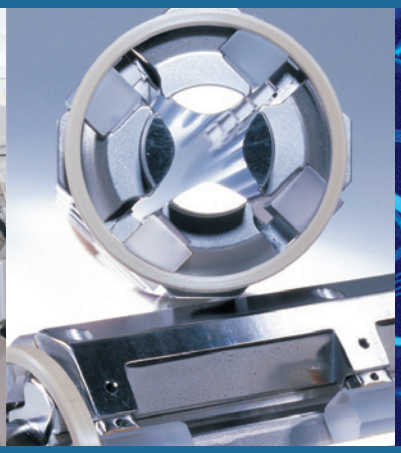
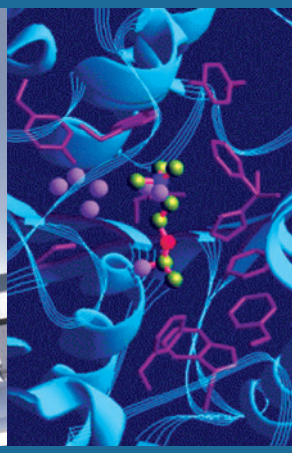
Thermo Fisher Scientific

Orbitrap Velos Pro

Getting Started
Tune Plus 2.7

Revision A - 1288300





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Software Version: Xcalibur 2.1 or higher, Tune Plus 2.7

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

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Read This First

Welcome to the Thermo Scientific Orbitrap Velos Pro system! The Orbitrap Velos Pro mass spectrometer is a member of the family of LTQ™ mass spectrometer (MS) detectors.

About This Guide

This *Orbitrap Velos Pro Getting Started* manual provides information on how to set up, calibrate, and tune the Orbitrap Velos Pro mass spectrometer. Procedures in Chapters 1–4 can be performed from the Xcalibur™ Tune Plus window.

Who Uses This Guide

This *Orbitrap Velos Pro Getting Started* manual is intended for all personnel that need to operate the Orbitrap Velos Pro mass spectrometer, especially the key operator. This manual should be kept near the instrument to be available for quick reference.

Scope of This Guide

Orbitrap Velos Pro Getting Started includes the following chapters:

- Chapter 1: "Introduction" provides general information about this manual.
- Chapter 2: "Tune Plus Window" provides information on the Tune Plus window.
- Chapter 3: "Calibrating the Instrument for FTMS Measurements" provides procedures to calibrate the Orbitrap Velos Pro mass spectrometer for FTMS measurements.
- Chapter 4: "Performing Diagnostics/Checks" describes several diagnostic procedures.
- Chapter 5: "Instrument Setup" describes the FTMS relevant topics of the data dependent settings in the Instrument Setup.
- Chapter 6: "Instrument Configuration" gives instructions about configuring the instrument.

- Chapter 7: "Orbitrap Velos Pro ETD Instruments"describes the Orbitrap analyzer relevant differences in instrument settings and procedures with respect to using a Orbitrap Velos Pro ETD instrument.
- Appendix A: "Miscellaneous Information" gives additional information about various topics.

Related Documentation

In addition to this guide, Thermo Fisher Scientific provides the following documents for Orbitrap Velos Pro and Orbitrap Velos Pro ETD mass spectrometers:

- *LTQ Orbitrap Series Preinstallation Requirements Guide*
- *Orbitrap Velos Pro Hardware Manual*
- Velos Pro manual set

You can access PDF files of the documents listed above and of this guide from the data system computer. The software also provides Help.

❖ **To view product manuals**

1. From the Microsoft™ Windows™ taskbar, choose **Start > Programs > Thermo Instruments > Manuals > *model***.
2. Click the PDF file that you want to view.

Contacting Us

There are several ways to contact Thermo Fisher Scientific.

Assistance

For technical support and ordering information, **visit us on the Web**:

www.thermoscientific.com/ms

Service contact details for customers in Europe are available under:

www.thermoscientific.com/euservicecontact

Customer Information Service

cis.thermo-bremen.com is the Customer Information Service site aimed at providing instant access to

- Latest software updates
- Manuals, application reports, and brochures

Thermo Fisher Scientific recommends that you register with the site as early as possible. To register, visit register.thermo-bremen.com/form/cis and fill in the registration form. Once your registration has been finalized, you will receive confirmation by e-mail.

Changes to the Manual

❖ **To suggest changes to this manual**

- Please send your comments (in German or English) to:

  Editors, Technical Documentation
  Thermo Fisher Scientific (Bremen) GmbH
  Hanna-Kunath-Str. 11

  28199 Bremen

  Germany

- Send an e-mail message to the Technical Editor at

  documentation.bremen@thermofisher.com

You are encouraged to report errors or omissions in the text or index. Thank you.

Typographical Conventions

This section describes typographical conventions that have been established for Thermo Fisher Scientific manuals.

Data Input

Throughout this manual, the following conventions indicate data input and output via the computer:

- Messages displayed on the screen are represented by capitalizing the initial letter of each word and by italicizing each word.
- Input that you enter by keyboard is identified by quotation marks: single quotes for single characters, double quotes for strings.
- For brevity, expressions such as "choose **File > Directories**" are used rather than "pull down the File menu and choose Directories."
- Any command enclosed in angle brackets < > represents a single keystroke. For example, "press **<F1>**" means press the key labeled *F1*.
- Any command that requires pressing two or more keys simultaneously is shown with a plus sign connecting the keys. For example, "press **<Shift> + <F1>**" means press and hold the <Shift> key and then press the <F1> key.
- Any button that you click on the screen is represented in bold face letters. For example, "click **Close**".

Topic Headings

The following headings are used to show the organization of topics within a chapter:

Chapter 1 Chapter Name

Second Level Topics

Third Level Topics

Fourth Level Topics

Safety and EMC Information

In accordance with our commitment to customer service and safety, these instruments have satisfied the requirements for the European CE Mark including the Low Voltage Directive.

Designed, processed, and tested in an ISO9001 registered facility, this instrument has been shipped to you from our manufacturing facility in a safe condition.

This instrument must be used as described in this manual. Any use of this instrument in a manner other than described here may result in instrument damage and/or operator injury.

Notice on Lifting and Handling of Thermo Scientific Instruments

For your safety, and in compliance with international regulations, the physical handling of this Thermo Scientific instrument *requires a team effort* for lifting and/or moving 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: If this instrument is used in a manner not specified by Thermo Fisher Scientific, the protection provided by the instrument could be impaired.

Notice on the Susceptibility to Electromagnetic Transmissions

Your instrument is designed to work in a controlled electromagnetic environment. Do not use radio frequency transmitters, such as mobile phones, in close proximity to the instrument.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear different from the main flow of text. Safety and special notices include the following:

Warning Warnings highlight hazards to human beings. Each Warning is accompanied by a Warning symbol. ▲

Caution Cautions highlight information necessary to protect your instrument from damage. ▲

Note Notes highlight information that can affect the quality of your data. In addition, notes often contain information that you might need if you are having trouble. ▲

Identifying Safety Information

This guide contains precautionary statements that can prevent personal injury, instrument damage, and loss of data if properly followed. Warning symbols alert the user to check for hazardous conditions. These appear throughout the manual, where applicable. The most common warning symbols that appear in Thermo Fisher Scientific manuals are shown below.

In addition, every instrument has specific hazards. So, be sure to read and comply with all precautions described in this guide. They will help ensure the safe, long-term use of your system.

Warning General Hazard. This general symbol indicates that a hazard is present that could result in injuries if it is not avoided. The source of danger is described in the accompanying text. ▲

Warning Electric Shock Hazard. High Voltages capable of causing personal injury are used in the instrument. The instrument must be shut down and disconnected from line power before service is performed. Do not operate the instrument with the top cover off. Do not remove protective covers from PCBs. ▲

Warning Burn Hazard. Treat heated zones with respect. Parts of the instrument might be very hot and might cause severe burns if touched. Allow hot components to cool before servicing them. ▲

Warning Corrosive Material. Wear gloves when handling toxic, carcinogenic, mutagenic, or corrosive/irritant chemicals. Use approved containers and procedures for disposal of waste solution. ▲

General Safety Precautions

Observe the following safety precautions when you operate or perform service on your instrument:

- The system should be operated by trained personnel only. Read the manuals before starting the system and make sure that you are familiar to the warnings and safety precautions!
- Accurate results can be obtained only, if the system is in good condition and properly calibrated.

- Service by the customer should be performed by trained qualified personnel only and should be restricted to servicing mechanical parts! Service on electronic parts should be performed by Thermo Fisher Scientific field service engineers only!
- Before plugging in any of the instrument modules or turning on the power, always make sure that the voltage and fuses are set appropriately for your local line voltage.
- Only use fuses of the type and current rating specified. Do not use repaired fuses and do not short-circuit the fuse holder.
- The supplied power cord must be inserted into a power outlet with a protective earth contact (ground). When using an extension cord, make sure that the cord also has an earth contact.
- Do not change the external or internal grounding connections. Tampering with or disconnecting these connections could endanger you and/or damage the system.
- The instrument is properly grounded in accordance with regulations when shipped. You do not need to make any changes to the electrical connections or to the instrument's chassis to ensure safe operation.
- Never run the system without the housing on. Permanent damage can occur. When leaving the system, make sure that all protective covers and doors are properly connected and closed, and that heated areas are separated and marked to protect for unqualified personnel!
- Do not turn the instrument on if you suspect that it has incurred any kind of electrical damage. Instead, disconnect the power cord and contact a Thermo Fisher Scientific field service engineer for a product evaluation. Do not attempt to use the instrument until it has been evaluated. (Electrical damage may have occurred if the system shows visible signs of damage, or has been transported under severe stress.)
- Damage can also result if the instrument is stored for prolonged periods under unfavorable conditions (for example, subjected to heat, water, etc.).
- Always disconnect the power cord before attempting any type of maintenance.
- Capacitors inside the instrument may still be charged even if the instrument is turned off.
- Never try to repair or replace any component of the system that is not described in this manual without the assistance of your Thermo Fisher Scientific field service engineer.

- Do not place any objects upon the instrument—especially not containers with liquids—unless it is requested by the user documentation. Leaking liquids might get into contact with electronic components and cause a short circuit.

Safety Advice for Possible Contamination

Hazardous Material Might Contaminate Certain Parts of Your System During Analysis.

In order to protect our employees, we ask you to adhere to special precautions when returning parts for exchange or repair.

If hazardous materials have contaminated mass spectrometer parts, Thermo Fisher Scientific can only accept these parts for repair if they have been properly decontaminated. Materials that due to their structure and the applied concentration might be toxic or that are reported in publications to be toxic are regarded as hazardous. Materials that will generate synergetic hazardous effects in combination with other present materials are also considered hazardous.

Your signature on the Health and Safety Form confirms that the returned parts have been decontaminated and are free of hazardous materials. Download the form from decon.thermo-bremen.com or order it from the Thermo Fisher Scientific field service engineer.

Parts contaminated by radioisotopes should not be returned to Thermo Fisher Scientific—neither under warranty nor within the exchange part program. If unsure about parts of the system possibly being contaminated by hazardous material, please make sure the Thermo Fisher Scientific field service engineer is informed before the engineer starts working on the system.

Contents

Figures

Tables

Procedures

Chapter 1 Introduction

This guide describes only the FTMS detector relevant settings and procedures of the Orbitrap Velos Pro software (Tune Plus 2.7). For ion trap relevant settings and procedures, refer to the *LTQ Series Getting Started* manual.

In addition to this guide, the Tune Plus Help gives information to specific topics. Nevertheless, it is recommended to read this manual entirely.

Chapter 2 Tune Plus Window

This chapter provides Orbitrap Velos Pro mass spectrometer specific information about the Tune Plus window. It contains the following topics:

Preliminary Remarks

The Tune Plus window shows the schematic view of the Orbitrap Velos Pro mass spectrometer and the instrument name. See Figure 2-1.

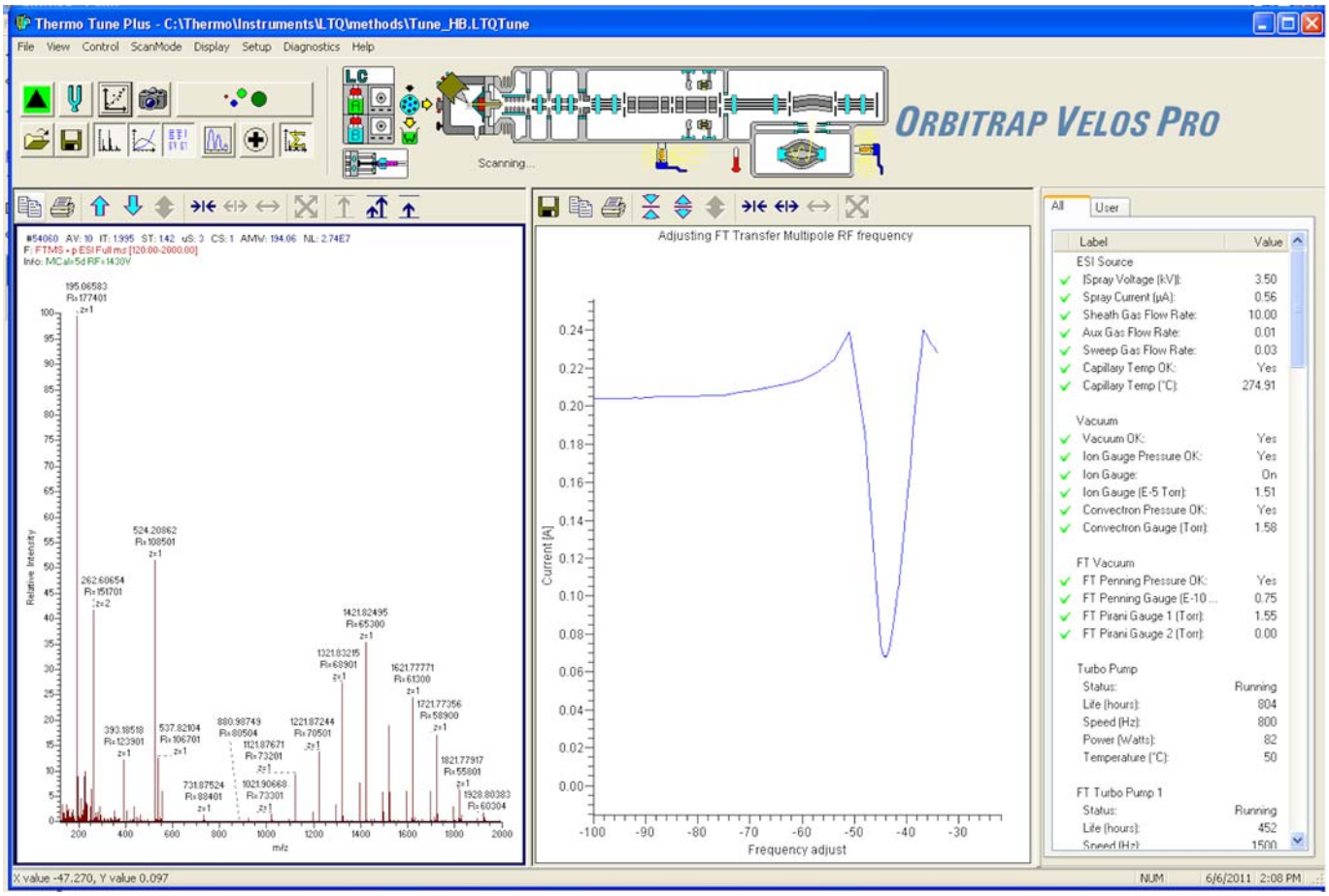


Figure 2-1. Tune Plus window

To access the functions of the Tune Plus window, use the menu commands, toolbar buttons, and display views. The FT relevant changes or additions of the menu commands, toolbar buttons, and display views are explained in the following chapters.

View Menu

This section describes those elements of the View menu that are different from the Velos Pro version of the Tune Plus window.

Spectrum View

The Spectrum view displays real-time ion trap or FT mass spectra depending on the analyzer type selected in the Define Scan dialog box. See Figure 2-2.

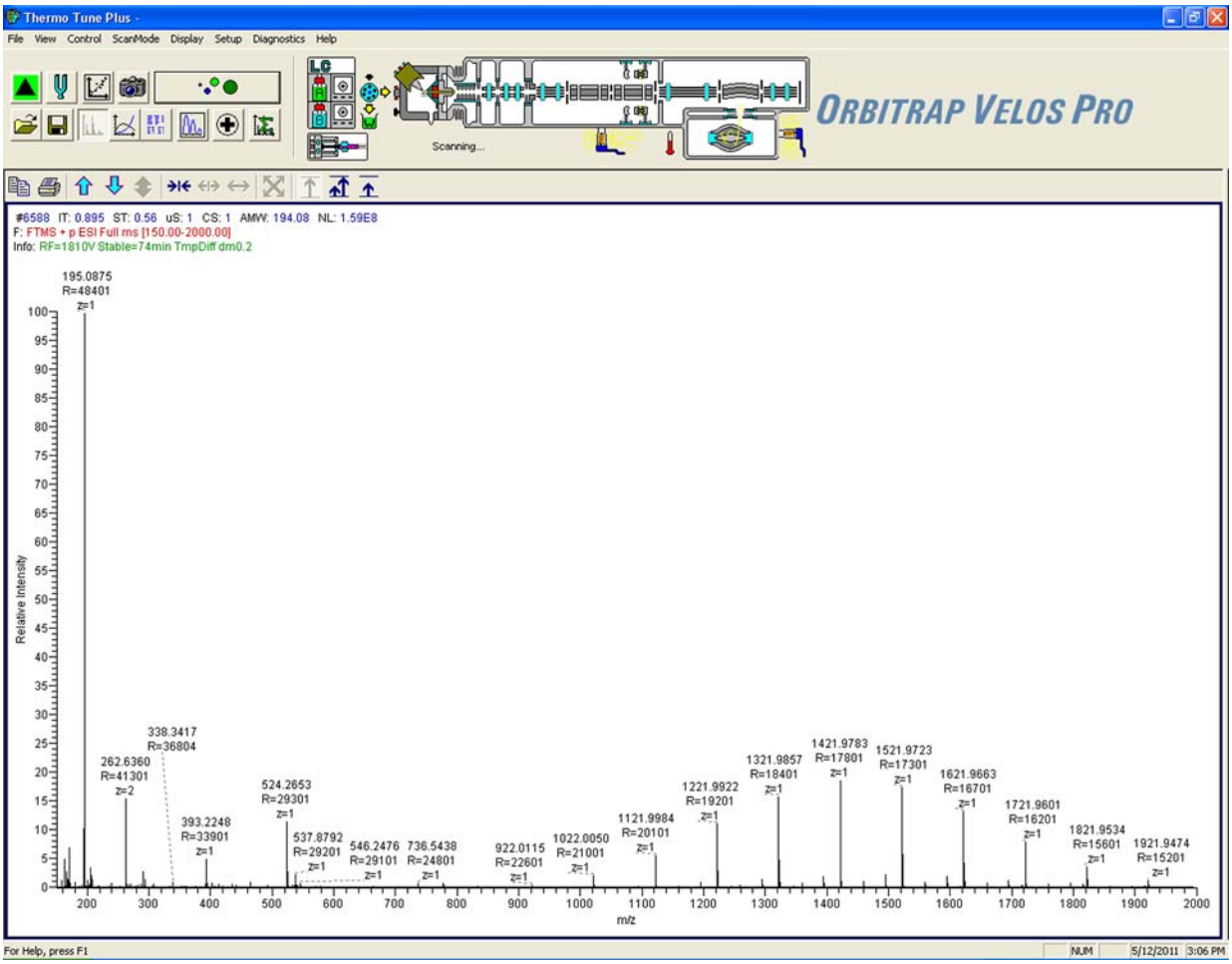


Figure 2-2. Spectrum View page

The Spectrum view page has a shortcut menu, which is displayed when you right-click anywhere on the page. To open the Spectrum Display Options dialog box, choose **Display Options**. The dialog box has two pages: the Ion Trap page and the FT page.

Use the FT page to determine the number of decimals shown on peak labels. See Figure 2-3 on page 2-4. To change the number of decimals, click the arrows in the spin box to increment [up arrow] or decrement [down arrow] the value. You can set the number of decimals to any value

from 0 to 5. Alternatively, you can enter a value in the spin box text field. The Orbitrap Velos Pro mass spectrometer changes the number of decimals when you click **Apply** or **OK**.

