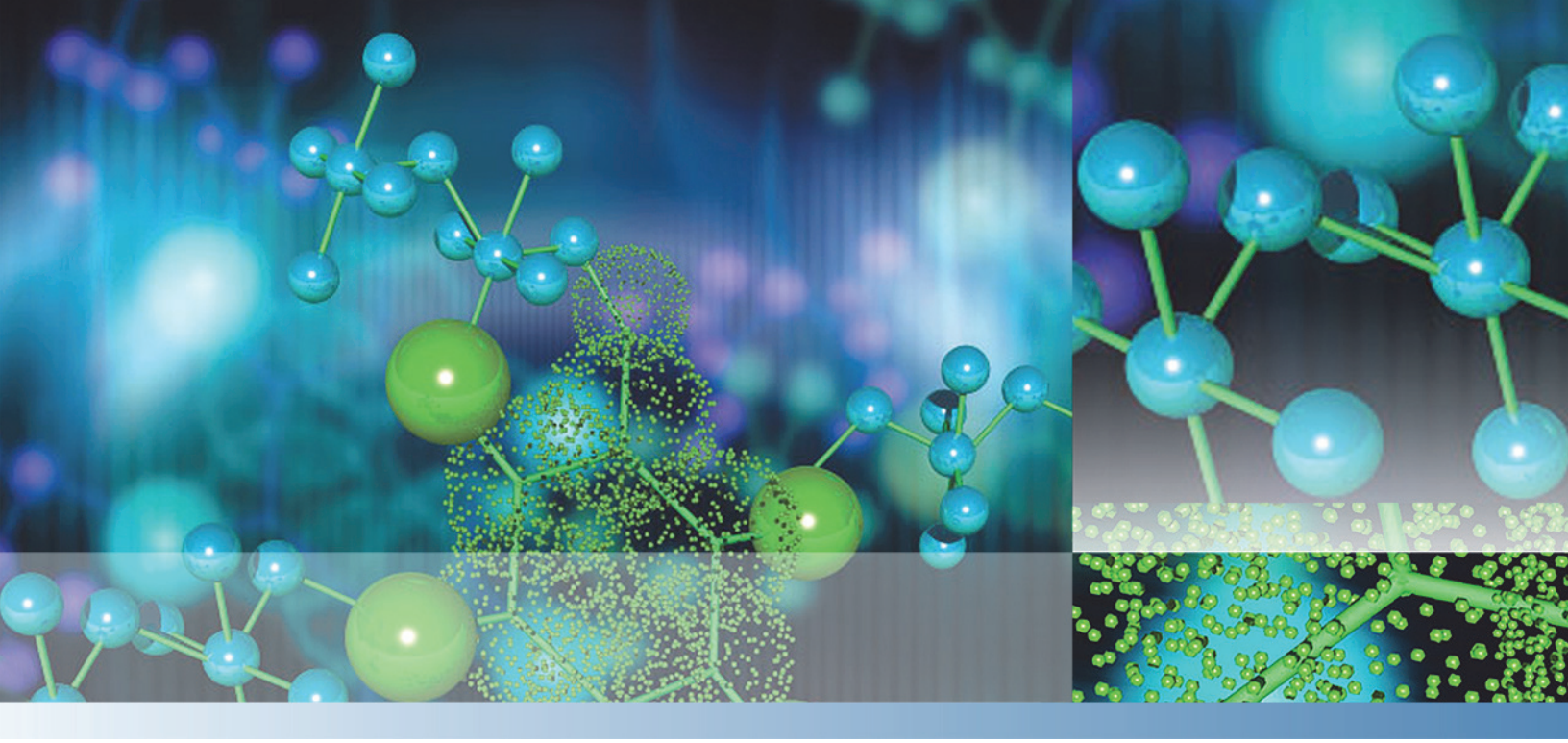

Achieving Peak Instrument Performance

Reference Manual

For the LTQ Velos, Velos Pro, LTQ Orbitrap Velos, Orbitrap Velos Pro, and Orbitrap Elite

97655-97003 Revision B September 2015

DOCUMENTATION SURVEY

Thermo SCIENTIFIC

Release history: Revision A, October 2012; Revision B, Sept 2015

Software version: Microsoft Windows 7 Professional (32-bit and 64-bit)—Thermo Foundation 2.0 and later, and Thermo Xcalibur 2.2 and later; Windows XP Workstation SP3—Foundation 1.0.2 SP2 or earlier, and Xcalibur 2.1 SP1 or earlier; Thermo LTQ Tune Plus 2.7 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 domestic and international regulations. When the system is delivered to you, it meets all pertinent electromagnetic compatibility (EMC) and safety standards as described in the next section or sections by product name.

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

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

- Velos Pro MS
- LTQ XL MS

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

Velos Pro MS

EMC Directive 2004/108/EC

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

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

Low Voltage Safety Compliance

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

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LTQ XL MS

EMC Directive 2004/108/EC

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

CFR 47, FCC Part 15, Subpart B, Class A: 2013
CISPR 11: 2009 + A1
CISPR 24: 2010
AS/NZS CISPR 22: 2009
ICES-003: 2012
EN 55011: 2009 + A1
EN 55024: 2010
EN 61326-1: 2013
EN 61000-3-2: 2006 + A1+ A2
EN 61000-3-3: 2008
EN 61000-4-2: 2009
EN 61000-4-3: 2006 + A1+ A2
EN 61000-4-4: 2004 + A1
EN 61000-4-5: 2006
EN 61000-4-6: 2009
EN 61000-4-11: 2004

Low Voltage Safety Compliance

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

FCC Compliance Statement

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

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

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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) in the UK and with the European Recycling Platform (ERP-recycling.org) in all other countries of the European Union and in Norway.

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

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

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

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

Directive DEEE

2012/19/EU

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WEEE Direktive

2012/19/EU

Thermo Fisher Scientific hat Vereinbarungen mit Verwertungs-/Entsorgungsfirmen in allen EU-Mitgliedsstaaten getroffen, damit dieses Produkt durch diese Firmen wiederverwertet oder entsorgt werden kann. Weitere Informationen finden Sie unter www.thermoscientific.de/rohs.

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Preface

The *Achieving Peak Instrument Performance Reference Manual* describes how to maintain a stable ionization spray; how to use the tune, calibrate, and diagnostic features in the Thermo Tune Plus application to achieve peak instrument performance; and how to clean the ion optic elements.

Use the information in this manual for these Thermo Scientific™ mass spectrometers:

- Stand-alone instruments: LTQ™ Velos™ and Velos Pro™
- Hybrid Orbitrap™ systems: LTQ Orbitrap Velos™, Orbitrap Velos Pro™, and Orbitrap Elite™

This manual focuses on the linear ion trap optics, with additional information for the hybrid Orbitrap systems in Chapter 7, "Orbitrap Systems."

Contents

❖ **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.

Related Documentation

Thermo Fisher Scientific provides additional documents for these instruments that are accessible from the data system computer. If you received your instrument before October 2012, contact your local Thermo Fisher Scientific sales representative about receiving the printed quick reference card, *Achieving Peak Performance* (P/N 97655-97002), that complements this reference manual.

❖ To view product manuals

To access the manuals for the mass spectrometer, from the Microsoft™ Windows™ taskbar, choose **Start > All Programs > Thermo Instruments > Manuals > *model***, where *model* is your specific model, and then click the applicable PDF file.

The application also provides Help.

❖ To view the data system Help

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

❖ To download user documentation from the Thermo Scientific website

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

Cautions and Special Notices

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

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

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

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

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

The *Achieving Peak Instrument Performance Reference Manual* contains the following caution-specific symbols (Table 1).

Table 1. Caution-specific symbols and their meanings

Symbol	Meaning
	**Chemical hazard:** Observe Good Laboratory Practices (GLP) when handling chemicals. Only work with volatile chemicals under a fume or exhaust hood.Wear gloves and other protective equipment, as appropriate, when handling toxic, carcinogenic, mutagenic, corrosive, or irritant chemicals. Use approved containers and proper procedures to dispose of waste oil and when handling wetted parts of the instrument.
	**Hot surface:** Before touching the API source assembly, allow heated components to cool.
	**Risk of electric shock:** This instrument uses voltages that can cause electric shock, personal injury, or both. Before servicing, shut down the instrument and disconnect it from line power. While operating the instrument, keep covers on.
	**Risk of eye injury:** Eye injury could occur from splattered chemicals or airborne particles. Wear safety glasses when handling chemicals or servicing the instrument.
	**Sharp object:** Avoid handling the tip of the syringe needle.

Read and understand the following cautions that are specific to the shutdown of the mass spectrometry system or to the removal of parts for cleaning.

CAUTION **If you must turn off the mass spectrometer in an emergency, turn off the main power switch located on the right-side power panel.** This switch turns off all power to the mass spectrometer, including the forepumps, without harming components within the system. However, do not use this method as part of the standard shutdown procedure. Instead, refer to "Shutting Down the Mass Spectrometer Completely" in Chapter 3 of the *LTQ Series Hardware Manual*.

To turn off the LC, autosampler, and data system computer in an emergency, use their respective on/off switch or button.

CAUTION To avoid an electrical shock, be sure to follow the instructions in "Shutting Down the Mass Spectrometer Completely" in Chapter 3 of the *LTQ Series Hardware Manual*.

CAUTION Do not turn the instrument on if you suspect that it has incurred any kind of electrical damage. Instead, disconnect the power supply cord and contact Thermo Fisher Scientific technical support for a product evaluation. Do not attempt to use the instrument until it has been evaluated. (Electrical damage might have occurred if the system shows visible signs of damage, or has been transported under severe stress.)

CAUTION Do not disconnect the power supply cord at the mass spectrometer while the other end is still plugged into the electrical outlet.

CAUTION Do not place any objects—especially containers with liquids—on top of the instrument, unless instructed to in the documentation. Leaking liquids might contact the electronic components and cause an electrical short circuit.

CAUTION **Hot surface.** Allow heated components to cool to room temperature (approximately 20 minutes) before servicing them.

Contacting Us

There are several ways to contact Thermo Fisher Scientific for the information you need. You can use your smartphone to scan a QR code, which opens your email application or browser.

Contact us	Customer Service and Sales	Technical Support
	(U.S.) 1 (800) 532-4752	(U.S.) 1 (800) 532-4752
	(U.S.) 1 (561) 688-8731	(U.S.) 1 (561) 688-8736
	us.customer-support.analyze@thermofisher.com 	us.techsupport.analyze@thermofisher.com 

❖ **To find global contact information or customize your request**

1. Go to www.thermoscientific.com.
2. Click **Contact Us**, select the **Using/Servicing a Product** option, and then type the product name.
3. Use the phone number, email address, or online form.

❖ **To find product support, knowledge bases, and resources**

Go to www.thermoscientific.com/support.

❖ **To find product information**

Go to www.thermoscientific.com/lc-ms.

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- Send an email message to Technical Publications (techpubs-lcms@thermofisher.com).
- Complete a survey at www.surveymonkey.com/s/PQM6P62.

1

Introduction

This chapter provides general information for achieving peak performance from the following Thermo Scientific stand-alone mass spectrometers and hybrid Orbitrap systems:
LTQ Velos, Velos Pro, LTQ Orbitrap Velos, Orbitrap Velos Pro, and Orbitrap Elite.

Contents

Pumping Down the Mass Spectrometer

To help optimize the performance of the mass spectrometers, make sure that you do the following:

- Stand-alone mass spectrometers: Pump down the vacuum system for at least 15 hours. For instructions, refer to "Starting the Mass Spectrometer" in Chapter 3 of the *LTQ Series Hardware Manual*.

  After two hours, you can view the mass spectrum to determine if the system is functioning correctly. However, the electron multiplier lifetime and gain calibration might be affected.

- Hybrid Orbitrap instruments: Bakeout the system for at least 8 hours. For instructions, refer to the getting started guide for your Orbitrap model.

IMPORTANT

- Pump-down times of less than 15 hours on new instruments or after venting the mass spectrometer might cause incorrect calibration of the transfer lenses and might increase the aging of the electron multipliers.
- If the instrument is new, you must calibrate the instrument after completing the pump-down time. For instructions, refer to Chapter 3 in the *LTQ Series Getting Started Guide*.

Instrument Control Application

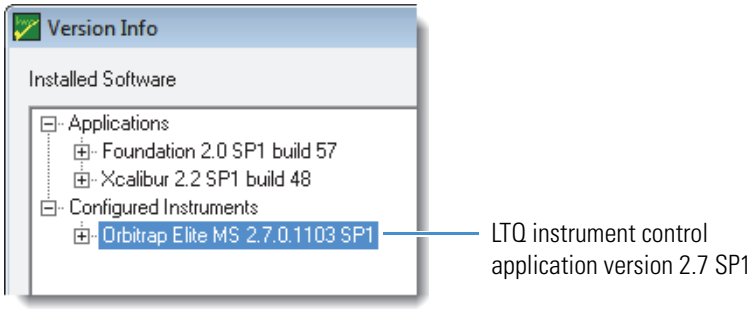
If your LTQ instrument control application is earlier than 2.7 SP1, the mass spectrometer might have reduced functionality or lack optimal tuning or calibration. Always verify that the instrument control application is up-to-date. After you upgrade the application, Thermo Fisher Scientific recommends that you run the automatic calibration.

Note (LTQ 2.7 SP1 and later) The option to Enable Sweep Gas While in Standby on the instrument configuration window's Ion Source page is the default selection with a default flow rate of 5 (arbitrary unit). This option improves robustness by minimizing the amount of particulate matter that enters the mass spectrometer.

❖ To identify the version level of the instrument control application

From the Windows taskbar, choose **Start > All Programs > Thermo Foundation *x.x* > Version Info**, where *x.x* is the installed version of Thermo Foundation™, to open the Version Info window (Figure 1).

Figure 1. Version Info window (example)



To obtain the latest version of the LTQ instrument control application, contact your local Thermo Fisher Scientific field service engineer for assistance.

Daily Operation

To ensure the proper operation of the instrument, Thermo Fisher Scientific recommends that you perform the daily preventive maintenance described in Chapter 4 of the *LTQ Series Hardware Manual*.

2

Ionization Spray Stability

This chapter identifies the various ion sources designed for the LTQ Series mass spectrometers, and describes how to establish and maintain a stable ionization spray.

Contents

Ion Source Types

Table 2 shows the Thermo Scientific atmospheric pressure ionization (API) sources that can attach to the LTQ Series mass spectrometers and defines their applicable ionization modes. For additional information, refer to the documentation provided with the API source and Appendix A, "Online Resources."

Table 2. Thermo Scientific API sources for the LTQ Series mass spectrometers (Sheet 1 of 2)

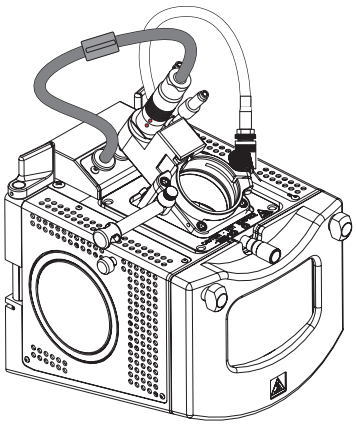
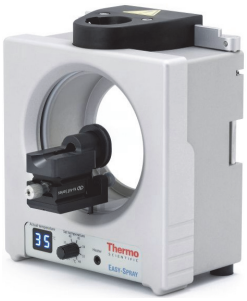
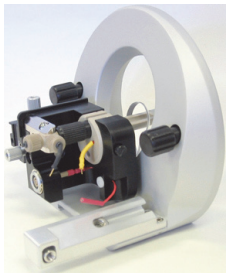
API source housing	Ionization mode	Definition
Ion Max™ (shown) or Ion Max-S[a] 	Electrospray (ESI)	A type of atmospheric pressure ionization that is currently the softest ionization technique available to transform ions in solution into ions in the gas phase.
	Heated-electrospray (H-ESI)	Converts ions in solution into ions in the gas phase by using ESI in combination with heated auxiliary gas.
	Atmospheric pressure chemical ionization[b] (APCI)	A soft ionization technique done in an API source operating at atmospheric pressure. Electrons from a corona discharge initiate the process by ionizing the mobile phase vapor molecules. A reagent gas forms, which efficiently produces positive and negative ions of the analyte through a complex series of chemical reactions.

Table 2. Thermo Scientific API sources for the LTQ Series mass spectrometers (Sheet 2 of 2)

API source housing	Ionization mode	Definition
EASY-Spray™ Series (EASY-Spray shown) 	Nanoelectrospray (nanospray, nanoESI, or NSI)	A type of ESI that accommodates very low flow rates of sample and solvent at 1–20 nL/min (for static nanospray) or 100–1000 nL/min (for dynamic nanospray, which is also called nanoESI nanoLC gradient separation). • EASY-Spray Series ion source—Provides an integrated, temperature controlled column-emitter solution that requires you to make just a single nanoViper™ connection between the LC and the MS source to achieve exceptional nanoflow LC/MS performance.
Nanospray Flex™ 		• Nanospray Flex ion source—Enables the use of nanoscale flow rates and maintains excellent spray stability to ensure efficient evaporation and ionization of liquid samples. The included DirectJunction™ adapter offers total flexibility with respect to column and emitter choices.
Nanospray (NSI-1 Dynamic Nanospray Probe with the NSI-1 Base[c]) 		• Nanospray ion source—Enables sensitive nanospray analysis with minimal dead volume. The Nanospray ion source is a comprehensive solution with a high-resolution imaging system for direct observation and optimization of the spray. A consumables kit accompanies each Nanospray probe.

[a] The Ion Max-S is identical to the Ion Max, except that the Ion Max has an adjustable probe mount and a front door with a view window.

[b] This manual excludes information for the APCI mode.

[c] For use with the Ion Max or Ion Max-S source housing

Maintaining Spray Stability

Before you perform any tune, calibration, or diagnostic procedure, make sure that you establish stable ionization spray conditions.

IMPORTANT Failure to maintain a stable spray might compromise the data quality or result in a poor tune, calibration, or diagnostic result.

Follow these procedures:

1. To infuse the calibration solution into the API source
2. To adjust the spray stability

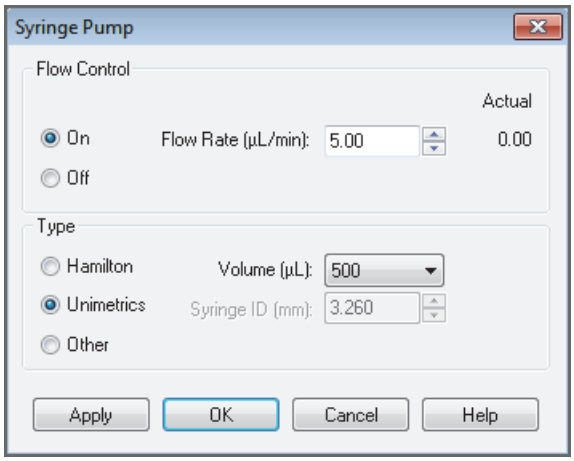
❖ To infuse the calibration solution into the API source

1. Set up the syringe pump to infuse a solution.

   You can use the calibration solution, but it is not required for this evaluation. For instructions, refer to "Setting Up the Syringe Pump for Tuning and Calibration" in Chapter 3 of the *LTQ Series Getting Started Guide*.

2. In the Tune Plus window, choose **Setup > Syringe Pump** to open the Syringe Pump dialog box (Figure 2).

   Figure 2. Syringe Pump dialog box



3. Under Flow Control, select the **On** option, and then enter an appropriate value in the Flow Rate (µL/min) box.

   - For ESI and H-ESI modes, enter **5.00**.
   - For NSI mode, refer to the product user guide.

4. Under Type, do the following:
   - Select the **Hamilton** or **Unimetrics** option.

     Note A 500 µL Unimetrics syringe is supplied with the LTQ Series mass spectrometer.

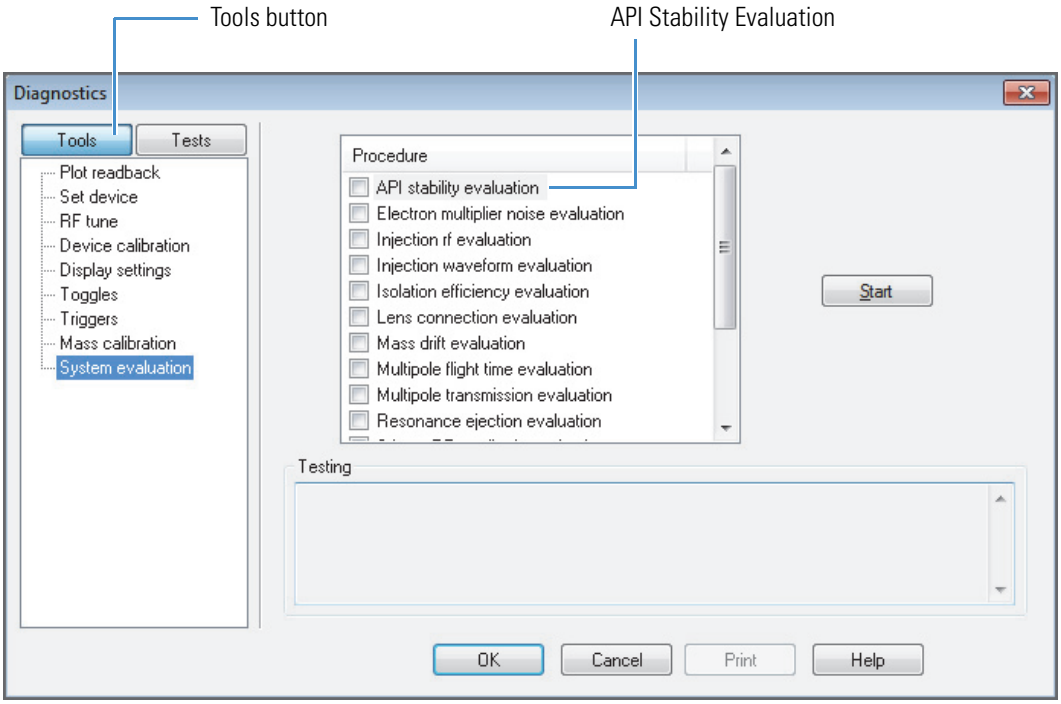
   - In the Volume (µL) list, select **250** or **500** as appropriate.
5. Click **Apply** or **OK** to start the syringe pump.

   You can also use the syringe pump on/off button that is located above the syringe pump.

❖ To adjust the spray stability

1. Run the API Stability Evaluation diagnostic as follows:
   a. In the Tune Plus application, choose **Diagnostics > Diagnostics**, click **Tools**, and then select **System Evaluation** (Figure 3).

