert valve can switch the solvent front, gradient endpoint, or any portion of the LC run to waste. [Figure 8](#) shows both of these configurations.

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

- Load (position 1-2)—The sample loop is isolated from the solvent stream. Solvent flow from the LC pump enters and exits the valve through ports 5 and 6, respectively. When you load the sample into port 2, the sample enters and exits the sample loop through ports 1 and 4, respectively. As you overfill the sample loop, the excess sample exits the valve through port 3 to waste.
- Inject (position 1-6)—The sample loop is open to the solvent stream. The solvent flow from the LC pump flushes sample out of the sample loop, and then exits through port 6 into the API source.

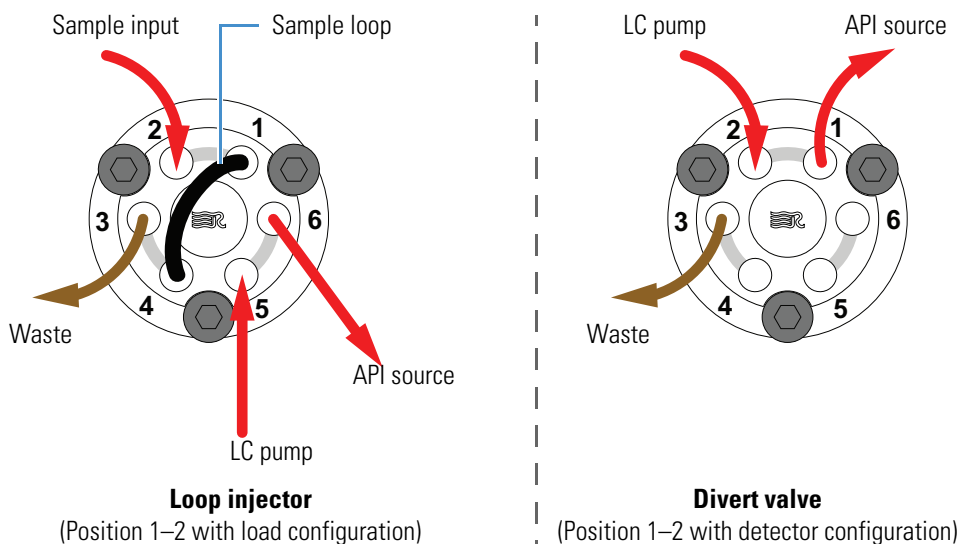
In the divert valve configuration, the valve switches between these two positions:

- Detector (position 1-2)—Solvent flow from the LC pump enters the valve through port 5 and exits through port 6 into the API source.
- Waste (position 1-6)—Solvent flow from the LC pump enters the valve through port 5 and exits through port 4 to waste.

4 Using the Syringe Pump and Divert/Inject Valve

Divert/Inject Valve

Figure 8. Divert/inject valve plumbed as a loop injector and as a divert valve

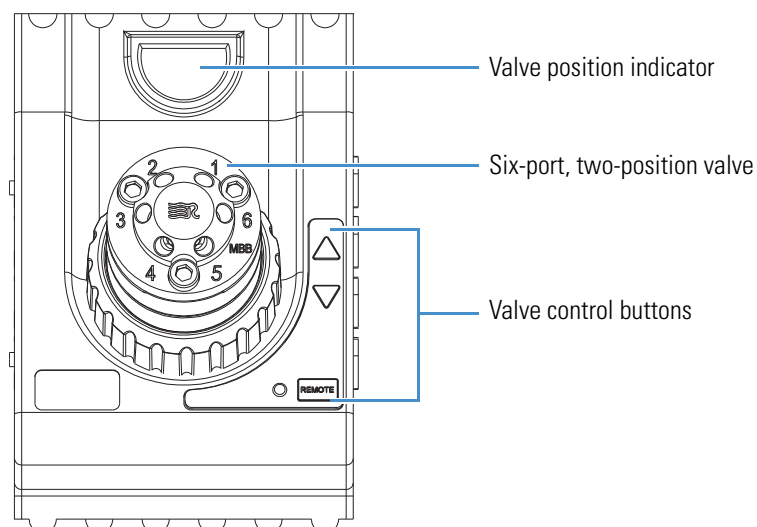


Controlling the Divert/Inject Valve

You can control the divert/inject valve as follows:

- Use the MS's data system to specify the parameters in the Divert Valve Properties pane in the Method Editor. For instructions, refer to the Method Editor Help.
- Use the valve's control buttons (Figure 9) to divert the LC flow between the MS and waste when the valve is in the divert valve configuration, or switch between load and inject modes when the valve is in the loop injector configuration. For instructions, refer to the manufacturer's manual.

Figure 9. Divert/inject valve (front view)



Preparing the System for Calibration

This chapter describes how to prepare the TSQ Endura or TSQ Quantiva system before you calibrate the MS.

IMPORTANT If the MS was turned off for an extended period of time, pump down the vacuum system for at least 15 hours before you start the instrument calibration process. For instructions, refer to Chapter 8, “Maintenance,” in the *TSQ Endura and TSQ Quantiva Hardware Manual*.

Contents

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

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 μ L syringe with 500 μ L of the ESI positive calibration solution.

For a list of provided solutions, see [Chemical 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 3](#)).

2. Follow the procedure [To set up the API source for direct infusion \(H-ESI mode\)](#).
3. Turn on the syringe pump's power switch (on the back of the device).

- 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.


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 API 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

- In the Tune window, place the MS in **On** mode, .
- Click **Profile (Centroid)** and select the profile data type.
- Click **Positive (Negative)** and select the positive ion polarity mode.
- Set the syringe pump parameters as follows:
 - Click **Syringe Off** to turn on the syringe pump.
The button name changes to Syringe On.
 - Click the arrow next to the Syringe On/Off button to open the syringe pump settings box (Figure 10), and then enter the following:

Flow Rate ($\mu\text{L}/\text{min}$): 5

Volume (μL): 500

Figure 10. Syringe pump settings box



Flow Rate ($\mu\text{L}/\text{min}$)	<input type="text" value="5"/>
Volume (μL)	<input type="text" value="500"/>
<input type="button" value="Apply"/>	
<input type="button" value="Prime"/>	

- Click **Apply**.

5. Verify that the inlet plumbing connections do not leak.
6. Open the syringe pump settings box, and press and hold **Prime** to prime the syringe at 100 $\mu\text{L}/\text{min}$.



7. Verify that the system readback is normal (see [page 59](#)).

This completes the setup for calibrating the MS. Go to [Chapter 6, “Establishing a Stable Ionization Spray.”](#)

5 Preparing the System for Calibration

Setting Up the Mass Spectrometer for Calibration

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 API source.

IMPORTANT

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

Contents

- [Determining the Initial API Source Settings](#)
- [Evaluating the Spray Stability](#)

Determining the Initial API Source Settings

Before you evaluate the spray stability, use the Tune application to determine the initial values for the API source parameters. These initial settings provide a starting point for both calibrating and optimizing system performance. The optimal settings for your application depend on the compounds of interest, the solvent matrix, and the chromatographic conditions.

❖ **To determine the initial API source settings**

1. In the Tune window, click the **Ion Source** tab.
2. In the Current LC Flow ($\mu\text{L}/\text{min}$) box, type the flow rate as noted in Table 6, and then click **Get Defaults**.


Table 6. Recommended LC flow rates

Solution type	Injection type	LC flow rate ($\mu\text{L}/\text{min}$)
Calibration	Syringe	5
Sample	Syringe	3–10
	Autosampler	200

3. Click **Apply**.

The Tune application makes a change record in the History pane. Go to the next section.

Evaluating the Spray Stability

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

❖ **To evaluate the spray stability**

1. Open the Tune window.
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 ($\mu\text{L}/\text{min}$): **5**
 Syringe pump settings box: **5** $\mu\text{L}/\text{min}$ 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 pane, verify the value in the Current LC Flow ($\mu\text{L}/\text{min}$) 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.


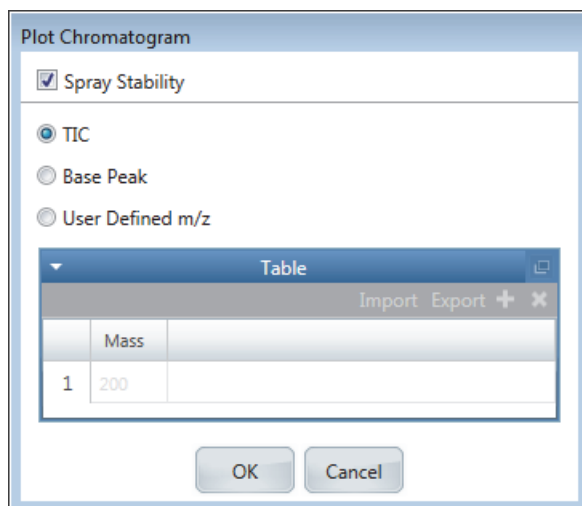
A real-time plot of the solution's mass spectrum appears.
7. Plot the full **total ion current (TIC)** and **relative standard deviation (RSD)** graphs as follows:
 - a. Click the **Plot Chromatogram** icon, , to open the Plot Chromatogram dialog box (Figure 11).