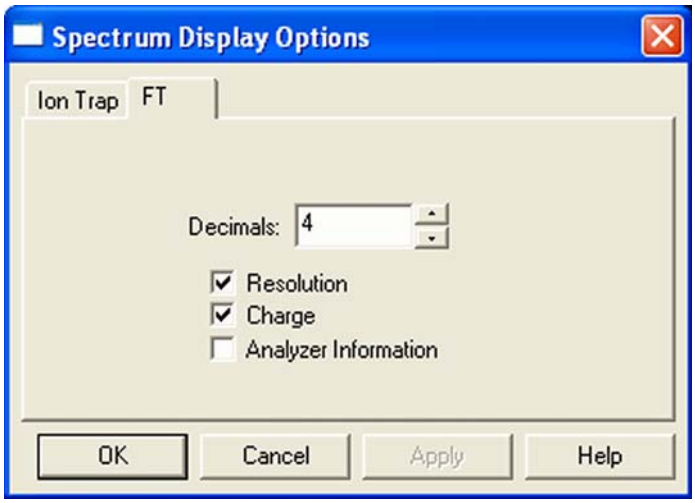


Figure 2-3. Spectrum Display Options dialog box—FT page

A check box allows showing additional analyzer information for FTMS scans. This information will be displayed above the spectrum graph if the box is selected. See "FT Analyzer Messages" on page A-3 for a list of items that may be displayed as analyzer information.

You can also decide whether or not to show the resolution and/or the charge state of peaks in the FT spectrum by clearing or selecting the corresponding check boxes.

If the FTMS analyzer is used, it is possible to display different diagnostic views in the Spectrum view. See Chapter 4: "Performing Diagnostics/Checks" for diagnostic features that involve the Spectrum view.

Graph View

The Graph view displays, in a variety of traces, real-time data generated during calibration, tuning, and diagnostic tests. For example, the right side of Figure 2-4 shows the progress of the transfer efficiency evaluation.

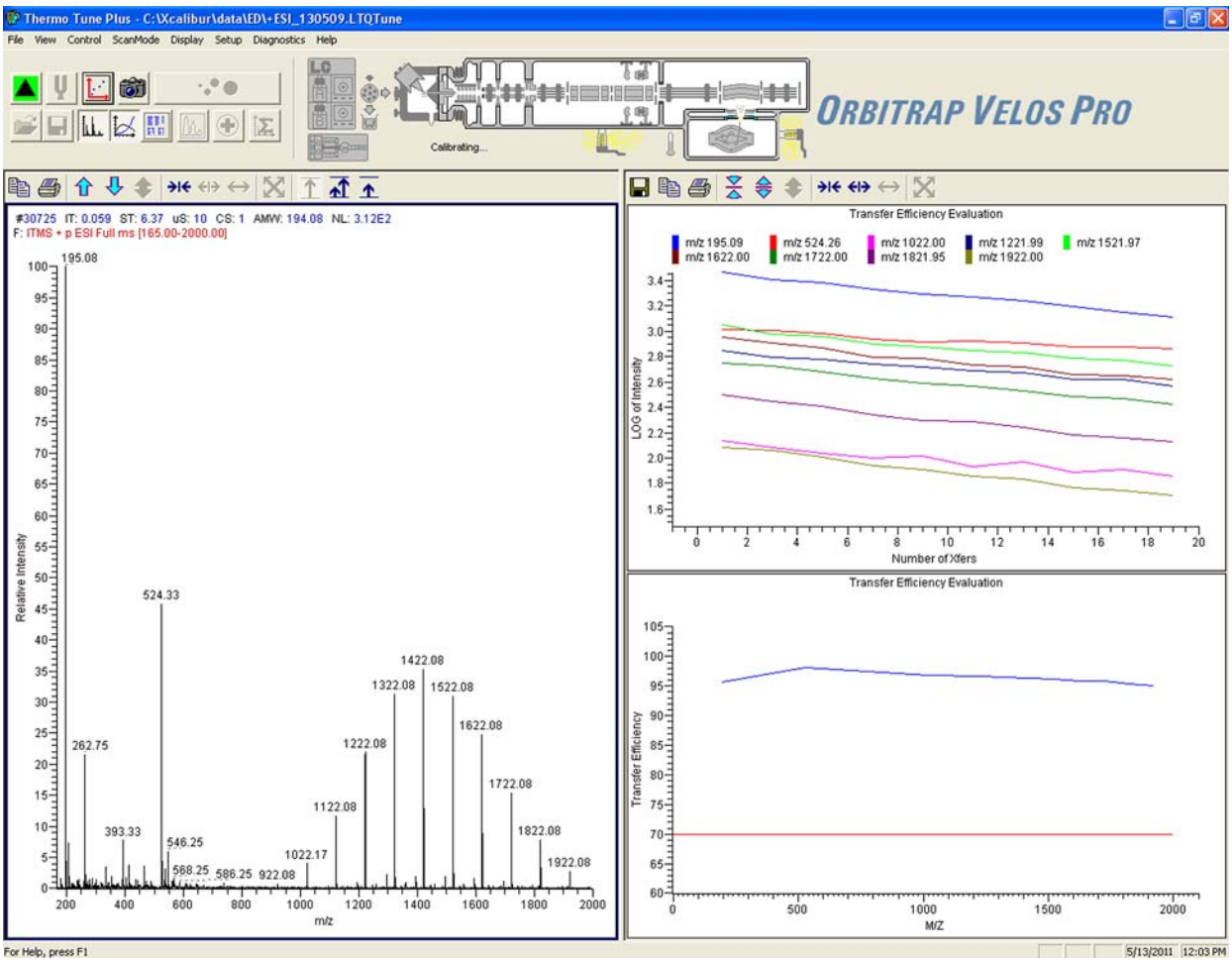


Figure 2-4. Graph view page

Status View

The Status view displays real-time status information for the Orbitrap Velos Pro mass spectrometer. See Figure 2-5 on page 2-6. The Status view has two pages: the All page and the User page. The All page displays the real-time status information for about 80 parameters of the Orbitrap Velos Pro mass spectrometer. You can scroll through the list to observe the status of the parameters. The User page displays real-time status information for Orbitrap Velos Pro mass spectrometer parameters that you have selected in the User Status Display Configuration dialog box. (See page 2-6.)

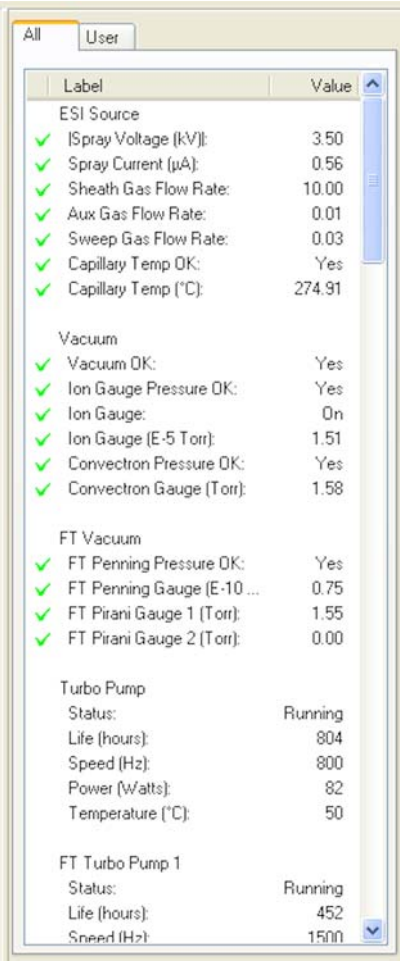


Figure 2-5. Status view—All page

User Status Display Configuration Dialog Box

Figure 2-6 on page 2-7 shows the User Status Display Configuration dialog box.

❖ **To configure the User page**

1. Choose **View > Display Status View**.
2. Click the User tab. Right-click the User page to display the shortcut menu.
3. Choose **Configure**. The User Status Display Configuration dialog box is displayed. See Figure 2-6.
4. Select the check boxes that represent the status parameters you want to have displayed on the User page.
5. Click **OK** to close the dialog box.

   The User page displays real-time status information for the selected parameters.

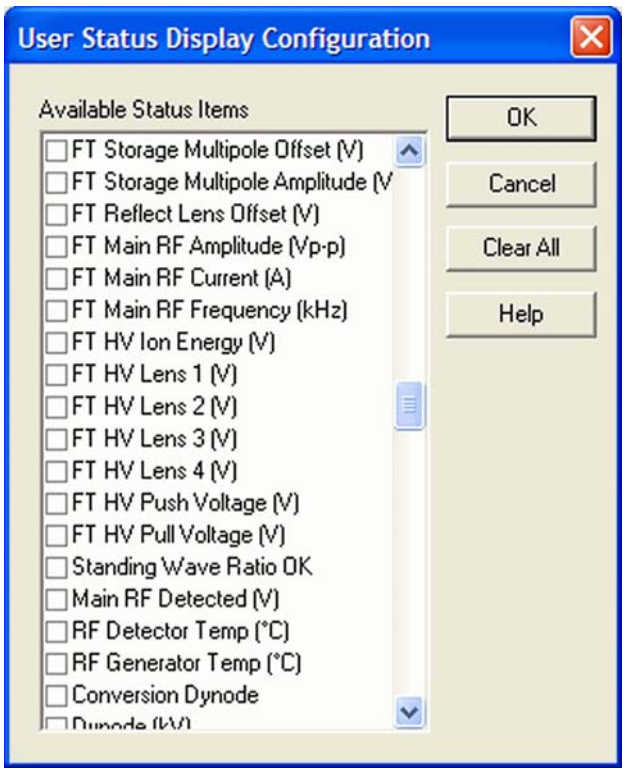


Figure 2-6. User Status Display Configuration dialog box

Scan Mode Menu

This section describes the elements of the Scan Mode menu that are different from the ion trap.

Define Scan

Choose **Define Scan** to display the Define Scan dialog box. Use the Define Scan dialog box to define a scan in various ways depending on the scan mode and scan type combination. Also, this dialog box allows choosing the ion trap or the Orbitrap (FTMS) as analyzer. Figure 2-7 shows the Define Scan dialog box with the Advanced Scan features. The Advanced Scan features can be activated in the Scan Mode menu of Tune Plus.

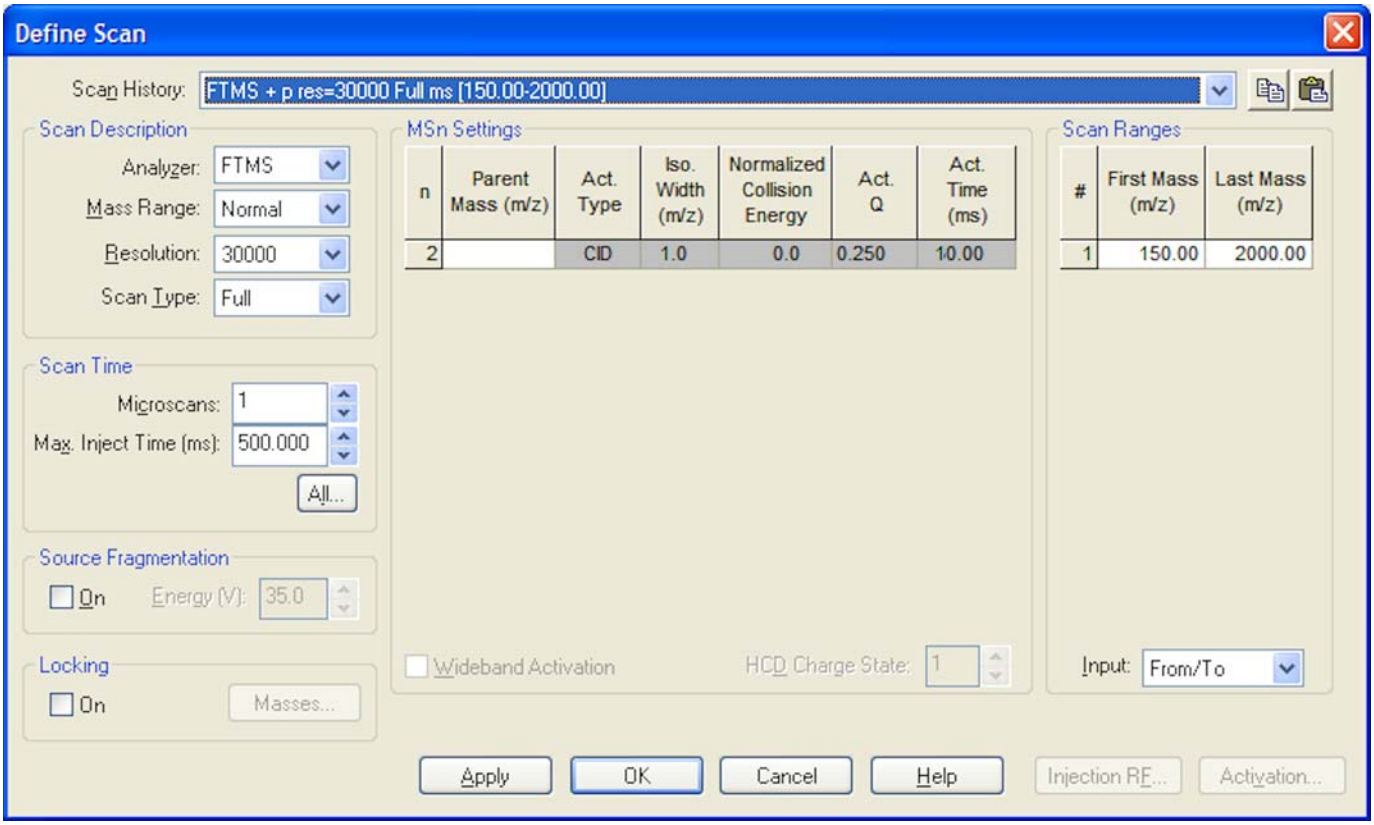


Figure 2-7. Define Scan dialog box (Advanced Scan Features enabled)

Scan Description

The Scan Description area contains the following elements:

Analyzer At the top, the Analyzer list box allows selecting the analyzer type used during the currently selected scan event. The following options are available:

- **FTMS** selects the Orbitrap analyzer.
- **Ion Trap** selects the ion trap analyzer.

Mass Range The following mass ranges are available:

- Low: *m/z* 15–200 for ion trap analyzer only
- Normal: *m/z* 50–2000 for ion trap analyzer and FTMS analyzer
- High: *m/z* 100–4000 for ion trap analyzer and FTMS analyzer

Scan Rate / Resolution When you have selected the entry Ion Trap in the Analyzer list box, this list box allows setting the scan rate (Normal, Rapid, Enhanced, Turbo, Zoom, UltraZoom).

When you have selected the entry FTMS in the Analyzer list box, this list box allows setting the resolution of the FT mass spectra.
Available resolution settings (FWHM at *m/z* 400) are 7500, 15000, 30000, 60000, and 100000.

Scan Type Usage of the scan types Full MS, SIM, SRM, or CRM is analogous to the ion trap. However, only one scan range is available for FTMS SIM, FTMS SRM, and FTMS CRM scans.

Scan Time

The Scan Time area contains the following elements:

Microscans The number of microscans determines how many spectra are averaged in one analytical scan. If FTMS is chosen as analyzer, transients are averaged for one analytical scan.

The number of microscans can be set individually for FTMS, Ion Trap MS, FTMS SIM, Ion Trap SIM, FT MS^n, Ion Trap MS^n, and Ion Trap Zoom.

Max Inject Time

The inject time is automatically controlled by the Automatic Gain Control™ (AGC™). The entry in this spin box limits the inject time to a maximum value. To ensure the high mass accuracy of the Orbitrap Velos Pro mass spectrometer, the maximum inject time should not be reached. Otherwise, the number of ions does not correspond to the AGC target value.

The maximum inject time can be set individually for FTMS, Ion Trap MS, FTMS SIM, Ion Trap SIM, FT MS^n, Ion Trap MS^n, and Ion Trap Zoom.

Note If the maximum inject time is reached, the number of ions does not correspond to the current AGC target value. This may affect the mass accuracy of FTMS spectra. ▲

Scan Time Settings

Click **All** to display the Scan Time Settings dialog box. See Figure 2-8. It allows displaying and setting all scan time settings for all scan types at the same time for both the ion trap and the FT analyzer.

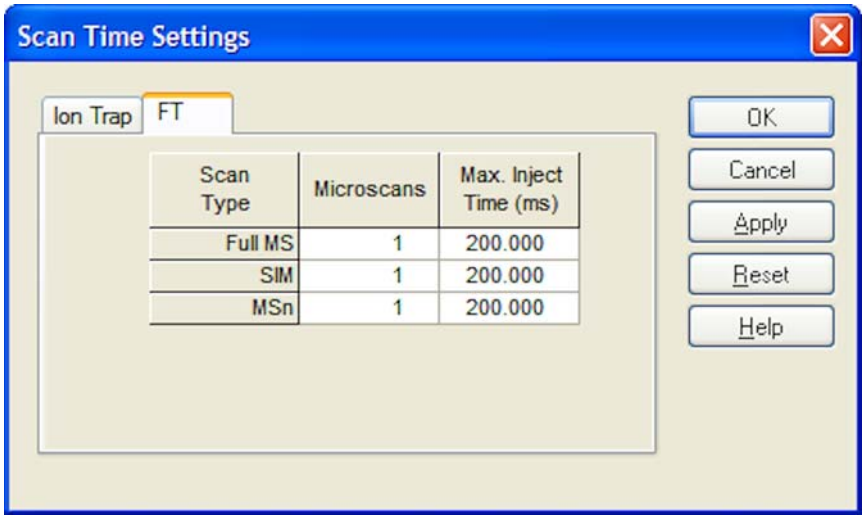


Figure 2-8. Scan Time Settings dialog box—FT page

Locking

Locking allows using one or more peaks in the spectrum as internal reference to improve mass accuracy. Locking is available for FTMS scans.

Select the On check box in the Locking area to enable locking. Then, click **Masses** to display a dialog box for entering and editing lock mass lists. See Figure 2-9.

Lock mass lists can consist of one or more lock masses. If the list contains lock masses that are (temporarily) not found in the spectrum, these lock masses are ignored (temporarily) and the instrument steps back to the external calibration. Thus, even when lock masses are used, the instrument should be external calibrated as well. For standard full scan experiments, it is expected that the spectrum shows at least one peak that corresponds to a lock mass.

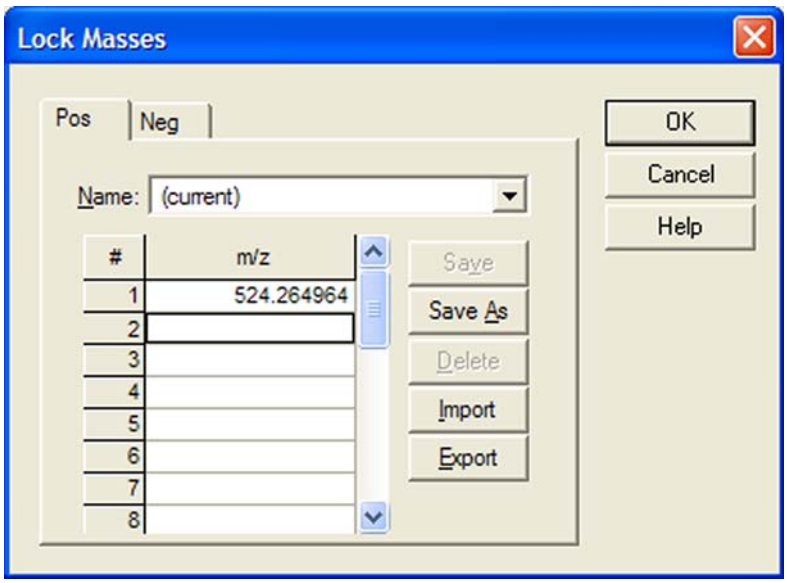


Figure 2-9. Lock Masses dialog box

There are two situations where the instrument makes use of a special mode to artificially mix the lock mass into the spectrum:

- If none of the given lock masses is 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 or SIM scan type, the instrument adds the lock mass by using SIM injections.

This way, lock masses can be used for all FTMS scan types and for varying lock mass abundances. There is no need for user interaction other than specifying a list of reference mass candidates.

Note Injection of lock masses is turned off completely, when you set the target value of the lock mass abundance to 0% on the Set device page of the Diagnostics dialog box. See page 4-13. ▲

See "FT Analyzer Messages" on page A-3 on how to view information about the instruments locking state. See "Using Locking in Automated Runs" on page 5-2 on how to set FTMS locking in Instrument Setup.

MS^n Settings

Use the table in this area to specify the parameters for each segment of an MS^n experiment.

Act. Type

The Activation Type list box becomes available when you enter a parent mass. It allows specifying how the ions are activated for fragmentation and has the following options:

- CID (Collision-induced dissociation)
- PQD (Pulsed-Q dissociation)
- ETD (electron transfer dissociation)[1]

  Use ETD to fragment peptides and proteins.
- HCD (higher energy CID)

  To use HCD you must select FTMS in the Analyzer list box. Use HCD to obtain triple quadrupole-like fragment ion spectra. HCD is available only for the final step in an MS^n experiment—it is not possible to set up an experiment where the first activation method is HCD and the second method is CID, for example. If you enter a new step after an HCD experiment, Tune Plus will change it to a CID experiment.