Figure 3. Diagnostics dialog box showing the System Evaluation page (Velos Pro)

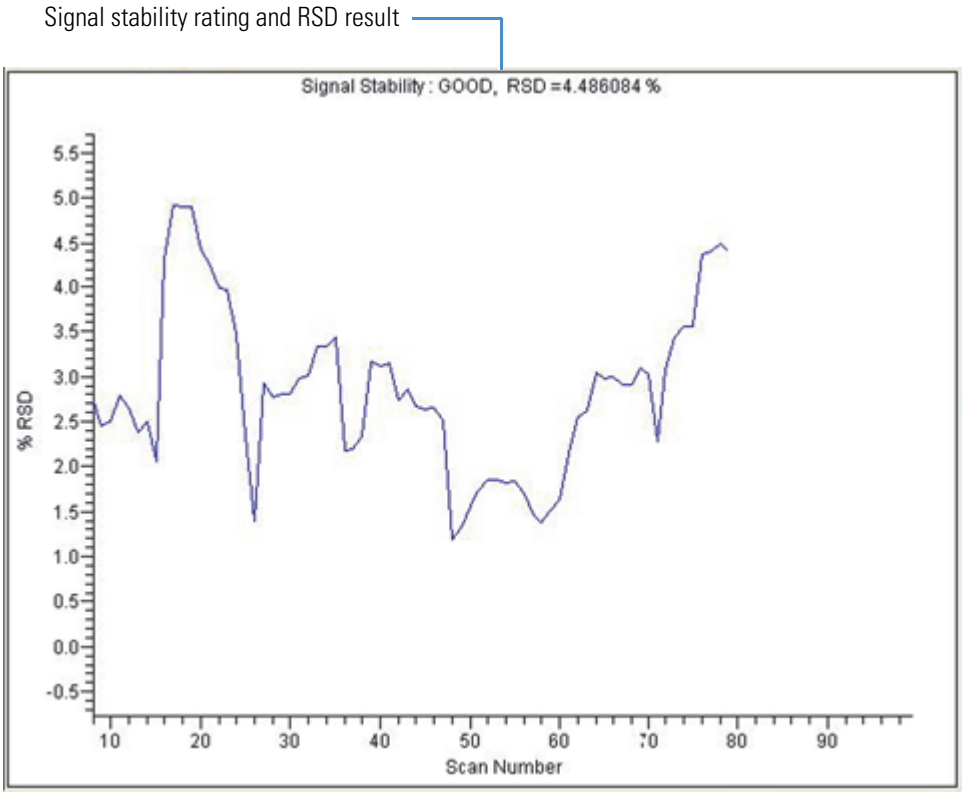


b. Select the **API Stability Evaluation** check box, and then click **Start**.

> **Note** Because the API stability evaluation runs indefinitely, when you are ready to end the evaluation, click Stop and then click OK, which closes the dialog box.

The API stability evaluation generates a real-time graph showing the relative standard deviation (RSD) of the total ion current (TIC) for a 10 Da selected ion monitoring (SIM) scan that is centered around the most abundant mass-to-charge ratio in the current spectrum. Figure 4 shows an example. When you change the parameters, such as the spray voltage, the RSD value shown in the graph decreases or increases, depending on whether the change makes the spray more or less stable. The actual RSD value and its rating (for example, excellent or good) appear above the graph.

Figure 4. API stability evaluation results (Orbitrap Elite, H-ESI probe, and SIM scan type)



2. In the Tune Plus window, choose **Settings > *type* Source**, where *type* is **ESI**, **Heated ESI**, or **NSI**, to open the source dialog box.

3. While you observe the real-time signal stability graph, adjust the following parameters in the *type* Source dialog box so that the value of the %RSD becomes less than 15%.

   Although the goal is 15% or less for the RSD of the TIC, you can often reduce the %RSD much more by adjusting these parameters.

   - Electrospray voltage—Use the following values.

ESI or H-ESI probe	EASY-Spray nanospray API source	Nanospray Flex API source or NSI-1 dynamic nanospray probe
4.5 kV	1.4–2.4 kV	1.5–2.5 kV

   - Sheath, auxiliary, and sweep gas flow rates—Refer to Table 3 in Chapter 1 of the *LTQ Series Getting Started Guide*.
   - (H-ESI only) Heater temperature—Use the range from Off to 50 °C. For additional information, refer to the Tune Plus Help (Heated ESI Source dialog box topic).

4. When you are ready, click **OK** in each open dialog box.

Note Before you run the calibrations or diagnostic tests that require the use of the calibration solution, Thermo Fisher Scientific recommends that the %RSD value be less than 15 percent.

3

Mass Spectrometer Calibration

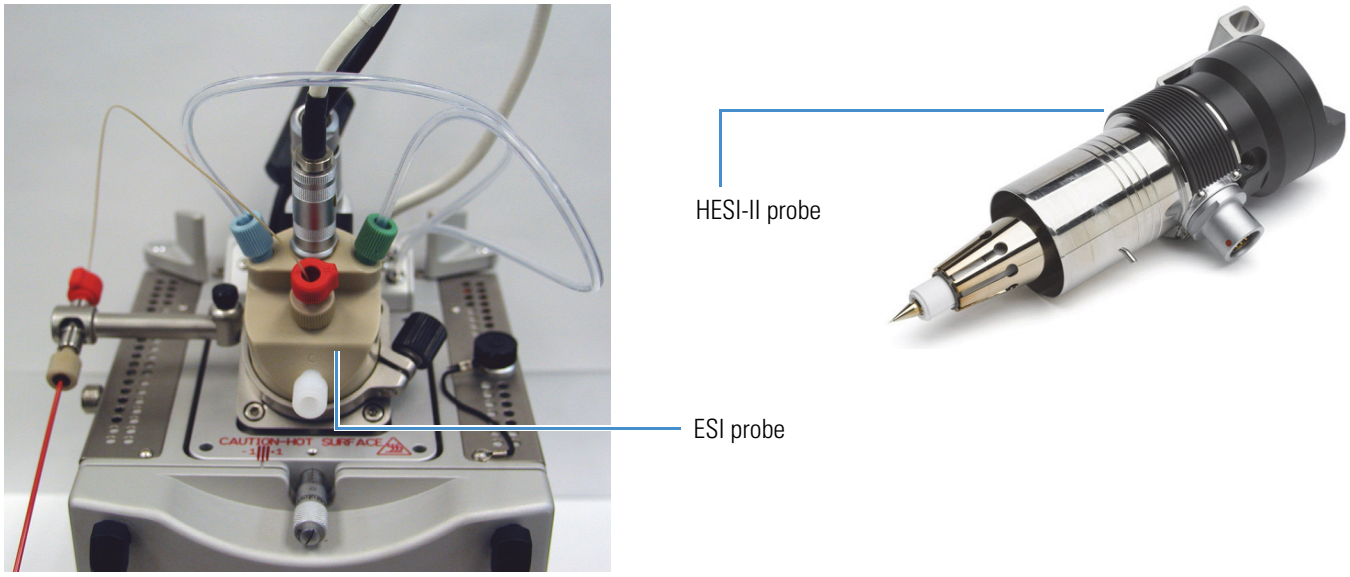
This chapter shows the probe types that you can use to calibrate the mass spectrometer, specifies the appropriate calibration mixtures (calmix), discusses two calibrations that have the most affect on sensitivity, and recommends the calibration checks that you should run. You must periodically calibrate the mass spectrometer to maintain peak performance over time. Before you calibrate the mass spectrometer, ensure that the ionization spray is stable by following the instructions in Chapter 2, "Ionization Spray Stability."

Contents

Probe Types for Calibration

When you calibrate the mass spectrometer, use only an ESI or H-ESI type probe (Figure 5). Do not attempt to calibrate the mass spectrometer with an NSI probe, which can affect the calibration results.

Figure 5. ESI probe (installed) and HESI-II probe

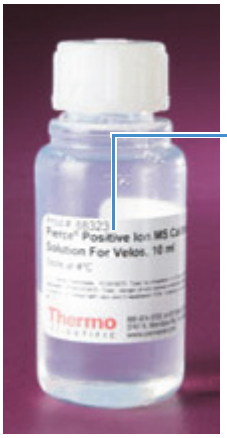
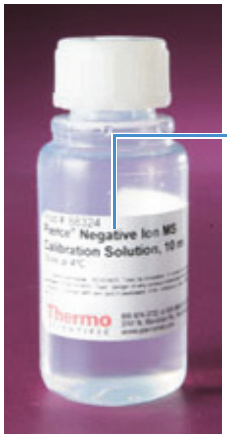


Calibration Mixture

Make sure that the calibration mixture (calmix) is fresh and that you have the correct solution for either the stand-alone mass spectrometer or the hybrid Orbitrap system. Use the calmix solution provided in the Velos Pro Preinstallation Kit (P/N OPTON-20042) or in the applicable LTQ Orbitrap Preinstallation Kit. You can also order the appropriate Pierce™ ready-made solution at www.thermo.com/pierce (see Table 3).

To order another preinstallation kit, which includes the positive calmix solution, contact your local Thermo Fisher Scientific field service engineer.

Table 3. Ordering information for the calibration mixtures

LTQ Velos and Velos Pro stand-alone mass spectrometers and hybrid Orbitrap systems	LTQ Orbitrap Velos, Orbitrap Velos Pro, and Orbitrap Elite systems
Pierce LTQ Velos ESI Positive Ion Calibration Solution (P/N 88323)	Pierce ESI Negative Ion Calibration Solution (P/N 88324)
 Positive	 Negative

Note

- For diagnosing instrument problems, use the positive ion calibration solution—even if you observe the problem in negative ion mode.
- For negative mode calibration of the hybrid Orbitrap systems, use the negative ion calibration solution.

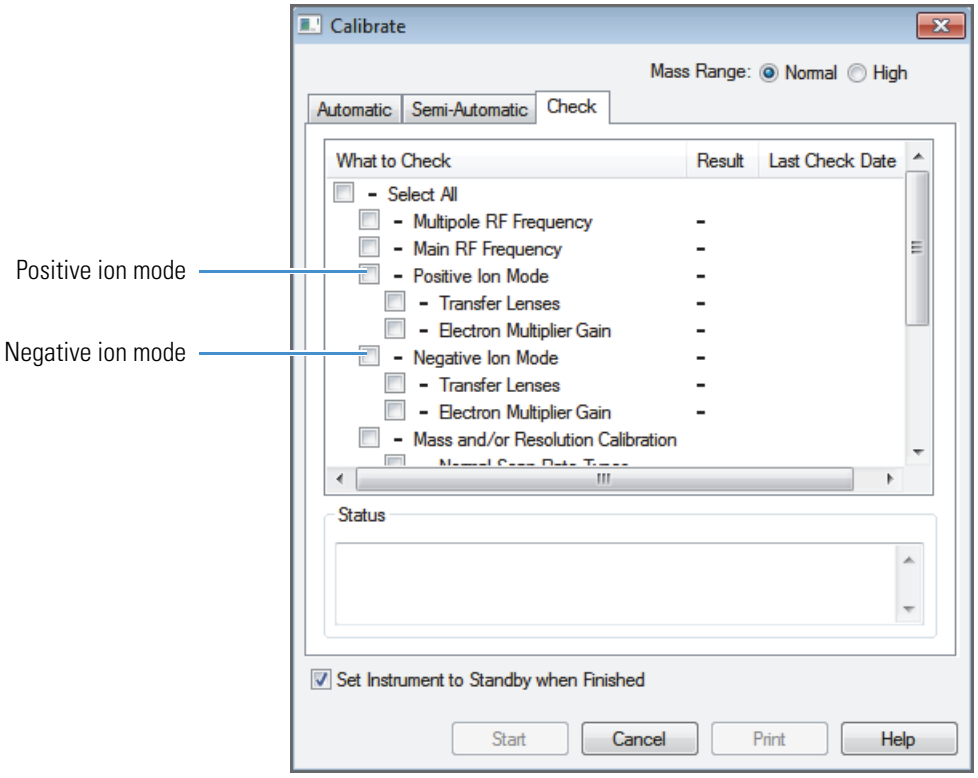
Types of Calibration

IMPORTANT You must pump down the ion trap vacuum system for at least 15 hours before running the calibrations.

In the Tune Plus application, the Calibrate dialog box has the following calibration categories (pages) for the mass spectrometer in the normal mass range (*m/z* 50–2000):

- Automatic—Performs an automatic optimization of all calibration parameters.
- Semi-Automatic—Performs an automatic calibration of all the calibration parameters or performs calibration of specific parameters.
- Check—Performs an automatic check of all the calibration parameters or performs a calibration check of specific parameters (Figure 6).

Figure 6. Calibrate dialog box showing the Check page



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

IMPORTANT To determine if the instrument calibration is within specifications, run all of the calibration checks once a week or monthly, depending on the instrument's condition and its usage.

Run the actual calibration for any calibration check that fails. If an actual calibration repeatedly fails, contact Thermo Fisher Scientific technical support for assistance.

Make sure that you check the calibrations for the Transfer Lenses and the Electron Multiplier Gain in both positive and negative ion modes (Figure 6) because these calibrations affect sensitivity and can change more often than other calibration parameters with instrument usage. (When you select either the Positive Ion Mode or Negative Ion Mode check box, the Tune Plus application automatically selects both calibrations in that mode.)

For calibration instructions, see "Evaluating the System Parameters Associated with Signal Intensity" on page 26.

4

Tuning the Mass Spectrometer

A proper instrument tune not only provides optimal sensitivity, it also provides for longer periods between cleaning cycles and more stable, long-term performance. Ensure that you periodically check the tune of the mass spectrometer to maintain peak instrument performance over time. This chapter describes the operational features of the tune files and the optimum tune settings for the ion optics.

During normal use of a mass spectrometer, residue can accumulate on the ion optic elements. Over time, this accumulated residue can alter the performance characteristics of the ion optics, which can result in reduced signal sensitivity. In some cases, this reduction can be significant and occur more rapidly depending on the extent of the sample cleanup, the extent of the instrument use, and most importantly whether you use nonoptimized tune values.

IMPORTANT To obtain high levels of sensitivity **over a longer period of time**, the ion optics settings must provide a sufficient voltage gradient that is appropriate for a range of optical element surface conditions. For details, see "Tuning the Ion Optic Elements" on page 17.

Note For the instrument control application, ensure that you have LTQ 2.7 SP1 or later installed on the data system computer. If the installed version is earlier than 2.7 SP1, you might have to manually set some of the ion optics parameters. To upgrade the application, contact your local Thermo Fisher Scientific field service engineer for assistance.

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Tune Files

The Tune Plus application uses tune files to store and set the values of various parameters that correspond to the installed API source and probe.

Note Each type of probe, such as APCI, H-ESI, or NSI, has its own tune file, and each tune file also has information for the different API sources.

Note the following about the tune files:

- Before you tune the instrument, make sure that you install the appropriate API source probe for your experiment.
- When you switch from one type of probe to another, the Tune Plus application **automatically loads the last saved tune parameters** for the newly installed probe and **overwrites** the tune values from the previously installed probe.
- If you make changes to the tune parameters and do not save them before switching to another type of probe, the changes are lost.
- If you make changes to the tune parameters, the title bar for the Tune Plus window updates only after you save the tune file.
- If you want to use any of the tune parameters determined by using a different probe:
  a. Write down the applicable parameters and their values (or save a screen capture of the dialog box).
  b. Install the new probe.
  c. Open the default tune file associated with the installed probe and manually enter the new parameters' values into the Ion Optics dialog box.
  d. Save the tune file with the new settings.

  Tip To more easily locate a tune file, you might want to include the probe type in the file name.

Note the following about tune files and ETD systems:

- For LTQ 2.6 SP3 or earlier, the Tune Plus application saves the ETD parameters in the tune file associated with a specific API source probe.
- For LTQ 2.7 and later, the Tune Plus application saves the ETD parameters (excluding the ETD reaction time) in an ETD-dedicated system file. This means, for ETD systems you can switch to another API source probe type without losing the current ETD parameters.

Tuning the Ion Optic Elements

Tune parameters are instrument parameters whose values can vary with the type of experiment. To achieve the highest sensitivity or the lowest limits of detection for an analyte of interest, tune the mass spectrometer with that analyte. In addition, appropriate tuning can improve long-term performance by ensuring that the voltage gradient on the ion optics is appropriate for a wide variety of conditions.

For additional information about tuning the ion optics, refer to the *LTQ Series Getting Started Guide*. For cleaning instructions, see Chapter 6, "Cleaning the Ion Optics."

These are the critical ion optic components and parameters to tune:

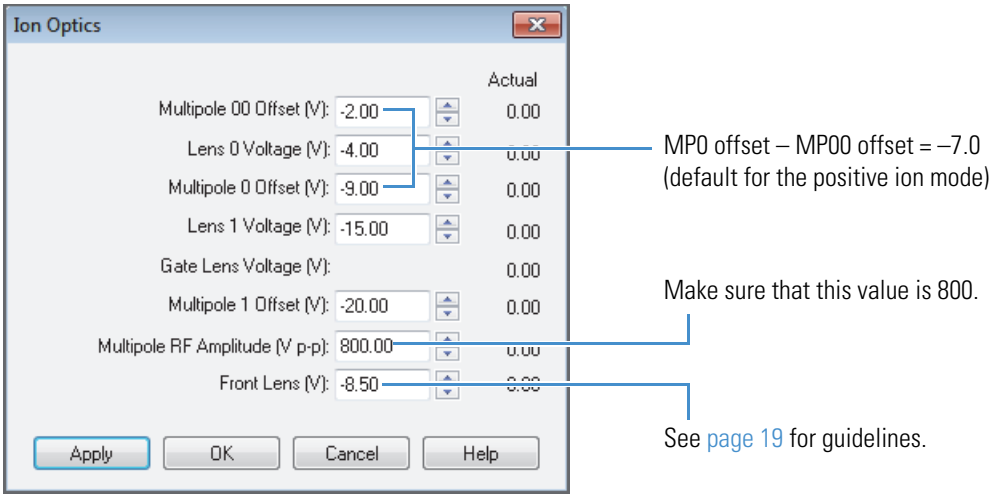
- Multipoles MP00 RF Lens and MP0
- Front Lens
- Multipole RF Amplitude

Multipoles MP00 RF Lens and MP0

You must tune the multipole MP00 rf lens (more commonly called MP00) and multipole MP0 offsets appropriately to achieve high sensitivity over long periods of run time. In particular, the voltage difference between these two optical elements is critical in addition to the absolute values.

In the Ion Optics dialog box (Figure 7), make sure that you set the voltage difference between the Multipole 0 Offset and Multipole 00 Offset (also called the MP0–MP00 gradient) to –5.5 V or greater (positive ion mode). This setting establishes an adequate voltage gradient to ensure that ions have sufficient ion kinetic energy to overcome any small potential barriers caused by normal levels of residue buildup on the optical surfaces. The recommended MP0–MP00 gradient and the values stored in the default tune files are –7.0 V (positive ion mode) and 7.0 V (negative ion mode) for LTQ 2.7 SP1 and later.

Figure 7. Ion Optics dialog box



❖ To set the ion optics parameters

1. Attach the ion source housing to the mass spectrometer, and then install an ESI, H-ESI, or NSI probe as appropriate for the experiment.

   For ESI and H-ESI modes, refer to Chapter 2 in the *LTQ Series Getting Started Guide*. For NSI mode, refer to the product documentation.

2. Establish a stable spray (see "Maintaining Spray Stability" on page 5).

3. In the Tune Plus window, click the **Open** icon, and then open the default tune file associated with the installed probe.

4. Save the tune file to a new name (for example, ESI_mytune_date).

5. Choose **Setup > Ion Optics** to open the Ion Optics dialog box.

6. Ensure that the ion optic values are as shown in Figure 7 on page 18. If they are not, manually type the values.

   Use negative voltages for positive ion mode and positive voltages for negative ion mode.

> **IMPORTANT** (For LTQ 2.7 SP1 and later versions only) The default ion optics values typically provide an optimum tune for both sensitivity and long-term instrument performance. However, because each system might have slight differences, follow the procedure, "To tune the front lens" on page 20, and use the optimized value.

7. Click **OK**.

8. Click the **Save** icon.

IMPORTANT Save the tune method before you change the type of probe. Because a tune file is source-type dependent, when you change the source type (for example, from H-ESI to NSI), you must repeat this tune procedure and save the tune file to another name.

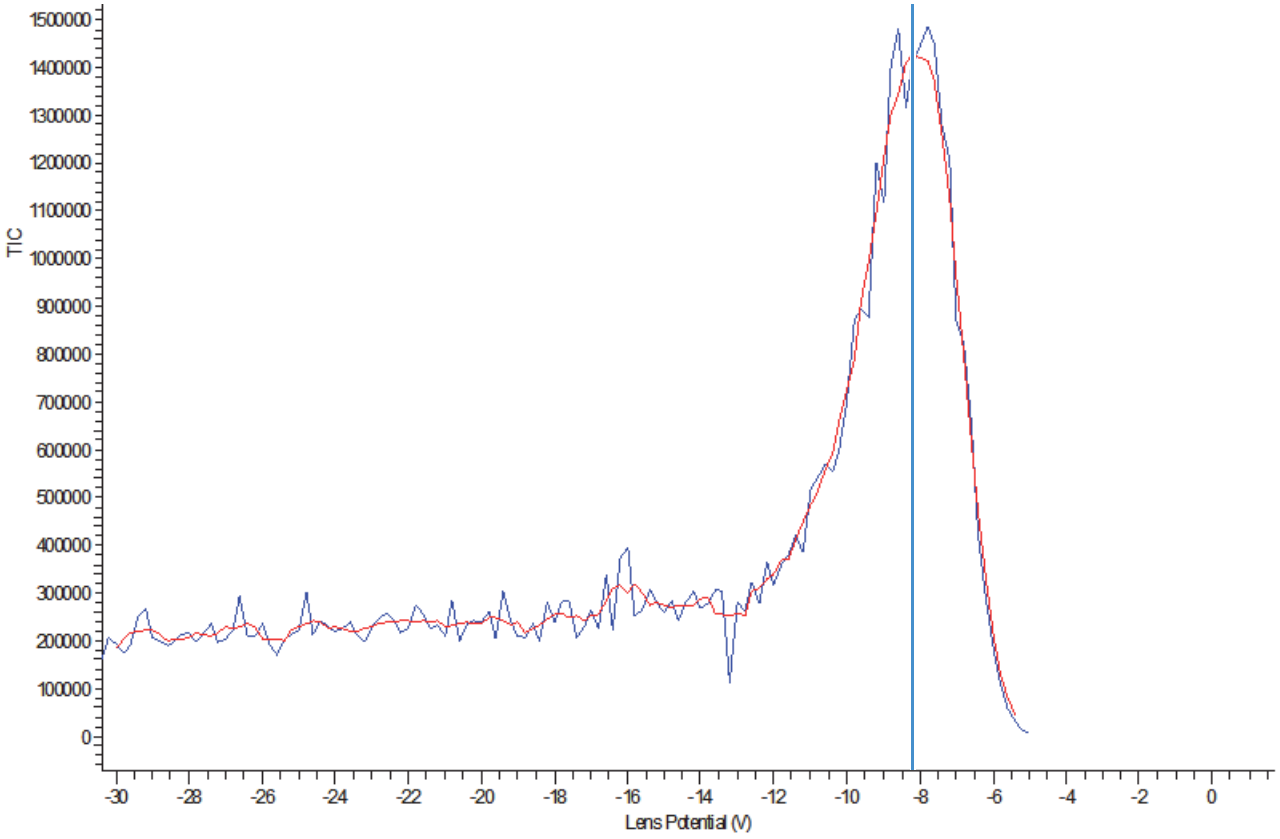
Front Lens

Because of the sharpness of its tuning curve, the front lens voltage is the next most critical setting that can affect the sensitivity and the long-term performance of the ion optics. The front lens voltage depends on the multipole MP0 offset. For the default MP0 offset range (–8 to –12 V), the front lens voltage optimizes at a value slightly more positive than the MP0 voltage. A more positive front lens voltage decelerates the ions, which allows more efficient trapping of the ions as they enter the high-pressure ion trap. The relationship between the MP0 voltage setting and the front lens varies from instrument to instrument. Therefore, you must tune the front lens in semi-automatic mode.