Figure 11. 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 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 7 lists the criteria for a stable spray in either positive or negative ion polarity mode.

6 Establishing a Stable Ionization Spray

Evaluating the Spray Stability

Table 7. 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, optimize the API source parameters (see [Chapter 8](#)).

10. Repeat this procedure for negative ion polarity mode.

This completes the spray stability evaluation.

Performing the Tune, Calibration, or Calibration Check

This chapter describes how to tune, calibrate, or check the calibration of the TSQ Endura or TSQ Quantiva MS in H-ESI mode. The tune, calibrate, and calibration evaluation procedures require that you infuse the calibration solution into the instrument at a steady flow rate. For instructions on how to prepare the calibration solution, see [Appendix C, “Preparing the Polytyrosine Calibration Solution.”](#)

IMPORTANT Follow these precautions against instrument errors or malfunctions:

- To reduce the risk of incorrect readings or instrument malfunctions, perform regular calibrations.
- To reduce the risk of potential errors, use quality control and internal standard (similar compound) monitoring at all times.



CAUTION Do not operate the system if it fails a calibration procedure. Contact a Thermo Fisher Scientific field service representative.

Contents

- [Calibration Solution Peak Values](#)
- [System Tune and Calibration](#)
- [Spray Stability Evaluation](#)
- [System Tune and Check](#)
- [Check Mass Position and Resolution](#)
- [Calibrate Mass Position and Resolution](#)
- [Electron Multiplier Calibration](#)

Calibration Solution Peak Values

The mass spectra of the calibration solution (polytyrosine 1-3-6) in positive (Figure 12) and negative (Figure 13) ion polarity modes have peaks at m/z values close to the following theoretical values:

- Positive mode: m/z 182.08, m/z 508.21, and m/z 997.40
- Negative mode: m/z 180.07, m/z 506.19, and m/z 995.38

IMPORTANT If you observe interfering peaks in the polytyrosine 1-3-6 spectrum that are within ± 10 Da of any of these calibration masses, follow the procedure [To flush the inlet components](#). Ensure that the interfering masses show less than 25 percent of the intensity of the calibrant ions.

Figure 12. Mass spectrum of the calibration solution in positive ion polarity mode

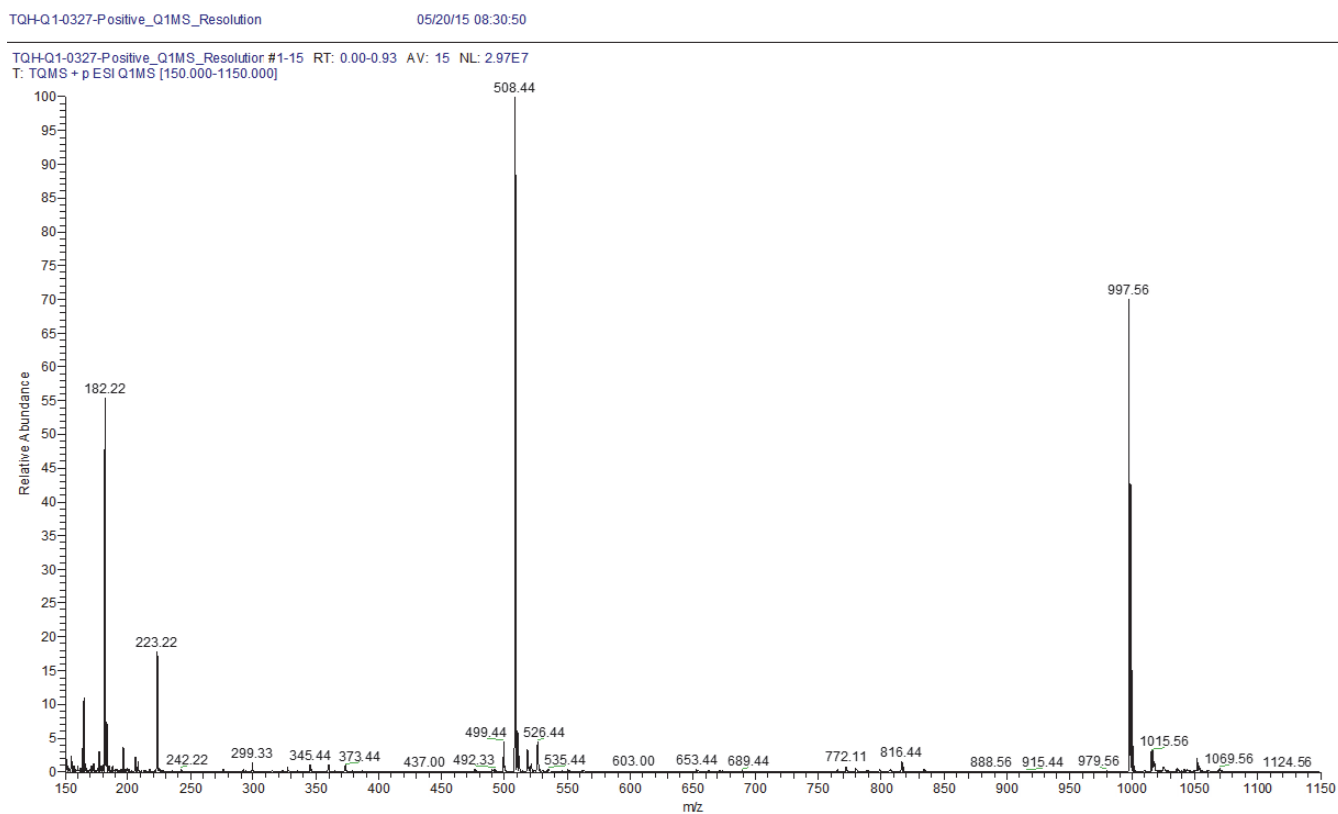
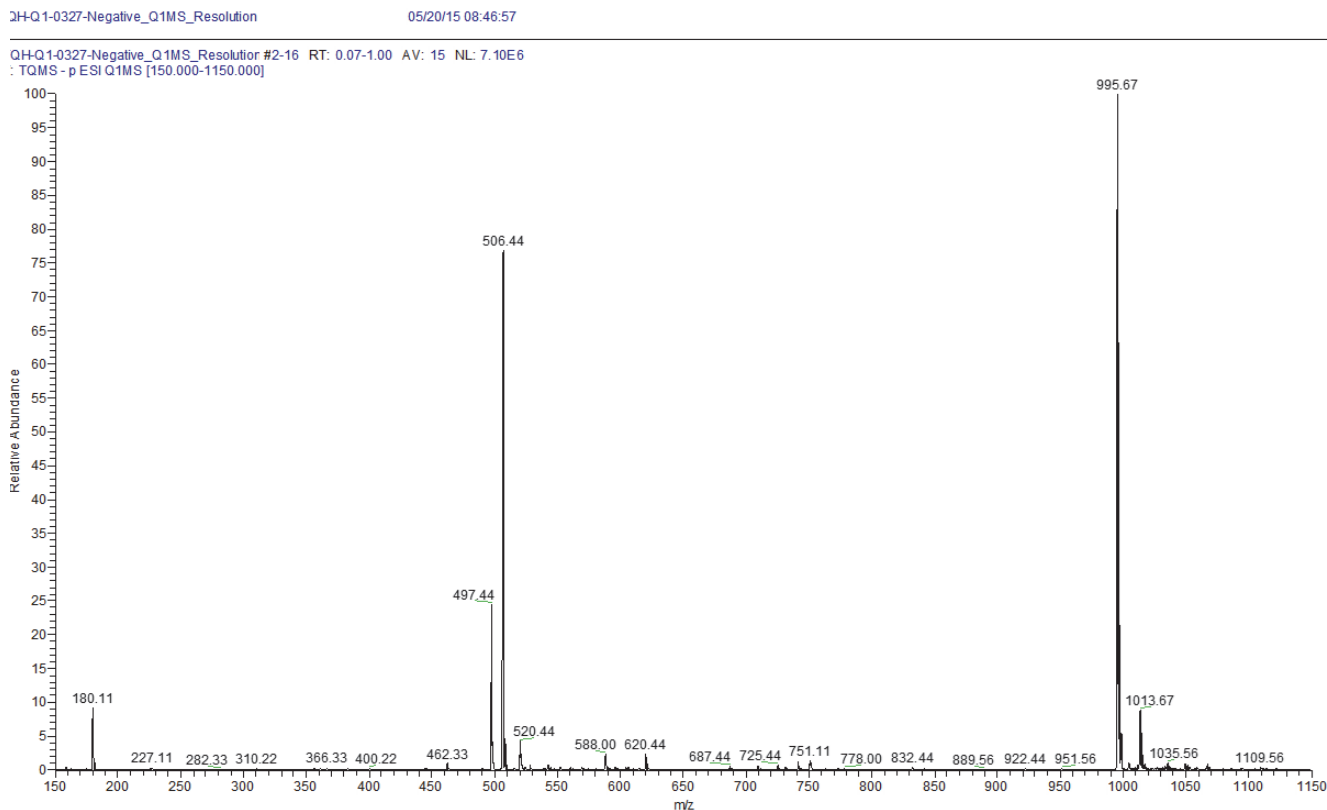


Figure 13. Mass spectrum of the calibration solution in negative ion polarity mode

System Tune and Calibration

Calibration parameters are instrument parameters that affect the mass accuracy and resolution. Calibrate the TSQ Endura and TSQ Quantiva MSs in H-ESI mode before acquiring data in H-ESI or APCI 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 analyzer.