Normalized Collision Energy

When ETD is selected in the Activation Type list box, the Normalized Collision Energy spin box is disabled. For ETD activation, no RF amplitude is used to fragment ions.[1]

[1]This feature is available only for the Orbitrap Velos Pro ETD mass spectrometer.

Act. Q

When HCD is selected in the Activation Type list box, the Activation Q spin box is disabled. For HCD activation, no Q value is used for calculating voltages or amplitudes.

The Activation Q spin box is also disabled for ETD activation.[1]

HCD Charge State

The required absolute collision energy for the fragmentation of precursor ions depends on their charge states. A lower collision energy is required for higher charge states. The algorithm for calculating the absolute collision energy is based on empirical data taken from measurements on peptides. For example, the required absolute energy to fragment $[M+2H]^{2+}$ ions is about 90% of that of the corresponding $[M+H]^{+}$ ions. For $[M+3H]^{3+}$ ions, the value goes down to 85%.

To take advantage of this, enter the charge state of the ions to be fragmented into the spin box. To change the displayed value, click the arrows in the spin box to increment [up arrow] or decrement [down arrow] the value. Alternatively, you can enter a value in the spin box text field. You can set the HCD charge state to any value from 1 to 99. The default value is 1.

The HCD Charge State spin box is available only if HCD is selected as activation type, regardless of the status of the Advanced Scan features. See Figure 2-10.

[1]This feature is available only for the Orbitrap Velos Pro ETD mass spectrometer.

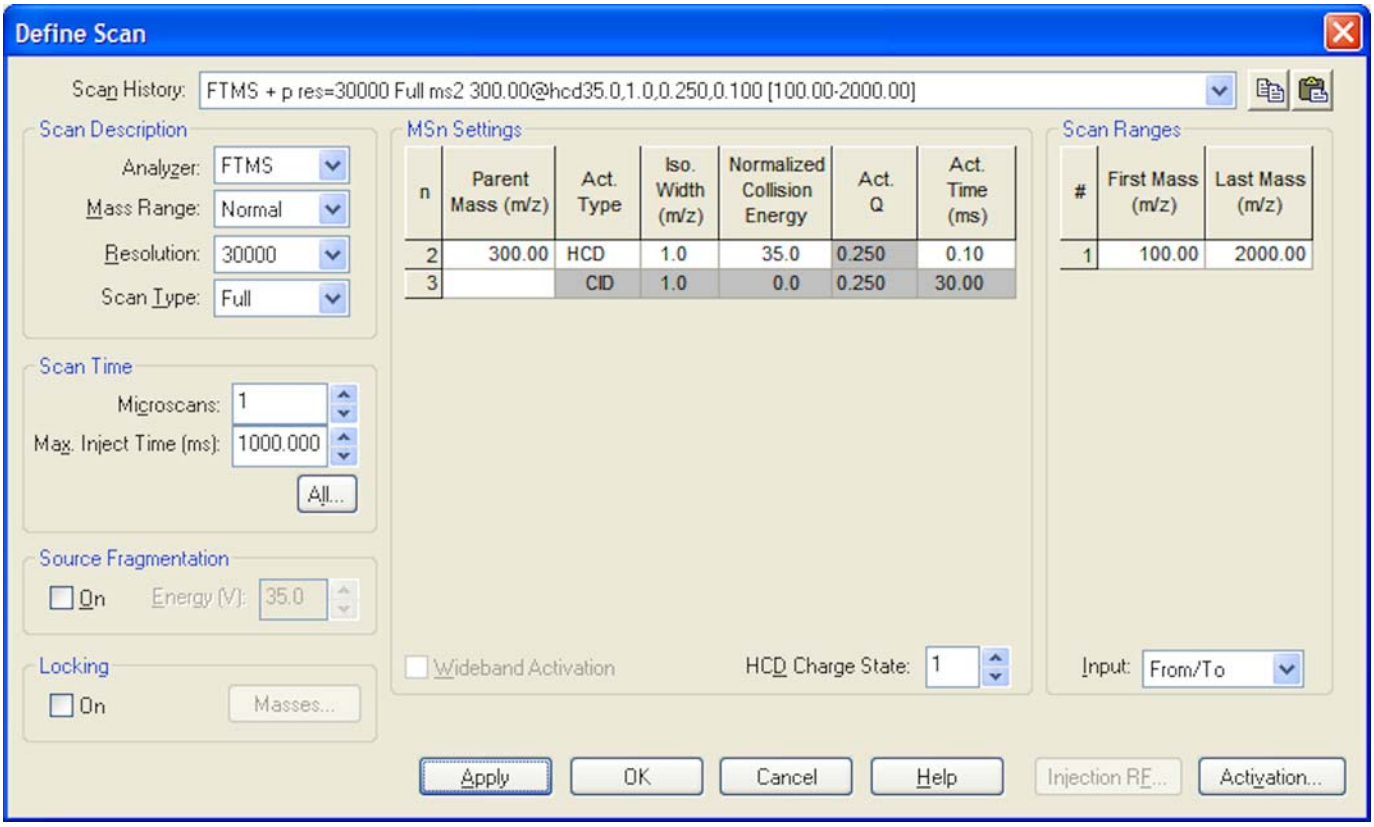
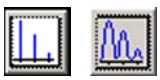


Figure 2-10. Define Scan dialog box with HCD selected as activation type

Scan Ranges

When HCD is selected as the activation method in the MS^n Settings area, the First Mass (*m/z*) is set to either 0.05×LastMass or 100, whichever is higher.

Centroid/Profile



Use this pair of buttons to switch between the Centroid and the Profile format. The Profile format for FTMS data is a compressed Profile format. "FT Profile Mode" on page 4-10 describes how to switch to full Profile format for FTMS data for diagnostic purposes.

For further information, see also "Data Size of FT Raw Files" on page A-4.

Positive/Negative

Use this pair of buttons to switch between positive ion and negative ion polarity. Different FT transfer, storage, and mass calibration parameters are used for the different polarities.

Display Menu

This section describes the elements of the Display menu that are different from the ion trap.

Spectrum Averaging

Use this toggle to switching on or off spectrum averaging. If spectrum averaging is enabled, the displayed spectrum is the moving average of several spectra before and is shown in red. Averaging FTMS scans is an averaging of transients. Use this functionality in analogy to ion trap scans.

❖ **To average FTMS scans**

1. In the Tune Plus window, choose **Display > Spectrum Averaging > Settings…** to display the Spectrum Averaging dialog box. See Figure 2-11.
2. Enter the number of transients to average into the spin box.
3. Click **OK** to save your changes and close the dialog box.

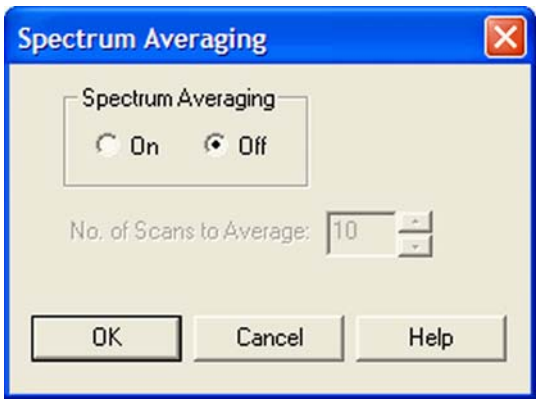


Figure 2-11. Spectrum Averaging dialog box

Setup Menu

This section describes the elements of the Setup menu that are different from the Velos Pro version of the Tune Plus window.

FT Transfer Optics

The FT transfer parameters are only changed by an FT transmission calibration, which is usually only necessary when the hardware of the system has been modified. This dialog box displays the actual FT readback values for the current scan mode. See Figure 2-12.

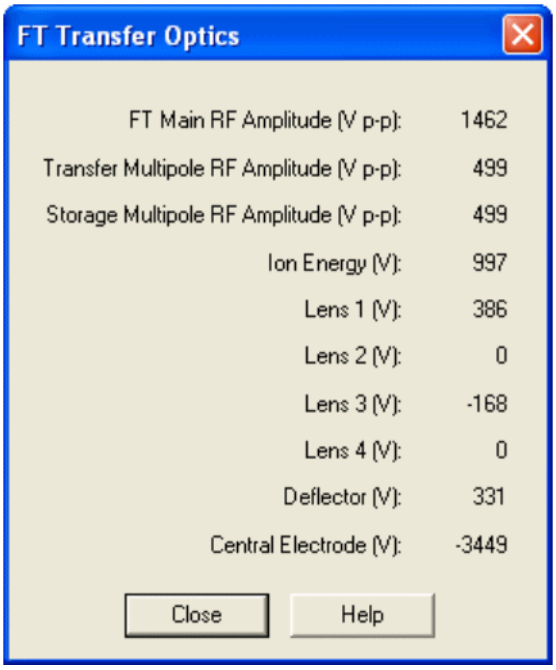
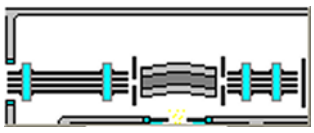


Figure 2-12. FT Transfer Optics dialog box

❖ **To open the FT Transfer Optics dialog box**

- From the Tune Plus window, choose **Setup > FT Ion Optics**, or
- click  in the Instrument Control toolbar.

FT Injection Control

Use the Injection Control dialog box to set the automatic gain control (AGC) target values. In addition, the Injection Control dialog box allows enabling or disabling the injection waveforms.

❖ **To open the FT Injection Control dialog box**

- From the Tune Plus window, choose **Setup > FT Injection Control**, or
- click  in the Instrument Control toolbar.

The Injection Control dialog box has two pages to enable the independent selection of target values for ion trap scans and FT scans.

Ion Trap Page

Recommended target values for the ion trap:

Full MS Target: 3e+04
SIM Target: 1e+04
MS^n Target: 5000
Zoom Target: 3000

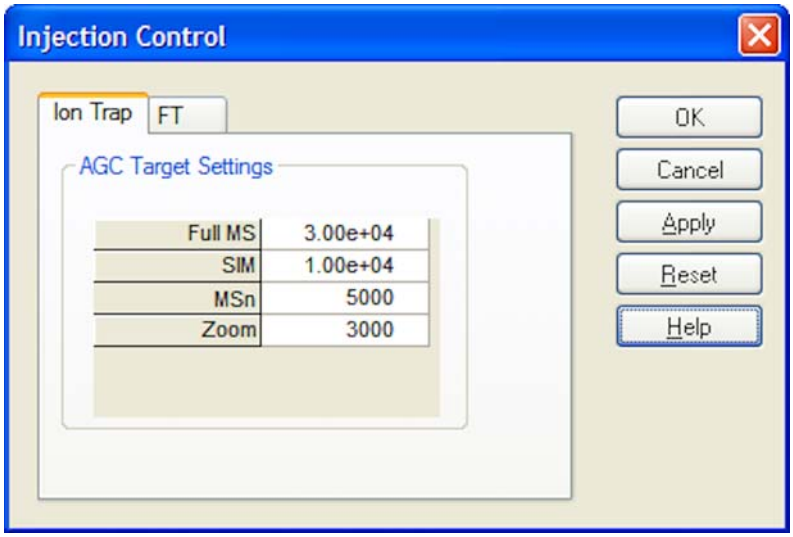


Figure 2-13. Ion Trap page of the Injection Control dialog box

FT Page

For FTMS measurements, only the Full MS target, the SIM target, and the MS^n target are used.

Recommended target values for the FT analyzer:

Full MS Target: 1e+06
SIM Target: 5e+04
MS^n Target: 5e+04

Note Lower target values than those listed above may be used to obtain shorter inject times. For MS^n scans, lower target values may also improve the isolation/fragmentation efficiency. Higher target values than those listed above can be used to improve the dynamic range. However, target values far above the recommended settings may affect isolation/fragmentation efficiency and mass accuracy for the FTMS analyzer. ▲

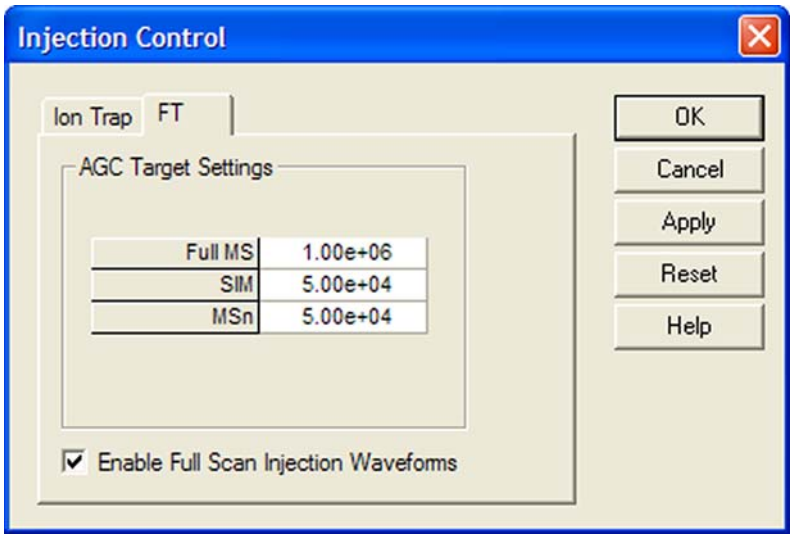


Figure 2-14. FT page of the Injection Control dialog box

Enable Full Scan Injection Waveforms

You can enable or disable the injection waveforms for FT scans. For ion trap scans, the injection waveforms are always enabled.

If the injection waveforms are enabled, a filter is applied on the ions that are injected into the ion trap. The ions above and below the selected ion or ion range selected are rejected. This option is often useful if the ion trap is being filled with ions of greater or lesser mass than the ion mass or ion mass range of interest. For example, this option can be used to remove high mass ions that are not of interest and to ensure that more target ions can enter the trap before the trap is full.

Note The FT injection waveforms option only applies to full scan MS scans performed with the Orbitrap analyzer. In FT SIM and FT MS^n scans, the injection waveforms are automatically enabled. ▲

FT Vacuum

Use the Vacuum dialog box to monitor the vacuum system parameters. The Vacuum dialog box has two pages to enable an independent selection of displaying the vacuum data of the ion trap or the FT part.

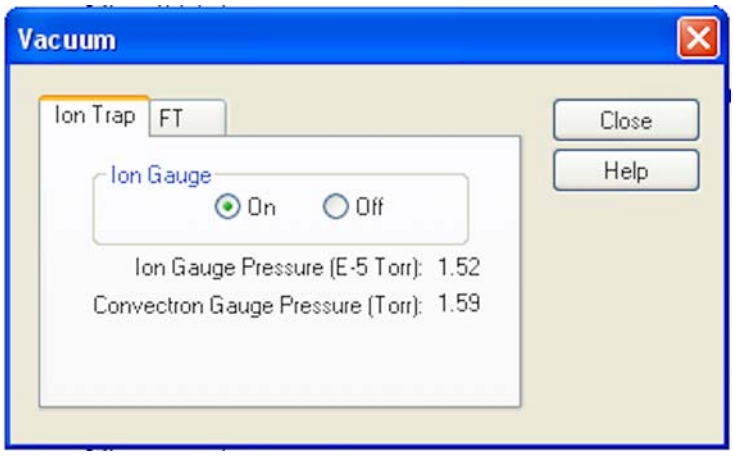


Figure 2-15. Ion Trap page of the Vacuum dialog box

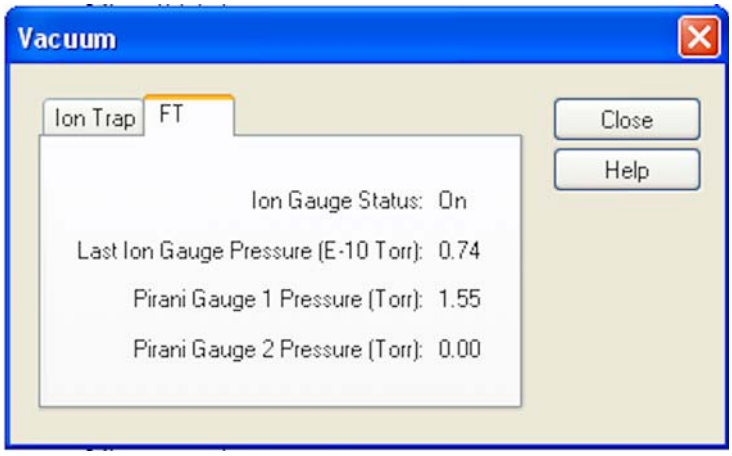


Figure 2-16. FT page of the Vacuum dialog box

❖ **To open the Vacuum dialog box**

- From the Tune Plus window, choose **Setup > Vacuum…**, or
- click  in the Instrument Control toolbar.

FT Temperature Monitor

Use the FT Temperature Monitor dialog box to view the status of the FTMS analyzer temperature regulation. Deviations of the actual temperature from the temperature setpoint can affect instrument performance. It is not possible to operate the instrument when the bakeout procedure is active.

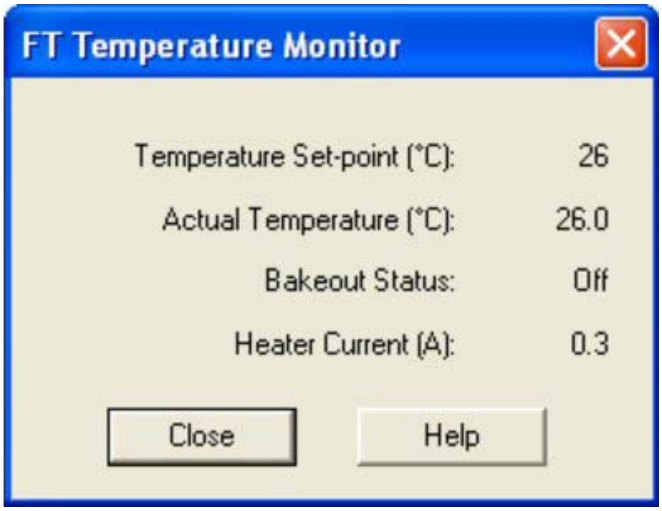


Figure 2-17. FT Temperature Monitor dialog box

❖ **To open the FT Temperature Monitor dialog box**

- From the Tune Plus window, choose **Setup > FT Temperature Monitor…**, or
- click  in the Instrument Control toolbar.

FT Lock Masses

Choose **Setup > FT Lock Masses** to display the Lock Masses dialog box. See Figure 2-9 on page 2-11. Use this dialog box to enter and edit lock mass lists for positive and negative ion mode. See “Locking” on page 2-11 for details.

Tune Methods

Several parameters, like the ion source parameters, ion trap ion optics parameters, or AGC target values are stored in tune methods. This topic points out for which parameters a differentiation between ion trap and FTMS is made. The Tune Plus title bar displays the name of the current tune method. If you are not currently editing a preexisting tune method, the title bar displays the word *Untitled*.

Parameters with Differentiation between Ion Trap Scans and FT Scans

A differentiation between ion trap scans and FTMS scans is made for the following tune parameters.

AGC Target Values

They can be set and saved independently for these experimental modes (no differentiation between positive and negative ion polarity mode):

- Ion Trap Full MS Target
- Ion Trap SIM Target
- Ion Trap MS^n Target
- Ion Trap Zoom Target
- FT Full MS Target
- FT SIM Target
- FT MS^n Target

Microscans and Maximum Inject Time

They can be set and saved independently for these experimental modes:

- Ion Trap Full MS, positive ion mode
- Ion Trap SIM, positive ion mode
- Ion Trap MS^n, positive ion mode
- Ion Trap Zoom, positive ion mode
- FT Full MS, positive ion mode
- FT SIM, positive ion mode
- FT MS^n, positive ion mode
- Ion Trap Full MS, negative ion mode

- Ion Trap SIM, negative ion mode
- Ion Trap MS^n, negative ion mode
- Ion Trap Zoom, negative ion mode
- FT Full MS, negative ion mode
- FT SIM, negative ion mode
- FT MS^n, negative ion mode

Inject Waveform Flags

The flag whether the inject waveform is enabled or disabled can be set and saved independently for

- Ion trap scans
- FT full scans.

Parameters without Differentiation between Ion Trap Scans and FT Scans

No differentiation between ion trap scans and FT scans is made for all ESI parameters, and for all ion source and ion optics parameters.

Parameters not saved in a Tune Method

All parameters that can be set in an instrument method are not saved in the tune method. Thus, the following parameters are not saved in a tune method:

- Analyzer (Ion Trap or FTMS)
- Mass Range (Low, Normal, or High)
- Scan Rate
- Resolution
- Scan Type (Full, SIM, SRM, CRM)
- Scan Range
- Polarity* (positive or negative)
- Data type* (centroid or profile)

* Only the data format (centroid or profile) and the ion polarity are saved in a tune file that are set after a new start of Tune Plus.