Figure 8 shows the result of the semi-automatic tune, indicating the sharpness of the optimum operating range and the determination of the optimum front lens voltage, which is positioned at the peak of the tuning curve (approximately –8.5 V for this example). (The red curve is a smoothed version of the blue curve.)

Tip Usually, a full automatic tune is unnecessary. However, you can run the automatic tune to ensure that the default values yield optimum results. If the automatic tune's performance results are within ±20 percent of the default values, Thermo Fisher Scientific recommends that you use the default values to ensure optimized long-term performance.

Figure 8. Front lens tune curve at *m/z* 1522 with MP0 offset at –8.5 V

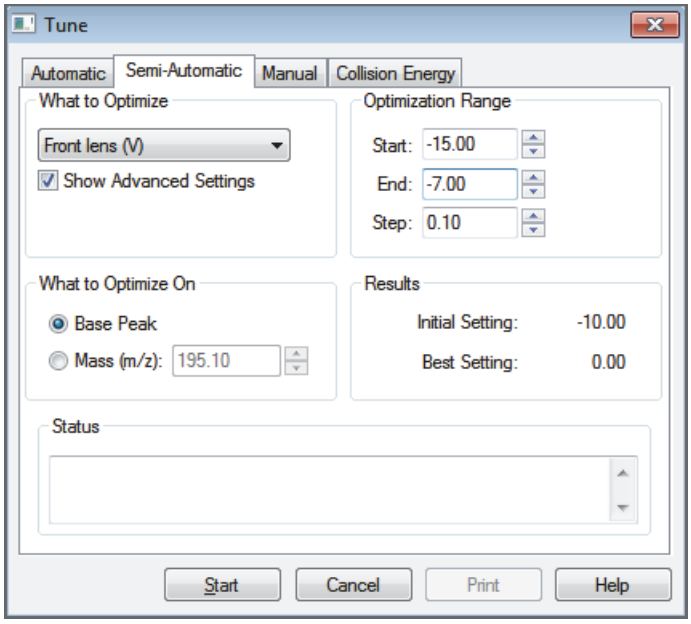


❖ To tune the front lens

1. Follow the procedure, "To set the ion optics parameters" on page 18.

2. In the Tune Plus window, click the **Tune** icon to open the Tune dialog box, and then click the **Semi-Automatic** tab.
3. In the What to Optimize list, select **Front Lens (V)** (Figure 9).

Figure 9. Tune dialog box showing the Semi-Automatic page



4. Under Optimization Range, ensure that the Start, End, and Step values are as shown in Figure 9.

   The optimum front lens voltage is directly dependent on the multipole MP0 offset, which has a default value of –9 V. Typically, you set the front lens voltage to a value within a few volts of the MP0 voltage. For an MP0 offset of –9 V, the front lens voltage is usually in the range of –7.0 to –10.0 V (default value is –8.5 V).

   Note Remember that the ion optic voltages are negative for positive ion mode and positive for negative ion mode.

5. Under What to Optimize On, do one of the following as applicable for the experiment:

   - Select the **Base Peak** option.

   –or–

   - Select the **Mass (*m/z*)** option, and then enter the mass of the analyte.

6. Click **Start**.

7. When the semi-automatic tune finishes, click the **Save** icon.

Multipole RF Amplitude

To contain and transport the ions effectively requires a multipole rf amplitude higher than 600 V_{p-p} (Figure 7 on page 18). This is especially true at the higher multipole offset values used in the new default tune files and the higher mass-to-charge ratio ions.

(LTQ Velos and Velos Pro only) For LTQ 2.7 and later, the default tune files set the multipole rf amplitude to the optimum value of 800 V_{p-p}. If you have an older version of the instrument control application, install LTQ 2.7 SP1 or later; otherwise, you must enter all recommended values manually.

Diagnostics for Signal Issues

This chapter provides a workflow chart to help you resolve certain signal issues, such as a loss of sensitivity or signal instability. Make sure to follow the order in which the tests are presented, and use the appropriate calibration solution (calmix) for your mass spectrometer (see page 10).

Record any test failures and submit the data to your local Thermo Fisher Scientific field service engineer, as noted in "Reporting Unresolved Issues" on page 53.

CAUTION For proper performance, operate the mass spectrometer at the proper vacuum levels. Operating the instrument with poor vacuum levels can cause reduced sensitivity, tuning problems, and reduced electron multiplier life.

Contents

Workflow for Resolving Signal Issues

If you suspect that there might be a problem with the signal, follow the diagnostic workflow charts shown in Figure 10 and Figure 11 on page 25.

Figure 10. Diagnostics workflow (Chart 1 of 2)

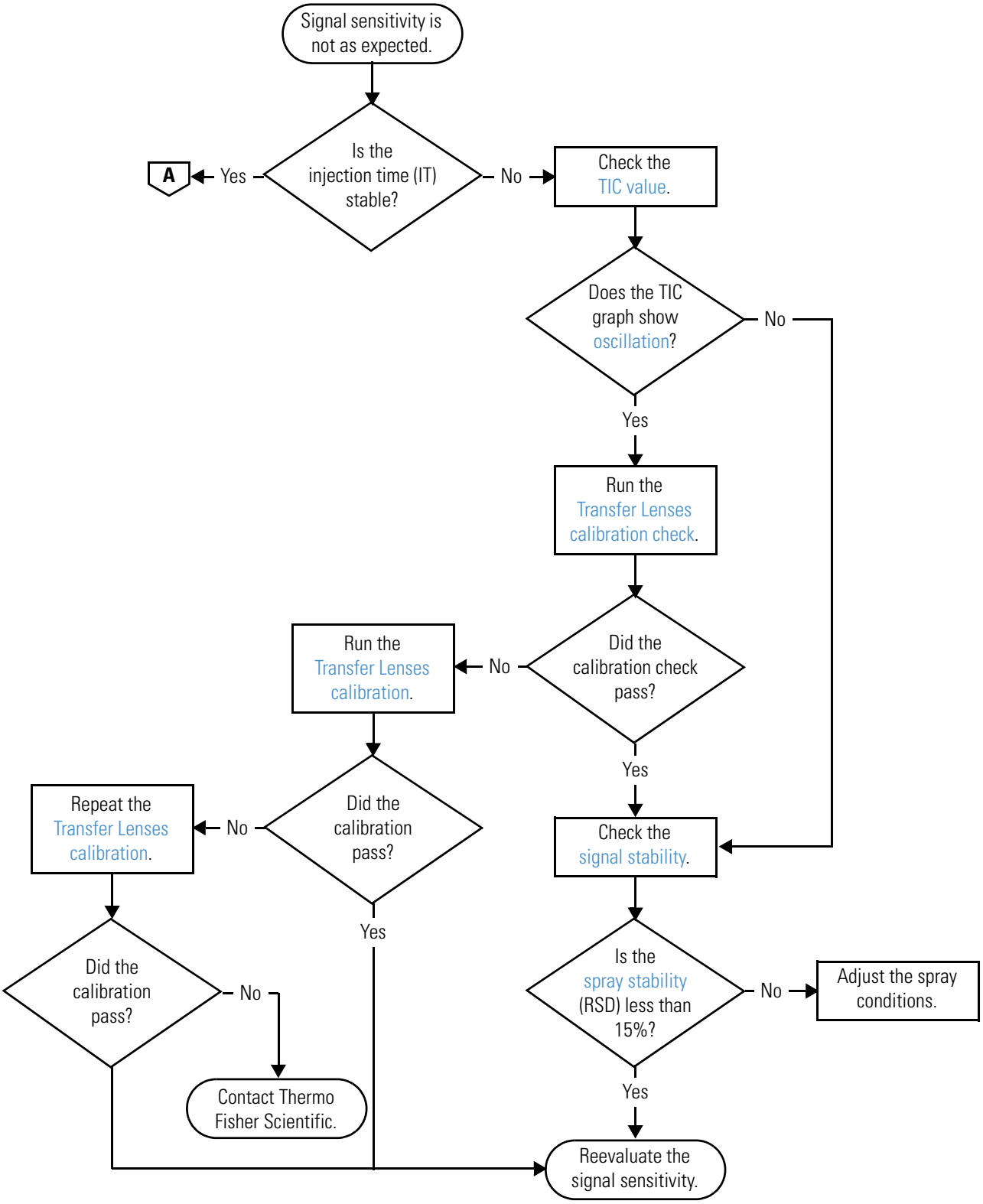
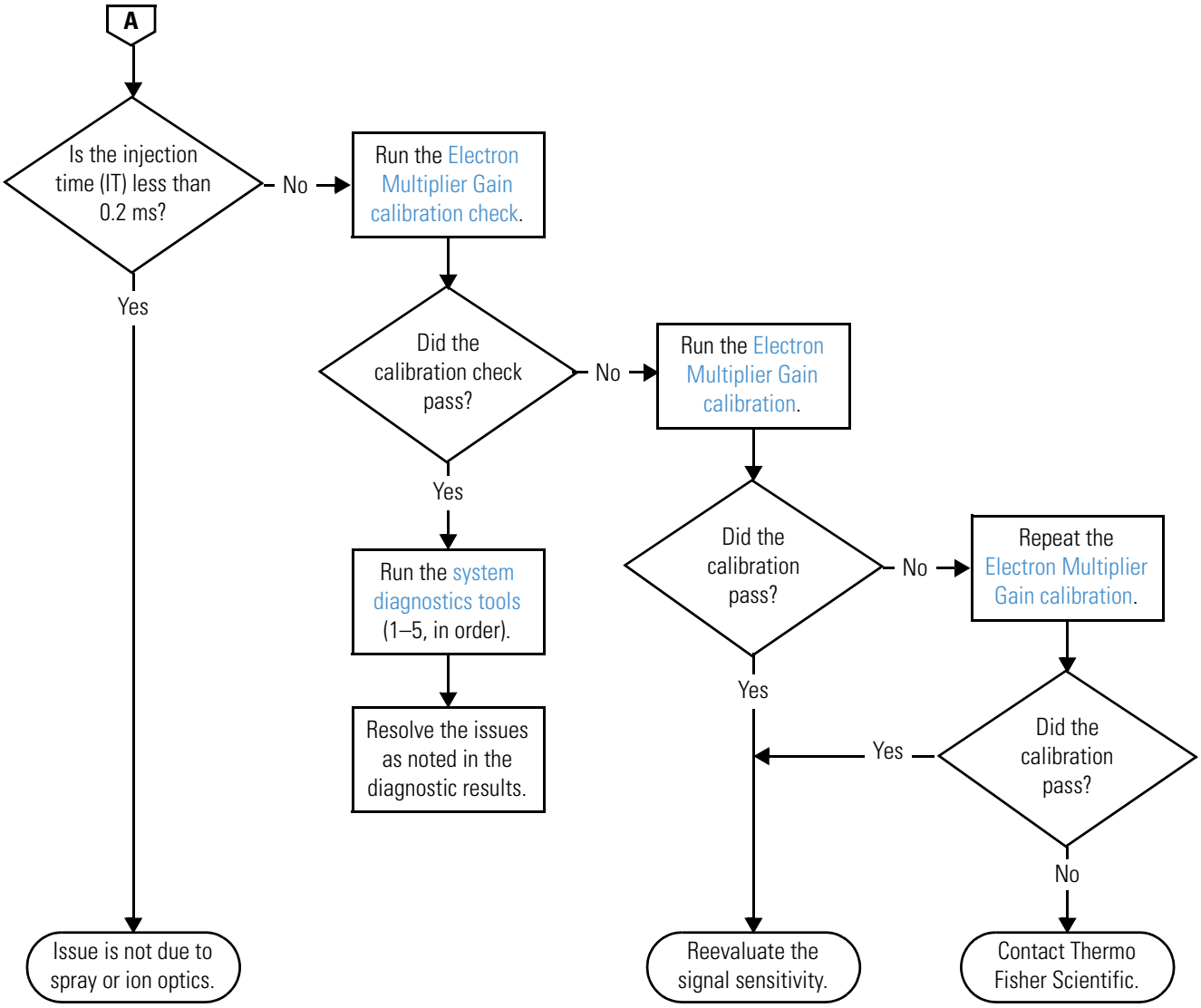


Figure 11. Diagnostics workflow (Chart 2 of 2)



Evaluating the System Parameters Associated with Signal Intensity

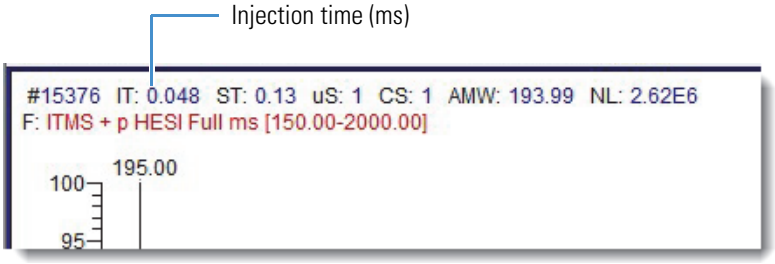
If a spectrum shows a reduction or loss in signal intensity, review the following parameters in this order:

1. Injection Time
2. Spray Stability
3. Manual TIC Tune
4. Electron Multiplier Gain
5. Transfer Lenses
6. System Evaluation Tools (consists of six diagnostic tests)

Injection Time

Injection time (IT) (also called ion injection time or ion time) is the amount of time, in milliseconds, that ions are allowed to accumulate in the ion trap mass analyzer. With Automatic Gain Control™ (AGC) on, the injection time is set automatically for each scan (up to the set maximum injection time) based on the AGC target value specified in the Injection Control dialog box. The actual injection time for a scan, which appears above the spectrum (Figure 12), fluctuates with each completed scan.

Figure 12. Location of the spectrum's injection time (IT)



Typical injection times when you infuse the normal calibration solution are less than 0.2 ms for an *m/z* 150–2000 full MS scan with a target value of 3×10^4. If the injection time is greater than 0.2 ms, try resolving the issue as follows:

- Make sure that the calibration solution is fresh and that you have the correct solution. (See "Calibration Mixture" on page 10.)
- Make sure that the spray is stable. (See "Maintaining Spray Stability" on page 5.)
- Make sure that you use the correct tune values for the ion optics.

- See the diagnostics workflow chart in Figure 11 on page 25.
- Clean or change the ion transfer tube.

CAUTION Hot surface. The ion transfer tube operates above 250 °C (482 °F). Allow the ion sweep cone and the ion transfer tube to cool to room temperature (approximately 20 minutes) before touching them. Be aware that if you remove the ion transfer tube when it is 200 °C or higher, you might damage the end of the tube.

You do not have to vent the system to remove the ion transfer tube. For instructions, refer to "Cleaning the API Ion Transfer Tube, Spray Cone, and Ion Sweep Cone" in Chapter 5 of the *LTQ Series Hardware Manual*. Also, read the cautions in "Cautions and Special Notices" on page xiv.

Spray Stability

Follow the procedure, "To adjust the spray stability" on page 6, to evaluate and then, if needed, adjust the spray stability.

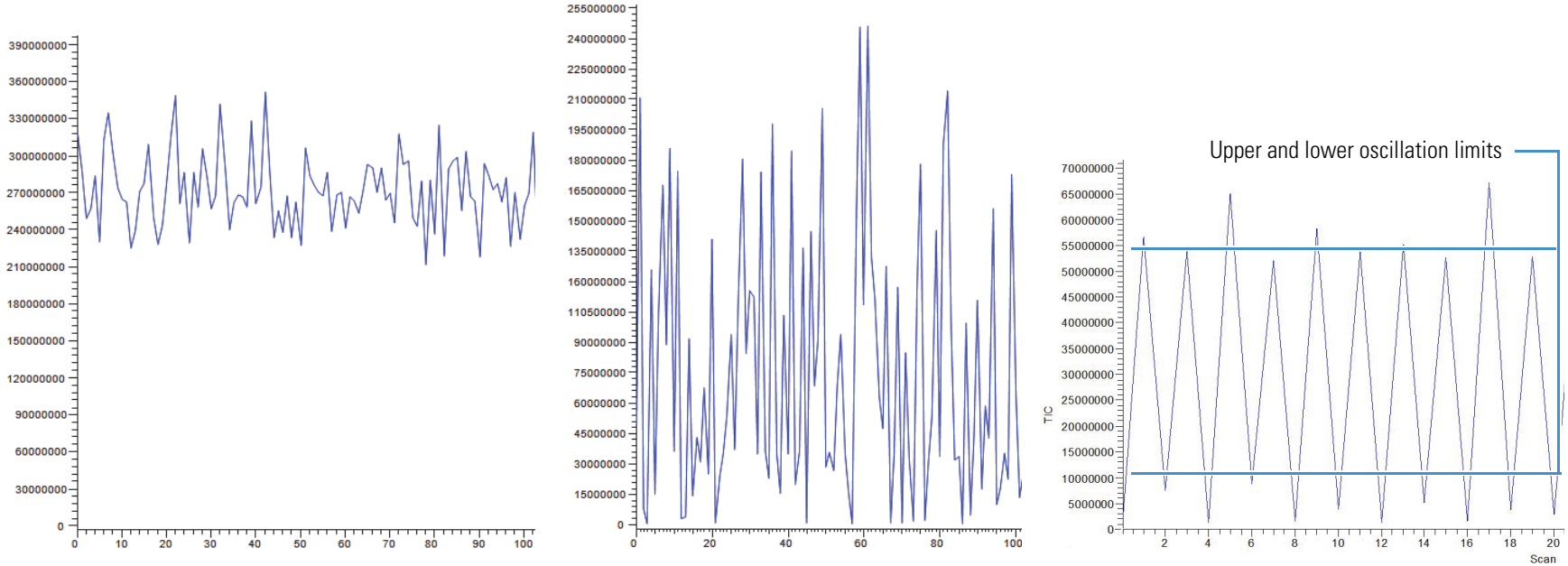
Manual TIC Tune

The total ion current (TIC) is the sum of the ion current intensities across the scan range in a mass spectrum. The TIC tune is a generic tool that generates a real-time graph of the TIC as a function of the scan number. Use the TIC manual tune to observe the signal stability and the effects of changes to various parameters.

The TIC tune can also help diagnose a particular issue that causes signal oscillation. TIC oscillations, as shown in Figure 13 (top, right graph), are direct scan-to-scan changes where the peak alternates between a high and low level. If you see TIC oscillation, it might indicate that the transfer lenses are out of calibration or that the ion trap might be contaminated; see the diagnostics workflow chart in Figure 11 on page 25.

Figure 13 shows TIC graphs with and without oscillation, and an unstable TIC graph without oscillation.

Figure 13. Examples of TIC graphs (Velos Pro)



TIC **Unstable TIC without oscillation** **TIC with oscillation**

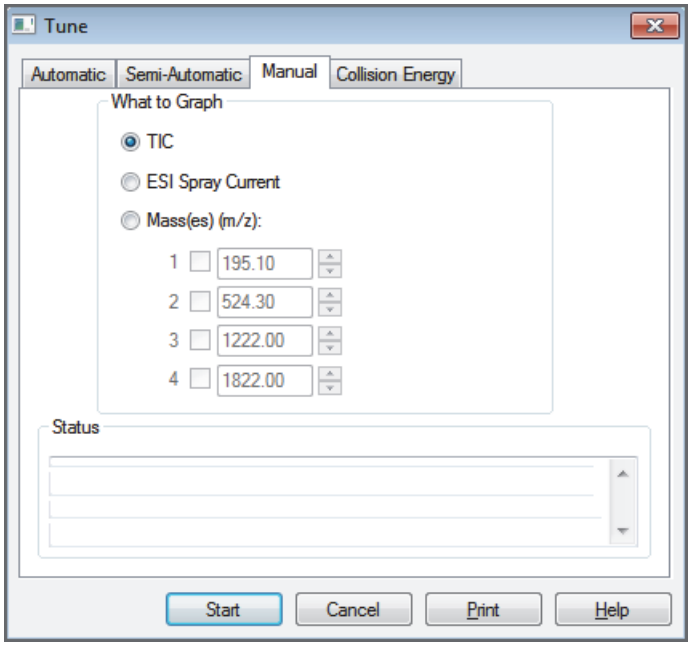
❖ To evaluate the TIC graph

1. Set up the syringe pump to infuse a solution.

   You can use the calibration solution, but it is not required for this evaluation.

2. In the Tune Plus window, click the **Tune** icon to open the Tune dialog box.
3. Click the **Manual** tab, and then select the **TIC** option (Figure 14).

   Figure 14. Tune dialog box showing the Manual page



4. Click **Start**.

   A message box appears.

5. Click **OK** when you are ready to continue.

6. In the Tune Plus window, click the **Display Graph View** icon.
7. Observe the TIC in the Graph view pane.

   Figure 13 on page 28 shows examples of TIC graphs.

8. If there is TIC oscillation, do the following:
   a. Follow the procedures on page 31 and page 32.
   b. Follow the procedures on page 33 and page 34.
   c. If the oscillation persists, the ion trap or its lenses might be contaminated. Save screen captures or save your observations to a raw data file (choose File > Save As).

> **IMPORTANT** Only a Thermo Fisher Scientific field service engineer can provide service on the ion trap.