Tune parameters are instrument parameters that affect the magnitude of the ion signal. There are two types of tune parameters: mass dependent and compound dependent.

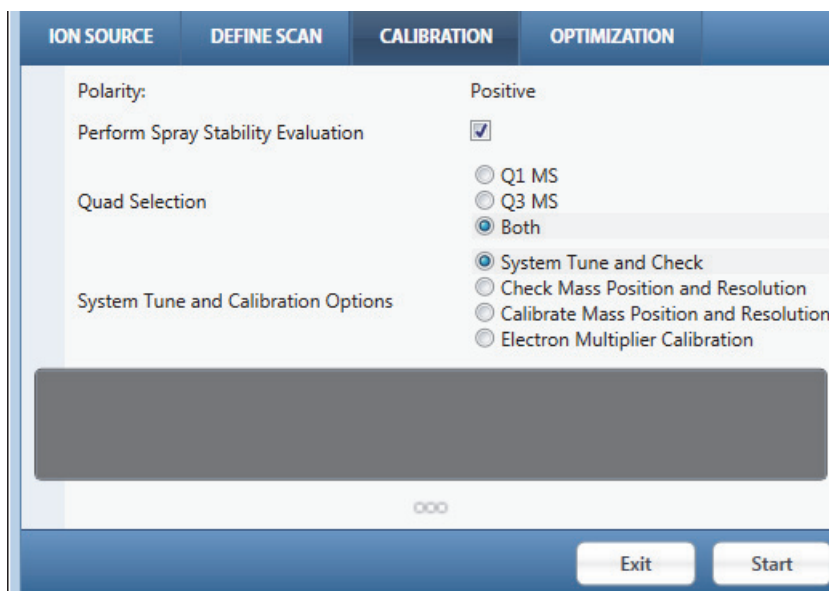
- Mass-dependent tune parameters include the RF voltage of the RF lens, the DC offset voltages of multipoles M00 and M0, and the DC offset voltages of lenses L11, L12, L21, L23, L31, L33, and L4.
- Compound-dependent tune parameters include the spray voltage (H-ESI or NSI mode) or spray current (APCI mode), sheath gas pressure, auxiliary gas pressure, sweep gas pressure, vaporizer temperature, and ion transfer tube temperature. Whenever you change experiments, use the Optimization pane to optimize API gas pressures for your compound and the spray voltage or spray current. See [Chapter 8, “Optimizing the System Parameters.”](#)

Note The Tune application writes the calibration parameters and the mass-dependent tune parameters to the calibration file. It writes the compound-dependent tune parameters to a change record in the History pane. You can rename and save the change record to the Favorites pane for future use in the Tune application or the Method Editor. See [Using the History Pane](#).

❖ **To perform system tune and calibration**

1. Set up to infuse the polytyrosine 1-3-6 calibration solution at 2 $\mu\text{L}/\text{min}$ into the API source. See [Setting Up the Syringe Pump for Direct Infusion](#).
2. Place the MS in **On** mode.
3. In the Define Scan pane, select **Full Scan (Q1)** or **Full Scan (Q3)**.
4. Click **Calibration** to display the Calibration Status page, and then click **Calibrate** to display the options on the Calibration pane ([Figure 14](#)).

Figure 14. Calibration procedures and options



5. Select the **Perform Spray Stability Evaluation** check box.
6. Under Quad Selection, select the **Q1MS**, **Q3MS**, or **Both** option.
7. Under System Tune and Calibration Options, select one of the following options:
 - [System Tune and Check](#)
 - [Check Mass Position and Resolution](#)
 - [Calibrate Mass Position and Resolution](#)
 - [Electron Multiplier Calibration](#)

- Click **Start**.
- When the procedure is complete, select a report generation option and click **OK**.

Note When it completes a calibration, the MS writes the calibration parameters to a calibration file, which overwrites the previous calibration file. You cannot replace or modify the calibration file.

Spray Stability Evaluation

The spray stability evaluation generates real-time graphs of both the TIC and the RSD of the TIC. See [Evaluating the Spray Stability](#). The %RSD must be less than 15 percent for the spray stability evaluation to pass.

System Tune and Check

When you select the System Tune and Check option ([Figure 14](#)), the MS maximizes the ion signal by optimizing the RF voltage of the RF lens, the DC offset voltages of multipoles M00 and M0, and the DC offset voltages of lenses L11, L12, L21, L23, L31, L33, and L4. The system optimizes for polytyrosine 1, 3, and 6, and for quadrupoles Q1 and Q3. After the optimizations are complete, the system performs a mass position and resolution test.

For a description of the multipoles and lenses, refer to the *TSQ Endura and TSQ Quantiva Hardware Manual*.

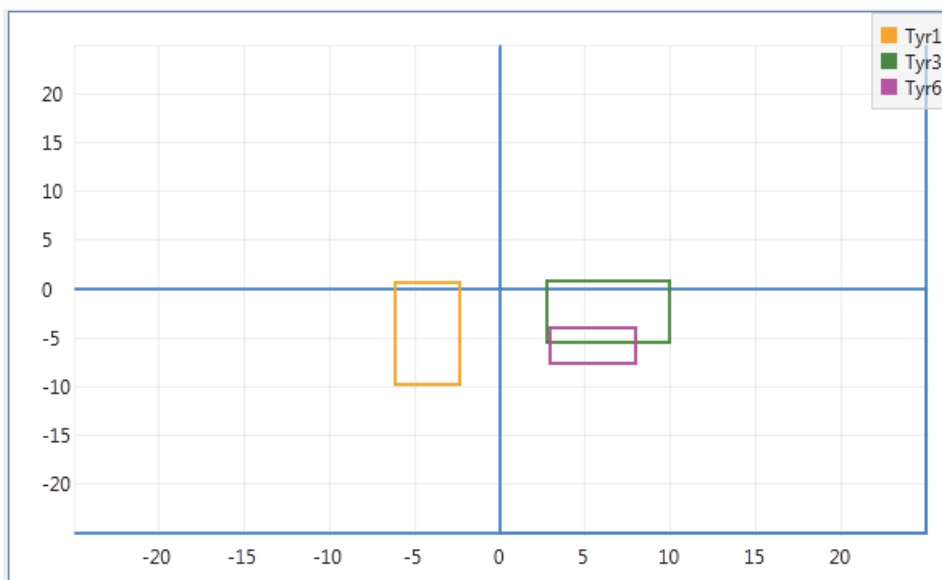
Check Mass Position and Resolution

On a regular basis, evaluate the mass position and resolution by selecting its check box ([Figure 14](#)). If the evaluation fails, run the mass position and resolution calibration. While checking the mass position and resolution evaluation, the Tune application compares the measured isotopic peaks of polytyrosine 1-3-6 (red curve) with the theoretical isotopic peaks (blue curve).

This procedure repeats for polytyrosine 1, 3, and 6, for 0.2 (TSQ Quantiva MS only), 0.4, and 0.7 mDa FWHM peak widths, and for quadrupoles Q1 and Q3.

[Figure 15](#) shows the error range boxes for polytyrosine 1, 3, and 6. The y axis is the error range of the peak widths from the expected peak width of m/z 0.4 (in mDa), and the x axis is the error range of the mass positions from the expected mass position (in mDa). The MS measures the peak width and mass position over a large number of scans. For the evaluation to pass, the error ranges boxes must be within ± 100 mDa from the expected peak width and mass positions.