Chapter 3 Calibrating the Instrument for FTMS Measurements

This chapter provides procedures to calibrate the Orbitrap Velos Pro mass spectrometer for FTMS measurements. It contains the following topics:

Preliminary Remarks

There are no specific tune procedures for the FTMS part. All FTMS ion transfer and excitation parameters are treated as calibration parameters and are determined in automatic calibration procedures.

In the automatic calibration, the FT transmission calibration and the FT mass calibration are automatically performed for all calibration ranges. In the semi-automatic calibration, it is possible to decide whether the transmission and/or mass calibration are performed only for the positive ion mode, only for the negative ion mode or for both polarities. See "Automatic Calibration Page" on page 3-15 and "Semi-Automatic Calibration Page" on page 3-17 for further details.

On the FT Manual page of the Calibrate dialog box, you can select your own calibration masses for FT ion transmission, storage transmission, and FT mass calibration. See "FT Manual Calibration Page" on page 3-22 for further details.

Note Thermo Fisher Scientific recommends using the semi-automatic calibration. ▲

Calibration Files and their Backups

After a successful or partly successful calibration, the ion trap and FT calibration parameters are saved automatically. All ion trap and FT calibration parameters are stored in the calibration file master.LTQCal, which is located in the folder:

C:\Thermo\Instruments\LTQ\system\msx

The calibration parameters for the reagent ion source in Orbitrap Velos Pro ETD mass spectrometers are stored in an additional calibration file master.LTQReagent, which is located in the same folder.

Backup Current Calibration

It is possible to create a backup of the current calibration file manually or by choosing **File > Backup Current Calibration** in the Tune Plus window. The Backup Current Calibration and Restore Backup Calibration commands work by copying the master.LTQCal to user.LTQCal and vice versa.

If a backup calibration user.LTQCal was already generated, the old user.LTQCal will be backed-up to a file named userXYZ.LTQCal. If you perform backup calibrations at regular intervals, then a history of your calibration files is generated in the folder:

C:\Thermo\Instruments\LTQ\system\msx

Using the Backup Calibration command regularly allows returning to previous calibrations in case a new calibration is suspected to have worsened instrument performance.

Restore Backup Calibration

Upon **Restore Backup Calibration**, the calibration values saved in user.LTQCal are automatically downloaded to the instrument. Therefore, it is recommended to generate a current backup after a successful calibration.

It is also recommended to use the **Restore Backup Calibration** command instead of the **Restore Factory Calibration** command because the backup calibration file is newer than the factory calibration file.

Note Pressing the reset button of the instrument loads the master.LTQCal file into the internal computer of the instrument. ▲

Calibration Solutions

This section provides information about preparing the calibration solutions for the Orbitrap Velos Pro mass spectrometer.

The *positive ion mode calibration solution* allows calibrating Thermo Scientific mass spectrometers with ESI or HESI II probe in positive ion mode. Supported instruments are the Orbitrap Velos Pro mass spectrometer, the Thermo Scientific LTQ Velos Series mass spectrometers, and the Thermo Scientific LTQ Orbitrap Velos Series mass spectrometers. The positive ion mode calibration solution covers a mass range from *m/z* 74 to *m/z* 1822 and is therefore usable for calibrations between *m/z* 50 and *m/z* 2000.

The *negative ion mode calibration solution* allows calibrating Thermo Scientific MS detectors with ESI source in negative ion mode. Supported instruments are the Orbitrap Velos Pro mass spectrometer, other LTQ based hybrid instruments (LTQ FT, LTQ FT Ultra, and LTQ Orbitrap Series), and Exactive Series instruments. The negative ion mode calibration solution covers a mass range from *m/z* 265 to *m/z* 1880 and is therefore usable for calibrations between *m/z* 50 and *m/z* 2000.

Obtaining Ready-to-Use Calibration Solutions

To free you from time-consuming mixing and dilution steps and to allow you to focus on data acquisition, Thermo Fisher Scientific provides ready-to-use calibration solutions. Table 3-1 shows the available calibration solution packages.

Table 3-1. Available calibration solutions packages

Calibration Solution	Product Name	Product Number
Positive ion mode	Pierce LTQ Velos ESI Positive Ion Calibration Solution, 10 mL	88323
Negative ion mode	Pierce ESI Negative Ion Calibration Solution, 10 mL	88324

You can order ready-to-use calibration solutions from www.thermo.com/pierce or www.fishersci.com. The prepared calibration solutions are shipped at ambient temperature and stable at 2–8 °C for 1.5 years.

Chemicals for Preparing Calibration Solutions

The n-butylamine, caffeine, MRFA, Ultramark 1621, sodium dodecyl sulfate, and sodium taurocholate needed to make the calibration solutions are supplied with your chemical accessory kit. When ordering replacements, use the information listed in Table 3-2.

Table 3-2. Calibration compounds for Orbitrap Velos Pro MS

Description	Quantity	Supplier Product Number
Supplier: Sigma Chemical Company, see below.		
n-Butylamine*	25 mL	471305-25ML
Caffeine Methanol Solution	1 mL	C6035-1ML
Met-Arg-Phe-Ala acetate salt	5 mg	M1170-5MG
Sodium Dodecyl Sulfate	10 g	L4509-10G
Sodium Taurocholate Hydrate	250 mg	T4009-250MG
Supplier: ABCR GmbH & Co. KG, see below.		
Ultramark® 1621 Mass Spec. Standard	250 mg	AB172435

* If ordering elsewhere, use only mass spec grade quality.

To order more of these compounds, contact:

Sigma Chemical Company
P. O. Box 14508
St. Louis, Missouri, USA 63178-9916
Phone (800) 325-3010 (in the U.S. & Canada)
(314) 771-3750 (U.K. & International)
Web site www.sigma-aldrich.com

or

ABCR GmbH & Co. KG
Im Schlehert 10
D-76187 Karlsruhe, Germany
Phone +49 (0)721 950 61-0
Fax +49 (0)721 950 61-80
Email info@abcr.de
Web site www.abcr.de/english.htm

Solvents and Modifiers

You can also order specific chemicals from Thermo Fisher Scientific, which are sold under its Fisher Chemical brand. As specified in Table 3-3 use only LCMS grade chemicals for calibrating your LTQ Orbitrap Series system.

Table 3-3. Recommended solvents and reagents

Solvent / Reagent	Specifications	Fisher Chemical P/N
Methanol	LCMS grade	A456-4
Acetonitrile	LCMS grade	A955-4
Water	LCMS grade	W6-4
Isopropyl alcohol	LCMS grade	A461-4
Acetic acid (modifier)	LCMS grade	A507-500 or A35-500
Formic acid (modifier)	99–100% (This acid must be supplied in a glass bottle.)	A117-50

For a complete selection of LCMS-grade consumables from Fisher Scientific, visit www.FisherLCMS.com.

Safety Advice

Potentially hazardous chemicals used in procedures throughout this chapter include the following:

- Glacial acetic acid
- Acetonitrile
- Methanol
- Formic acid

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

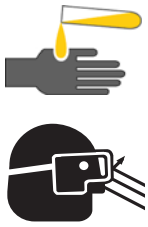
Caution Please consider the following cautions when preparing calibration solutions:

- Do not filter solvents. Filtering solvents can introduce contamination.
- Do not use plastic pipettes to prepare your tuning and calibration standards. Plastic products can release phthalates that can interfere with your analyses. ▲

Preparing Stock Solutions

Use the chemicals described in the previous section to prepare the calibration solutions from the following stock solutions:

- "Caffeine Stock Solution", next topic
- "MRFA Stock Solution" on page 3-7
- "Ultramark 1621 stock solution" on page 3-8
- "N-Butylamine Stock Solution" on page 3-8
- "Sodium Dodecyl Sulfate Stock Solution" on page 3-8
- "Sodium Taurocholate Stock Solution" on page 3-8



Warning Avoid exposure to potentially harmful materials. Always wear protective gloves and safety glasses when you handle solvents or corrosives. Also contain waste streams and use proper ventilation. Refer to your supplier's Material Safety Data Sheet (MSDS) for proper handling of a particular solvent. ▲

Caffeine Stock Solution

A 1 mg/mL stock solution of caffeine in 100% methanol is provided with your LTQ Orbitrap Series MS detector. You can also order this solution through Sigma. The Sigma product number for this solution is C6035.

MRFA Stock Solution

❖ **To prepare the MRFA stock solution**

1. Obtain the vial of L-methionyl-arginyl-phenylalanyl-alanine acetate × H_2O (MRFA) in your accessory kit. In this form, the MRFA sample has an average molecular weight of 523.7 u. Carefully weigh 3.0 mg of the MRFA sample.
2. Dissolve the MRFA sample in a total volume of 1.0 mL of 50:50 methanol:water. Mix the solution (5.0 nmol/µL) thoroughly.
3. Transfer 50 µL of the 5 nmol/µL solution into a clean polypropylene tube.
4. Add 1.45 mL of 50:50 methanol:water to the tube. Mix this solution (166.7 pmol/µL) thoroughly.
5. Label the tube *MRFA stock solution* and store it in a freezer until it is needed.

Ultramark 1621 stock solution

❖ **To prepare the Ultramark 1621 stock solution**

1. Obtain the vial of Ultramark 1621 in your accessory kit.
2. Using a syringe, measure out 10 µL of Ultramark 1621, and dissolve it in 10 mL of acetonitrile.
3. Mix the solution thoroughly.
4. Label the vial *Ultramark 1621 stock solution* and store it in a freezer until it is needed.

N-Butylamine Stock Solution

❖ **To prepare the n-butylamine stock solution**

1. Using a syringe, transfer 5 µL of n-butylamine to a 25 mL (minimum) volumetric glass flask.
2. Add 9995 µL of 50:50 methanol/water to the flask.
3. Mix the solution thoroughly.
4. Transfer the solution to a vial.
5. Label the vial *N-butylamine stock solution*.

Sodium Dodecyl Sulfate Stock Solution

❖ **To prepare the sodium dodecyl sulfate stock solution**

1. Obtain the vial of sodium dodecyl sulfate. In this form, the sample has an average molecular weight of 288.4 u.
2. Prepare the stock solution of sodium dodecyl sulfate by dissolving 2.88 mg in 10 mL of 50:50 methanol:water.
3. Mix the solution (1.0 nmol/µL) thoroughly.
4. Label the vial *Sodium Dodecyl Sulfate stock solution (1 nmol/µL)*.

Sodium Taurocholate Stock Solution

❖ **To prepare the sodium taurocholate stock solution**

1. Obtain the vial of sodium taurocholate. In this form, the sample has an average molecular weight of 537.7 u.
2. Prepare the stock solution of sodium taurocholate by dissolving 5.38 mg in 10 mL of 50:50 methanol:water.

3. Mix the solution (1.0 nmol/µL) thoroughly.
4. Label the vial *Sodium Taurocholate stock solution (1 nmol/µL)*.

LTQ/FT-Hybrid Positive Ion Mode Calibration Solution

The LTQ/FT-hybrid positive ion mode calibration solution consists of caffeine, MRFA, Ultramark 1621, and n-butylamine in an acetonitrile:methanol:water solution containing 1% acetic acid.

❖ **To prepare the positive ion mode calibration solution**

1. Pipet 20 µL of the caffeine stock solution into a light-protected, clean, dry 10 mL volumetric flask.
2. Pipet 100 µL of the MRFA stock solution into the flask.
3. Pipet 100 µL of the Ultramark 1621 stock solution into the flask.
4. Pipet 100 µL of the stock solution of n-butylamine into the flask.

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

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

LTQ/FT-Hybrid Negative Ion Mode Calibration Solution

The LTQ/FT-hybrid negative ion mode calibration solution consists of sodium dodecyl sulfate, sodium taurocholate, and Ultramark 1621 in an acetonitrile:methanol:water solution containing 1% acetic acid.

❖ **To prepare the negative ion mode calibration solution**

1. Pipet 100 µL of the sodium dodecyl sulfate stock solution into a light-protected, clean, dry 10 mL volumetric flask.

2. Pipet 100 µL of the sodium taurocholate stock solution into the flask.
3. Pipet 100 µL of the Ultramark 1621 stock solution into the flask.

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

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

Applicable Calibration Solutions for FT Manual Calibration

Because the FT Manual page of the Calibrate dialog box allows using your own calibration masses, it is possible to use custom calibration solution here. However, there are some requirements for the calibration masses. The scan ranges of the instrument need to be covered properly by the given masses.

Calibration and Tuning of the Ion Trap

This section describes the calibration and tuning of the ion trap for FT measurements.

Calibration of the Ion Trap

The ion trap has to be successfully calibrated before an FT calibration is performed. It is very important that the electron multiplier gain is correctly calibrated because the AGC prescan is performed in the ion trap. Thus, the electron multiplier gain calibration should be checked before an FT calibration is performed.

For the ion trap calibrations, the positive ion mode calibration solution or the negative ion mode calibration solution can be used.

Tuning the Ion Trap for Positive Ion Mode

For the positive ion mode, it is recommended to perform an automatic tune of *m/z* 524 at a Full MS Target of 1e4–3e4. Use the positive ion mode calibration solution or the negative ion mode calibration solution with the settings in the Define Scan dialog box that are shown on Figure 3-1 on page 3-12.

The spectrum should look similar to the spectrum shown in Figure 3-2 on page 3-12 and Figure 3-3 on page 3-13. Make sure the peaks at *m/z* 74, 138, 195, 524, and the highest Ultramark peaks are all present, ideally above 30% of the base peak.

The inject time should be stable and less than 150 µs (if a Full MS target of 3e4 is used). Do not forget to save the tune method after a successful tuning.

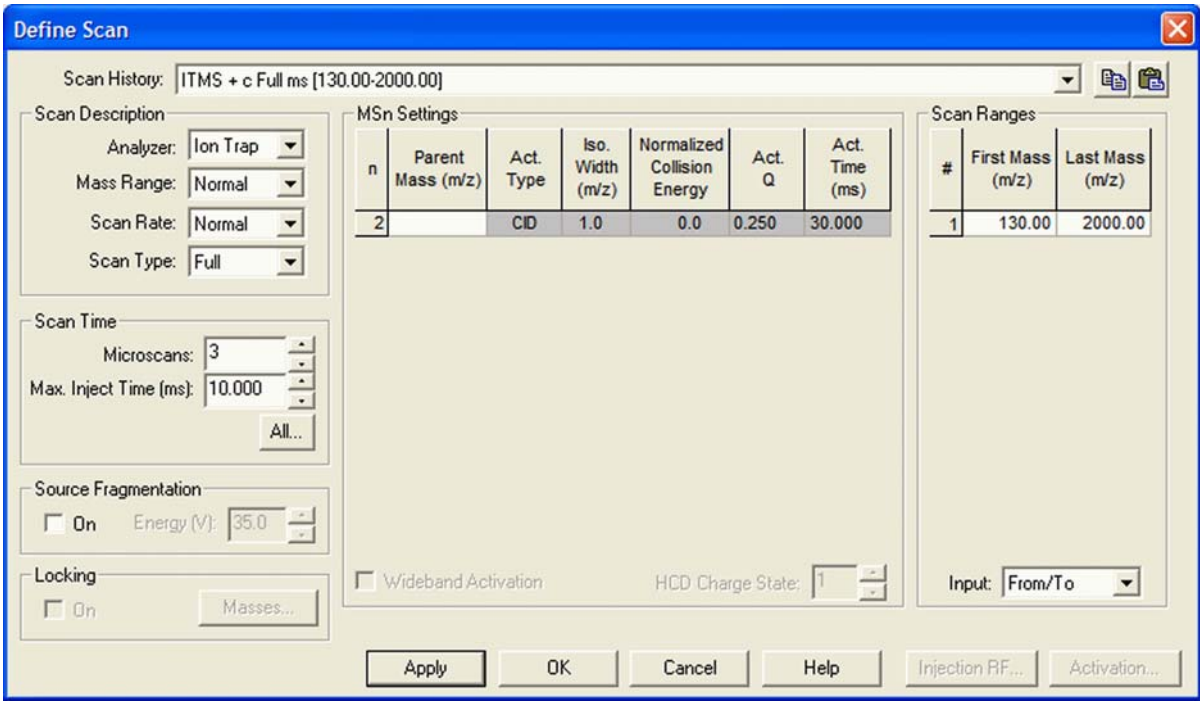


Figure 3-1. Recommended settings in the Define Scan dialog box for an automatic tune of the ion trap

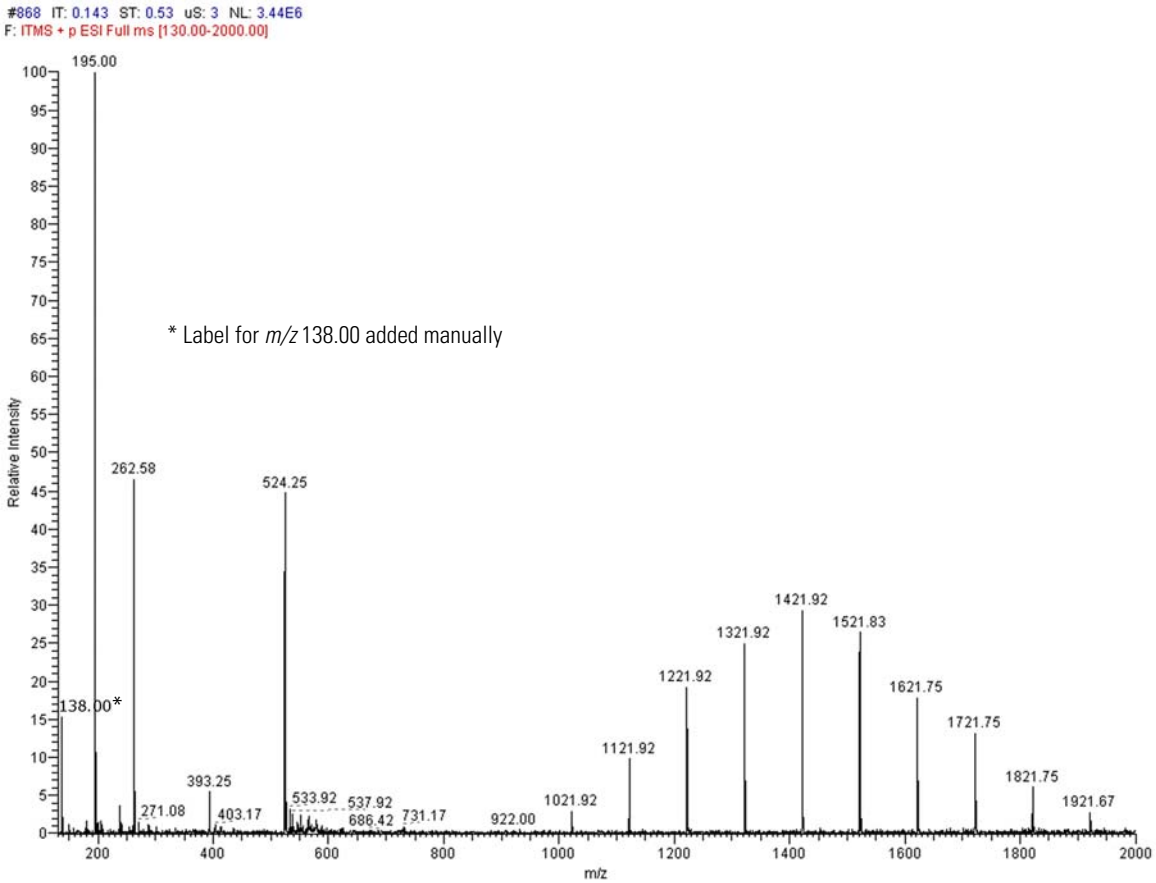


Figure 3-2. Ion trap spectrum of positive ion mode calibration solution, scan range *m/z* 130–2000, positive ion polarity mode