Electron Multiplier Gain

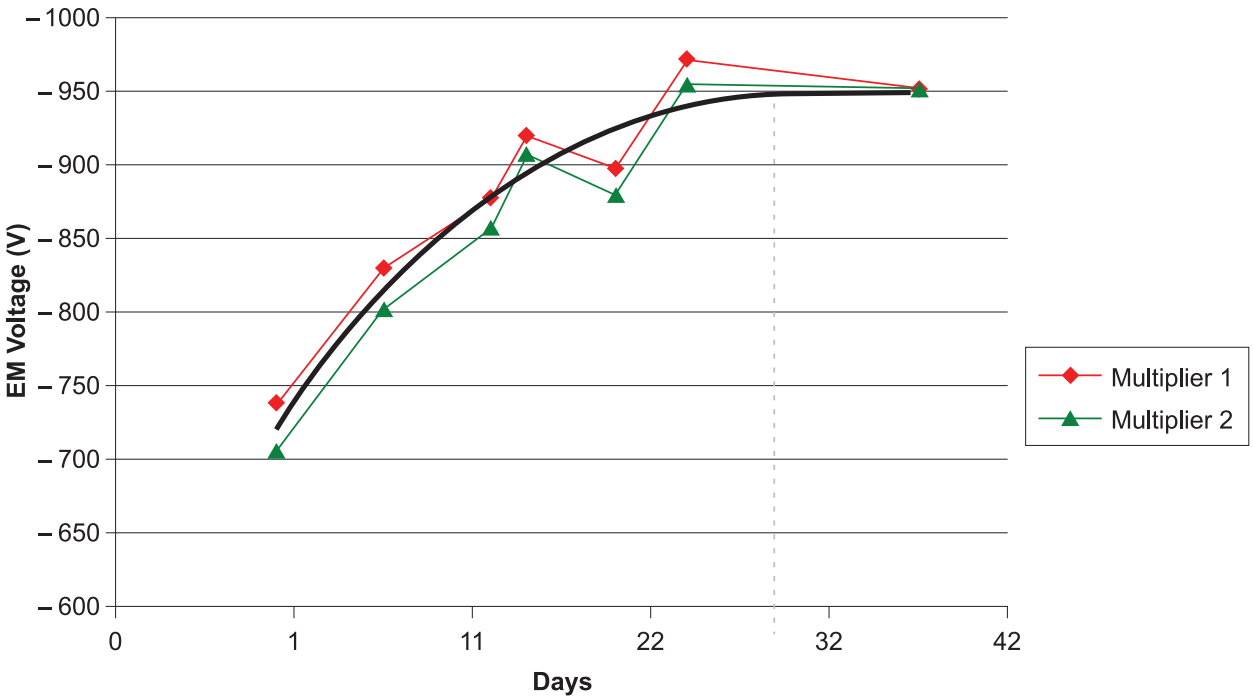
The calibration check for the electron multiplier (EM) gain determines if the multipliers have achieved the expected gain setting. The EM gain is the ratio of the signal out to the signal in. The EM gain is critical to ensure that the proper number of ions have accumulated in the ion trap and for proper quantitative performance. As the multipliers age, you must adjust the operating voltage to maintain a fixed gain. Because the multipliers age more rapidly when they are new, to ensure proper gain, calibrate the new multipliers every 3 days for the first month or so of operation or until the multipliers' voltages begin to flatten out (plateau).

As the multipliers' voltages start to flatten out, the EM gain changes more slowly. The amount of time to reach the voltage plateau varies with conditions, treatment, and instrument use. For example, multipliers in heavily used instruments plateau faster than instruments that are used only occasionally.

After the multipliers' voltages flatten out, you can run the EM gain calibration check less often, for example, every 2–4 weeks. If the results of the calibration check indicate a failure, run the actual calibrations on the Semi-Automatic page of the Calibrate dialog box.

The graph in Figure 15 shows an example of the rate of voltage change for two new electron multipliers in an LTQ Velos mass spectrometer. In this example, approximately 28 days passed before the voltages started to flatten out.

Figure 15. New electron multipliers' voltages over time (LTQ Velos example, fairly continuous usage)



❖ To evaluate the electron multiplier gain

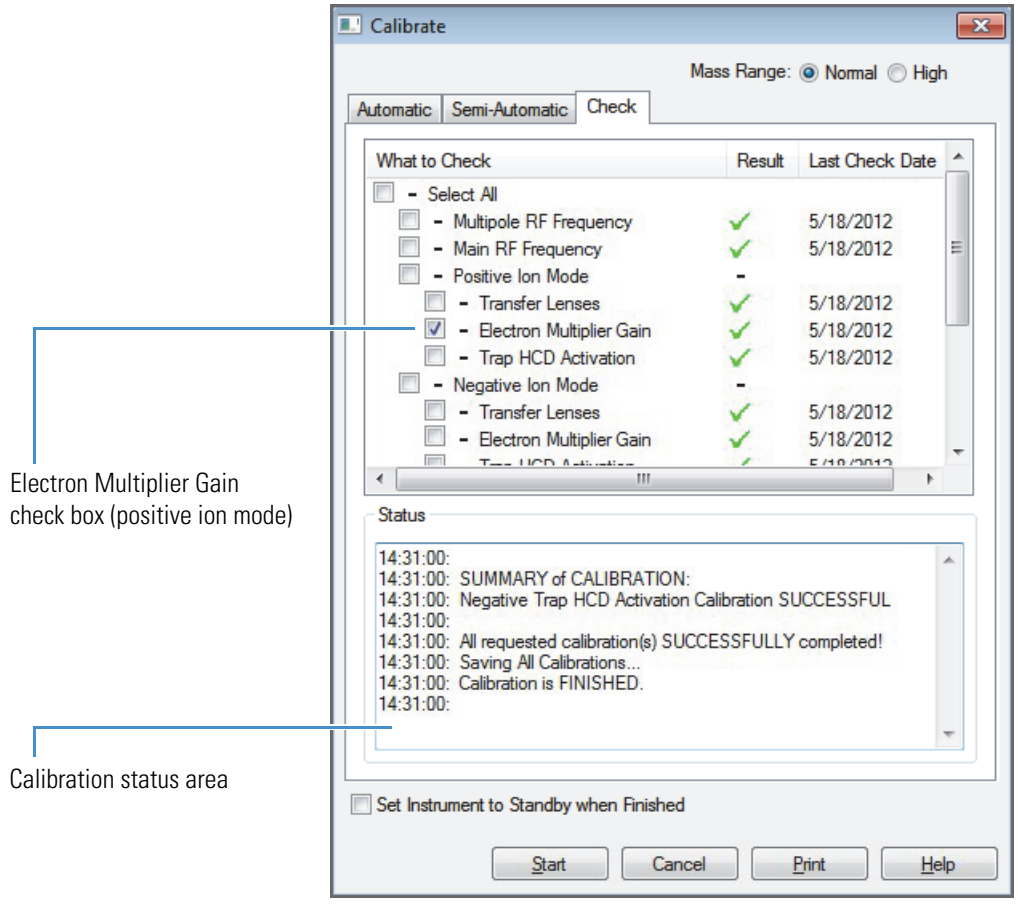
Note Run this calibration check, and if needed the actual calibration, once a week.

1. Set up the syringe pump to infuse the calibration solution.
2. Make sure that the tune voltages are correct (see "Tuning the Ion Optic Elements" on page 17) and that the spray is stable (see "Maintaining Spray Stability" on page 5).

3. In the Tune Plus window, click the **Calibrate** icon to open the Calibrate dialog box.
4. For the Mass Range, select the **Normal** option.
5. Click the **Check** tab, and then select the **Electron Multiplier Gain** check box under Positive Ion Mode (Figure 16) or Negative Ion Mode, as applicable.

   Do not run the electron multiplier gain for both polarities at the same time.

Figure 16. Calibrate dialog box showing the Check page (Velos Pro[1])



[1] The Trap HCD Activation option appears only when the stand-alone Velos Pro mass spectrometer has an activated Trap-HCD license.

6. Click **Start**.

   A message box appears.

7. Click **OK** when you are ready to continue.

   The calibration check starts. When it is completed, review the Result column and the bottom status information. See the note on page 12 for an explanation of the Result column.

8. If the calibration check fails, do the following:

   a. Save a screen capture of the dialog box. (See "Reporting Unresolved Issues" on page 53.)

   b. See the diagnostics workflow chart in Figure 11 on page 25, and follow the next procedure, To calibrate the electron multiplier gain.

❖ To calibrate the electron multiplier gain

1. In the Calibrate dialog box, click the **Semi-Automatic** tab.
2. Select the **Electron Multiplier Gain** check box under the appropriate polarity mode.
3. Click **Start**.

   A message box appears.

4. Click **OK** when you are ready to continue.

   The calibration starts. When it is completed, review the Result column and the bottom status information. See the note on page 12 for an explanation of the Result column.

5. If the calibration fails, do the following:

   a. Save a screen capture of the dialog box. (See "Reporting Unresolved Issues" on page 53.)

   b. Make sure that the ionization spray is stable. (See "Maintaining Spray Stability" on page 5.)

   c. Repeat this calibration.

      - If the calibration succeeds, follow the procedure, "To run the ejection and multiplier gain ratio" on page 53.
      - If the problem persists, contact Thermo Fisher Scientific technical support for assistance.

Transfer Lenses

The transfer lenses calibration check determines if the ion transmission through the transfer lenses is within tolerance. Run this check once a month (in the applicable polarity mode) or more often if you suspect that there is charging contamination. After an actual calibration, the efficiency of the ion transmissions is typically above 90 percent.

Calibration check results that are less than 70 percent indicate a failure of the ion transmission efficiency. In this case, run the actual calibration on the Semi-Automatic page of the Calibrate dialog box. If an actual calibration repeatedly fails, there might be some contamination of components within the ion trap assembly. Contact Thermo Fisher Scientific technical support for assistance.

❖ To evaluate the transfer lenses

Note Run this calibration check, and if needed the actual calibration, once a month.

1. Set up the syringe pump to infuse the calibration solution.

2. In the Tune Plus window, click the **Calibrate** icon to open the Calibrate dialog box.
3. For the Mass Range, select the **Normal** option.
4. Click the **Check** tab, and then select the **Transfer Lenses** check box under the appropriate ion mode (Figure 16 on page 31).
5. Click **Start**.

   A message box appears.
6. Click **OK** when you are ready to continue.

   The calibration check starts. When it is completed, review the Result column and the bottom status information. See the note on page 12 for an explanation of the Result column.
7. If the calibration check fails, do the following:
   a. Save a screen capture of the dialog box. (See “Reporting Unresolved Issues” on page 53.)
   b. See the diagnostics workflow chart in Figure 11 on page 25, and follow the next procedure, To calibrate the transfer lenses.

❖ To calibrate the transfer lenses

1. In the Calibrate dialog box, click the **Semi-Automatic** tab.
2. Select the **Transfer Lenses** check box under the appropriate ion mode.
3. Click **Start**.

   A message box appears.
4. Click **OK** when you are ready to continue.

   The calibration starts. When it is completed, review the Result column and the bottom status information. See the note on page 12 for an explanation of the Result column.
5. If the calibration fails, repeat this calibration procedure.

   If the problem persists, contact Thermo Fisher Scientific technical support for assistance.

System Evaluation Tools

IMPORTANT If you run more than one diagnostic test at a time, the four below tests are run in alphabetical order instead of the specified order and the data shown in the graphs is not saved.

Run all of the following diagnostic tools[2] one at a time and in the stated order:

1. Multipole Gradient Evaluation
2. Source Optics Flight Time Evaluation
3. Multipole MP0 Flight Time Evaluation
4. Ion Optics Charging Evaluation

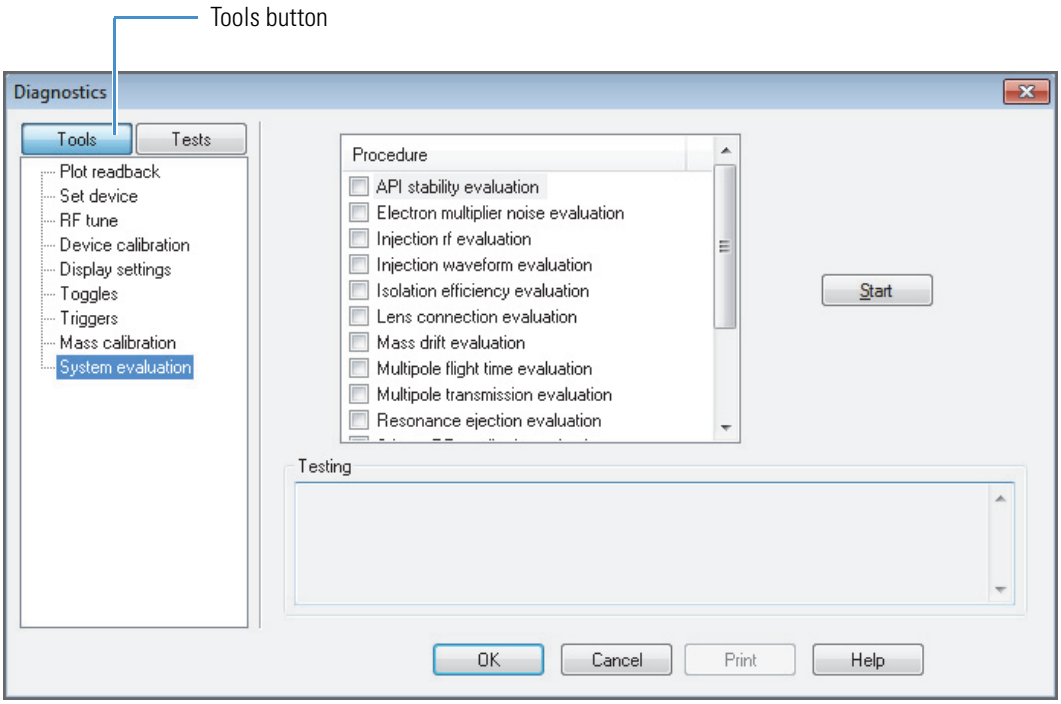
   IMPORTANT This diagnostic evaluation can discharge the system. Therefore, run this evaluation in this stated order and be aware that running this test a second time might not show the same behavior.
5. Ejection and Multiplier Gain Ratio

[2] This list of system evaluation tools is only available with the LTQ application, 2.7 SP1 and later.

❖ **To view the list of system evaluation procedures**

1. In the Tune Plus window, choose **Diagnostics > Diagnostics** to open the Diagnostics dialog box.
2. Click **Tools**, and then select **System Evaluation** (Figure 17).

Figure 17. Diagnostics dialog box showing the System Evaluation page (Velos Pro)

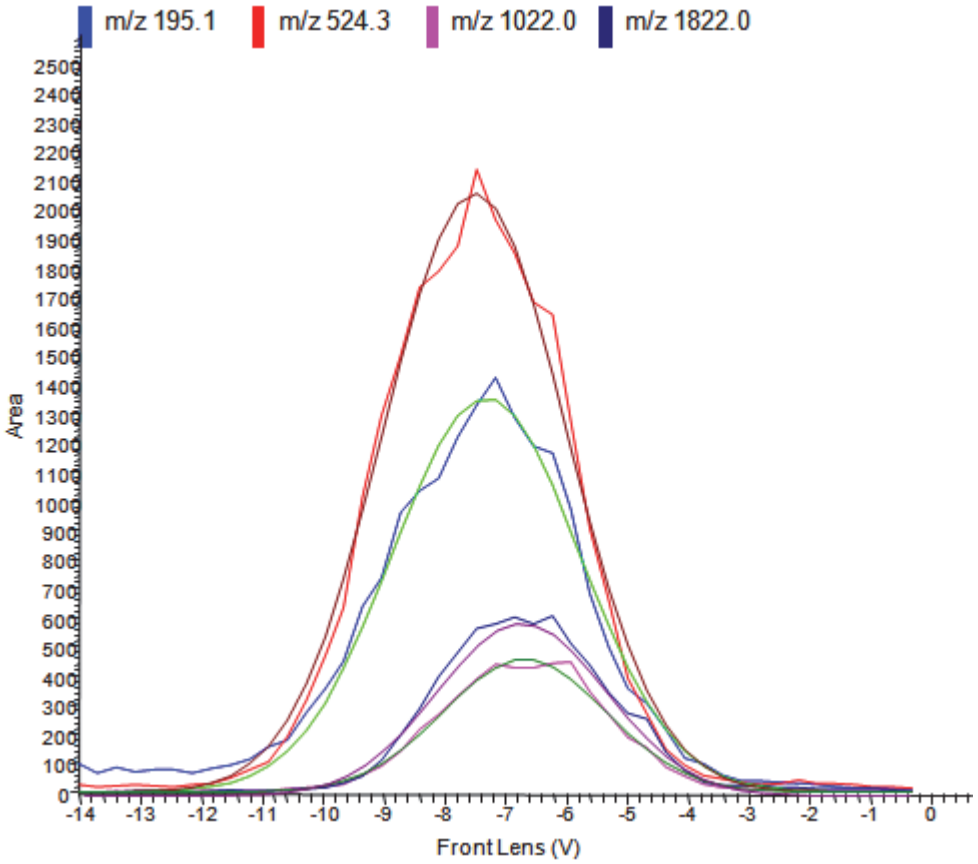


Multipole Gradient Evaluation

The multipole gradient evaluation is a diagnostic tool that provides information about the sensitivity of the system as a function of the multipole MP0–MP00 voltage gradient (see page 17), which can help determine the condition (level of contamination) of the ion optic elements.

This diagnostic evaluation sets the optics to their standard default settings, turns off the AGC, and changes the MP0 offset to various preset voltages, which then changes the MP0–MP00 gradient. With each new MP0 offset value, the Tune Plus application optimizes the front lens voltage at various mass-to-charge ratios, as shown in Figure 18.

Figure 18. Graph: signal intensity versus the front lens voltages



To determine the condition of the ion optics, review the generated real-time graph of the normalized signal intensity versus the MP0–MP00 gradient. Example graphs that show clean and contaminated systems are shown in Figure 19 and Figure 20 on page 38, respectively. Loss of signal at the lower gradients (Figure 20) indicates that the optics have some level of contamination and might possibly need cleaning when the voltage gradients become too high.

Figure 19. Graph: normalized signal intensity versus the MP0–MP00 gradient for a clean system

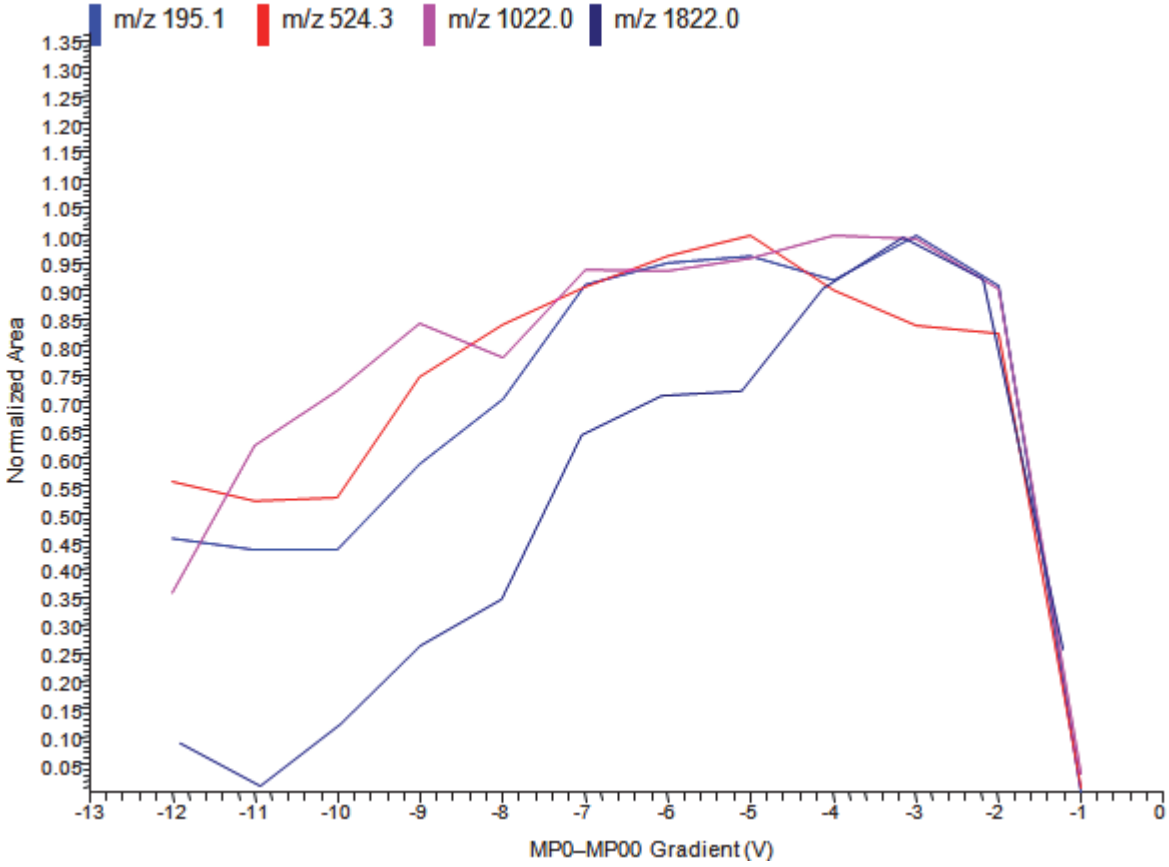
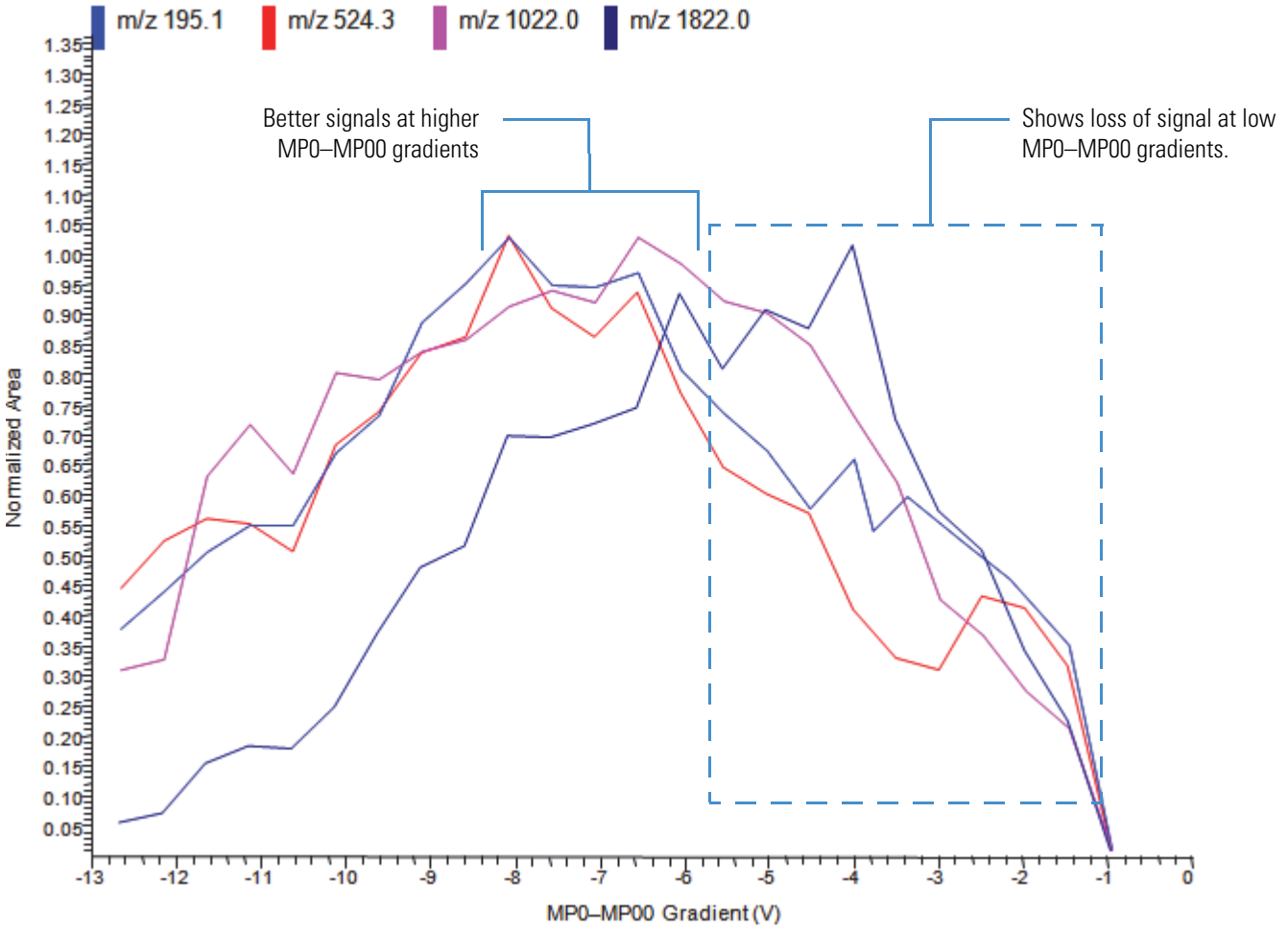


Figure 20. Graph: signal intensity versus the MP0–MP00 gradient for a contaminated system



❖ To run the multipole gradient evaluation

Note If the instrument is in negative ion mode, this diagnostic test automatically sets the mode to positive before starting.