Figure 15. Error range of the polytyrosine 1-3-6



Calibrate Mass Position and Resolution

Use the Calibrate Mass Position and Resolution option (Figure 14) if the mass position and resolution evaluation fails. During the mass position and resolution calibration, the calibration procedure varies the RF and DC voltages that it applies to quadrupoles Q1 and Q3 to best fit the measured peak widths and positions of the polytyrosine isotopic peaks (displayed red curve) to the theoretical peak widths and positions of those peaks (displayed blue curve). This procedure repeats for polytyrosine 1, 3, and 6, for 0.2 (TSQ Quantiva MS only), 0.4, and 0.7 mDa FWHM peak widths, and for quadrupoles Q1 and Q3.

Electron Multiplier Calibration

The electron multiplier gain decreases as the multiplier ages. This calibration procedure increases the voltage on the electron multiplier to maintain a gain of 5×10^5 for MS mode and 2×10^6 for MS/MS mode. Select this option (Figure 14) if you notice a falloff of the ion signal intensity.

If you have flushed the inlet components of the MS, you can now start using your analyte solution for data acquisition.

IMPORTANT Before you start using your analyte, follow the procedure [To flush the inlet components](#).

Optimizing the System Parameters

After you complete the calibration procedures in [Chapter 7](#), you can further optimize the TSQ Endura or TSQ Quantiva MS for your specific analyte. Use the Optimization pane in the Tune application to optimize all at once the API source and the instrument parameters, such as the RF lens and the collision energy.

Contents

- [Setting Up the Plumbing for Compound Optimization](#)
- [Optimizing Parameters in the Optimization Pane](#)

Setting Up the Plumbing for Compound Optimization

With the high-flow infusion technique, you can introduce the analyte into the MS's API source. The high-flow infusion technique uses a Tee union to direct the analyte from the syringe pump into an LC flow that is appropriate (flow rate and composition) for your experiment. For plumbing instructions, see [Setting Up the Inlet for High-Flow Infusion Without an Autosampler](#).

Optimizing Parameters in the Optimization Pane

IMPORTANT Thermo Fisher Scientific recommends that you optimize the API source parameters if the spray becomes unstable and also after restarting the sample flow into the system, which can be multiple times daily.

The RF lens transfers ions from the API source to the ion optics. The Optimization pane can find the optimum RF lens voltage for transmitting a precursor ion through the RF lens.

Note The RF lens voltage is a mass-dependent tune parameter until you run the RF lens voltage optimization. After that, it is a compound-dependent tune parameter.

The magnitude of the RF lens voltage affects the mass spectrum:

- Decreasing the RF lens voltage does the following:
 - Decreases the transmission of high m/z ions through the RF lens and increases the transmission of low m/z ions.
 - Might decrease the amount of fragmentation of fragile ions in the RF lens.
- Increasing the RF level does the following:
 - Increases the transmission of high m/z ions through the RF lens and decreases the transmission of low m/z ions.
 - Might increase the amount of fragmentation of fragile ions in the RF lens.

The collision energy determines the product ion intensities from collision-induced dissociation (CID) of the precursor ions in an MS/MS experiment. Use the Optimization pane to find the optimum collision energy for the MS/MS transitions of a single precursor ion.

❖ **To optimize the API source and compound-optimization parameters**

1. Verify that the syringe has a sufficient volume of sample solution.
2. In the Tune window, click **Syringe Off** to turn on the syringe pump.

The button name changes to Syringe On. If your LC/MS system includes an LC device, prepare an LC method before you turn on the liquid flow to the API source.

3. In the Optimization pane (Figure 16), do the following:
 - a. In the Sample Injection Type list, select **Syringe**.


Note You cannot optimize the source parameters with the autosampler injection type. However, if you select the autosampler injection type for compound optimization only, remember to remove the column from the autosampler plumbing.

- b. In the Mass List Type list, make a selection.

Note For descriptions of the Optimization parameters, refer to the Tune Help.

- c. In the Source Optimization list, select **On** and then select the **Spray Voltage**, **Sheath Gas**, **Aux Gas**, and **Sweep Gas** check boxes that appear.
 - d. For a SIM scan, select the **Source Fragmentation**, **RF Lens**, and **Precursor Ion Mass** check boxes. For an SRM scan, also select **On** in the Product Ion Optimization list.

Note Under System Settings, the default values are sufficient for most experiments.

- e. Click the **Add/Remove Table Column** icon, , next to the parameters that you want to customize per compound. Otherwise, the MS applies the parameter setting to all compounds.



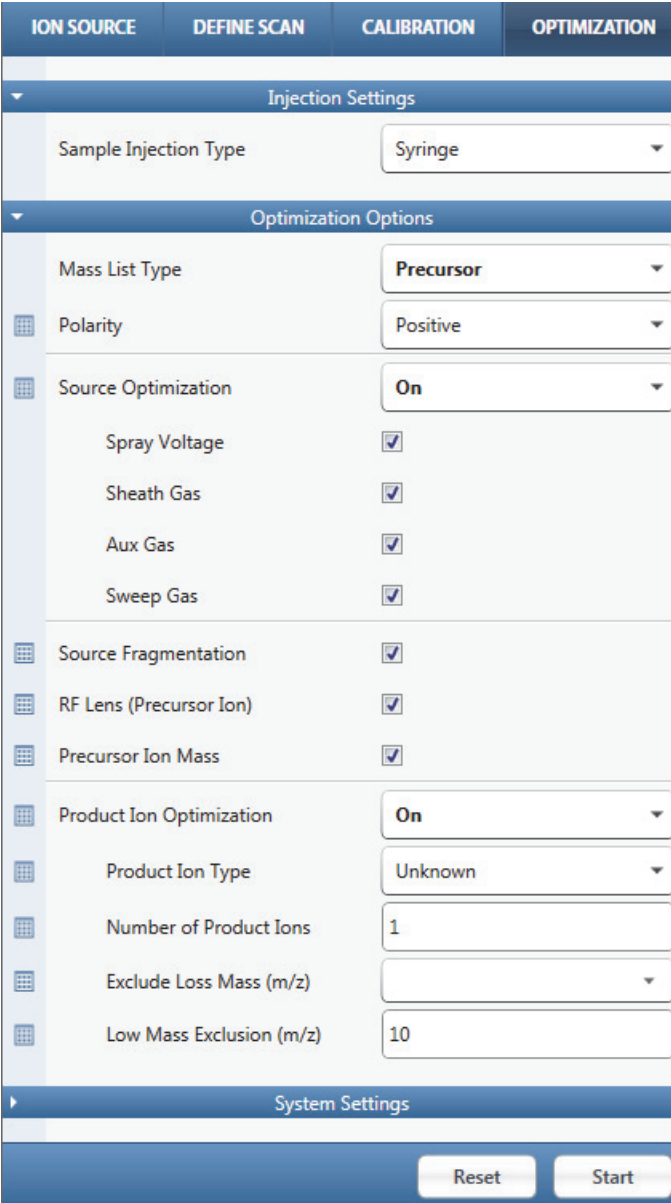
When selected, the icon changes color, from  to , and the parameter appears in the Optimization Table (not shown here).