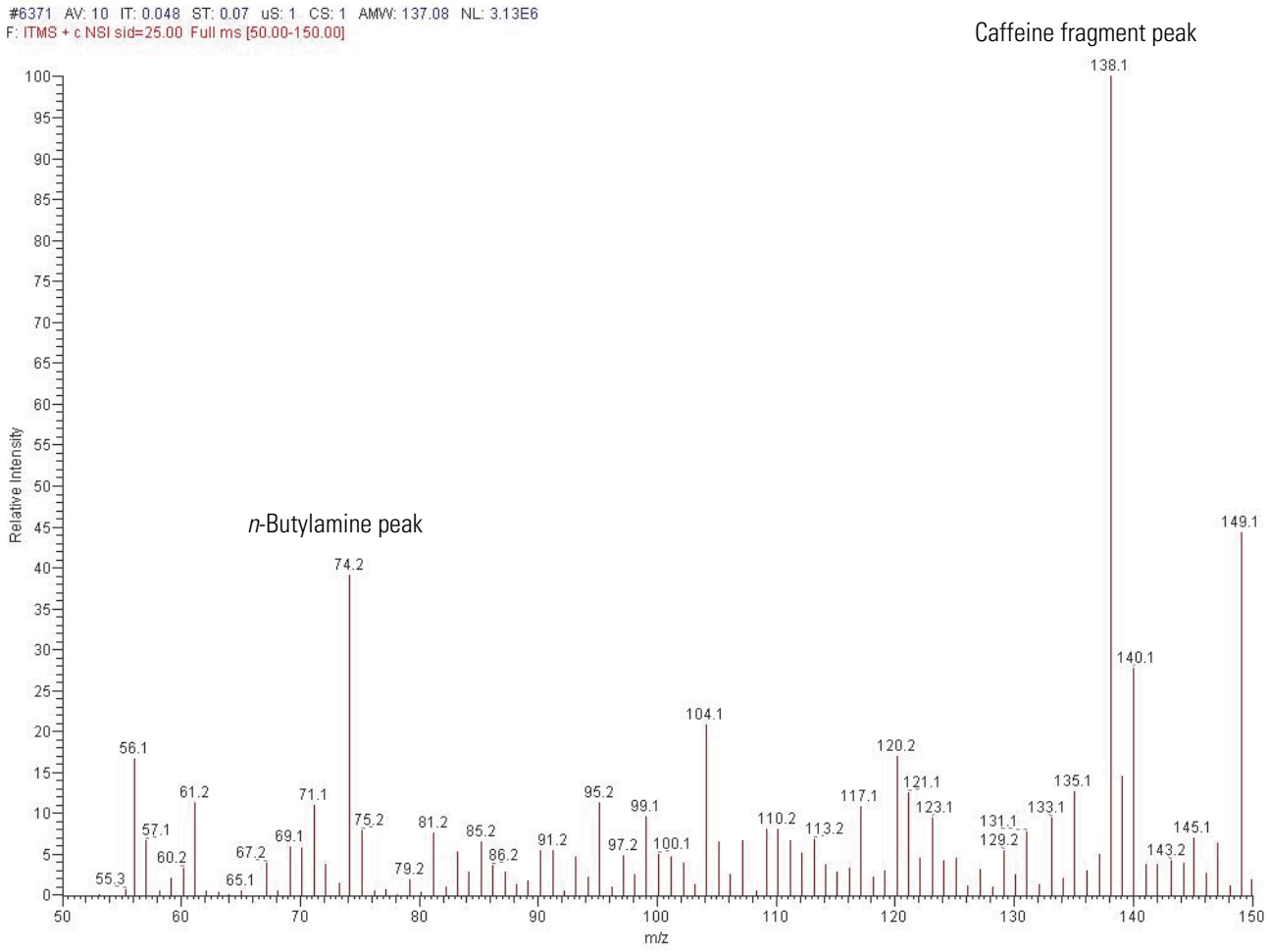


Figure 3-3. Ion trap spectrum of the positive ion mode calibration solution (lower range), positive ion polarity mode

Tuning the Ion Trap for Negative Ion Mode

For the negative ion mode, it is recommended to perform an automatic tune of *m/z* 514 at a Full MS Target of 1e4–3e4. Use the negative ion mode calibration solution with the settings shown in Figure 3-1 on page 3-12.

After the automatic tuning, a manual adjustment of the S-lens RF level should be used to get an ion trap spectrum in the scan range *m/z* 150–2000. At *m/z* 265 is the base peak (100%) and the highest Ultramark adduct ion peaks are at about 80%, as shown in Figure 3-4 on page 3-14.

The S-lens RF level affects the mass spectrum as follows:

- Decreasing the S-lens RF level will decrease the amount of fragmentation of fragile ions in the S-lens.
  Decreasing the S-lens RF level will decrease the transmission of high *m/z* ions through the S-lens and increase the transmission of the low *m/z* ions.

- Increasing the S-lens RF level will increase the amount of fragmentation of fragile ions in the S-lens.
  Increasing the S-lens RF level will increase the transmission of high *m/z* ions through the S-lens and decrease the transmission of the low *m/z* ions.

If the S-lens RF level is set very low, in-source fragmentation of the Ultramark adduct ions may occur. Thus, if you observe rather ions at *m/z* 906, 1006, … than ions at *m/z* 1280, 1380, … the S-lens RF level has to be increased. The inject time should be stable and less than 1 ms (if a target of 1e4 is used). Do not forget to save the tune method after a successful tuning.

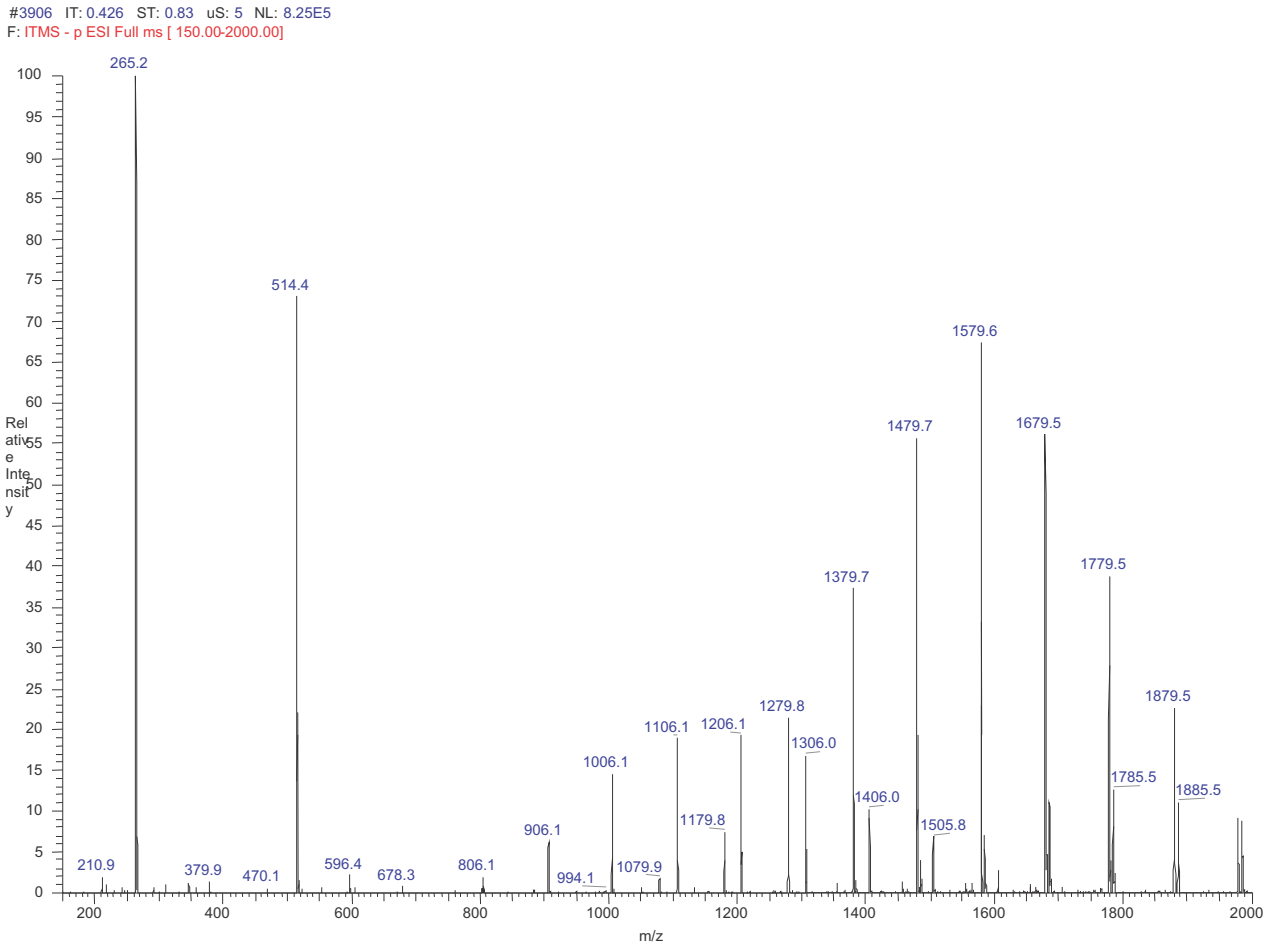


Figure 3-4. Ion trap spectrum of the negative ion mode calibration solution, scan range *m/z* 150–2000, negative ion polarity mode

Automatic Calibration Page

Use the Automatic page of the Calibrate dialog box to perform an automatic calibration of all the calibration parameters, including all ion trap calibrations and all FT calibrations. See Figure 3-5.

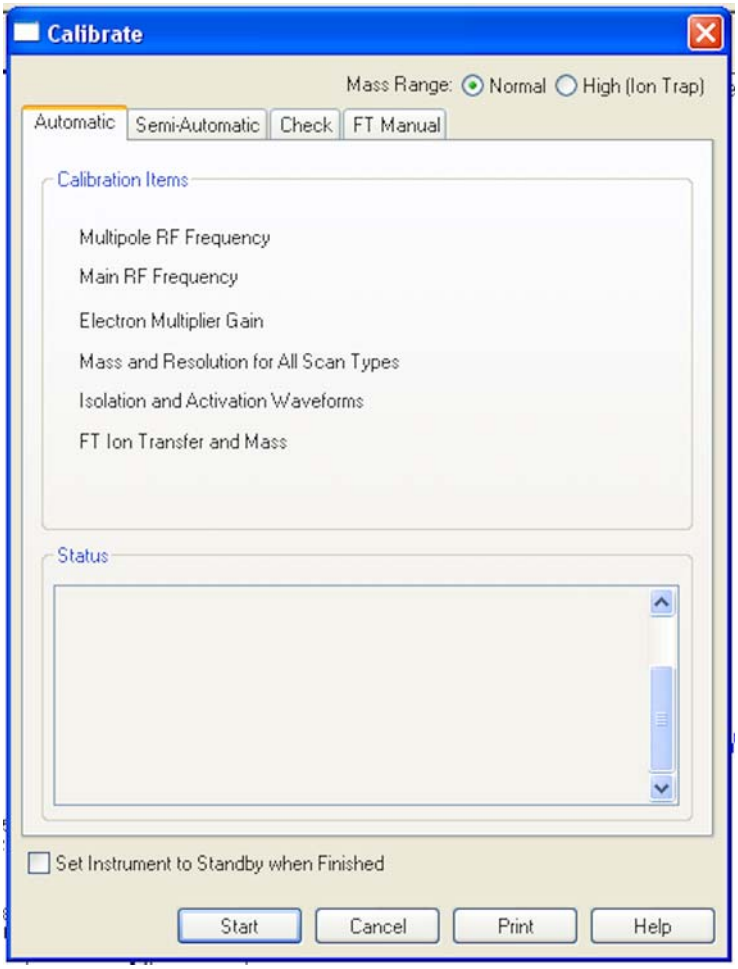


Figure 3-5. Automatic page of the Calibrate dialog box

In an automatic calibration, the FT calibration procedures are performed automatically one after another, following the ion trap calibration. To perform an automatic calibration, the negative ion mode calibration solution has to be used.

The calibration masses and all experimental parameters (for example, target values, scan ranges, or resolution settings) are set automatically and cannot be influenced by the user.

Note Usually, you do not need to perform a complete ion trap calibration or an FT ion transmission calibration unless the hardware is modified in some way. However, it is necessary to repeat the electron multiplier calibration and the FT mass calibration on a regular basis. Thus, in the most cases it is not recommended to perform an automatic calibration of the Orbitrap Velos Pro mass spectrometer. In this case, all calibrations are performed, which takes about 1 hour. To run a multiplier gain calibration or an FT mass calibration (which takes only some minutes), it is recommended to use the semiautomatic calibration. ▲

Semi-Automatic Calibration Page

Use the Semi-Automatic page of the Calibrate dialog box to select specific calibration parameters to calibrate, for example only the ion trap calibrations or only the FT calibrations. (See Figure 3-6.) For FT calibrations, it is also possible to differentiate between positive and negative ion mode.

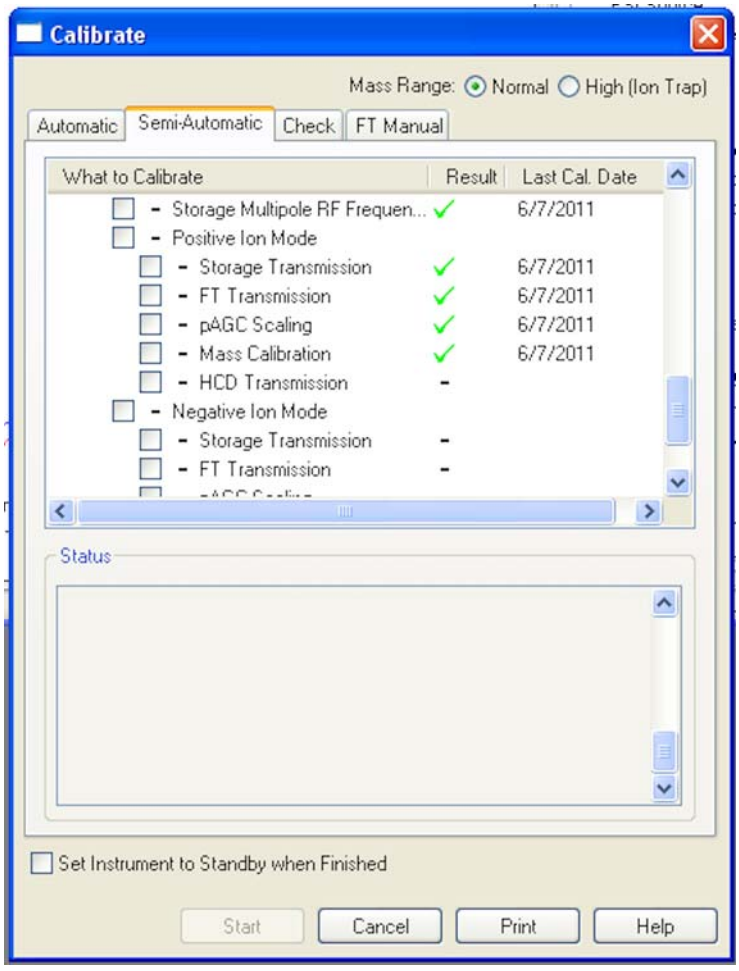


Figure 3-6. Semi-Automatic page of the Calibrate dialog box

To calibrate one or more selected parameters, clear the Select All check box to make the individual calibration parameters available. Select the parameter(s) you want to calibrate, then click **Start**.

For example:

- To run a complete automatic calibration (ion trap and FT), select the Select All check box. Then, click **Start**. This is analogous to the automatic calibration. As already described before, it is not recommended to perform an automatic calibration of the Orbitrap Velos Pro mass spectrometer if not necessary because all calibrations are performed, which takes about 1 hour.

- To run an automatic calibration of the ion trap, select the Select All—Ion Trap check box. Then, click **Start**.
- To run an automatic calibration of the FT part, select the Select All—FT check box. Then, click **Start**.
- To run an FT mass calibration, select the Mass Calibration check box. Then, click **Start**.

In a semi-automatic calibration, the selected FT calibration procedure(s) are performed automatically one after another.

All calibrations apart from the FT calibrations for the negative ion mode can be performed with either the positive ion mode calibration solution or the negative ion mode calibration solution. To run the FT ion transmission and/or the mass calibration for the negative ion mode, the negative ion mode calibration solution has to be used.

The calibration masses and all experimental parameters like target values, scan ranges, resolution settings, inject waveforms etc. are set automatically and cannot be influenced by the user.

HCD Transmission

Use this check box to specify whether to perform a calibration of the higher energy collision-induced dissociation (HCD) transmission efficiency of the Orbitrap mass analyzer in positive ion mode.

Check Calibration Page

Use the Check page of the Calibrate dialog box to automatically check several calibration settings. See Figure 3-7.

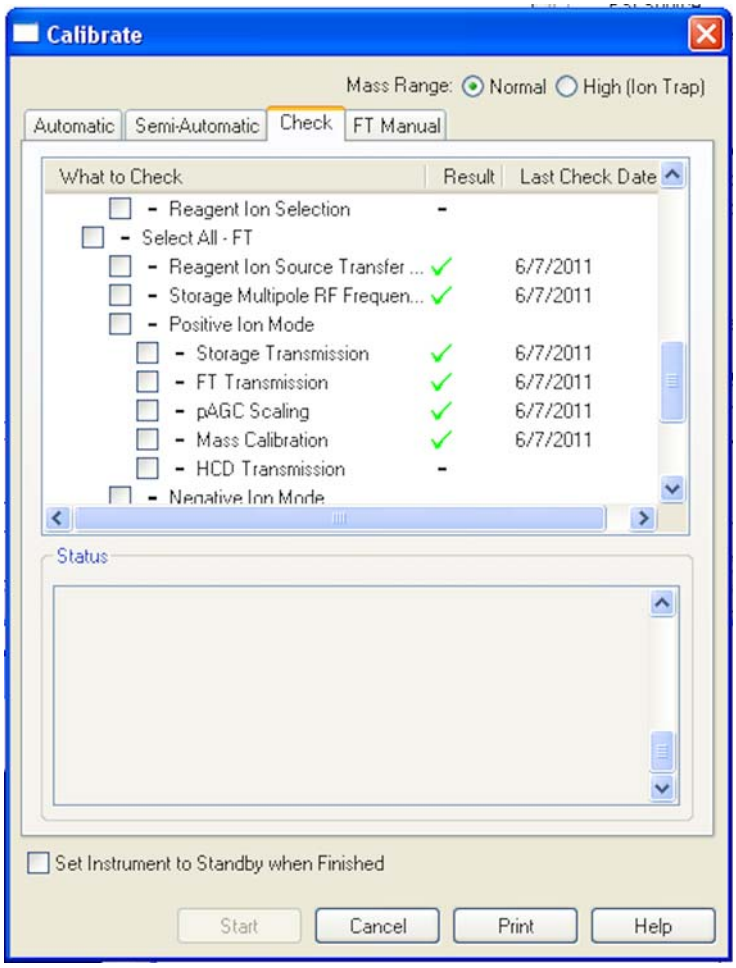


Figure 3-7. Check page of the Calibrate dialog box

All calibration checks apart from those for the FT negative ion mode can be performed with either the negative ion mode calibration solution or the positive ion mode calibration solution. To check the FT calibrations in the negative ion mode, the negative ion mode calibration solution has to be used.

The calibration masses and all experimental parameters like target values, scan ranges, resolution settings, etc. are set automatically.

At the conclusion of the check procedure, the Orbitrap Velos Pro mass spectrometer displays a message that indicates whether the parameter(s) are calibrated properly or not.

Using the Check page of the Calibrate dialog box, you can select the following parameters:

Select All	Use this check box to specify whether or not to check all calibration parameters. To check all calibration parameters, select the Select All check box. In this case, all ion trap calibration parameters and all FT calibration parameters are checked. You can also check each calibration parameter individually. To make the individual calibration parameters available, clear the Select All check box.
Select All Ion Trap	Use this check box to specify whether or not to check the calibration of only the ion trap parameters.
Reagent Ion Selection[1]	Use this check box to specify whether or not to check the reagent ion source selection.
Select All-FT	Use this check box to specify whether or not to check the calibration of only the FT ion transfer optics and mass analyzer.
Reagent Ion Source Transfer Multipole RF Frequency[1]	Use this check box to specify whether or not to check the frequency of the RF voltage of the transfer multipole in the reagent ion source optics.
Transfer Multipole RF Frequency	Use this check box to specify whether or not to check the frequency of the RF voltage of the transfer multipole in the FT transfer ion optics.
Storage Multipole RF Frequency	Use this check box to specify whether or not to check the frequency of the RF voltage of the storage multipole in the FT transfer ion optics.
Positive Ion Mode	Use this check box to specify whether or not to check the FT ion transmission calibration and FT mass calibration for the positive ion mode.
Negative Ion Mode	Use this check box to specify whether or not to check the FT ion transmission calibration and FT mass calibration for the negative ion mode.

[1]This feature is available only in the Orbitrap Velos Pro ETD mass spectrometer.