1. Set up the syringe pump to infuse the positive calibration solution.
2. Open the System Evaluation diagnostics page (see page 35), and then select the **Multipole Gradient Evaluation** check box.
3. Click **Start**.

   A message box appears.
4. Click **OK** when you are ready to continue.

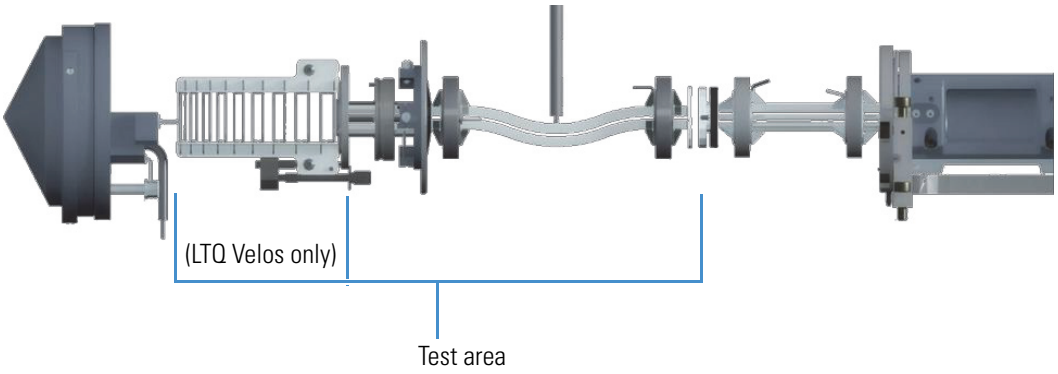
   The multipole gradient evaluation generates a real-time graph of the signal intensity versus the MP0–MP00 gradient (Figure 19 on page 37 and Figure 20).

5. (Optional) Save a screen capture of the diagnostic test results and the Tune Plus window.
6. Follow the procedure in the next section.

Source Optics Flight Time Evaluation

The source optics flight time evaluation is a diagnostic tool that measures the flight time of ions with various mass-to-charge ratios from the exit lens through multipole MP0 (Figure 21). The flight time can help determine the condition (level of contamination) of the ion optics. For the LTQ Velos mass spectrometer and LTQ Orbitrap Velos system, this evaluation includes the flight time through the S-lens.

Figure 21. Ion optics showing the test area for the source optics flight time evaluation



Charging and other issues can cause the flight times to be long, especially at low MP0–MP00 gradients.

The following figures display different graphs for this diagnostic test:

- Figure 22—Shows fast flight times to reach the peak heights, which indicate no or low levels of contamination.
- Figure 23 on page 41—Shows a lightly used instrument.
- Figure 24 on page 42—Shows a heavily used instrument.

Figure 22. Example graphs of the MP00/L0/MP0 flight times (Velos Pro)

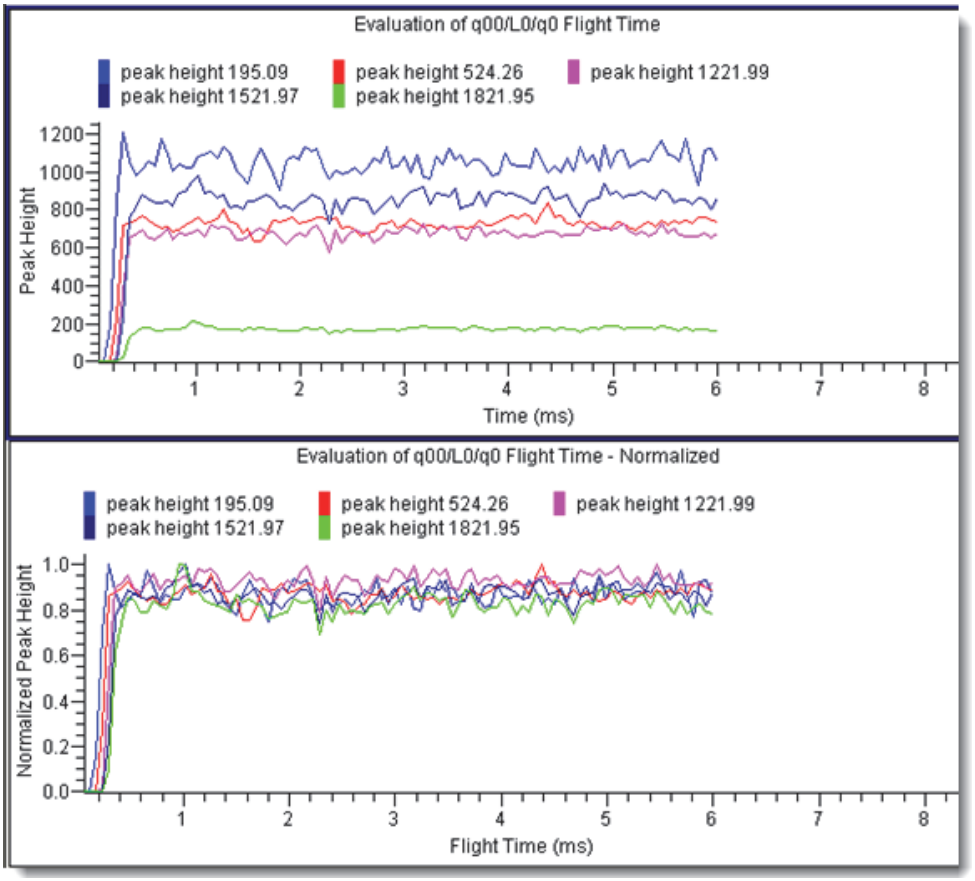


Figure 23. Graph of the source optics flight time evaluation (Velos Pro, light use)

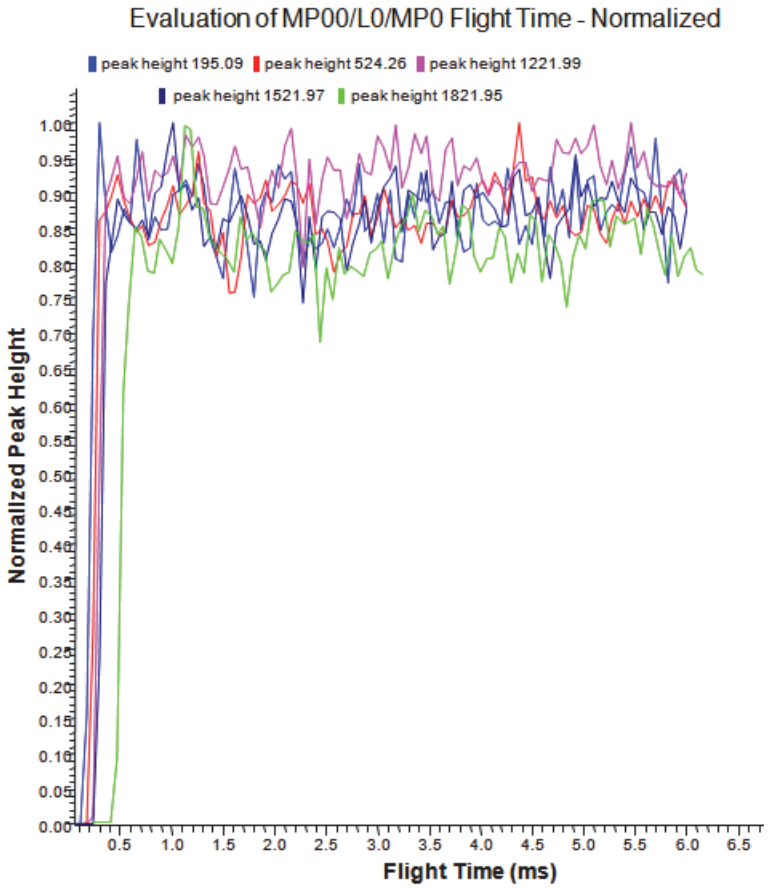
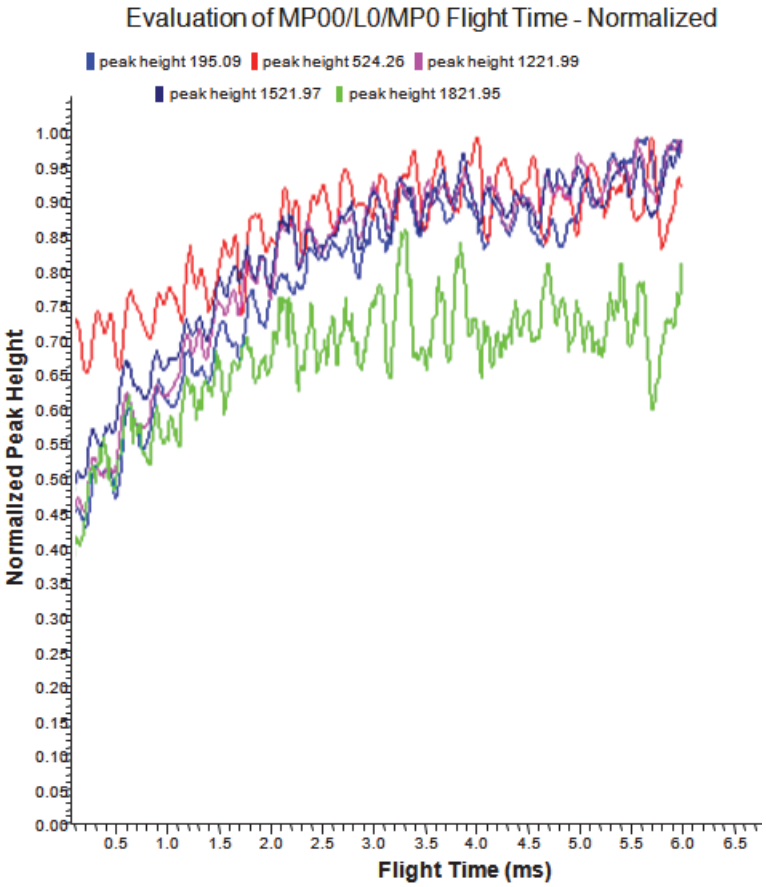


Figure 24. Graph of the source optics flight time evaluation (Velos Pro, heavy use)



❖ To run the source optics flight time evaluation

Note If the instrument is in negative ion mode, this diagnostic test automatically sets the mode to positive before starting.

1. Set up the syringe pump to infuse the positive calibration solution.
2. Open the System Evaluation diagnostics page (see page 35), and then select the **Source Optics Flight Time Evaluation** check box.
3. Click **Start**.

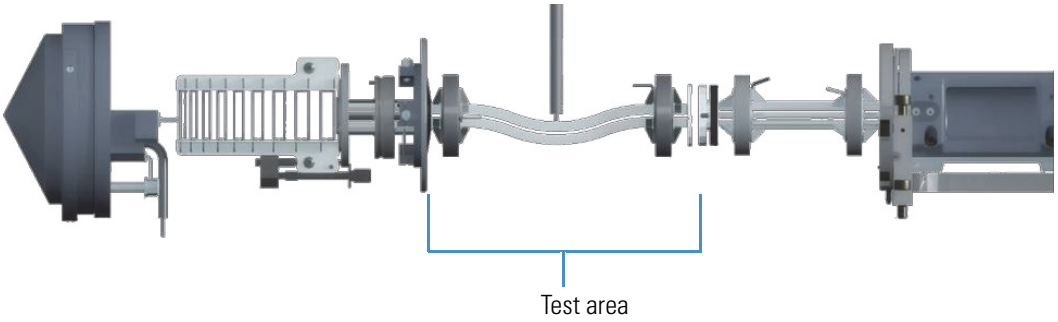
   A message box appears.
4. Click **OK** when you are ready to continue.

   This evaluation generates a real-time graph of the signal intensity at various mass-to-charge ratios as a function of time delay (Figure 22 on page 40).
5. (Optional) Save a screen capture of the diagnostic test results and the Tune Plus window.
6. Follow the procedure in the next section.

Multipole MP0 Flight Time Evaluation

The multipole MP0 flight time evaluation is a diagnostic tool that measures the flight time of various mass ions from lens L0 through multipole MP0 (Figure 25), which can help determine the condition (level of contamination) of MP0.

Figure 25. Ion optics showing the test area for the multipole MP0 flight time evaluation



Charging, and other issues, can cause the flight times to be long, especially at low MP0–MP00 gradients.

The following figures display different graphs for this diagnostic test:

- Figure 26—Shows fast flight times to reach the peak heights, which indicate no or low levels of contamination.
- Figure 27—Shows a lightly used instrument.

Figure 26. Example graphs of the multipole MP0 flight time (Velos Pro)

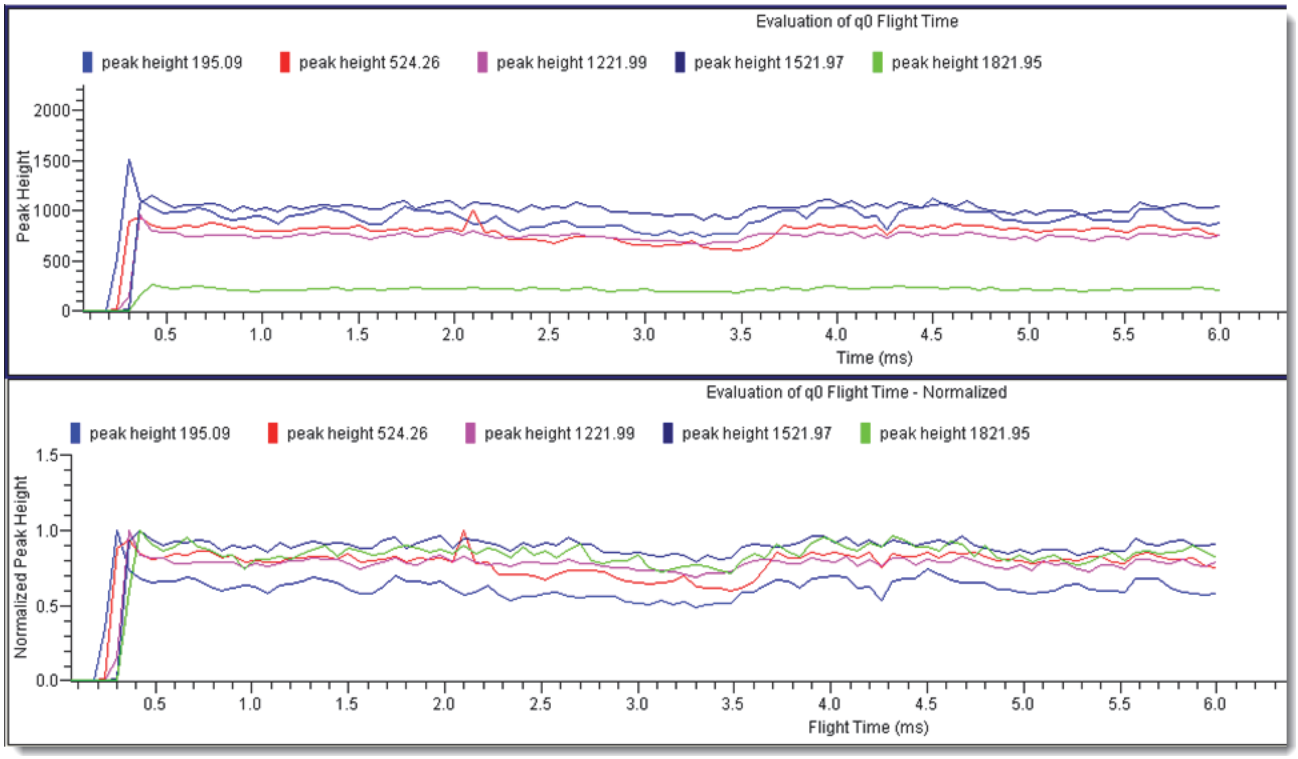
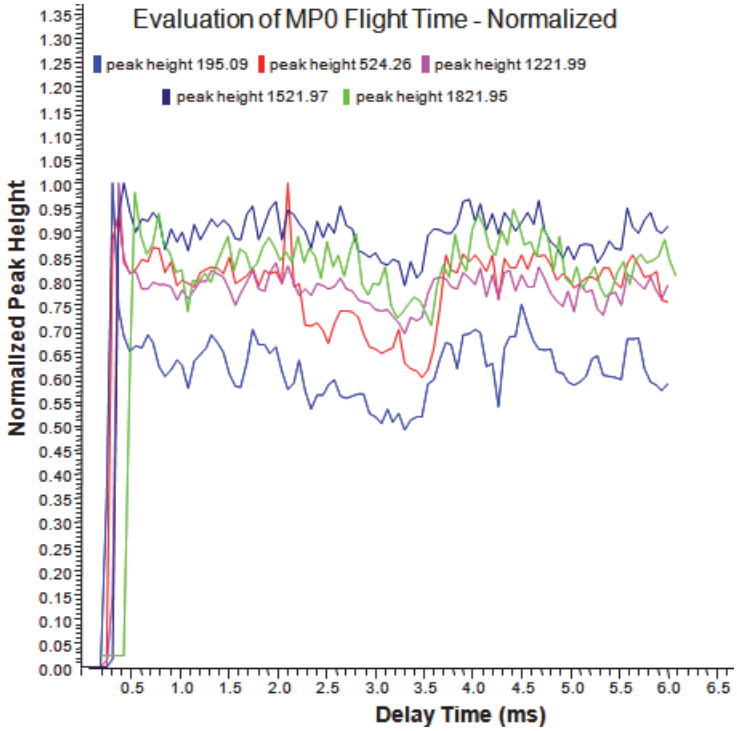


Figure 27. Graph of the multipole MP0 flight time evaluation (Velos Pro, light use)



❖ To run the multipole MP0 flight time evaluation

Note If the instrument is in negative ion mode, this diagnostic test automatically sets the mode to positive before starting. Be aware that changing the polarity can affect any charging conditions such that the results of subsequent diagnostic tests might be affected. Therefore, the diagnostic tests that you conduct after this one might not show the symptoms of charging.

1. Set up the syringe pump to infuse the positive calibration solution.
2. Open the System Evaluation diagnostics page (see page 35), and then select the **Multipole MP0 Flight Time Evaluation** check box.
3. Click **Start**.

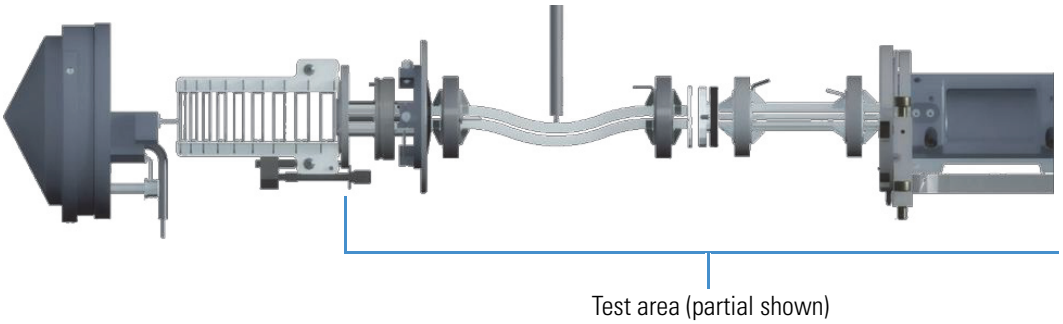
   A message box appears.
4. Click **OK** when you are ready to continue.

   This evaluation generates a real-time graph of the signal intensity at various mass-to-charge ratios as a function of the time delay (Figure 26 on page 44).
5. (Optional) Save a screen capture of the diagnostic test results and the Tune Plus window.
6. Follow the procedure in the next section.

Ion Optics Charging Evaluation

The ion optics charging evaluation is a diagnostic tool that sequentially tests each of the ion optic elements, from the exit lens up to and including the center lens (Figure 28 [center lens is not shown]), to determine if any specific optical elements might be charging and, therefore, need cleaning.

Figure 28. Ion optics showing the test area for the ion optics charging evaluation



This diagnostic evaluation uses the Turbo Scan rate to help speed up the evaluation. At the start of the test, the graph plots the TIC in positive ion mode for 30 seconds, the instrument changes to negative mode and transmits a negative ion beam (100 ms injection time) for 80 seconds to the optical element being tested, and then the instrument changes back to positive mode before again plotting the positive TIC for 30 seconds.

If an optical element has a charge, the top graph in the Tune Plus Graph view shows an intensity spike after the element's exposure to the negative ions. As you review the graph, notice any spikes that indicate which elements you might have to clean. The positive ion-to-negative ion flux ratio appears in the graph because this ratio can affect the results of the evaluation and, therefore, should be taken into account.