Figure 16. Optimizing the source parameters in the Optimization pane



4. In the Optimization Table, add a row for each compound and modify the column values as applicable.

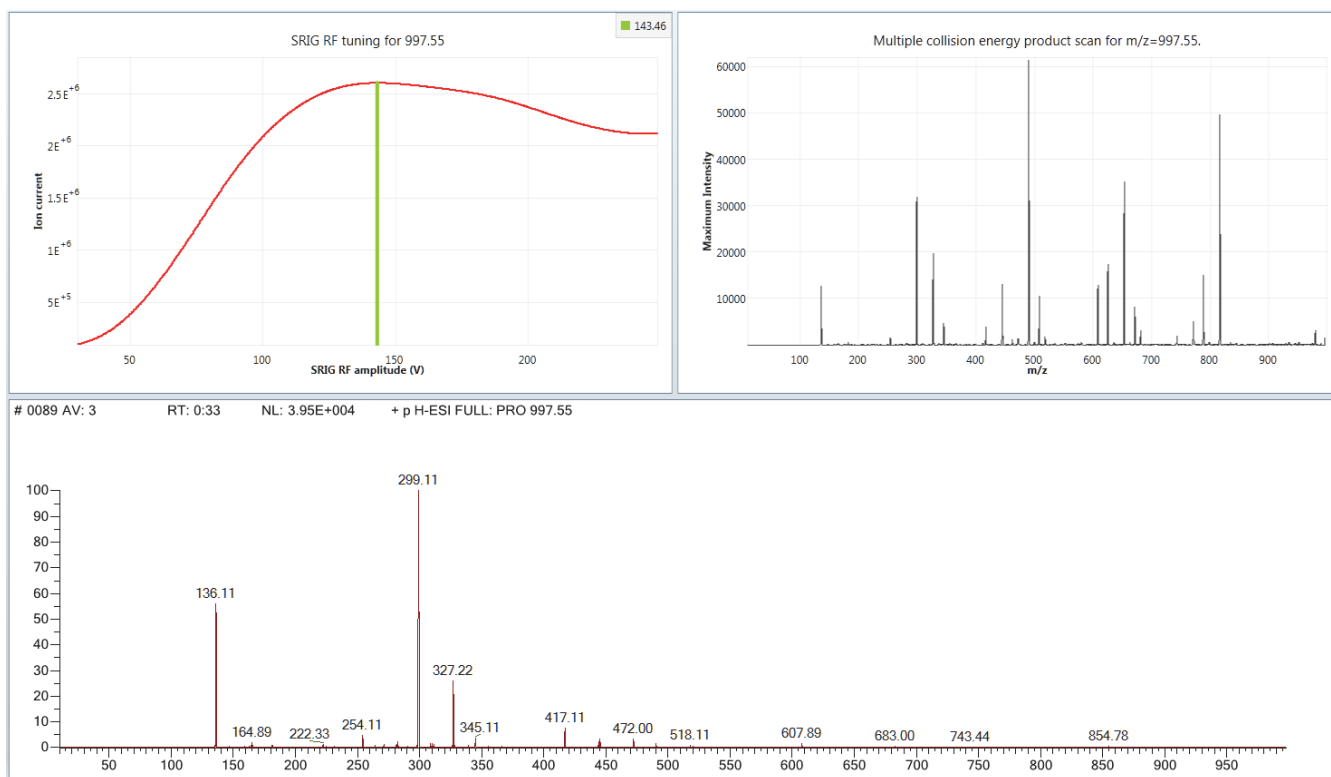
Tip If there are multiple syringes, enter the syringe number in the Injection ID column.

5. Place the system in **On** mode, and then click **Start**.

After the compound optimization is complete, you can view and save the generated report for each compound.

Figure 17 shows an example of “in progress” graphs and spectra.

Figure 17. Example optimization of the RF lens (SRIG RF) tuning for m/z 997.55



After the optimization is complete, the Acquisition Table appears, which contains links to an optimization report and raw data file for each optimized compound. The Optimization Results and Source Optimization Results tables also appear, displaying the optimized parameter values. Figure 18 shows an example of these tables.

Figure 18. Examples of the optimization results tables

Acquisition Table				
Progress	Compound	Report	Raw File	
1	 Tyr6	View	Open	

Optimization Results									
Select	Compound	Polarity	Precursor (m/z)	Product (m/z)	Collision Energy (V)	Intensity	Source Fragmentation (V)	RF Lens (V)	
1	<input checked="" type="checkbox"/> Tyr6	Positive	997.55	490.165	30.68	1939516.56	0	171.39	

Source Optimization Results							
Select	Compound	Polarity	Spray Voltage (V)	Sheath Gas (Arb)	Aux Gas (Arb)	Sweep Gas (Arb)	
1	<input checked="" type="radio"/> c1	Positive	0	2.8	0	0	

8 Optimizing the System Parameters

Optimizing Parameters in the Optimization Pane

Acquiring Sample Data

To manually acquire sample data, use the Tune application. However, to set the start (trigger) instrument before running an instrument method to acquire that data, use the Xcalibur data system. 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, scan type, mass range, and more.

Note 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 *TSQ Endura and TSQ Quantiva Getting Connected Guide*.

Contents

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

Using Tune to Acquire Sample Data

❖ To acquire a sample data file

1. Open the Data Acquisition pane ([Figure 19](#)), and then do the following:



- a. (Optional) To change the destination folder for the raw data file, click the **Browse** icon.

The default folder location is in *drive:\Thermo\Data*.

- b. In the File Name box, type **reserpine** (or the name of the analyte).

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).

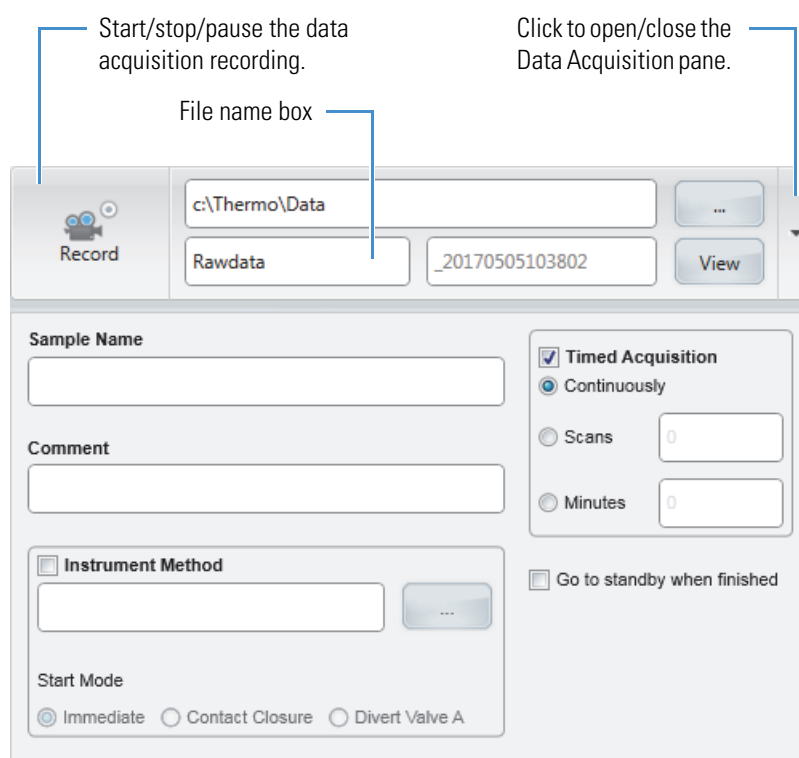
- d. In the Comment box, type a comment about the experiment.

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 19. 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 *Thermo Xcalibur Qual Browser User Guide* or the Qual Browser Help.

Using the Xcalibur Data System to Acquire Sample 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 the 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.

Follow these procedures:

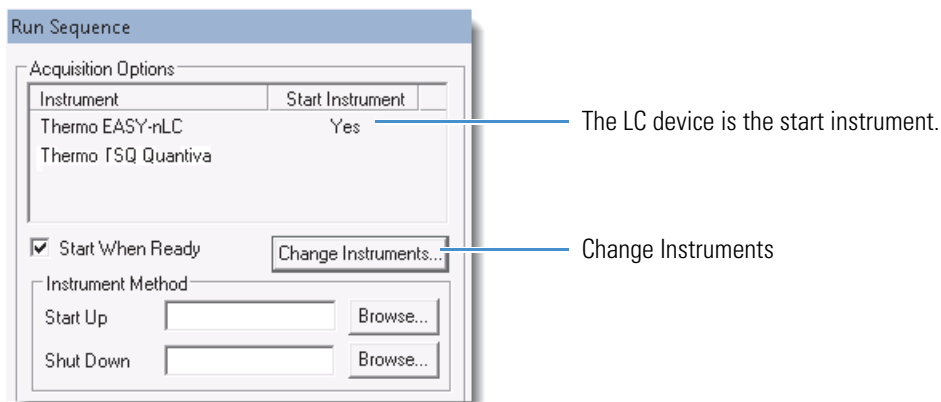
1. To select the external start instrument
2. To acquire a data file by using the Xcalibur data system

❖ 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 20).

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

Figure 20. Run Sequence dialog box (partial) showing the selected start instrument

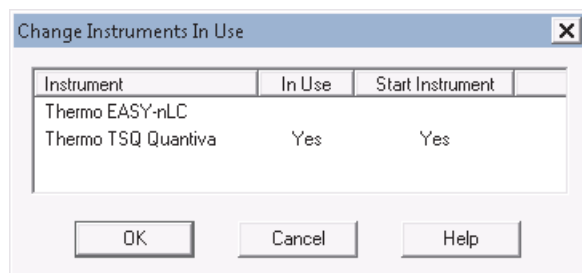


9 Acquiring Sample Data

Using the Xcalibur Data System to Acquire Sample Data

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 21).

Figure 21. Change Instruments In Use dialog box showing the MS as the start instrument



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

This completes the start instrument setup.

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

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

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.