Storage Transmission	Use this check box to specify whether or not to check the ion storage transmission calibration. The storage transmission is checked by transferring ions form the ion trap to the ion storage device and back, then scanning in the ion trap. The FT storage transmission calibration can be checked for the positive and negative ion mode independently.
FT Transmission	Use this check box to specify whether or not to check the FT ion transmission calibration. The ion transmission from the ion trap to the Orbitrap is checked by means of the calibration masses in SIM experiments at different AGC target values. The FT transmission calibration can be checked for the positive and negative ion mode independently.
Mass Calibration	Use this check box to specify whether or not to check the mass calibration of the Orbitrap mass analyzer. By doing so, the current mass calibration is checked, that is a check of the external mass calibration. The FT mass calibration can be checked for the positive and negative ion mode independently.
HCD Transmission	Use this check box to specify whether or not to check the HCD transmission calibration. To check the HCD transmission calibration, first make this check box available by clearing the Select All check box. Then, select the HCD Transmission check box.

The Last Check Date readback column gives the date of the last successful check for each item. If a check is performed that fails, the last successful check date still appears in the Last Check Date readback column. The last successful check continues to be in effect in the instrument. However, the result column will show a red x mark indicating that the current attempt check has failed or was aborted.

FT Manual Calibration Page

Use the FT Manual page of the Calibrate dialog box to perform or check an FT mass calibration with user-defined calibration masses. See Figure 3-8.

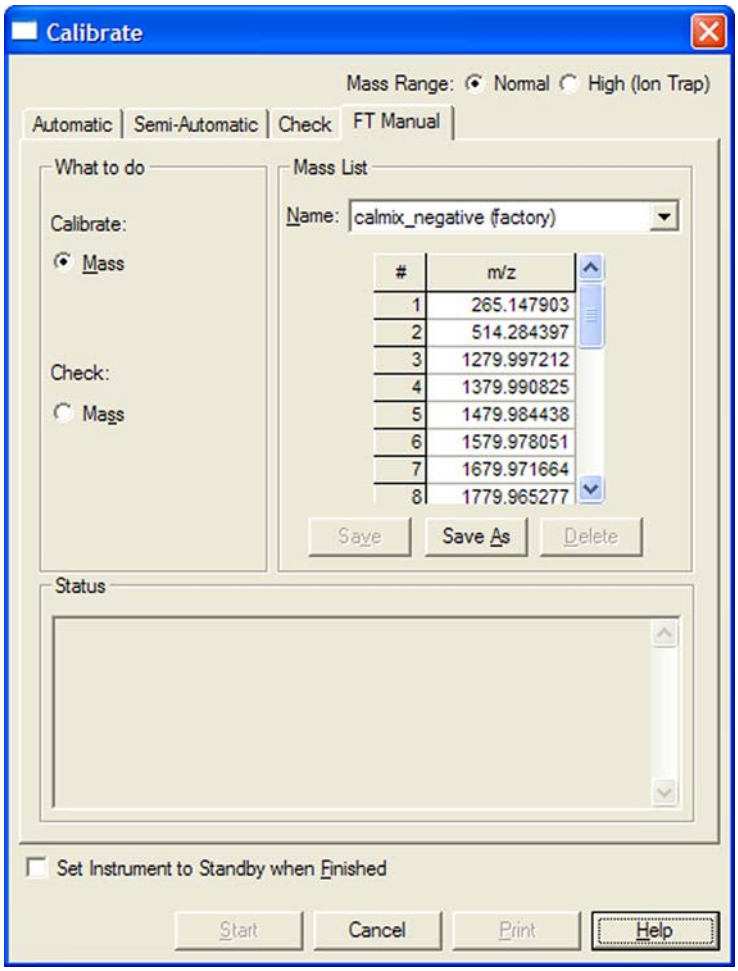


Figure 3-8. FT Manual page of the Calibrate dialog box

All experimental parameters like target values, scan ranges, resolution settings, etc. must be set manually.

Note When starting from the FT Manual page, the calibration is performed for the currently selected polarity only. ▲

Mass List Area

The calibration masses for manual calibration can be defined in the corresponding mass list on the FT Manual page of the Calibrate dialog box. Mass lists can be imported and exported via the Instrument Configuration page. See further details in Chapter 6: "Instrument Configuration".

Note Ensure that you have calculated the calibration masses with sufficient accuracy (sub ppm). ▲

Name This list box lists the names of the factory supplied and user created mass lists.

Mass List This table lists the mass-to-charge ratios of the ions that you are using to calibrate the Orbitrap mass analyzer. You can select an existing mass list in the Name list box, or you can create or modify a mass list by clicking on it and editing the entries in the Mass List table.

Save Click **Save** to save the mass list with the name that is selected in the Name list box.

Save As Click **Save As** a to save the mass list with a new name.

Delete Click **Delete** to delete the mass list that is selected in the Name list box.

Factory-Supplied Mass Lists

There are also two factory supplied mass lists, *calmix_positive (factory)* and *calmix_negative (factory)*. They contain the exact masses of all main ion peaks, which should appear if the negative ion mode calibration solution is used in positive or negative ion mode, respectively.

Chapter 4 Performing Diagnostics/Checks

This chapter describes several diagnostic procedures for the Orbitrap Velos Pro mass spectrometer. It contains the following topics:

System Evaluation Procedures

Use the System evaluation page in the Diagnostics dialog box to evaluate the system performance. See Figure 4-1.

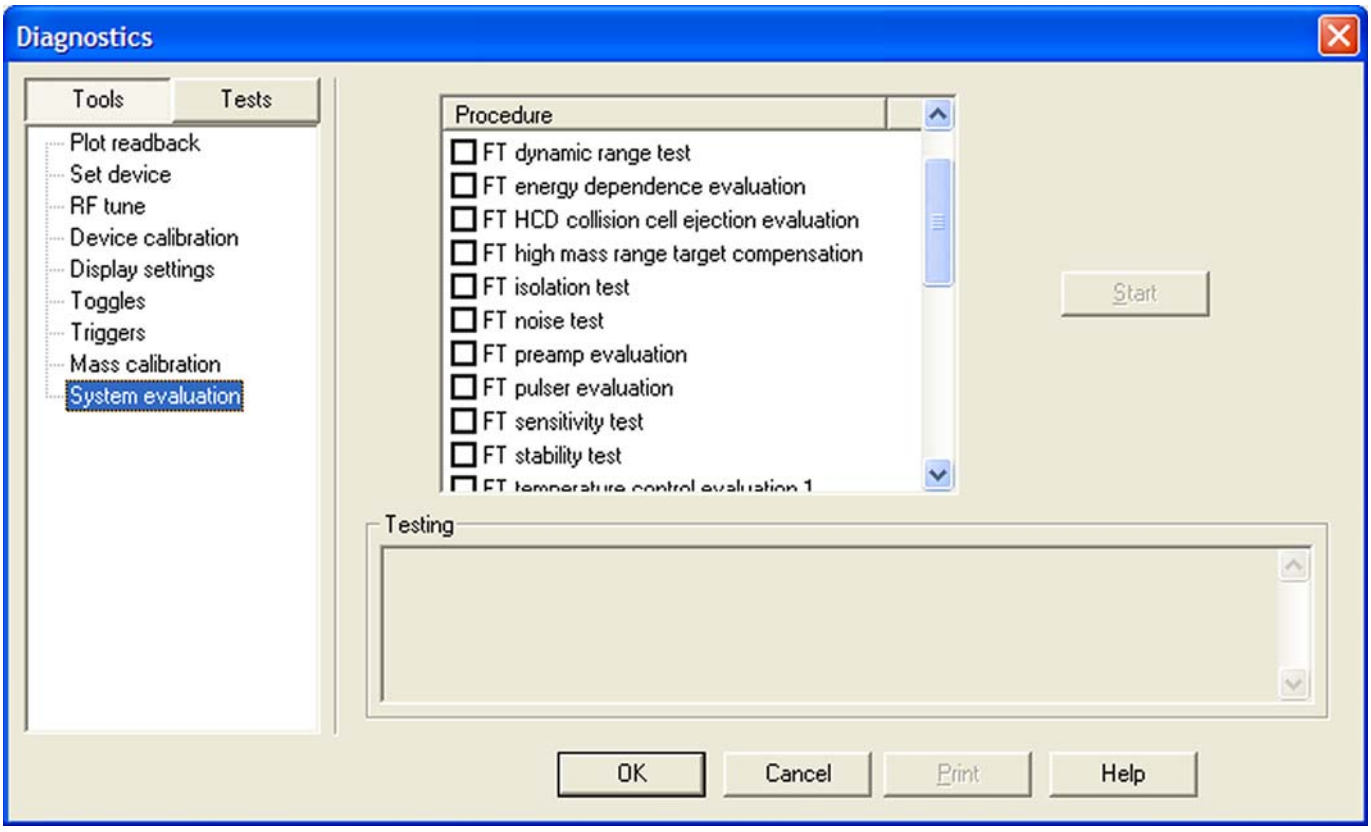


Figure 4-1. System evaluation page of the Diagnostics dialog box (Orbitrap Velos Pro MS)

❖ **To display the System evaluation page**

From the Tune Plus window, choose **Diagnostics > Diagnostics > Tools > System evaluation**.

In addition to several ion trap relevant system evaluation procedures, you can perform various FT system evaluation procedures, which are described in the following topics.

FT CLT-RF Pulser Evaluation

This procedure[1] determines the pulser timing characteristics of the CLT RF board. This procedure requires the infusion of the calibration solution in positive ion polarity mode.

[1]This feature is available only in the Orbitrap Velos Pro ETD mass spectrometer.

FT Dynamic Range Test

This test is only applicable for an infusion experiment with a solution containing MRFA, for example the positive ion mode calibration solution or a MRFA alone solution (for example 5×10^{-6} M in 100% methanol/water, 1% acetic acid). This test determines the signal-to-noise ratio of an isolated MRFA signal.

FT Energy Dependence Evaluation

This procedure determines the change in mass calibration when varying the ion energy (HV Offset). This procedure can help evaluating the FTMS analyzer components.

FT ETD Fragmentation Efficiency

This procedure[1] determines the ETD fragmentation efficiency, which is the percentage of ions that fragment relative to the number of parent ions. The test can run in both ion trap modes and FTMS modes. To run the test, you must infuse a solution of Angiotensin I to generate a spectrum that shows a triply-charged Angiotensin I ion at *m/z* 433 with an intensity of greater than 5E+5.

FT HCD Collision Cell Ejection Evaluation

This procedure analyzes the dynamics of ions in the HCD collision cell. This procedure can help evaluating the HCD collision cell components.

FT HCD Multipole Evaluation

This procedure evaluates the linearity and maximum amplitude of the HCD multipole.

FT High Mass Range Target Compensation

This procedure determines an AGC target compensation factor, which ensures that the FT mass calibration is still valid if the instrument is set into the high mass range mode. The resulting compensation factor will be saved in the calibration file. Usually, it is sufficient to run this procedure once. It is not necessary to repeat this procedure on a regular basis. It is recommended to use the positive ion mode calibration solution for this test. However, you can also use any other solution that gives reasonable ion signals at $1000 < m/z < 2000$.

[1]This feature is available only in the Orbitrap Velos Pro ETD mass spectrometer.

FT Isolation Test

This test is only applicable for an infusion experiment with a solution containing MRFA, for example the positive ion mode calibration solution or a MRFA alone solution (for example 5×10^{-6} M in 100% methanol/water, 1% acetic acid). This test is analogous to the "Check of the ion isolation waveform" on the Check page of the Calibrate dialog box. Here, the isolation of *m/z* 525.3 is performed at an AGC target of 2e+03 and analyzed by the ion trap. In contrast to this, the FT isolation test is performed at higher targets and uses the FT analyzer. Thus this test determines the maximum AGC target value that allows performing a unit isolation of *m/z* 525.3 at the presence of *m/z* 524.3 and 526.3.

FT Noise Test

This test determines resistant noise peaks in the selected scan range. In this test ions are "switched off" automatically. At the conclusion of the FT noise test, a list of resistant noise peaks is displayed in the Testing text.

FT Preamp Evaluation

Use this evaluation to check the basic FTMS analyzer signal detection path. The instrument needs to run in FTMS analyzer mode. Thermo Fisher Scientific recommends switching to diagnostic transient view. See "FT Include Transients" on page 4-9.

During the evaluation, the preamplifier input protection switches are activated with a period of 100 ms. This switching can be observed as periodic incidences in the transient if the electronic signal path is operational.

FT Pulser Evaluation

This procedure tests characteristics of the high voltage pulser. This procedure can help evaluating the FTMS analyzer electronics.

FT Reagent Ion Source Drift Time Evaluation

This evaluation[1] determines the drift time of reagent ions from the reagent ion source to the linear trap mass analyzer. This procedure requires the reagent ion source and filament emission to be on.

[1]This feature is available only in the Orbitrap Velos Pro ETD mass spectrometer.

FT Reagent Ion Source Transfer Multipole Evaluation

This procedure[1] evaluates the linearity and maximum amplitude of the reagent ion transfer multipole.

FT Sensitivity Test

The FT sensitivity test is only applicable for an infusion experiment with reserpine. The test assumes that a reserpine solution of 5×10^{-9} M (100% methanol, 1% acetic acid) is used. The following test are performed one after another:

1. SIM of *m/z* 609.3 using the ion trap as analyzer and an AGC target of 2e+03.
2. SIM of *m/z* 609.3 using the Orbitrap detector as analyzer and an AGC target of 5e+03.
3. SIM of *m/z* 609.3 using the Orbitrap detector as analyzer and an AGC target of 5e+04.
4. MS/MS of *m/z* 609.3 using the Orbitrap detector and an AGC target of 5e+04.

The test fails

- if the inject time, which is necessary to reach the selected AGC target value, is too high;
- if the ratio of the reserpine signal to the overall signal inside the SIM window is too low;
- if the ion transmission from the ion trap to the Orbitrap detector is too low, or
- if the intensity of the product ions of reserpine is too low.

FT Stability Test

This test is applicable for an infusion experiment with any sample solution. This test procedure checks the stability of the FT TIC (total ion current) detected in the selected scan range by means of 600 scans. In principle, the test can be performed at any experimental conditions. It is recommended, however, to perform this test in Full scan mode using one microscan, a resolution setting of 60000 and a FT Full MS Target of 5e+05 or 1e+06. At the conclusion of the FT stability test, the AGC stability and the corresponding signal variation is displayed. See Figure 4-2.

[1]This feature is available only in the Orbitrap Velos Pro ETD mass spectrometer.

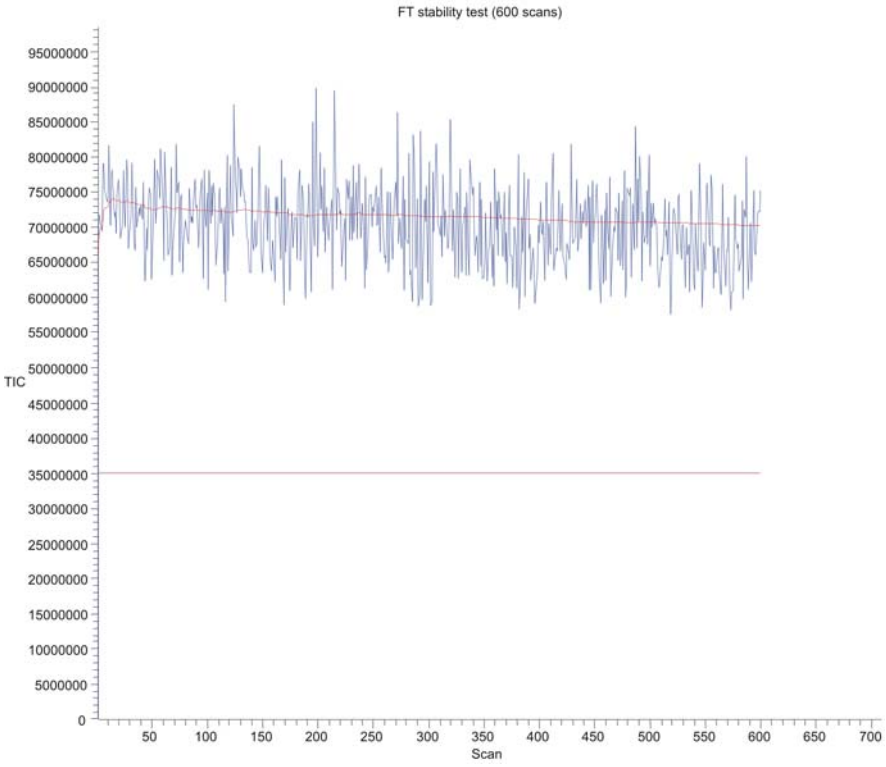


Figure 4-2. Result of the FT stability test displayed in the Graph View

FT Temperature Control Evaluation

Use this evaluation procedure to examine the temperature regulation behavior of the instrument by intentionally driving temperatures to extreme values.

Note The evaluation will usually take more than 12 hours where no measurements can be done. After stopping the evaluation, the instrument needs to stabilize temperatures for several hours before high mass accuracy measurements can be started. ▲

FT Temperature Monitor

Mass accuracy of the Orbitrap detector with external mass calibration depends on a stable temperature of the analyzer and the electronic components. This evaluation plots a history of the temperature regulation results to the Graph view. See "Graph View" on page 2-5.

Reagent CI Gas Pressure Evaluation

This evaluation procedure[1] plots the CI gas pressure of the reagent ion source against the anion intensity; the instrument then calculates the optimum reagent gas pressure. Enter this value into the CI Gas Pressure spin box of the Reagent Ion Source dialog box. See Figure 7-5 on page 7-6.

Note Thermo Fisher Scientific recommends performing this procedure after replacing the filament and/or the ion volume. ▲

[1]This feature is available only in the Orbitrap Velos Pro ETD mass spectrometer.

Toggles

Use the Toggles page in the Diagnostics dialog box to switch a subsystem from one state to another state. See Figure 4-3.

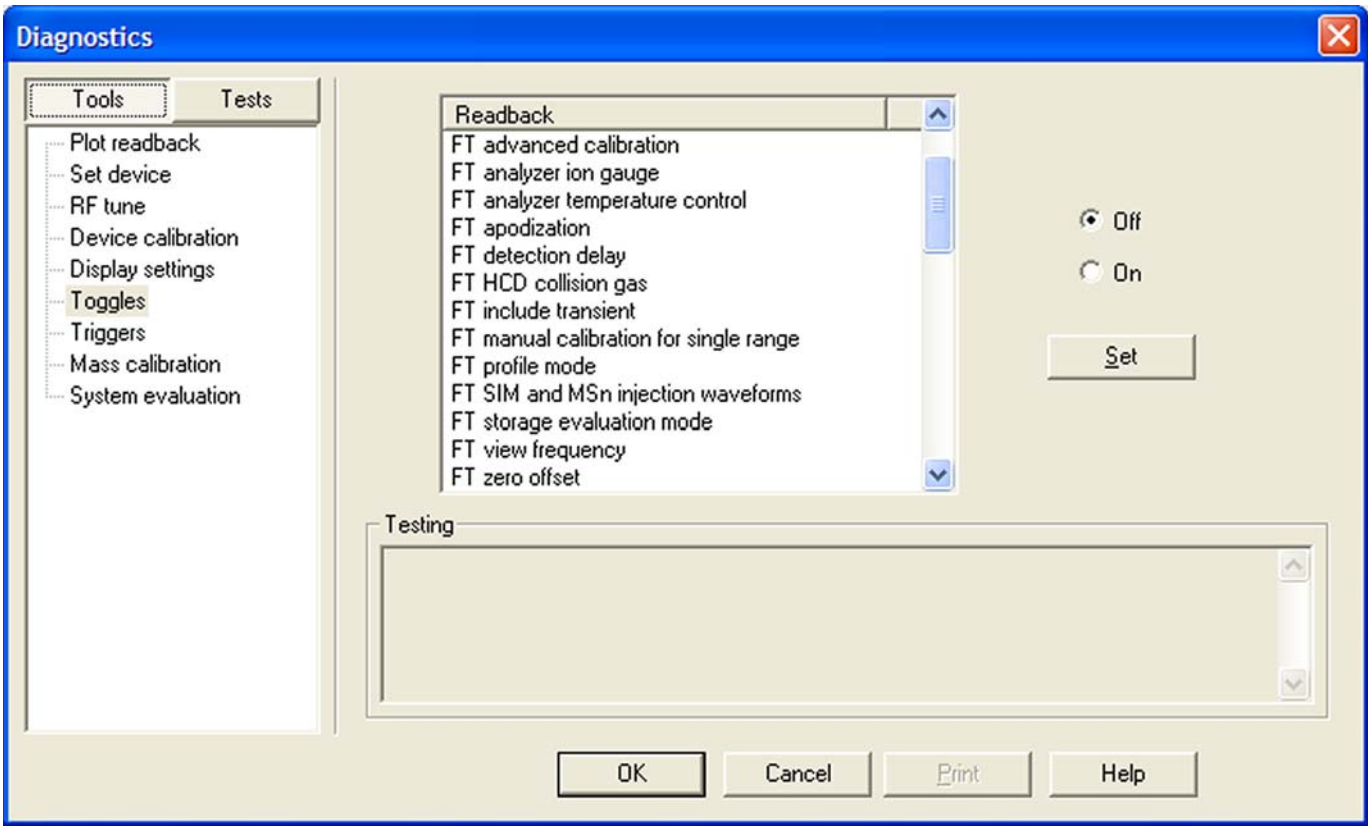


Figure 4-3. Toggles page of the Diagnostics dialog box

❖ **To display the Toggles page**

From the Tune Plus window, choose **Diagnostics > Diagnostics > Tools > Toggles**.