A high flux ratio is due to low negative ion flux and a low negative ion flux is due to the following:

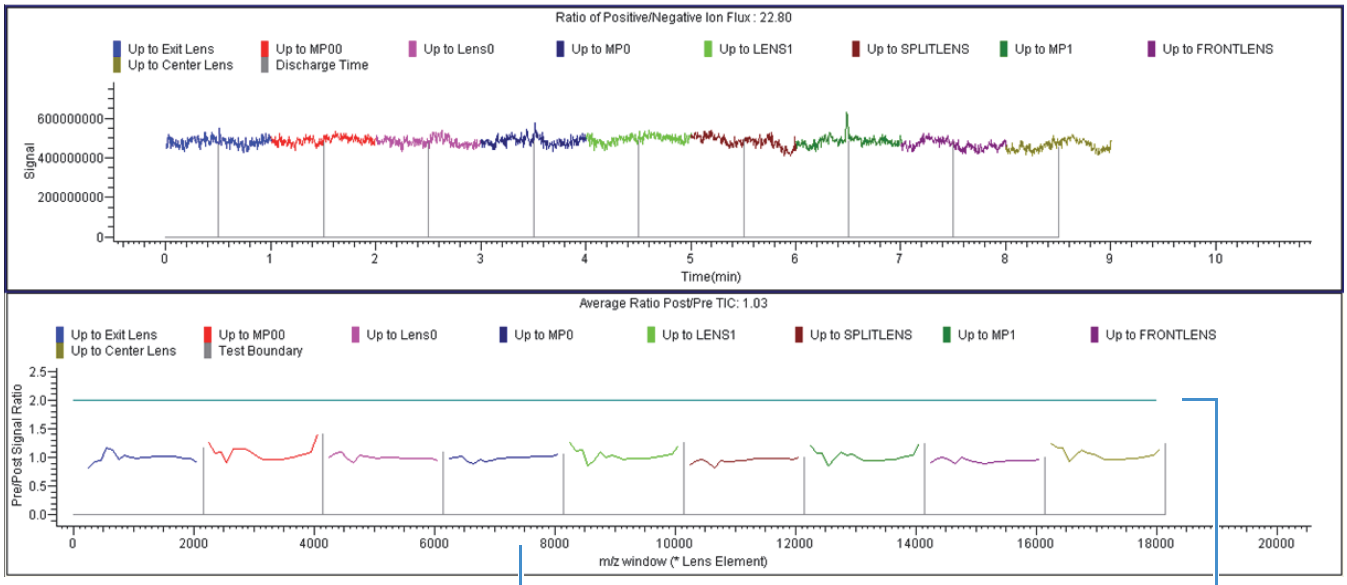
- The multiplier gain and transfer lenses have never been calibrated in negative ion mode.
- The instrument has never been tuned for negative ions.
- The sheath gas is not appropriate for negative ion mode.

The bottom Tune Plus graph shows the ratio of each optical element's signal before and after being exposed to the negative ions as a function of the mass-to-charge ratio. Because charging effects can be very dependent on the mass-to-charge ratio, this graph provides a very sensitive test of any potential optic issues. Signal ratios above the reference threshold of 2.0 indicate significant charging effects and further indicate the need to clean the element.

The following figures display different graphs for this diagnostic test:

- Figure 29—Shows a clean system.
- Figure 30 on page 48—Shows a contaminated MP00 rf lens.
- Figure 31 on page 49—Shows a contaminated MP0.
- Figure 32 on page 50—Shows a contaminated exit lens.

Figure 29. Graphs of the ion optics charging evaluation for a clean system (Velos Pro)



The *x* axis represents the cumulative mass range 0–2000 for each ion optic element.

Threshold set at 2.0

Figure 30. Graphs of the ion optics charging evaluation showing an example of a contaminated MP00 rf lens

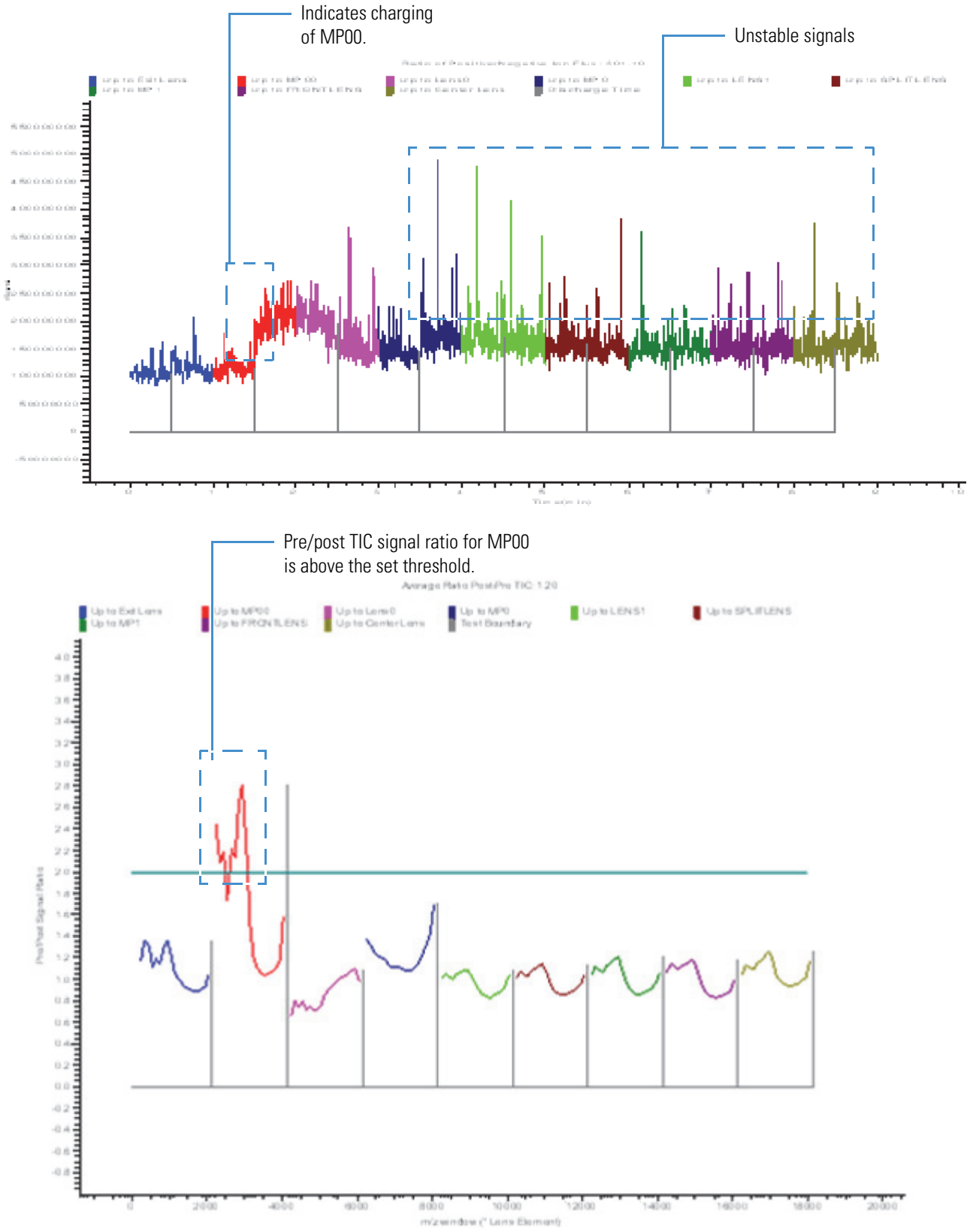


Figure 31. Graphs of the ion optics charging evaluation showing an example of a contaminated MP0

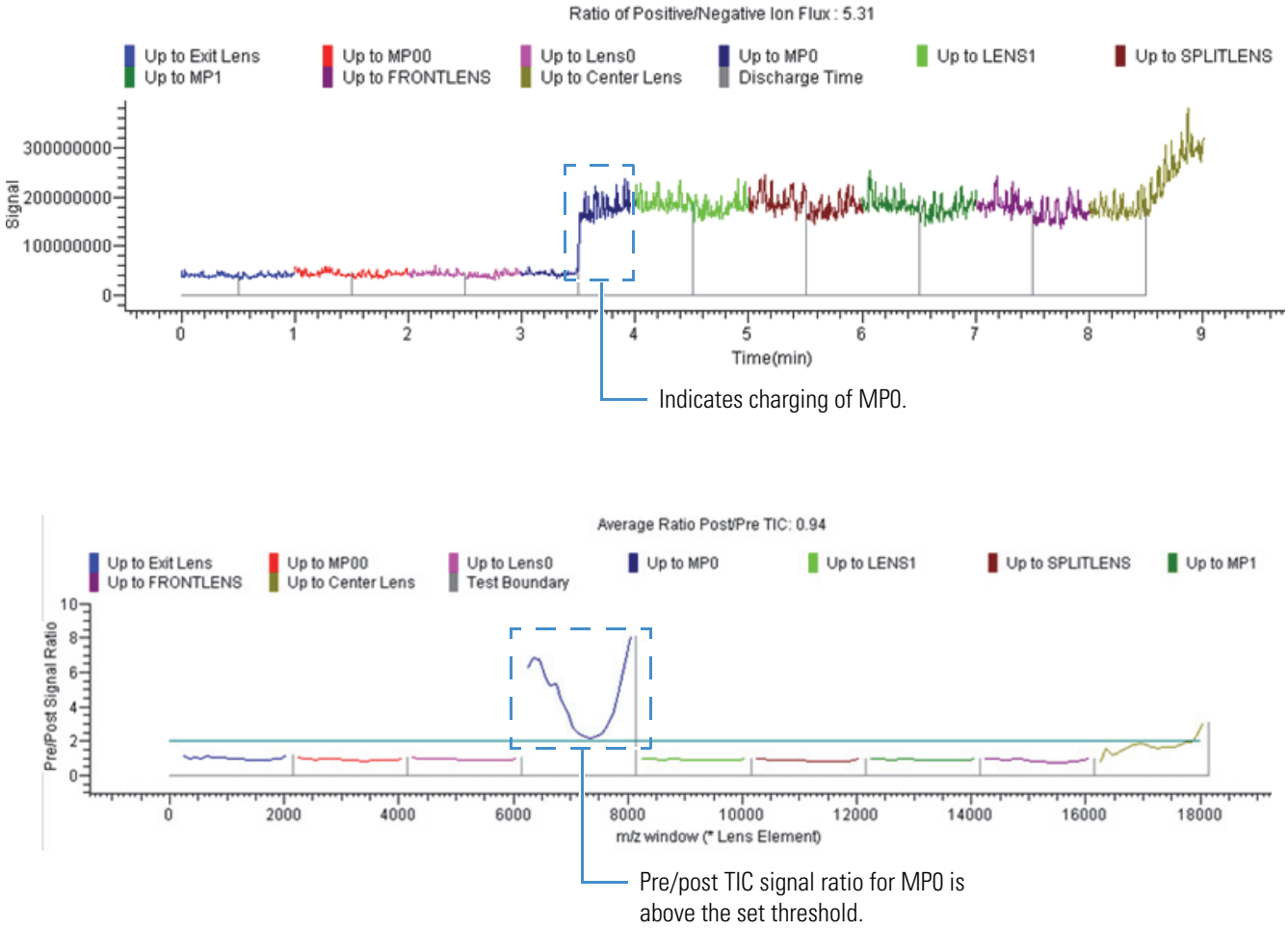
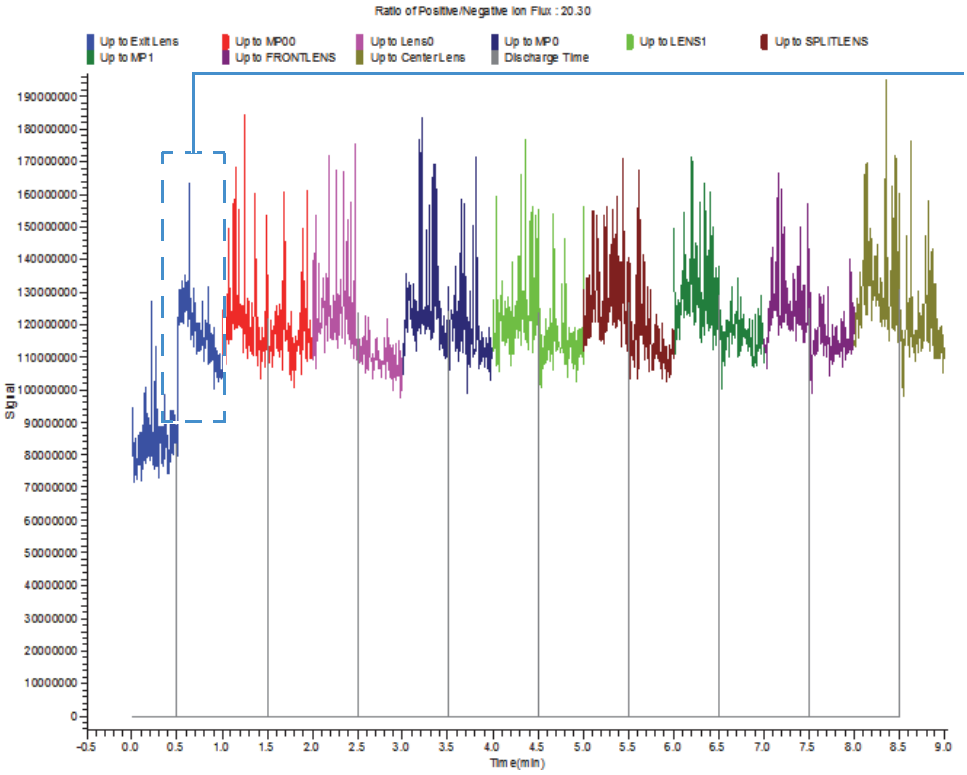
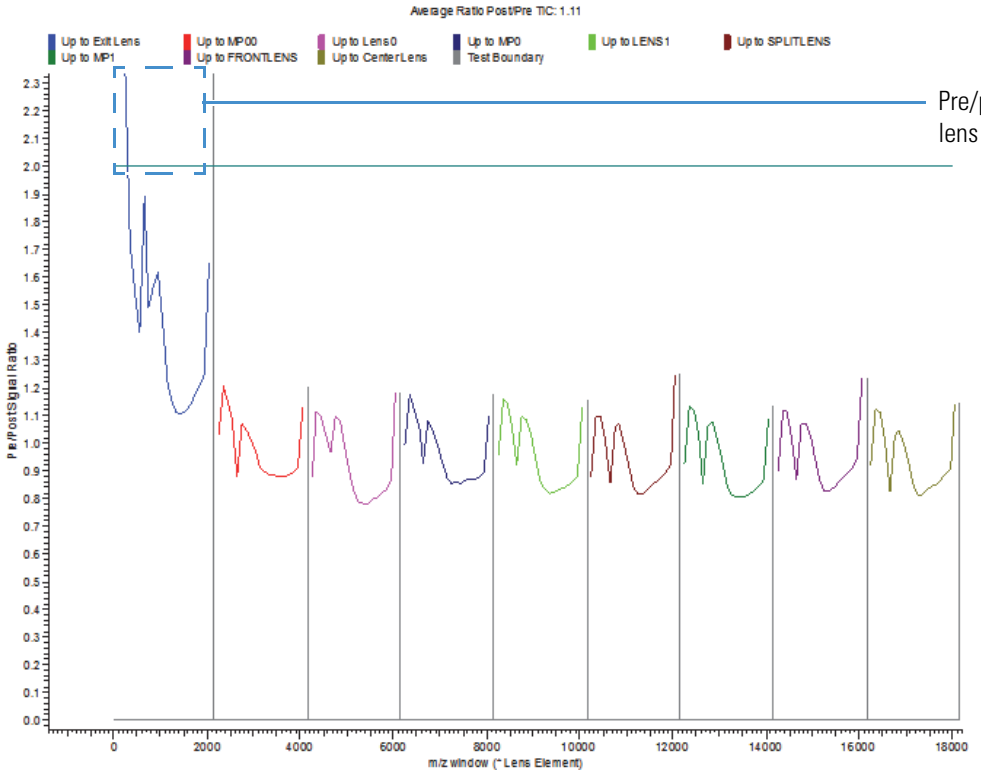


Figure 32. Graphs of the ion optics charging evaluation showing an example of a contaminated exit lens



Indicates charging of the exit lens.



Pre/post TIC signal ratio for the exit lens is above the set threshold.

❖ To run the ion optics charging evaluation

Note If the instrument is in negative ion mode, this diagnostic test automatically sets the mode to positive before starting.

1. Set up the syringe pump to infuse the positive calibration solution.
2. Open the System Evaluation diagnostics page (see page 35), and then select the **Ion Optics Charging Evaluation** check box.
3. Click **Start**.

   A message box appears.
4. Click **OK** when you are ready to continue.

   This evaluation generates two graphs. The top graph is a real-time graph of the total ion current (TIC) before and after the various ion optical elements are exposed to a negative ion beam (see the description on page 45). The bottom graph shows the signal intensity ratio (before and after negative ion exposure) as a function of the mass-to-charge ratio for each ion optics element (Figure 29 on page 47 through Figure 32 on page 50).
5. (Optional) Save a screen capture of the diagnostic test results and the Tune Plus window.
6. Follow the procedure in the next section.

Ejection and Multiplier Gain Ratio

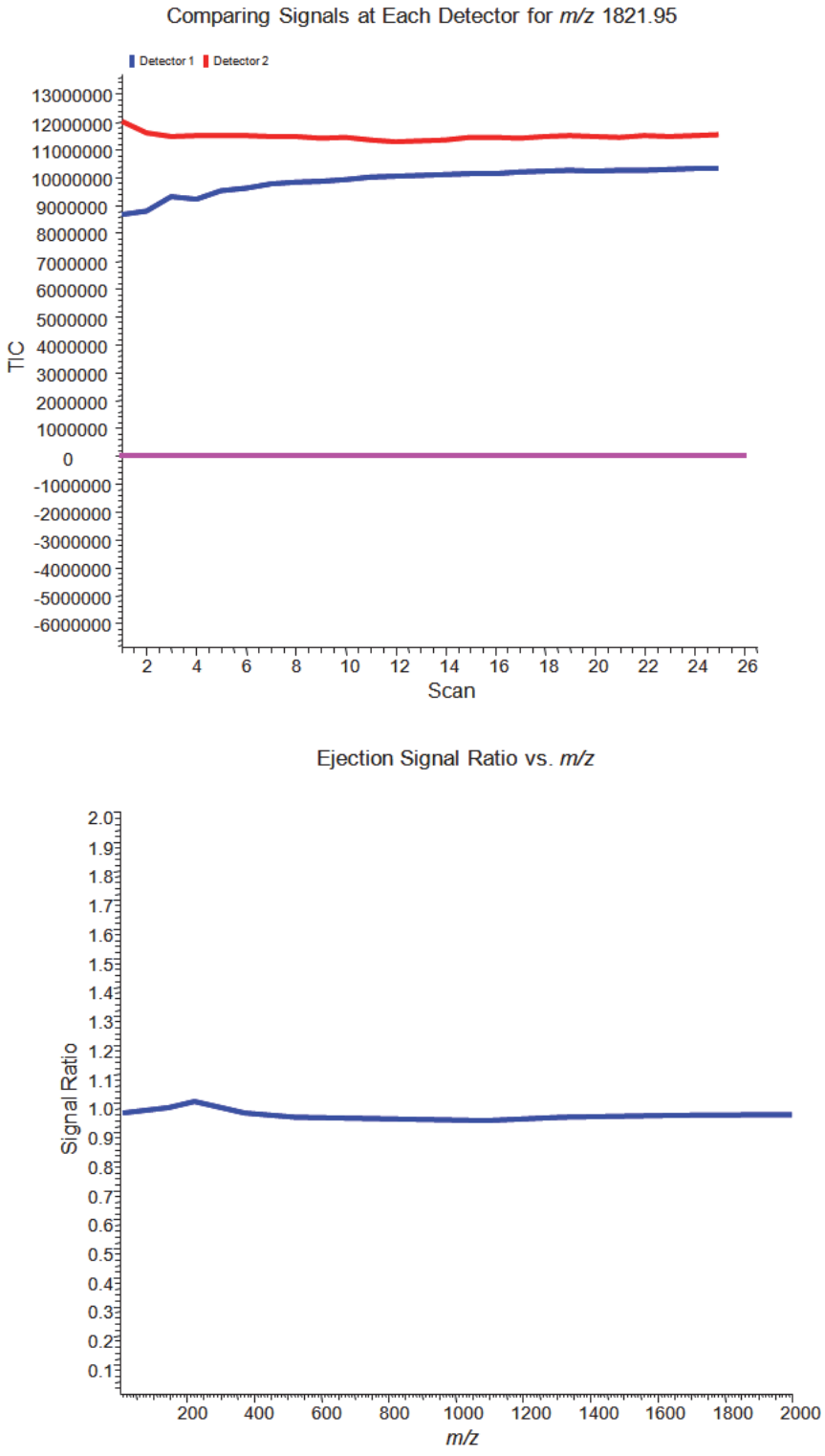
The ejection and multiplier gain ratio is a diagnostic tool that measures the ratio of the signals produced by the ions that are ejected out of one side of the ion trap compared to the other side. This signal ratio can indicate an asymmetry of the ejection slot geometry, a blockage of the slot by foreign material, or an electron multiplier gain mismatch.

This diagnostic evaluation independently measures the signal intensity for various masses on the detectors on both sides of the ion trap, calculates the ratio of the signal, and then generates a real-time graph as a function of the mass-to-charge ratio. The top graph in Figure 33 shows multiple measurements of the signal (*x* axis) to each detector, and the bottom graph shows the signal ratio as a function of the mass-to-charge ratio at nearly 1.0 for all points.

Mismatches in signal are often due to ion trap geometries or contaminated slots, which cause asymmetric ejection even at an optimum resonance ejection phase. To eliminate a gain mismatch as the cause of a signal ratio failure, Thermo Fisher Scientific recommends that you run this diagnostic evaluation after you run the multiplier gain calibration.

Note The ejection and multiplier gain ratio is no longer a part of the multiplier gain calibration check.

Figure 33. Graphs of the ejection and multiplier gain ratio evaluation (Velos Pro)



❖ To run the ejection and multiplier gain ratio

Note This diagnostic procedure runs in either positive or negative ion mode.

1. Set up the syringe pump to infuse the calibration solution.
2. Open the System Evaluation diagnostics page (see page 35), and then select the **Ejection and Multiplier Gain Ratio** check box.
3. Click **Start**.

   A message box appears.
4. Click **OK** when you are ready to continue.

   This evaluation generates a real-time graph (Figure 33 on page 52, lower graph) of the signal intensity ratio as a function of the mass-to-charge ratios.
5. (Optional) Save a screen capture of the diagnostic test results and the Tune Plus window.
6. When you are ready, click **OK** to close the dialog box.

This completes the recommended diagnostic testing. You can now begin to resolve any found issues, as recommended in this manual, and clean components as necessary.

Reporting Unresolved Issues

Be sure to report any tune, calibration, or diagnostic test failures to Thermo Fisher Scientific technical support.