Contents

- [Opening the Tune Window](#)
- [Setting the Instrument System Controls](#)
- [Setting the Instrument Power Mode](#)
- [Checking the Instrument Readback Status](#)
- [Setting the Tune Preferences](#)
- [Using the Mass List Table in the Define Scan Pane](#)
- [Using the History Pane](#)
- [Using the Favorites Pane to Save System Settings](#)

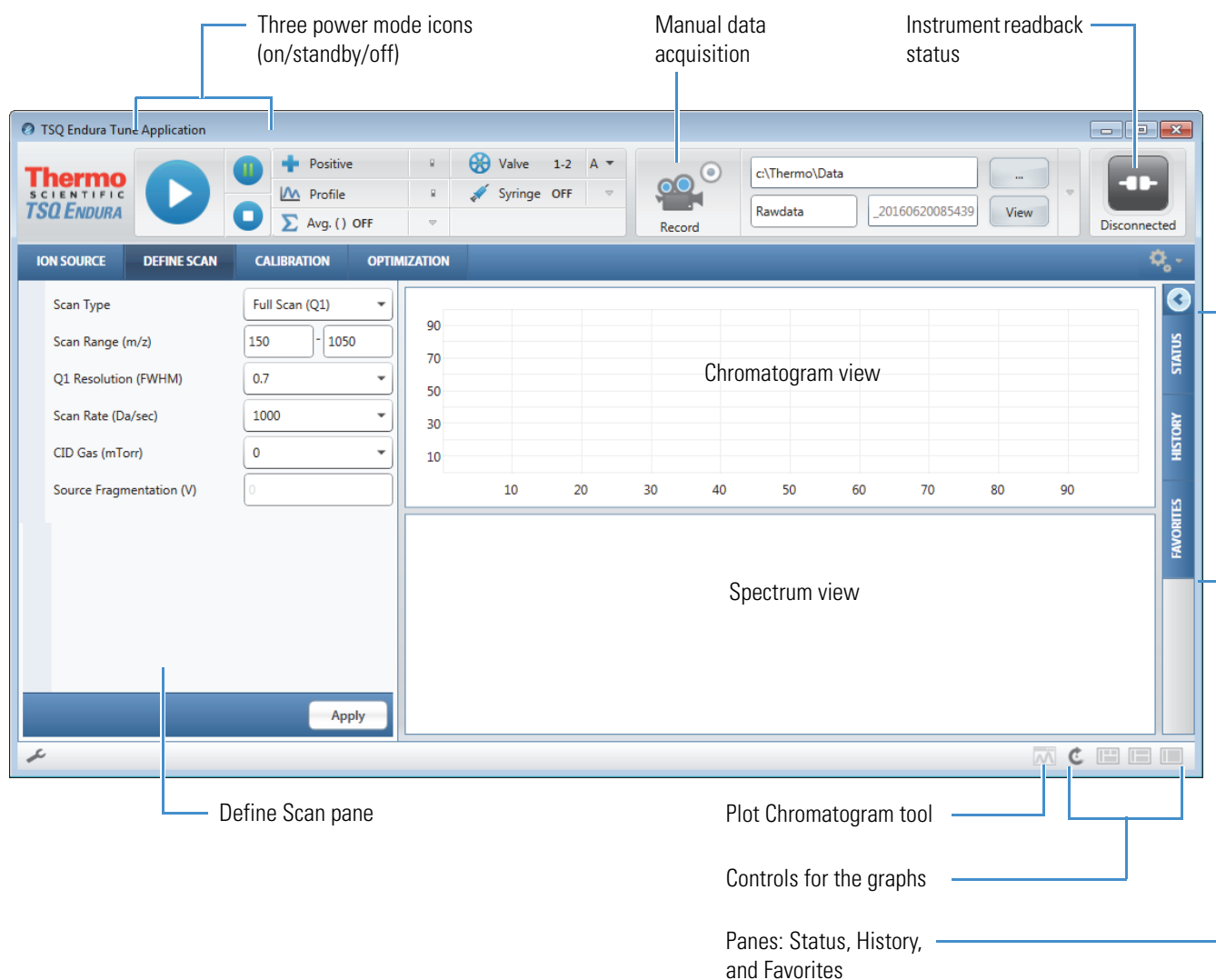
Opening the Tune Window

❖ **To open the Tune window**

From the Windows taskbar, choose **Start > All Programs > Thermo Instruments > model x.x Tune** (Figure 22).

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

Figure 22. Tune window showing the Define Scan pane



Setting the Instrument System Controls

Table 8 shows the options for each of the system control buttons at the top of the Tune window.

Table 8. Procedures for using the instrument control buttons (Sheet 1 of 2)



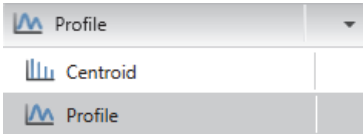

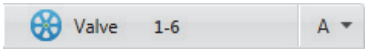
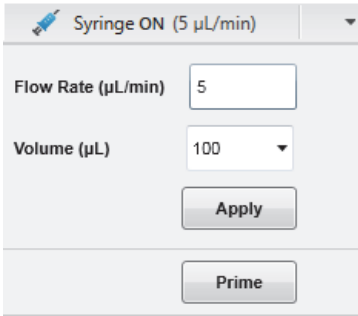
Button	Function	Procedure
	Power mode	<ul style="list-style-type: none"> ❖ To set the instrument power mode <p>Click the icon for the applicable power mode.</p> <p>The center of the selected icon changes from white to green. See Setting the Instrument Power Mode.</p>
	Ion polarity mode	<ul style="list-style-type: none"> ❖ To set the ion polarity mode <p>Click Positive (Negative) and select the ion polarity mode.</p>
	Data type	<ul style="list-style-type: none"> ❖ To set the data type <p>Click Centroid (Profile) and select the data type.</p>
	Spectrum scan averaging	<ul style="list-style-type: none"> ❖ To turn the scan averaging on or off <p>Click the button to switch between on and off.</p> <ul style="list-style-type: none"> ❖ To set the scan averaging value <p>Click the down arrow to enter the value, and then click Apply.</p>

Table 8. Procedures for using the instrument control buttons (Sheet 2 of 2)

Button	Function	Procedure
	Divert/inject valve	<ul style="list-style-type: none"> ❖ To set the divert/inject valve module and position <p>Click the down arrow to select the valve, and then click Valve to set the position.</p>
	Syringe pump	<ul style="list-style-type: none"> ❖ To turn the syringe pump on or off <p>Click Syringe On (Off) to switch between on and off.</p> <ul style="list-style-type: none"> ❖ To set the syringe pump parameters <ol style="list-style-type: none"> 1. Click the down arrow, and then type the 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.

Setting the Instrument Power Mode

Use the three power mode icons in the Tune window (Figure 22) to set the MS's power mode (on, standby, or off).







When you remove the API 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 API source, main RF voltage, and ion optic RF voltages. The auxiliary, sheath, and sweep gas flows remain on and return to their standby default settings (2 arbitrary). For a list of the on/off status of the MS components under varying power conditions, refer to Chapter 6 in the *TSQ Endura and TSQ Quantiva Hardware Manual*.

Checking the Instrument Readback Status

The system readback icon is located in the top right of the Tune window. [Table 9](#) lists the various readback states.

Table 9. 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 API 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


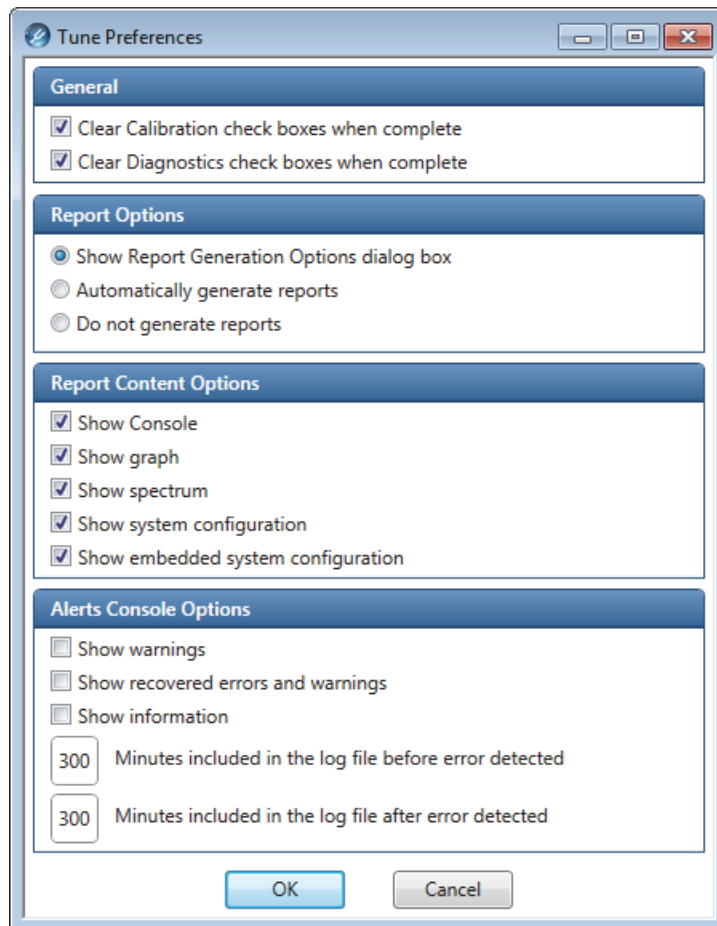
1. Click the **Options** icon, , and then choose **Preferences** to open the Tune Preferences dialog box (Figure 23).