Caution All toggles should only be used for diagnostic purposes. The functionality of the Orbitrap Velos Pro mass spectrometer may be harmed if a toggle is switched to a status that differs from its default value. ▲

If one of the FT toggles is (accidentally) different from its default value during data acquisition, the FT Analyzer Settings of the Scan Header of a raw file contains a reference to this. See Appendix A: "Miscellaneous Information" for further details.

Note The status of a toggle is not saved in the tune method and is set back to its default value after an instrument reset. The toggle state shown by the radio buttons next to the list box does not necessarily correspond to the actual settings. ▲

FT Advanced Calibration

With this toggle, the Advanced Calibration Features are switched on. The default setting is **Off** (disabled).

FT Analyzer Ion Gauge

With this toggle, the ion gauge for the FT analyzer vacuum can be disabled manually for diagnostic purposes. The default setting is **On** (enabled).

FT Analyzer Temperature Control

With this toggle, the FT analyzer temperature control regulation electronics can be disabled for diagnostic purposes. The default setting is **On** (enabled).

FT Apodization

With this toggle, the apodization can be switched on or off. The default setting is **On**.

FT Include Transients

If this toggle is on, it is possible to display transients in the Spectrum view by choosing **Show FT Transient** in the shortcut menu of the Spectrum view. The menu is displayed when you right-click anywhere on that page. See "Spectrum View" on page 2-3 for further details.

Note A transient view is only possible if profile (instead of centroid) is chosen as data format. ▲

During transient display in Spectrum view, the x-coordinate is misleadingly labeled with *m/z* instead of milliseconds. The default setting is **Off**.

Note It is not possible to acquire transients into an Xcalibur raw file. ▲

FT Manual Calibration for Single Range

This toggle can be used to influence the behavior of the FT manual calibration procedures. See "FT Manual Calibration Page" on page 3-22. In the default behavior, the FT manual calibration procedures calibrate the whole scan mass range for the actual polarity.

To be able to use non-standard calibration substances that cover a limited mass range only, advanced users may enable this toggle. With this toggle enabled, there is no check for mass range coverage of reference mass lists. Instead, the instrument stays in the chosen mass range and calibrates this range only. With this toggle enabled, the user is responsible to cover the whole mass range needed, possibly by calibrating manually in several steps with different substances. If the performed FT manual calibration is not suitable for the scan settings used in an FTMS analyzer data acquisition, the scan header of a raw data file contains a reference to this. See Chapter A: "Miscellaneous Information" for further details.

FT Profile Mode

Use this toggle to select whether the FT profile mode corresponds to a Full Profile format or to a Reduced Profile format. It is recommended to use the Reduced Profile Mode for data acquisition because the data size of the raw file is significantly decreased by using the Reduced Profile. The default setting is **Reduced**. For further information, see also "Data Size of FT Raw Files" on page A-4.

FT SIM and MS^n Injection Waveforms

Usually, for FT SIM scans and FT MS^n scans the injection waveforms are automatically enabled. It is not possible to change this setting in the Injection Control dialog box. With this toggle, it is possible to disable or enable the injection waveforms manually for diagnostic purposes. The default setting is **On**.

FT Storage Evaluation Mode

Use this toggle to test the characteristics of ion transfer components for the FTMS analyzer. The default setting is **On** (enabled).

FT View Frequency

If this toggle is switched on, the FT spectrum is shown as a frequency spectrum. If the system is on and the FT is chosen as analyzer, the frequency spectrum is displayed in the Spectrum view. The default setting is **Off**.

Note The x-coordinate is misleadingly labeled with *m/z* instead of kHz. ▲

This toggle is for diagnostic purposes only. Therefore, it is not possible to acquire frequency spectra.

FT Zero Offset

If this toggle is switched on, an offset is added to the spectrum. This enables to view the full noise band. The default setting is Off, if the Reduced Profile format is used. The setting is On, if the Full Profile format is used.

Isolate Reagent Ion

Use this toggle[1] to enable manually adjusting the Back Multipole DC Offset voltage. See "Tuning the Quadrupole Mass Filter" on page 7-21. The default setting is **Off**.

Reagent Ion AGC

If this toggle[1] is switched on, the instrument automatically performs an AGC scan of the reagent ion source in regular intervals. The default setting is **Off**.

[1]This feature is available only in the Orbitrap Velos Pro ETD mass spectrometer.

Set Device

Use the Set device page of the Diagnostics dialog box to select devices or experimental parameters from the list and to set the value for that device or parameter. See Figure 4-4.

❖ **To set a device**

1. Select the device or parameter you would like to set from the Device list box.
2. Enter the device parameter's value in the text box below the Device list box.
3. Click **Set** to apply the change to the device value or parameter.

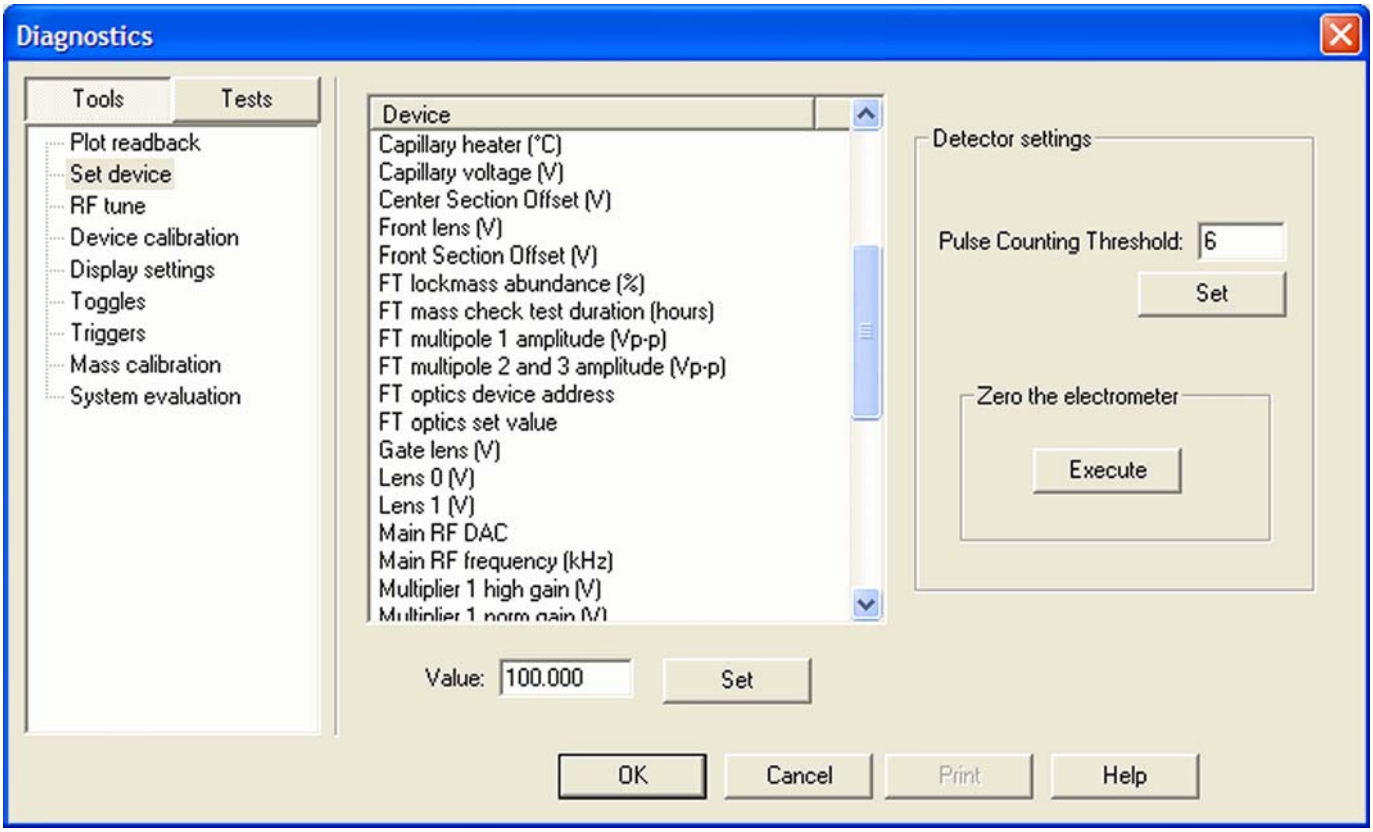


Figure 4-4. Set device page of the Diagnostics dialog box

Note The value in the text box below the Device list box, which is displayed after the call of this page, does not necessarily correspond to the actual value. ▲

The following topics describe several FT relevant parameters that may be changed from this page.

Note After an instrument reset, the manual settings are overwritten with the corresponding calibration parameters. ▲

Caution Changing the instrument settings can harm the functionality of the Orbitrap Velos Pro mass spectrometer, especially if followed by saving the calibration parameters (manually or at conclusion of a calibration procedure). Thus, this option should only be used by very advanced users. ▲

FT Lockmass Abundance

Use this device to change the target value of an injected lock mass relative to the actual FT scan target value. The default is 10%. The recommend target value is 0%, which means that injection of lock masses is turned off completely. Also see "Locking" on page 2-11.

FT Mass Check Test Duration

Use this device to change the duration of FT Manual mass calibration checks. See "FT Manual Calibration Page" on page 3-22. By changing this value, a long-term mass stability evaluation can be run. The default procedure of the FT manual mass calibration check is to perform 100 scans checking the mass accuracy. The test duration may be extended to up to 72 hours. The default procedure can be restored by setting the duration to zero. If the duration is set between two and 24 hours, the FT manual mass calibration check will specially control the syringe pump to allow running long-term test with a single syringe filling. For durations above 24 hours, it is assumed that an external syringe pump is used.

Setting new FT Transfer Optics Parameters

Two set device items can be used to overwrite the FT optics values:

- FT Optics Device Address
- FT Optics Set Value

These values are originally determined during instrument calibration and set automatically. Overwriting calibration values will influence instrument performance and should only be done for diagnostic purposes.

Note If you have changed FT transfer optics settings, Thermo Fisher Scientific recommends performing a full FT instrument calibration afterwards to assure good instrument performance. ▲

Display Settings

Use the Display Settings page in the Diagnostics dialog box to select a variety of instrument settings for display. See Figure 4-5. Select the instrument settings you want to display and click **Start**. The Orbitrap Velos Pro mass spectrometer displays the requested instrument settings in the Testing text box.

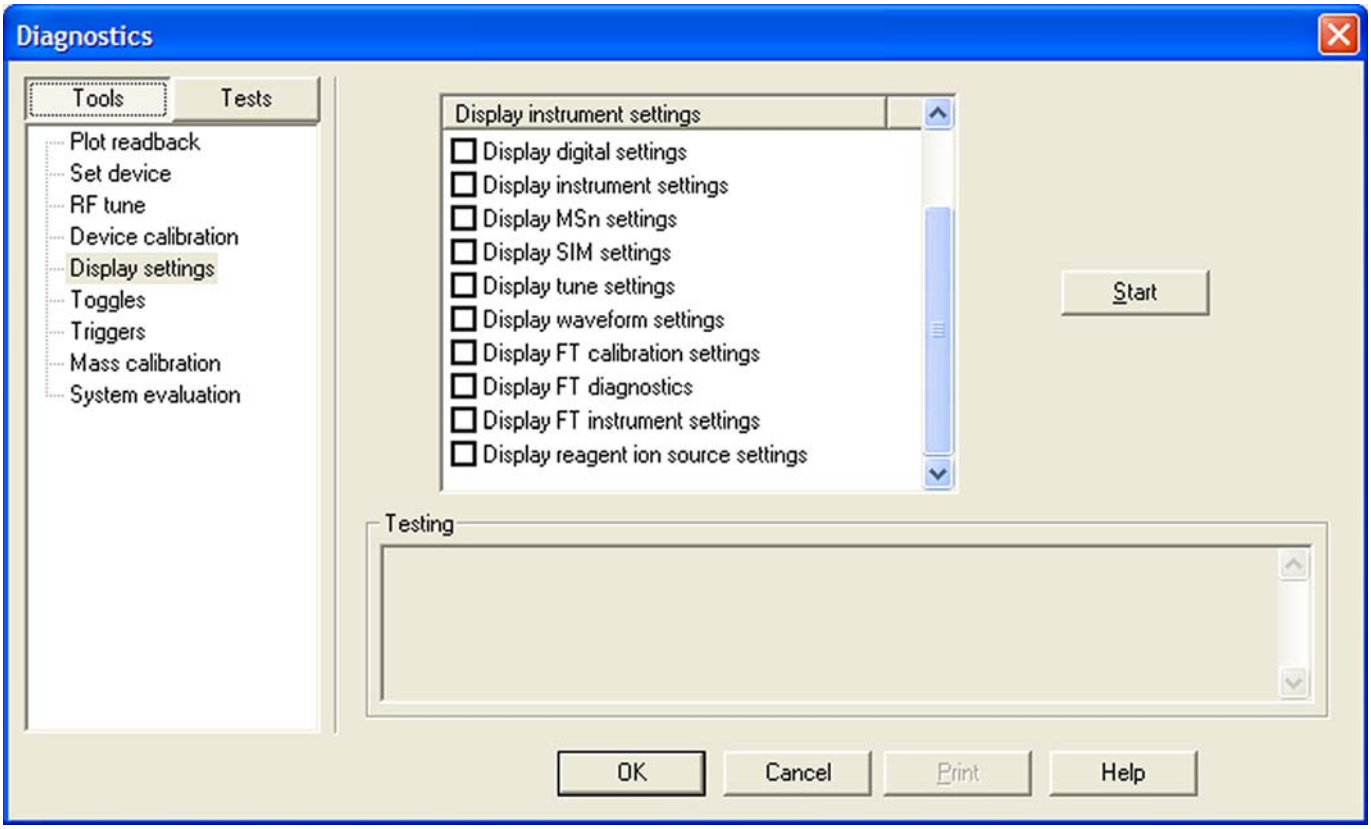


Figure 4-5. Display settings page of the Diagnostics dialog box

The following FT relevant instrument settings can be displayed:

Display FT calibration settings Displays all FT relevant calibration parameters in the diagnostics text box.

Display FT diagnostics Displays current diagnostic readback values of the FT electronic boards.

Display FT instrument settings Displays the current values of those FT instrument settings that depend on the scan range and ion polarity mode and can be changed manually on the Set Device page of the Diagnostics dialog box.

In the Orbitrap Velos Pro ETD mass spectrometer, an additional item is available:

Display reagent ion source settings	Displays the current values of the reagent ion source parameters.

Chapter 5 Instrument Setup

This chapter explains the "Locking" feature in automated runs and describes the FT relevant topics of the data dependent settings in the Instrument Setup. It contains the following topics:

Using Locking in Automated Runs

To use locking in an automated run, use the Instrument Setup program. See Figure 5-1.

Note See "Locking" on page 2-11 for a basic description on using locking with FTMS analyzer scans. ▲

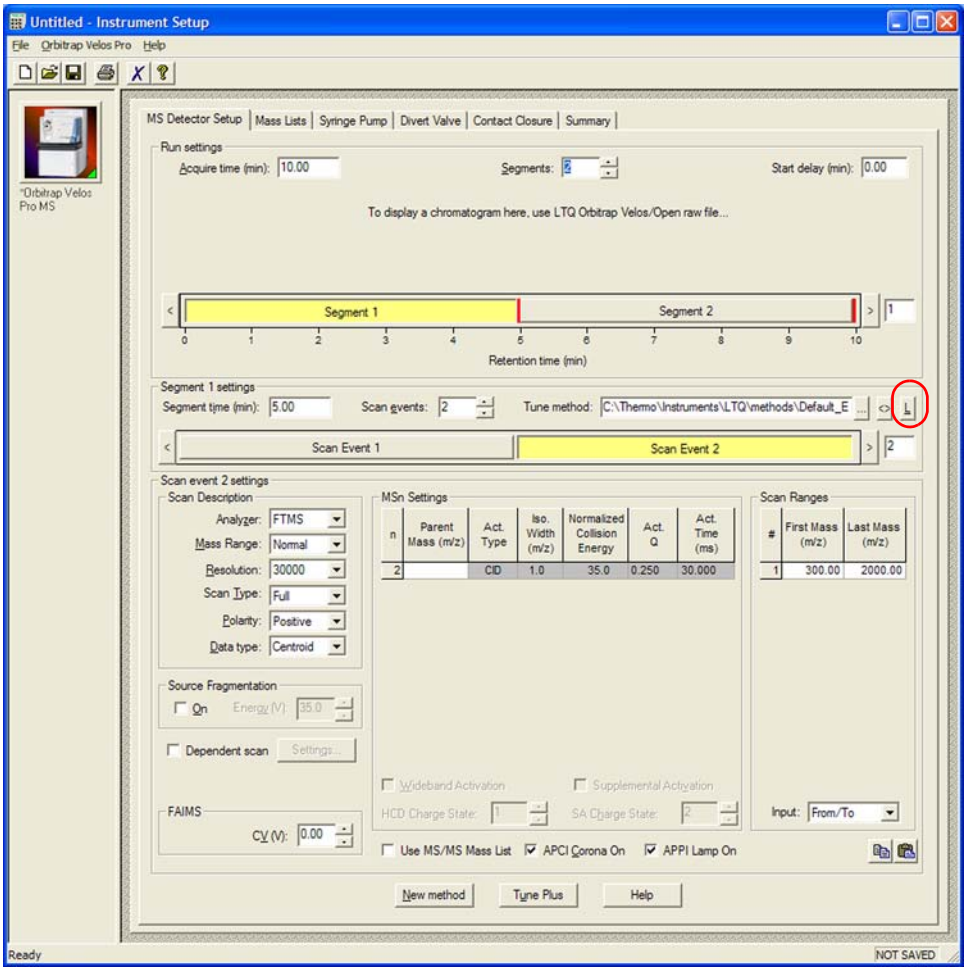


Figure 5-1. MS Detector Setup View—MS Detector Setup Page

The Lock Mass List button on the right side of the Segment settings area displays a lock mass list editor dialog. See Figure 2-9 on page 2-11. Segment-related lock masses can be entered here. There are separate lists for positive ion and negative ion mode. If the lock mass for a segment is empty, no locking will be applied in the run and the external mass calibration will be used.

Data Dependent Settings

This section describes the FT relevant topics of the data dependent settings in the Instrument Setup.

Using Masses instead of Mass-to-Charge Ratios

Figure 5-2 shows the Global page of the Data Dependent Settings dialog box. Use the page to select dependent scan settings that apply to all dependent scans.