❖ To report unresolved diagnostic issues

1. Take screen captures of the failing results.

   You can use your computer keyboard (PRINT SCREEN for the entire desktop or ALT+PRINT SCREEN for only the active window) or use the Snipping Tool provided with Microsoft Windows 7 and later.
2. Copy all of the text generated in the Status or Testing area of the applicable dialog box.
3. Open a text editor and paste the Tune Plus images and text into a new document.
4. Fully describe the problem, and then save the document.

5. Send an email message to Thermo Fisher Scientific technical support (see page xvii) with the following:

   - Attachments:
     - The text document that contains the screen captures, readback text, and description of the problem. Also state when the problem started and its duration.
     - Any supportive raw data files that demonstrate the problem.
     - The last few LOG files that are stored in the data system computer here:

       C:\Thermo\Instruments\LTQ\system\logs

   - Instrument information:
     - Model name (for example, Velos Pro or Orbitrap Elite)
     - Serial number (If you have an Orbitrap system, provide the serial numbers for both the front and back instruments.)

       The serial number label is located on the back of each instrument. The front instrument has a second label located on the inside chassis behind the front door.
   - Your contact information
   - Your request for service

6

Cleaning the Ion Optics

This chapter describes how to clean the exit lens and S-lens from the API source interface; lens L0 from the MP00 rf lens assembly; and the split gate lens, lens L1, and multipoles MP0 and MP1 from the MP0 and MP1 ion guides.

Contents

Guidelines

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

- Proceed methodically.
- Always wear a new pair of lint- and powder-free gloves when handling internal components. Never reuse gloves after you remove them because the surface contaminants on them recontaminate clean parts.
- Always place the components on a clean, lint-free surface.
- Never overtighten a screw or use excessive force.

IMPORTANT

- Put on a new pair of lint- and powder-free gloves before starting each removal, cleaning, and reinstallation procedure.
- Make sure that you do not introduce any scratches or surface abrasions while handling the ion optic components. Even small scratches can affect performance if they are close to the ion transmission path. Avoid using tools, such as metal pliers, that might scratch these components.

Work Area Preparation

Make sure the surrounding area is neat and clean before preparing the work area.

❖ **To prepare the work area**

Do the following:

- Prepare a clean work surface by covering the area with lint-free paper or a large sheet of clean aluminum foil.
- Have nearby the necessary tools, supplies, and replacement parts (when applicable).

Tools and Supplies

The mass spectrometer requires very few tools to perform routine maintenance procedures. You can remove and disassemble many of the components by hand. Table 4 lists the necessary chemicals, tools, and equipment for maintaining the instrument. (Two of the tools are already in the kits.) In addition, you can use the contents of the Preventive Maintenance (PM) Cleaning Kit (P/N 97455-62051).

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 Sheet (SDS). 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 4. Chemicals, tools, and equipment (Sheet 1 of 2)

Description	Part number
Chemicals	
Acetone (for aluminum foil)	
HPLC grade	Fisher Scientific™ A949
GC Resolv	Fisher Scientific A928-4 (amber glass, 4)
Detergent (for example, Liquinox™)	(Liquinox) Fisher Scientific: • 50-821-299 (1 quart) • 50-821-298 (1 gallon)
Methanol, LC/MS-grade	Fisher Scientific A456-1
Nitrogen gas, clean and dry	–
Water, LC/MS-grade	Fisher Scientific W6-1
Tools	
Fused-silica cutting tool	–
Hex ball driver, 3 mm[a]	00725-00048
Hex driver (or ball driver), 1/4 in.	–

Table 4. Chemicals, tools, and equipment (Sheet 2 of 2)

Description	Part number
Hex ball driver set: 0.050 in., 1/16 in., 5/64 in., 3/32 in., 7/64 in., 1/8 in., 9/64 in., 5/32 in., and 3/16 in.	00025-03025
Ion transfer tube removal tool[b]	70111-20258
Screwdriver, Phillips #2 (M3)	–
Screwdrivers, slotted: large and small	–
Wrenches, open-end: 5/16 in., 3/8 in., and 1/2 in.	–
(Optional) Toothbrush, soft (or similar tool)	–
(Optional) Tweezers, plastic (or similar tool)	–
Equipment	
Aluminum foil, heavy gauge[c]	Fisher Scientific 01-213-104
Beaker or graduated cylinder (for use with methanol)	–
Chamois-tipped swabs	00301-01912
Cotton-tipped applicators	Fisher Scientific A030102000
Gloves, lint-free and power-free	Fisher Scientific 19-120-2947[d] Unity[SM] Lab Services: • 23827-0008 (size medium) • 23827-0009 (size large)
Industrial tissues, lint-free	–
Magnification device	–
MICRO-MESH™ polishing swabs (SWB-01), 6000 grit (light purple color), 2.25 in. long[e]	–
Sonicator	–

[a] Provided in the HESI-II Probe Kit

[b] Provided in the MS Accessory Kit

[c] Rinse each sheet with acetone before use.

[d] Multiple sizes are available.

[e] Provided in the optional PM Cleaning Kit

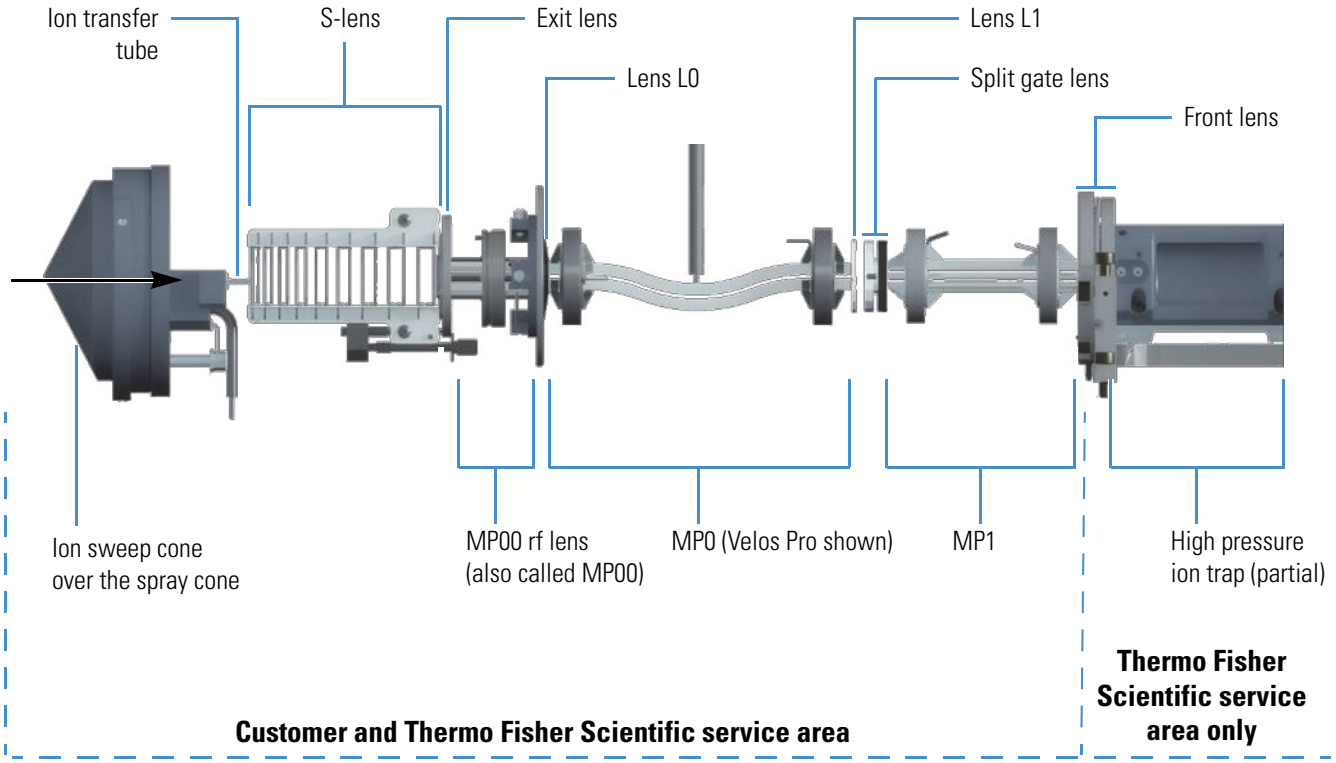
Ion Optics

Use the procedures in this section to clean the S-lens, exit lens, lens L0, lens L1, split gate lens, and multipoles MP0 and MP1. These components are located from the beginning of the ion path up until the front lens, as shown in Figure 34. For instructions about how to remove these components from the LTQ Velos or Velos Pro mass spectrometer, refer to Chapter 5 in the *LTQ Series Hardware Manual*.

Note Before you continue, read the cautions in "Cautions and Special Notices" on page xiv.

CAUTION If the diagnostic results indicate problems with the front lens or ion trap, contact Thermo Fisher Scientific technical support. To prevent accidental damage to the ion trap, do not attempt to service these areas yourself.

Figure 34. Ion path through the Velos Pro ion optics (illustrated side view)



Cleaning the S-Lens, Exit Lens, and Lens L0

When directed by the diagnostic results, follow the procedure in this section to clean the S-lens, exit lens, or lens L0, which are shown in Figure 35 and Figure 36.

For instructions about how to remove these components from the ion source interface, refer to the sections "API Source Interface Maintenance" and "MP00 RF Lens Maintenance" in Chapter 5 of the *LTQ Series Hardware Manual*.

Note Before you continue, read the cautions in "Cautions and Special Notices" on page xiv.

Figure 35. Exit lens and S-lens removed from the API source interface cage

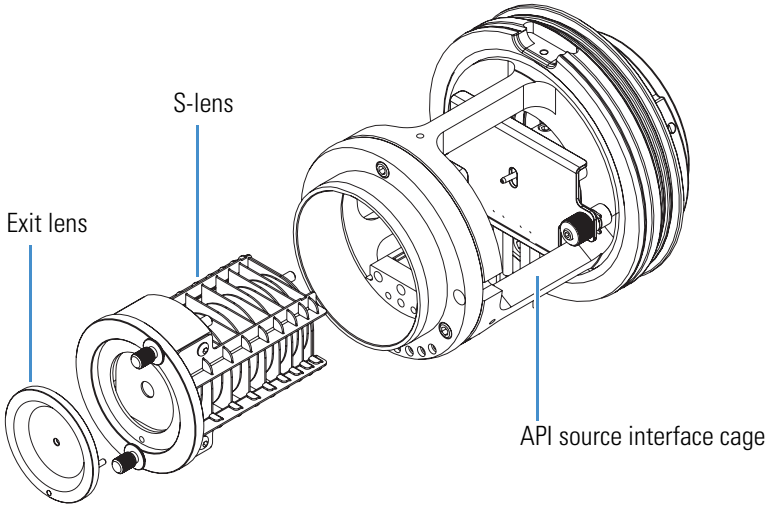
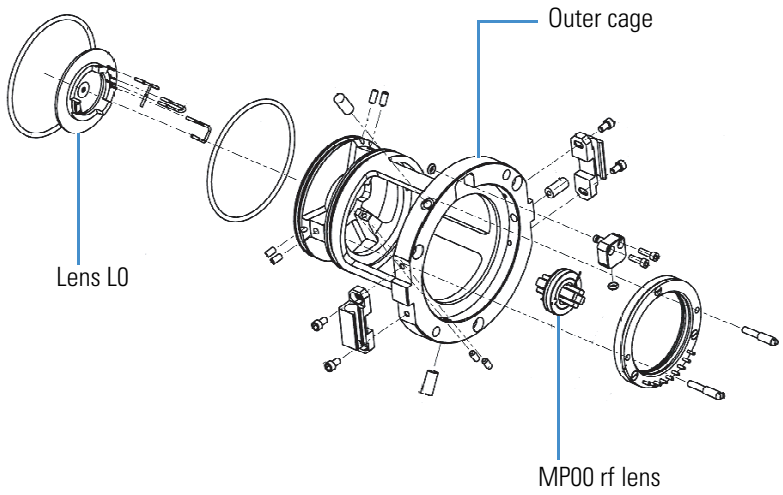


Figure 36. Lens L0 removed from the MP00 rf lens assembly



To remove and clean these components require the following tools and supplies.

Tools	Supplies
Ion transfer tube removal tool	Chamois-tipped swabs
Magnification device	Detergent (for example, Liquinox)
Slotted screwdriver, small	Gloves, nitrile
Sonicator	Industrial tissues, lint-free
(Optional) Soft toothbrush (or similar tool)	Methanol, LC/MS-grade
(Optional) Tweezers, plastic (or similar tool)	Micro-Mesh polishing swab, 6000 grit
(Optional) Wrenches, open-ended, large	Nitrogen gas
–	Water, LC/MS-grade

❖ To clean the S-lens, exit lens, and lens L0

CAUTION Do not clean the lenses with abrasives, acidic or caustic substances, or detergents not stated in this chapter.

IMPORTANT Always use LC/MS-grade methanol and LC/MS-grade water.

1. Using a magnification device, inspect the components for any lint, particulates, and sample buildup or coatings.
2. For 10 minutes, sonicate the components in either a 50:50 solution of methanol/water or a 1% solution of Liquinox in water.
3. If a sonicator is not available, do the following:
   - To clean the S-lens, use chamois-tipped swabs with a 1% solution of Liquinox in water. To clean the areas that you cannot reach with the swab, use the 6000 grit MICRO-MESH polishing swabs.
   - To clean the exit lens, use a soft toothbrush with a 1% solution of Liquinox in water.
4. For the exit lens and lens L0, use the 6000 grit MICRO-MESH polishing swabs to clean the bore.
5. Rinse the components thoroughly with water.
6. Sonicate the components in water for 10 minutes.
7. Sonicate the components in methanol for 10 minutes.
8. Rinse the components with methanol.

9. Dry the components with nitrogen gas to make sure that the solvent evaporates.
10. Using a magnification device, inspect the components for any residual lint or particulates.

Note Inspect the orifices to confirm that no lint or particulates are present in the bore of the orifices. Use plastic tweezers or a similar tool to remove any lint or particulate.

Cleaning the Split Gate Lens, Lens L1, and Multipoles MP0 and MP1

When directed by the diagnostic results, follow the procedures in this section to clean the split gate lens, lens L1, or multipoles MP0 and MP1, which are shown in Figure 37.

For instructions about how to remove these components from the vacuum manifold's top cover plate, refer to the section "MP0 and MP1 Ion Guides Maintenance" in Chapter 5 of the *LTQ Series Hardware Manual*.

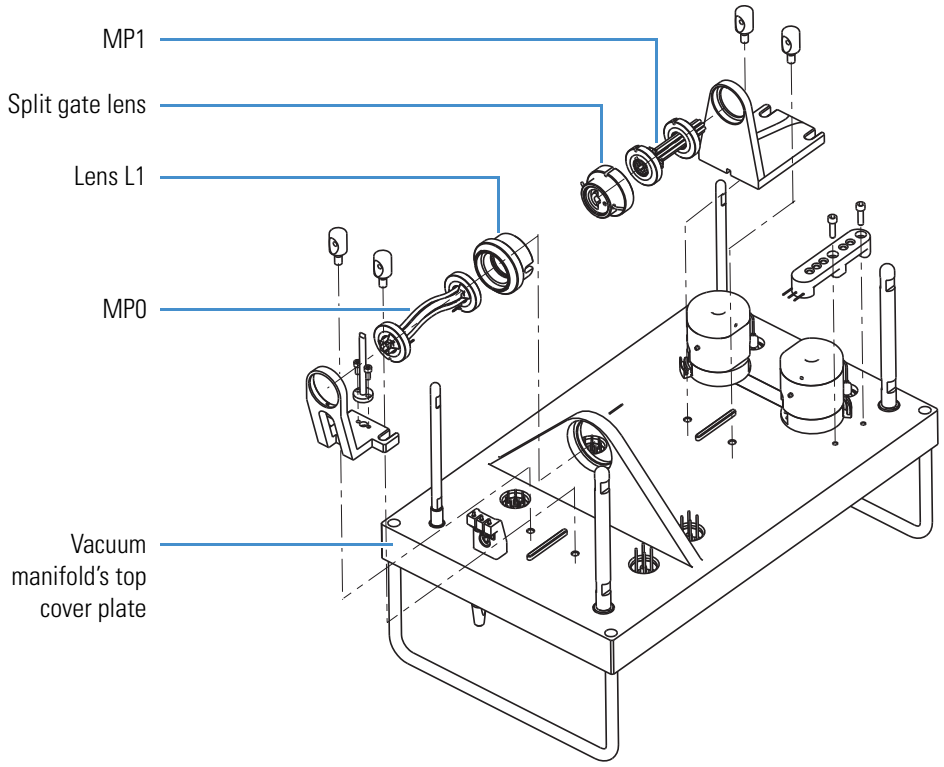
Note Before you continue, read the cautions in "Cautions and Special Notices" on page xiv.

IMPORTANT After you remove the top cover plate from the vacuum manifold, cover the opening with a large, lint-free tissue or a large, clean sheet of aluminum foil to keep the ion trap clean.

Follow these procedures, as applicable:

- To clean multipoles MP0 and MP1
- To clean the split gate lens and lens L1

Figure 37. MP0 and MP1 ion guides for the Velos Pro



To remove and clean these components require the following tools and supplies.

Tools	Supplies
1/4 in. hex driver	Beaker or graduated cylinder
5/64 in. hex ball driver	Chamois-tipped swabs
Magnification device	Detergent (for example, Liquinox)
Phillips screwdriver	Gloves, nitrile
Sonicator	Industrial tissues, lint-free
(Optional) Tweezers, plastic (or similar tool)	Methanol, LC/MS-grade
–	Nitrogen gas
–	Water, LC/MS-grade

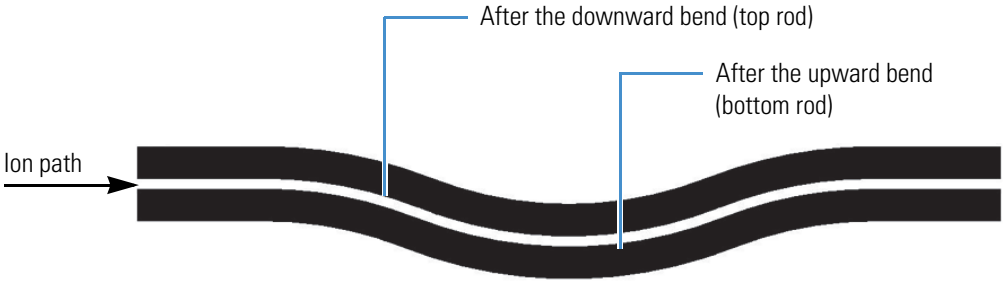
❖ To clean multipoles MP0 and MP1

IMPORTANT Always use LC/MS-grade methanol and LC/MS-grade water.

1. Using a magnification device, inspect the components for any lint, particulates, and sample buildup or coatings.
2. Sonicate the components in a 1% solution of Liquinox in water for 10 minutes.
3. Soak chamois-tipped swabs in a 1% solution of Liquinox in water, and then clean the components.

   Because multipole MP0 has a bent design, the areas of ion collisions on the inner surface of the rods are more concentrated after the multipole changes direction. When cleaning MP0, spend extra time at the areas shown in Figure 38.

   Figure 38. Common contamination areas on the inner surface areas of MP0 (Velos Pro)



4. Rinse the components thoroughly with water.
5. Sonicate the components in water for 10 minutes.
6. Sonicate the components in methanol for 10 minutes.
7. Soak chamois-tipped swabs in methanol, and then clean the components.
8. Rinse the components with methanol.
9. Dry the components with nitrogen gas to make sure that all the solvent evaporates.
10. Using a magnification device, inspect the components for any residual lint or particulates.

Note Inspect the inside surfaces and edges to confirm that no lint or particulates are present. Use plastic tweezers or a similar tool to remove the lint or particulate.

❖ To clean the split gate lens and lens L1

IMPORTANT Always use LC/MS-grade methanol and LC/MS-grade water.

1. Using a magnification device, inspect the components for any lint, particulates, and sample buildup or coatings.

   Note After use, the surfaces can be discolored, which is normal and not to be confused with sample buildup or coatings.

2. Soak lint-free tissues or chamois-tipped swabs in a 50:50 solution of methanol/water, and then clean the components.
3. Sonicate the components in methanol for 10 minutes.

   Note If using buffers or salt solutions in the mass spectrometer, you might need to use an aqueous solution for cleaning. If using an aqueous solution, flush the items with LC/MS-grade water and then with LC/MS-grade methanol.

4. Dry the components with nitrogen gas to make sure that all the solvent evaporates.
5. Using a magnification device, inspect the components for any residual lint or particulates.

   Note Inspect the inside surfaces and edges to confirm that no lint or particulates are present. Use plastic tweezers or a similar tool to remove the lint or particulate.

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Orbitrap Systems

This chapter provides additional information for achieving peak performance from the hybrid Orbitrap systems.

Contents

Additional Calibrations for the Orbitrap System

In addition to running the calibration checks for the Electron Multiplier Gain and Transfer Lenses, you should run two or three more semi-automatic calibrations, depending on which Orbitrap model you have (see Table 5 on page 69).

IMPORTANT Run these calibrations once per week as applicable for your model.