Figure 23. Tune Preferences dialog box



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


Using the Mass List Table in the Define Scan Pane

The mass list table appears when you select the SIM Scan (Q1), SIM Scan (Q3), or SRM scan type in the Define Scan pane. Use this table to specify scan parameters. To set different scan parameters for the precursor ions, add the parameters to the table.


- To add a row to the table
- To delete a row from the table
- To delete multiple rows from the table
- To add or remove scan parameters from the table

❖ To add a row to the table


Do one of the following:

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

❖ To delete a row from the table

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

❖ To delete multiple rows from the table

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

A Using Basic Tune Functions

Using the Mass List Table in the Define Scan Pane

❖ To add or remove scan parameters from the table


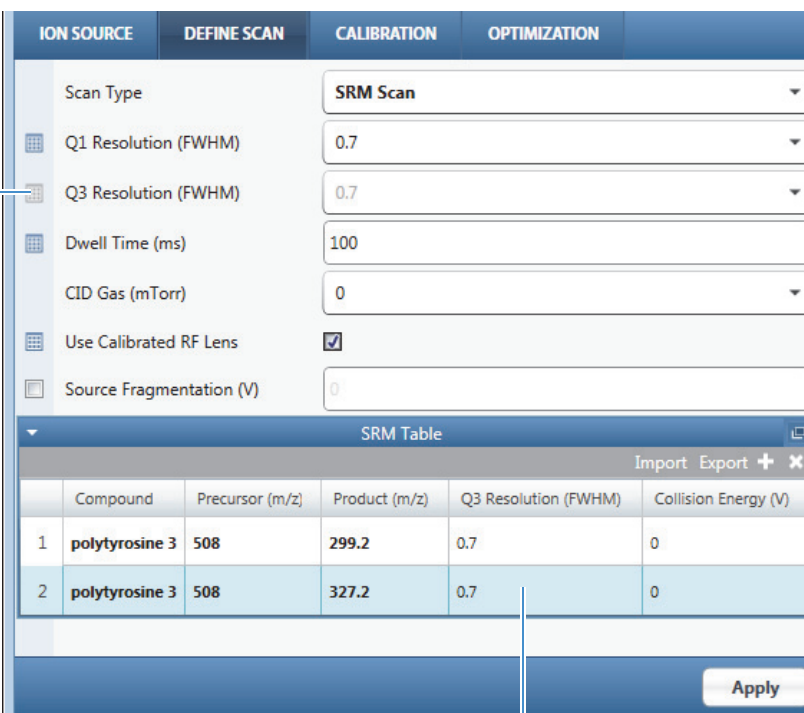
Click the **Table** icon, , once to add the adjacent scan parameter to the table. Click it again to remove the parameter from the table.

Figure 24 shows an example with Q3 Resolution added to the SRM Table.

Figure 24. Q3 Resolution selected and added to the SRM Table



The screenshot shows the 'DEFINE SCAN' pane with various parameters. The 'Q3 Resolution (FWHM)' parameter is selected and highlighted in gray. Below the parameters is the 'SRM Table' with the following data:

	Compound	Precursor (m/z)	Product (m/z)	Q3 Resolution (FWHM)	Collision Energy (V)
1	polytyrosine 3	508	299.2	0.7	0
2	polytyrosine 3	508	327.2	0.7	0

Annotations: A callout points to the 'Q3 Resolution (FWHM)' parameter in the parameter list, stating 'Q3 Resolution is selected (appears gray)'. Another callout points to the 'Q3 Resolution (FWHM)' column in the SRM Table, stating 'Q3 Resolution appears in the table.'

❖ 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.

❖ 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**.

Using the History Pane

When you click **Apply** in the Ion Source or the Define Scan pane, the Tune application adds a change record to the History pane. The change record records all changes to the instrument state that originated from the Tune application.

Change records in the History pane work as follows:

- The Tune application creates a change record when you change parameters in the Ion Source or Define Scan pane and then click **Apply**.
- The History pane displays the change records as subitems under the date that they were created. The maximum number of change records is 100.
- You can display a change record's parameters: either double-click the record or right-click it and choose **Load**. Parameters that are colored red differ from their default values.
- You can submit a change record's parameters to the MS: either double-click the record or right-click it and choose **Apply**.
- A change record is inactive if the API source type of the change record differs from the current API source type.

Using the Favorites Pane to Save System Settings

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

- [To create a favorite state](#)
- [To load settings only or apply a favorite state](#)
- [To rename a favorite state](#)
- [To delete a favorite state](#)

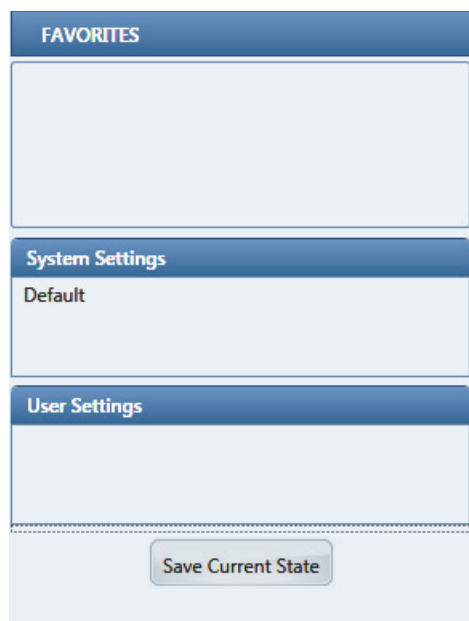
❖ **To create a favorite state**

1. In the Tune window, modify the parameters in the Ion Source or Define Scan pane.
2. Click **Apply** or **Export**.
3. Click the **Favorites** tab to display the Favorites pane ([Figure 25](#)).

A Using Basic Tune Functions

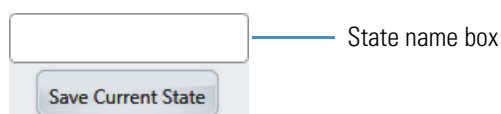
Using the Favorites Pane to Save System Settings

Figure 25. Favorites pane



4. Click **Save Current State**, and then type a unique name in the box (Figure 26).

Figure 26. State name box



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

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

❖ To load settings only or apply a favorite state

Under User Settings, right-click the state name, and then choose one of the following:

- **Load** to only display the key parameters in the applicable parameter boxes.
- **Apply** to submit the key parameters to the MS.

You can click Apply without first loading the parameters.

❖ To rename a favorite state

1. Under User Settings, right-click the state name, and then choose **Rename**.
2. Type a different name and press ENTER.

❖ To delete a favorite state

Under User Settings, right-click the state name, and then choose **Delete**.

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

For instructions on how to clean the ion sweep cone, spray cone, and ion transfer tube, refer to Chapter 8 in the *TSQ Endura and TSQ Quantiva Hardware Manual*.



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

Contents

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

Supplies

Table 10 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 10. Flushing and cleaning supplies

Description	Part number
Gloves, nitrile	Fisher Scientific™ 19-120-2947 ^a Unity Lab Services: <ul style="list-style-type: none"> • 23827-0008 (size medium) • 23827-0009 (size large)
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 API source at a flow rate of 200–400 $\mu\text{L}/\text{min}$ 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.

B Flushing the Inlet Components

Flushing the Inlet Components after Calibration

Preparing the Polytyrosine Calibration Solution

This appendix describes how to prepare the polytyrosine 1-3-6 calibration solution for H-ESI mode. The procedure calls for potentially hazardous chemicals, including methanol (Table 11) and polytyrosine (see contents of the [Chemical Kit](#)).



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.

IMPORTANT

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

Table 11. Recommended supplies (Sheet 1 of 2)

Description	Part number
Chemicals	
Formic acid (modifier), Optima™ LC/MS grade	A117-10X1AMP
Methanol, Optima LC/MS grade	Fisher Scientific: A456-1
Water, Optima LC/MS grade	Fisher Scientific: W8-1

Table 11. Recommended supplies (Sheet 2 of 2)

Description	Part number
Equipment	
Gloves, nitrile	Fisher Scientific™ 19-120-2947 ^a
	Unity Lab Services: <ul style="list-style-type: none"> • 23827-0008 (size medium) • 23827-0009 (size large)
Protective safety glasses or goggles	–

^a Multiple sizes are available.



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

❖ **To prepare the polytyrosine calibration solution**

1. Under a fume hood, remove the lid from the polytyrosine 1-3-6 vial.
2. Add 20 mL of 0.1% formic acid in 50:50 methanol/water to the vial.
3. Put on the lid and shake the vial until all solids have dissolved.