- pAGC Scaling
- Mass Calibration
- Advanced Signal Processing

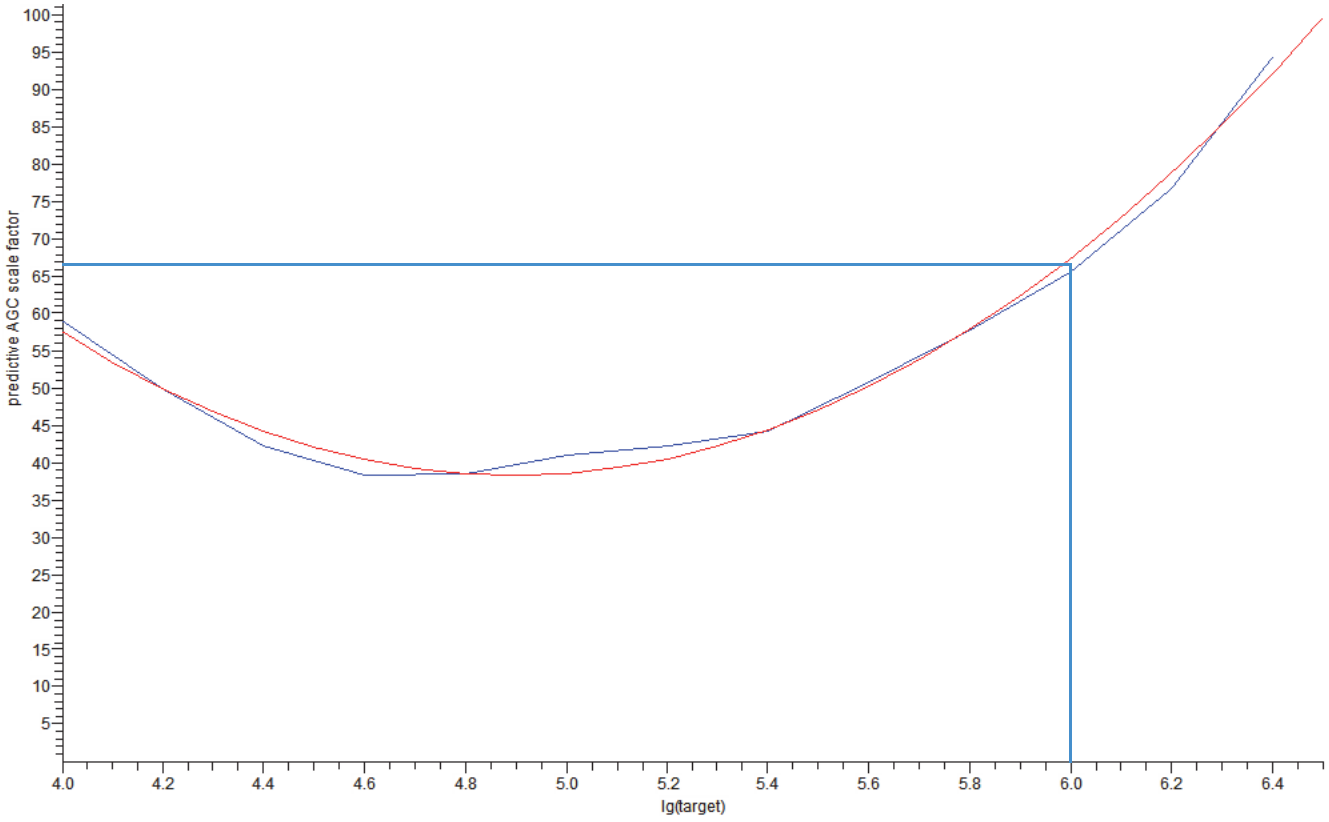
pAGC Scaling

The predictive Automatic Gain Control (pAGC) predicts the injection time for precursor ions based on its relative signal and the injection time of the previous full MS scan. The pAGC scaling calibration generates a real-time graph (Figure 39) of the pAGC scaling factor versus the base 10 logarithm of the target value, written as "lg(target)." The graph starts from a target of 1×10^4 and ends with a target of 5×10^6, which scales the abundance (ion trap [IT])/abundance (Fourier transform [FT]). (The red curve in the figure is a smoothed version of the blue curve.)

Note the following about the pAGC scaling calibration:

- It eliminates time spent with a prescan execution.
- The injection time for data-dependent scans is predicted based on the abundance of the precursor ions in the master scan, which can be an IT or FT full MS scan.
- Before you run LC-MS/MS experiments by using pAGC, you must scale the FT abundance.
- The scaling value at lg(6) (target is 1×10^6) should be in the range of 60 to 100.

Figure 39. Graph: pAGC scale factor versus lg(target)



Mass Calibration

The mass calibration performs a mass calibration of the Orbitrap mass analyzer.

Advanced Signal Processing

The Orbitrap Elite system includes Advanced Signal Processing (ASiP). ASiP uses the two types of information provided by the Fourier transformations: the magnitude and the phase component. The Orbitrap Elite with the ASiP calibration increases the resolving power by a factor of approximately 2. The LTQ Orbitrap Velos and Orbitrap Velos Pro systems do not use the phase component; instead, they use only the magnitude information.

To use phase information to enhance resolution, all of the ions must have the same phase, which occurs when the ions are injected into the hybrid Orbitrap. Therefore, the synchronization of the injection and detection of ions is the critical step. With the Orbitrap Elite, the injection of the ions from the C-trap into the Orbitrap mass analyzer and the start of the transient recording is synchronized.

The start time of the transient recording is very close to zero but not exactly. This inaccuracy is in the range of tens of nanoseconds and comes from, for example, a time-of-flight effect during injection and delays in the electronics. Because the exact start time is unknown, the ASiP calibration extrapolates backwards to time zero and then gives you the estimated start time and initial phase.

Calibrating the Orbitrap System

Note Follow this procedure once a week.

❖ To calibrate the Orbitrap system

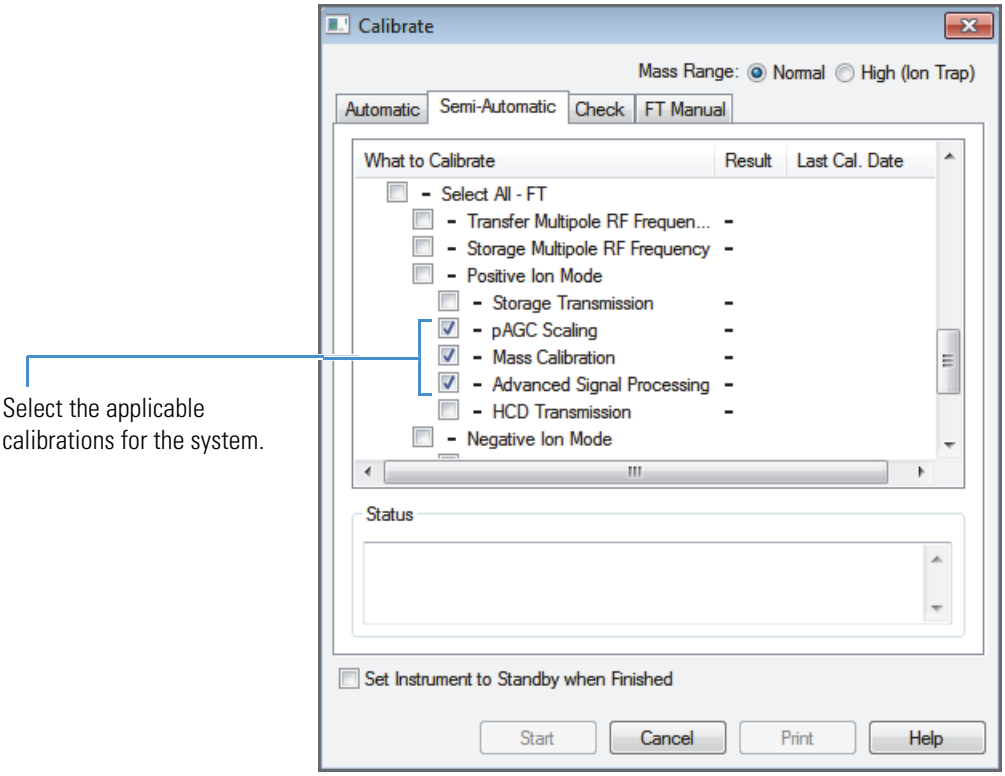
1. Set up the syringe pump to infuse the calibration solution.
2.  In the Tune Plus window, click the **Calibrate** icon to open the Calibrate dialog box.
3. For the Mass Range, select the **Normal** option.
4. Click the **Semi-Automatic** tab, and then under the appropriate ion mode select the applicable check boxes for your model as listed in Table 5.

   Figure 40 lists the applicable calibrations for the various models. For the Orbitrap Elite system, when you select the Mass Calibration check box, the Advanced Signal Processing check box is automatically selected.

Table 5. Semi-automatic calibrations for the hybrid Orbitrap systems

Semi-automatic calibration	LTQ Orbitrap Velos and Orbitrap Velos Pro	Orbitrap Elite
pAGC Scaling	✓	✓
Mass Calibration	✓	✓
Advanced Signal Processing		✓

Figure 40. Calibrate dialog box showing the Semi-Automatic page (Orbitrap systems)



5. Click **Start**.

   The following message appears: Please ensure that the syringe pump is full.

6. Click **OK**.

   The calibration starts. When it is completed, review the Result column and the bottom status information. See the note on page 12 for an explanation of the Result column.

7. If the calibration fails, repeat this calibration procedure.

   If the problem persists, contact Thermo Fisher Scientific technical support for assistance.

Checking and Improving the Mass Accuracy

This section provides a few tips for checking and improving the mass accuracy of an Orbitrap spectrum.

- Chemical Background Ions
- Scatter Plot in Thermo Proteome Discoverer
- Lock Masses and Lock Mass Abundance

Chemical Background Ions

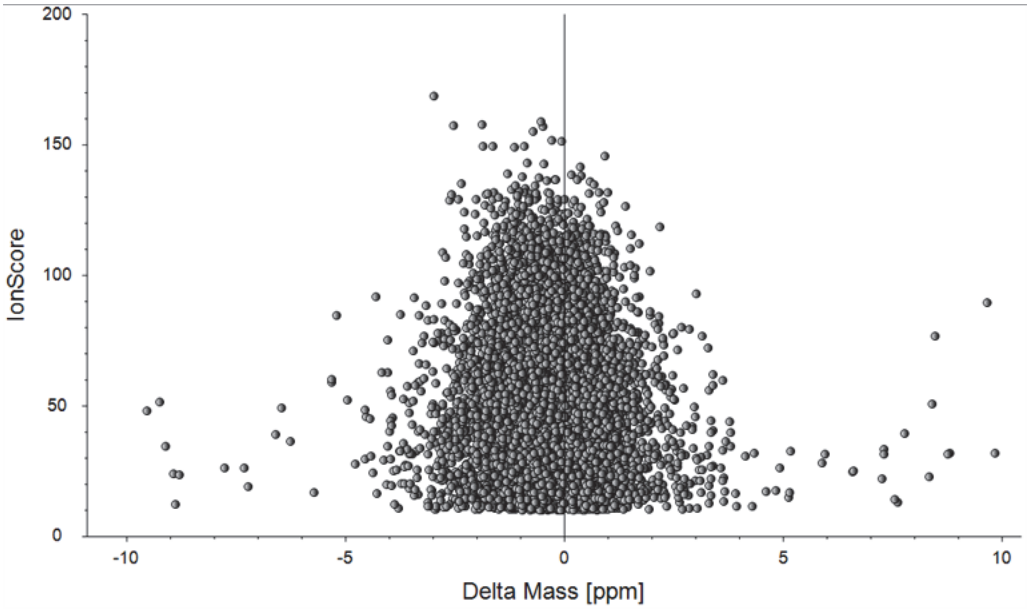
Check the mass accuracy of the known chemical background ions. If their mass accuracy is acceptable, the mass accuracy of the analyte ions are also acceptable.

Scatter Plot in Thermo Proteome Discoverer

If you are using the Thermo Scientific Proteome Discoverer™ application for peptide and protein mass spectrometry analyses, open and display the Report Item Distribution chart as a scatter plot. For instructions, refer to "Displaying the Report Item Distribution Chart as a Scatter Plot" in the Proteome Discoverer Help.

The average mass accuracy in the example plot shown in Figure 41 is –0.19 ppm with a standard deviation of 0.81 ppm. In this example, the plot shows both very high accuracy and precision due to the on-the-fly recalibration by using the lock mass option. In general, if the scatter plot points are significantly off-center to either side, run an FT mass calibration.

Figure 41. Report Item Distribution chart's scatter plot[1] (with lock mass correction)



[1] Generated with the Mascot™ search engine and the Proteome Discoverer application

Lock Masses and Lock Mass Abundance

Thermo Fisher Scientific recommends that you use a lock mass to improve mass accuracy and precision.

- Lock Masses
- FT Lock Mass Abundance

Lock Masses

You can use chemical background ions as lock masses in the spectrum for the mass spectrometer to use as references for internal mass calibration. When you specify lock masses in the Lock Masses dialog box, you improve both the mass accuracy and precision of the mass measurements. If you do not specify any lock masses, the instrument uses the external mass calibration. Therefore, regardless of whether you specify lock masses, you must also externally calibrate the instrument.

When there are analytes with high abundant signal intensities, the lock mass ions can be suppressed and, therefore, not present in the spectrum. If the instrument does not find the lock mass in one FTMS full scan, it applies the correction from a previous scan where the lock mass was found. The system then keeps this correction until the lock mass is found again in the spectrum. Using the correction is advisable because the slow drift of the Orbitrap electronics causes the drift of the mass accuracy of the Orbitrap mass analyzer. For example, at the beginning of a liquid chromatography (LC) run, background ions such as *m/z* 445 (polysiloxane) are present, which the instrument can use as the lock mass.

When you set the lock mass abundance to a value greater than zero percent, the lock mass is artificially mixed into the spectrum.

- If no lock masses are found in the full spectrum, the instrument tries to improve the abundance of the lock mass by performing additional SIM injections of the specified lock mass.
- If the given lock mass cannot be found in the spectrum because the instrument runs in MS^n mode or as a SIM scan type, the instrument adds the lock mass by using SIM injections.

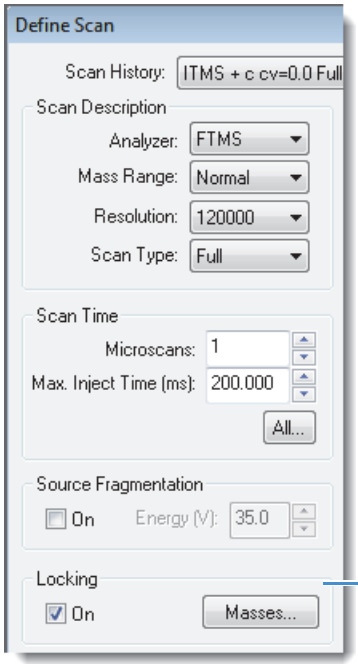
In either situation, you can use lock masses for all FTMS scan types and varying lock mass abundances. All that is required is enabling mass locking and specifying the list of reference mass-to-charge ratios.

❖ To define a scan with mass locking

1. In the Tune Plus window, click the **Define Scan** icon to open the Define Scan dialog box.
2. Under Scan Description, in the Analyzer list, select **FTMS**.
3. Under Locking, select the **On** check box (Figure 42).

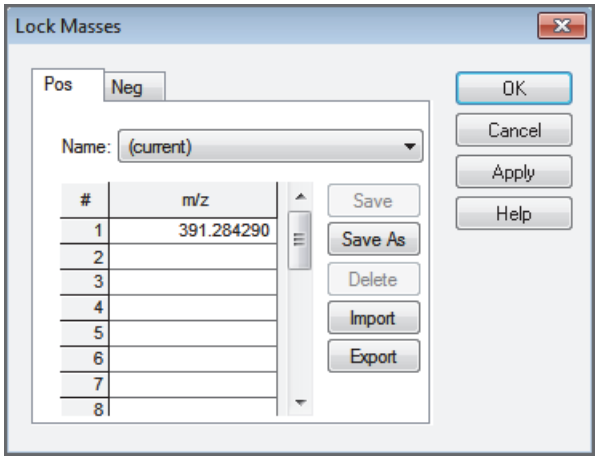
   The locking feature is available with the FTMS analyzer only.

Figure 42. Define Scan dialog box (Orbitrap Elite, partial)



4. Click **Masses** to open the Lock Masses dialog box (Figure 43).

Figure 43. Lock Masses dialog box



5. Enter one or more masses on the Pos or Neg page (as appropriate for the experiment), and then click **OK**.
6. In the Define Scan dialog box, set the remaining parameters to define the scan.

   For additional information, refer to the getting started guide for your Orbitrap system.
7. Click **OK**.

FT Lock Mass Abundance

You can find the setting for the FT Lockmass Abundance on the Set Device page under Tools in the Diagnostics dialog box (Figure 44). For methods with FTMS selected as the detector of the fragment ions (Figure 42 on page 73) and with the lock mass abundance set to zero percent, the system uses the lock mass correction from the full Orbitrap FTMS scan.

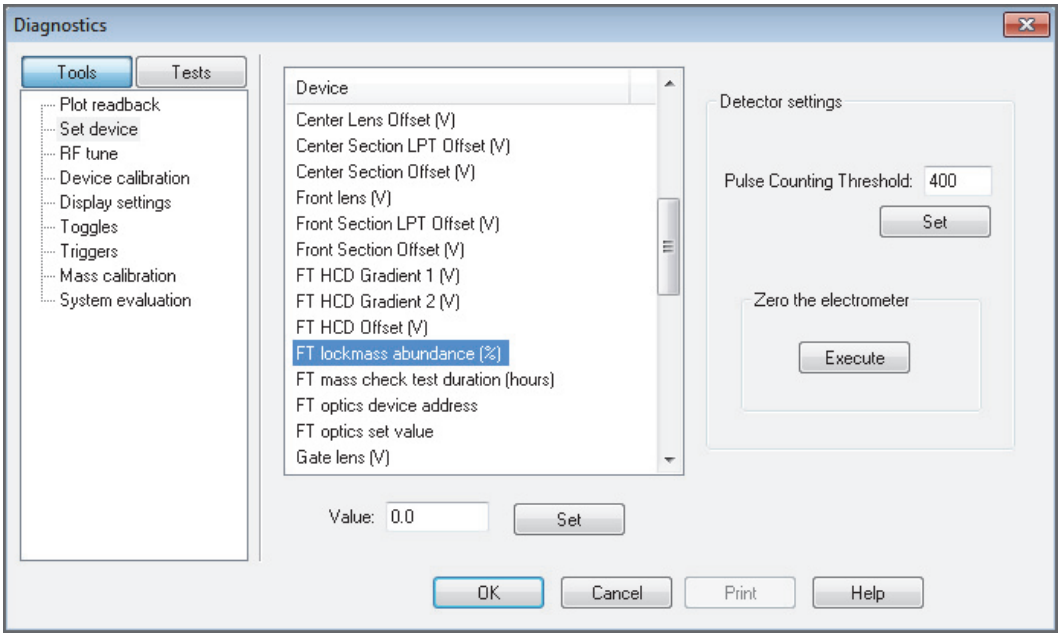
When you set the lock mass abundance to greater than zero percent, the instrument performs additional SIM scans to increase the signal of the lock mass ions. For LTQ 2.7 and later, the default lock mass abundance value is zero percent.

Tip For best results, Thermo Fisher Scientific recommends that you use the lock mass abundance value of zero percent.

❖ To set the FT lock mass abundance

1. In the Tune Plus window, choose **Diagnostics > Diagnostics**, click **Tools**, and then select **Set Device**.
2. Select **FT Lockmass Abundance (%)** (Figure 44).

Figure 44. Diagnostics dialog box showing the Set Device page



3. Make sure that the Value box is set to **0**, and then click **Set**.

   The default lock mass abundance value is zero percent.

4. Click **OK**.

Troubleshooting

Table 6 lists some problems that might occur with the mass spectrometer and their possible solutions.

Table 6. Troubleshooting solutions (Sheet 1 of 3)

Problem	Possible solutions
Complete loss of signal	• In the Tune Plus application, check the Status View for any faults. If necessary, run the appropriate diagnostics tests. • Make sure that the ion transfer tube is not restricted; clean or replace it if needed. • Check the source spray conditions by using direct infusion of the calibration solution. • Open a different tune file because the current tune file might have become corrupt (see page 16). • Press the reset button on the mass spectrometer (refer to Chapter 3 in the *LTQ Series Hardware Manual*). • If the problem persists, check the LC system.
Oscillating signal intensity	• Make sure that the spray is stable (see "Maintaining Spray Stability" on page 5). • If the problem persists, do the following: – Run the Transfer Efficiency Evaluation diagnostic. – If the evaluation fails, run the Transfer Lenses calibration for the appropriate ion mode. – If the calibration consistently fails, contact Thermo Fisher Scientific technical support for assistance. See "Reporting Unresolved Issues" on page 53.

Table 6. Troubleshooting solutions (Sheet 2 of 3)

Problem	Possible solutions
Loss of signal intensity	• Make sure that you pumped down the instrument for at least 15 continuous hours since the last time you vented the instrument. Failure to do so might cause incorrect calibration. • Make sure that the ion transfer tube is not restricted; clean or replace it if needed. • Check the tune. Make sure that the MP0–MP00 gradient is at least –5.5 V. • Check the signal by using direct infusion of the calibration solution. • Check the electron multiplier gain calibration. • Run the following charging diagnostics[a] (see page 34), and then clean the ion optics as directed by the results: a. Multipole Gradient Evaluation b. Source Optics Flight Time Evaluation c. Multipole MP0 Flight Time Evaluation d. Ion Optics Charging Evaluation (run last) • If the problem persists, check the LC system.
Failure of the electron multiplier gain calibration—the signal is too weak	• Make sure that the spray is stable (see "Maintaining Spray Stability" on page 5). • Make sure that the calibration mixture (calmix) is fresh and that you have the correct solution for either the stand-alone or Orbitrap instrument (www.thermo.com/pierce). – Stand-alone: Pierce LTQ Velos ESI Positive Ion Calibration Solution (P/N 88323) – Orbitrap: Pierce ESI Negative Ion Calibration Solution (P/N 88324) • Manually set the multiplier gain to a higher voltage through the Set Device page on the Diagnostics dialog box, and then repeat the calibration. • If the gain is above 2500 V, contact Thermo Fisher Scientific technical support to replace the electron multipliers.

Table 6. Troubleshooting solutions (Sheet 3 of 3)

Problem	Possible solutions
Failure of the ejection and multiplier gain evaluation	• Make sure that the spray is stable (see "Maintaining Spray Stability" on page 5). • Repeat the electron multiplier gain calibration. • If the problem persists, contact Thermo Fisher Scientific technical support for assistance.
Failure of the transfer lenses calibration	• Repeat the transfer lenses calibration in the mode that failed. • If the problem persists, contact Thermo Fisher Scientific technical support for assistance.

[a] Available with the LTQ application, 2.7 SP1 or later

A

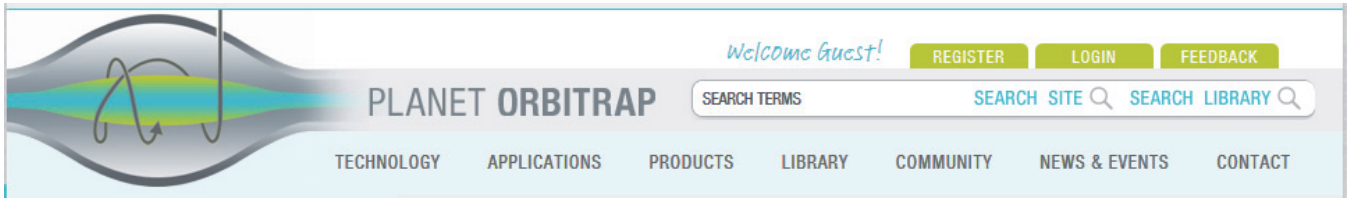
Online Resources

This appendix provides additional online resources.

Planet Orbitrap

Visit the Thermo Scientific website dedicated to Orbitrap systems:

PlanetOrbitrap.com



Online Product Information

Item		Website
API sources, various	Ion Max	Thermo Scientific
	EASY-Spray	Planet Orbitrap and Thermo Scientific
	Nanospray Flex	Thermo Scientific
	Nanospray	Thermo Scientific
Consumables	Fisher Scientific, Chemicals and Bioreagents	
	Unity™ Lab Services	

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