Installation Kits

The TSQ Endura or TSQ Quantiva MS ships with several kits. This appendix lists the necessary components for the instructions in this guide. For a full list of the kits and their contents, refer to the *TSQ Endura and TSQ Quantiva Hardware Manual*.

Contents

- [Chemical Kit](#)
- [MS Calibration Kit](#)
- [Performance Specification Kit](#)

Chemical Kit


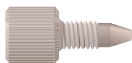
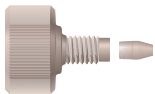
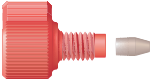
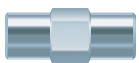

IMPORTANT Be aware of the following storage precautions for the calibration and reserpine solutions: Refrigerate the containers after opening. For long-term storage, keep refrigerated at 2–8 °C (36–46 °F).

Table 12. Chemical Kit (P/N 80100-62006)

Item	Quantity	Part number
Polytyrosine 1-3-6 standard, solid (CS Bio Company Inc., CS0272S)	1	00301-22925
TSQ Reserpine Solution Kit (contains reserpine standard, 100 pg/μL, 5 × 1 mL, P/N HAZMAT-01-00081)	1	80100-62033
LCMS Functionality Test Kit (for field service use only)	1	HAZMAT-01-00044

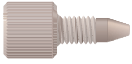



MS Calibration Kit

Table 13. MS Calibration Kit (P/N 80000-62013)

Image	Item	Quantity	Part number
	Ferrule, fingertight, natural PEEK	2	00101-18196
	Fitting, fingertight, one-piece natural PEEK, 10-32	1	00109-99-00016
	Fitting, fingertight, two-piece natural PEEK, two wings, 10-32	2	00101-18081
	Fitting, fingertight, two-piece, one wing, 10-32	2	00101-18195
	Grounding union, zero-dead-volume (ZDV), stainless steel, 1/16 in. orifice, 0.010 in. (0.25 mm) thru-hole, 10-32	1	00101-18182
	HPLC union, black PEEK, 10-32, 0.01 in. thru-hole	1	00101-18202
—	Syringe, GC, gas tight, 500 µL, 51 mm long	1	00301-01-00040
—	Tubing, natural PEEK, 1/16 in. OD, 0.0025 in. ID, 28 cm (11 in.) long	2	80000-22032
Note Use this tubing with the calibration solutions and for flow rates less than 50 µL/min.			
—	Tubing, red PEEK, 1/16 in. OD, 0.005 in. ID, 0.6 m (2 ft) long	1	00301-22912
—	Tubing, red PEEK, 1/16 in. OD, 0.005 in. ID, 18 cm (7.1 in.) long	2	80000-22053
Note Use this tubing for flow rates equal to or greater than 50 µL/min.			
—	Tubing, Teflon™ FEP, 1/16 in. OD, 0.03 in. ID, 3 cm (1.2 in.) long	1	00301-22915

Performance Specification Kit

Table 14. 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

D Installation Kits

Performance Specification Kit

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

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

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

E

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

electrospray (ESI) A 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 ($\mu\text{L}/\text{min}$) for the current sample, as defined in the current experiment method.

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

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

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

H

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

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.

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 $C_7H_7^{2+}$, $m/z = 45.5$.

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.

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

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

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

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

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

S

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

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

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

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

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.



Index

A

- APCI mode
 - description 3
 - direct infusion, optional connection 21
- API source
 - solvent waste container, connecting 11
 - spray insert 13
- APPI mode
 - description 4
 - spray insert, selecting 13
- auto-loop injection, schematic 20

B

- buffers, description 6
- buttons, on/standby/off 56

C

- calibration
 - parameters 41
 - precautions 39
- calibration solution
 - peak values 40
 - preparing 69
- Change Instruments In Use dialog box 54
- chemicals, storage precautions 71
- chromatograph, displaying 37
- cleaning the inlet components 65
- collision energy, description 46
- compliance
 - FCC iii
 - regulatory iii
- contacting us xvi
- contamination, preventing 18, 31

D

- data acquisition
 - button 52
 - Tune, using 51
 - Xcalibur, using 54
- data type, setting 57
- direct infusion, description 21
- directive, WEEE v
- directives
 - regulatory iii
- divert/inject valve
 - description 28
 - valve position indicator 29–30
- divert/inject valve, setting 58
- documentation, accessing xiv

E

- electromagnetic compatibility iii
- experiment templates, category list 7

F

- favorite parameter states 63
- FCC compliance iii
- figures, list of xi
- flow rates, setting 4
- flow-injection analysis, description 24

G

- gas flow rates, adjusting for LC flow rate 4
- grounding (ZDV) union 72
- grounding union (figure) 26

H

- H-ESI mode
 - description 2
 - plumbing connection, direct infusion 21

Index: |

high-flow infusion technique, schematic 20
HPLC with autosampler injection, schematic 20

I

infusion line, connecting to grounding union 21, 23
inlet components, flushing 65
Instrument Setup window 7
ion polarity mode, setting 57
ion transfer tube temperature, adjusting for LC flow rate 4

K

kits
 Chemical 71
 MS Calibration 72
 Performance Specification 73

L

LC pump connection, schematic 20
LC union 72
LC/MS experiments, connecting the plumbing 22
LC/MS operational guidelines
 APCI mode 5
 H-ESI mode 5
 NSI mode 5

M

manual-loop injection, schematic 20
mass list
 exporting from Tune 62
 importing into Tune 62
mass spectrometer
 calibration parameters 41
 flow rates, setting 4
 plumbing connections 17
 power modes, setting 58
 tune parameters 41
MSDS 6

N

NSI mode, description 4

O

optimization
 API source and compound-optimization parameters 46
 API source parameters 46
 special notice 45
optimization graphs (example) 48

optimization results (table examples) 49
optimum performance, flow rate range 2–3

P

panes
 Calibration 42
 Status page 42
 Data Acquisition 52
 Define Scan 56
 Favorites 64
 History 63
 Ion Source 5
 Optimization 47
PEEK tubing, special notice 17
plumbing connections, inlet 17
polarity mode
 See ion polarity mode, setting
polytyrosine 1-3-6
 See calibration solution 40
procedures
 API source
 installing or removing 11
 preparing for MS calibration 13
 spray insert
 adjusting the position 15
 installing or removing 13
 calibration tests, preparing the MS beforehand 31
 favorite states, using 63
 inlet
 components, flushing 67
 setting up for sample introduction 19
 MS, preparing for the API source 9
 sample introduction techniques
 direct infusion, connecting the plumbing 21
 high-flow infusion, connecting the plumbing 23
 HPLC with autosampler, connecting the
 plumbing 22
 manual-loop injection, connecting the plumbing 25
 spray stability, evaluating 36
 start instrument, selecting in Xcalibur 53
 syringe pump
 controlling 58
 setting up 19
 syringe, cleaning 67
 Tune preferences, setting 60
pumping down the MS vacuum system 31

R

readback status, description 59
Ready Out cable 51
Record button 52

regulatory compliance [iii](#)
 RF lens, description [45](#)
 Run Sequence dialog box (acquisition options) [53](#)

S

safety standards [iii](#)
 sample data, acquire by using Tune [51](#)
 sample input (figure) [26](#)
 sample introduction techniques, schematics [20](#)
 sample loss, preventing [51](#)
 sample tube (figure) [21](#), [26](#)
 SDS [6](#)
 separation techniques [2–3](#)
 sequence run, start instrument in Xcalibur [53](#)
 Skip Spray Stability Evaluation check box [42](#)
 solvents
 description [6](#)
 waste [11](#)
 start instrument, configuring with Xcalibur [53](#)
 supplies [66](#), [69](#)
 symbols, meaning [xiv](#)
 syringe
 adapter assembly [18](#)
 avoid contamination [31](#)
 cleaning [67](#)
 priming [27](#)
 syringe adapter assembly, drawing [28](#)
 syringe pump
 default flow rate [27](#)
 description [27](#)
 syringe, priming [33](#), [58](#)

T

Thermo Fisher Scientific website, user documents [xiv](#)
 time stamp, raw data file [51](#)
 Tune application
 basic functions [55](#)
 opening [56](#)
 Preferences dialog box [60](#)
 tune parameters
 compound dependent [41](#)
 mass dependent [41](#)

U

union types, plumbing
 grounding (ZDV) [72](#)
 LC [72](#)
 Tee [73](#)

V

vaporizer temperature, adjusting for LC flow rate [4](#)
 viewing documents [xiv](#)

W

WEEE directive [v](#)

X

Xcalibur file type, raw data (.raw) [51](#)
 Xcalibur file type, sequence (.sld) [53](#)

Z

ZDV union [72](#)