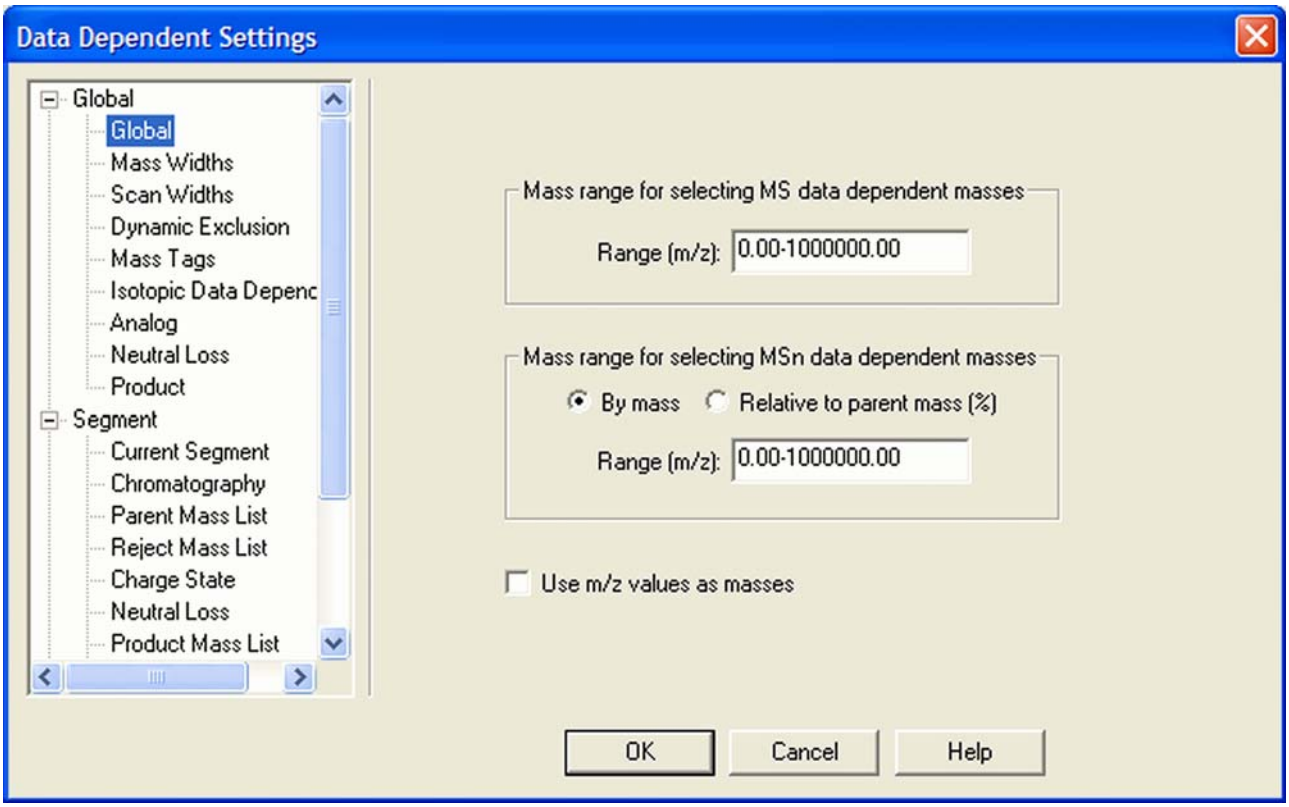


Figure 5-2. Data Dependent Settings dialog box—Global page

Instrument Setup allows using mass units in the input fields of dialog boxes instead of mass-to-charge ratios. To enable this feature, select the check box on the Global page and click **OK**. From now on, you can enter just the uncharged masses of the most intensive peaks. It is not necessary anymore to consider all possible charge states. The Orbitrap Velos Pro mass spectrometer determines the charge state from the full scan and converts these masses to mass-to-charge ratios. Enabling this feature affects the parameters in the data dependent settings as described below.

Mass Units in Global Parameters

Global	Mass Range is interpreted as masses. Therefore, a mass range of *m/z* 500–2000 will allow selecting a M^{2+} peak at *m/z* 251, but will ignore a M^{2+} peak at *m/z* 1001.
Mass Widths	Exclusion mass widths are interpreted as masses. Note that the isotope exclusion functionality will be based on masses. Therefore, if both a M^{2+} and M^{3+} peak show up for the same peptide in a full scan, only the more abundant charge state will be selected. Parent mass widths are interpreted as masses. Reject mass widths are interpreted as masses.
Dynamic Exclusion	Dynamic exclusion mass widths are interpreted as masses. When a peak is dynamically excluded, the neutral mass is put on the list. Therefore, other charge states of the same mass will be excluded in future cycles.
Mass Tags	Mass deltas are interpreted as masses. The mass deltas are converted back to mass-to-charge ratios based on the detected charge state of the selected peak. The partner must also have the same charge state. Mass tolerances are based on exclusion mass widths.
Isotopic Data Dependence	Mass differences are interpreted as masses. The mass differences are converted back to mass-to-charge ratios based on the detected charge state of the selected peak.
Neutral Loss	Neutral loss mass widths are interpreted as masses.
Product	Product mass widths are interpreted as masses.

Mass Units in Segment Parameters

Parent Mass List	Input will be interpreted as neutral masses—not $[M+H]^+$ or $[M-H]^+$. If "MS Charge State" is specified in the mass list, this value is used to convert the mass back to a mass-to-charge ratio. If this is not specified, masses are converted to mass-to-charge ratios based on all charge states that are not on the rejection list. "4 and up" is an allowed charge state, but the search is only performed for 4, and not 5, 6, etc. If more than one charge state is identified, only the most abundant is selected.
Reject Mass List	Input will be interpreted as neutral masses—not $[M+H]^+$ or $[M-H]^+$.
Charge State	Because masses must be converted back to mass-to-charge ratios, the charge state must be known for any peak that is analyzed. Therefore, rejection of unassigned charge states is automatically enforced.
Neutral Loss	Masses are interpreted as masses. The charge state used to convert back to mass-to-charge ratio is based on the peak being analyzed. The charge state of the potential partner must match the charge state of the peak.
Product Mass List	Input will be interpreted as neutral masses—not $[M+H]^+$ or $[M-H]^+$.
Add/Sub	Mass is interpreted as a mass and is converted to mass-to-charge based on the charge state of the selected peak.

Preview Mode

Figure 5-3 shows the Current Segment page of the Data Dependent Settings dialog box.

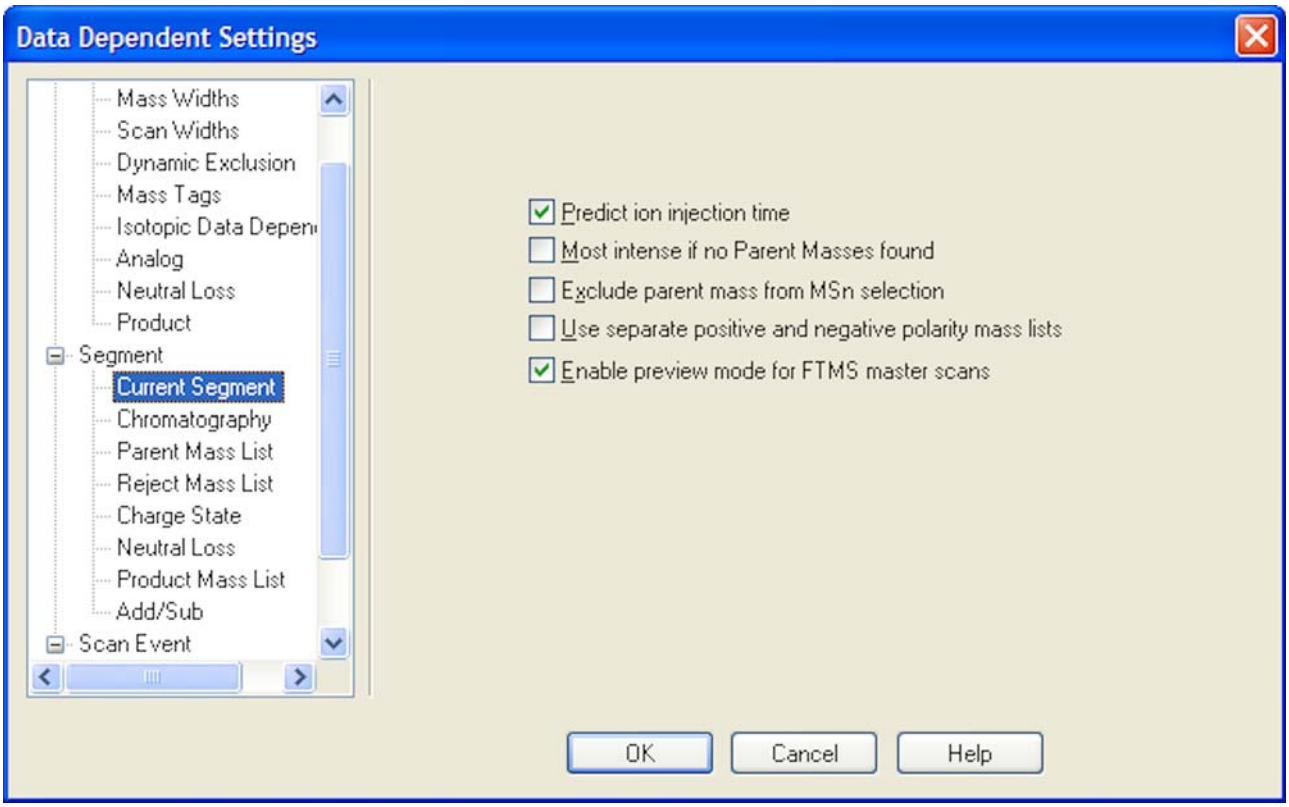


Figure 5-3. Data Dependent Settings dialog box—Current Segment page

If the preview mode for FTMS master scans is enabled on the Current Segment page, the data dependent decision is made on the basis of the FT master scan with lower resolution to increase the duty cycle. The resolution of the FTMS scan itself is not changed. Because the high resolution is usually not required to make the data dependent decision, it is recommended to enable the preview mode.

To prevent making data dependent decisions on basis of lower-resolution preview spectra, disable this option. For example, if there are ions with high charge states to be examined, and the data dependent settings require charge state recognition of precursor ions, this might be a reason to turn off this option. Otherwise, the high charge state clusters may not be resolved and charge states will not be recognized in preview mode.

Monoisotopic Precursor Selection

Figure 5-4 shows Charge State page of the Data Dependent Settings dialog box.

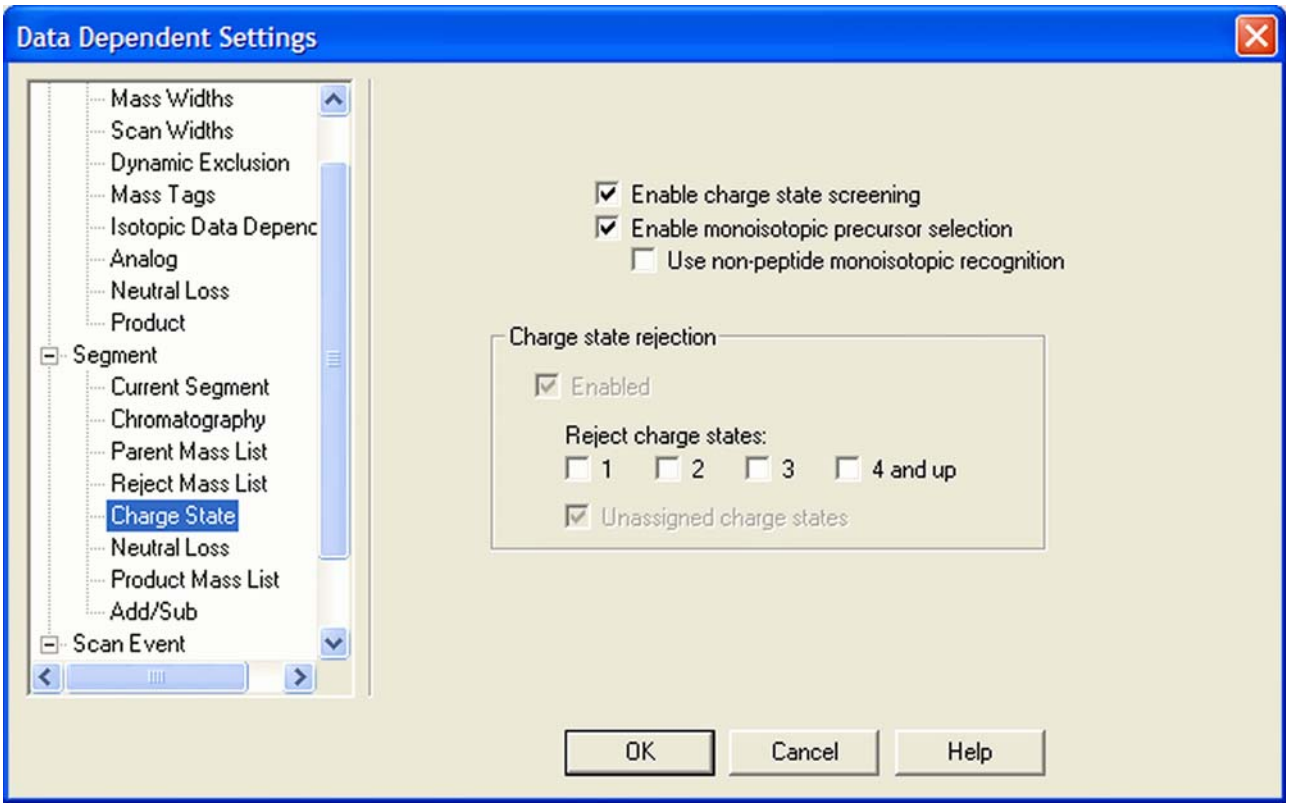


Figure 5-4. Data Dependent Settings dialog box—Charge State page (Advanced Features on)

If the monoisotopic precursor selection is enabled on the Charge State page, the data dependent scan is only performed for one molecular ion of the corresponding overall isotopic distribution if Dynamic Exclusion is enabled.

This check box is only available on the Charge State page if the Advanced Features are turned on in the Orbitrap Velos Pro menu of the Instrument Setup.

Note The algorithm for isotopic cluster recognition will handle correctly only such isotope distributions where the third peak (A + 2) is lower in intensity than the second peak (A + 1). For more complex isotope patterns (for example, ion containing Sn, Br, or multiple Cl atoms), it is recommended to clear the Enable monoisotopic precursor selection check box. ▲

Use Non-Peptide Monoisotopic Recognition

This check box is only available if monoisotopic precursor selection is active. If monoisotopic precursor selection is active and this box is not checked, precursor ions in FT master scans must match peptide-type isotopic distribution to identify the monoisotopic peak. If this box is selected, monoisotopic peaks will also be identified for small molecules and precursor ions with non-peptide-type isotopic distributions.

Enabling Charge State Dependent ETD Time

For data-dependent scans that use ETD activation, you can allow the instrument to adjust the reaction time according to the charge state of the ions. Select this check box[1] to have the instrument reduce the reaction time for highly charged ions and increase it for lowly charged ions.

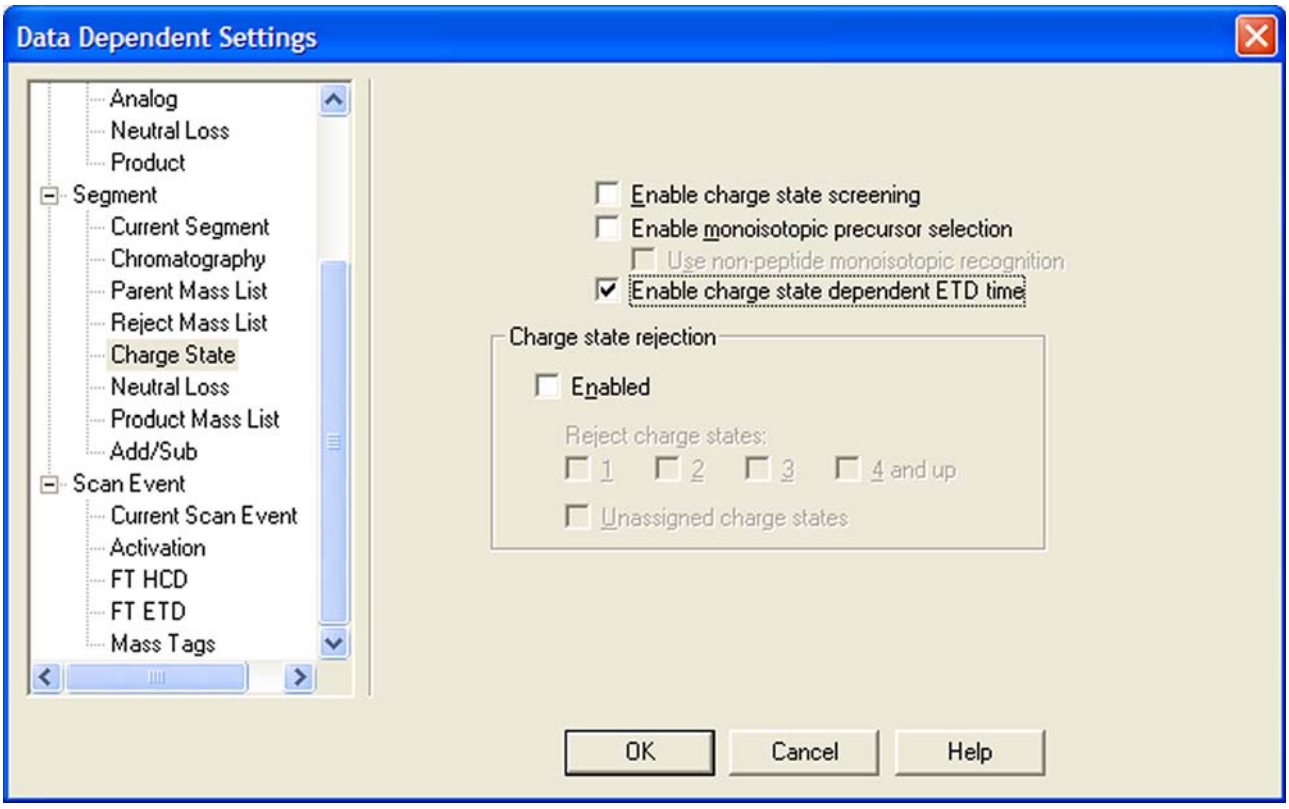


Figure 5-5. Enabling charge state dependent ETD time

[1]This feature is available only for the Orbitrap Velos Pro ETD mass spectrometer.

Data Dependent FT SIM Scans

A data dependent FT SIM scan is performed around the center mass determined in a previous reference scan event if the check box "Same MS order as referenced scan event" is selected on the Current Scan Event page as shown in Figure 5-6.

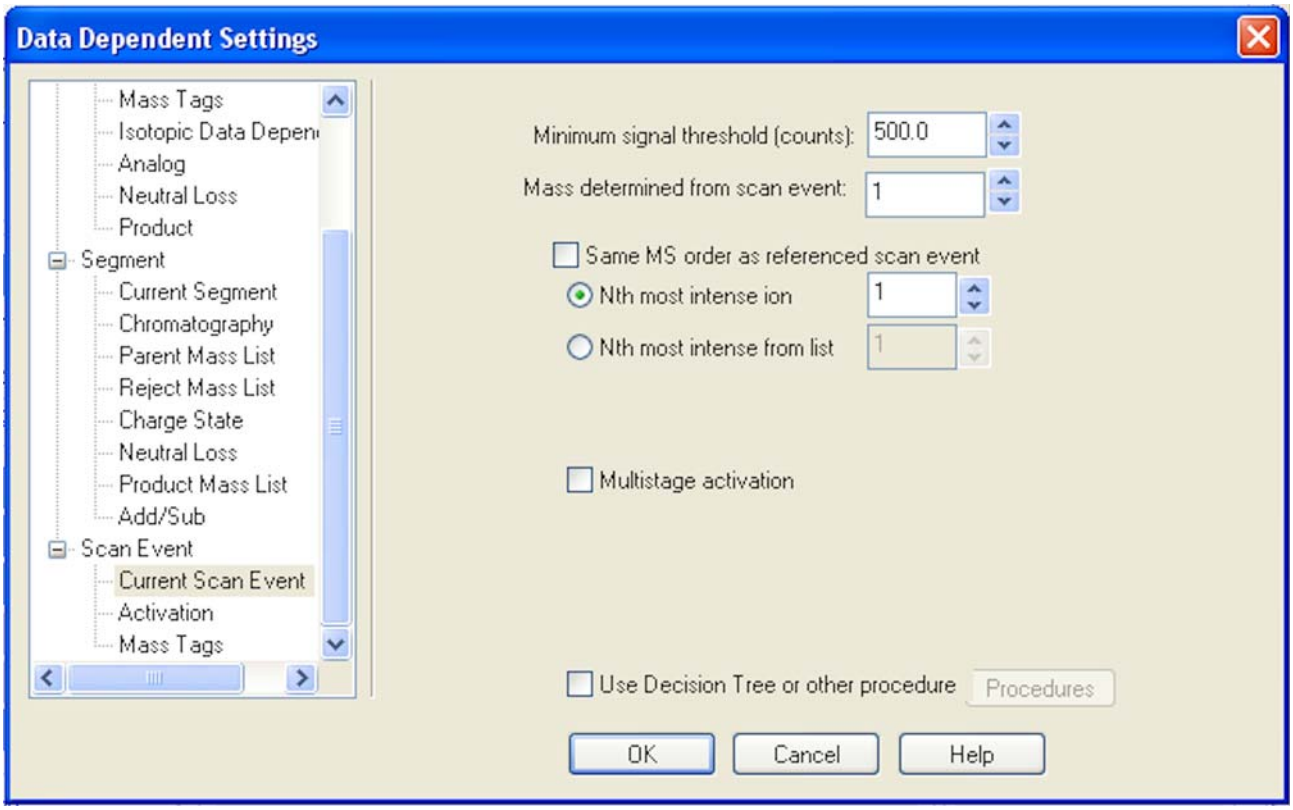


Figure 5-6. Data Dependent Settings dialog box—Current Scan Event page

Repeat previous Scan Event with HCD

The Repeat previous scan event with HCD check box will only be available when all of the following requirements are fulfilled:

- Select an experiment type other than data dependent triple play, data dependent neutral loss MS3, or data dependent product MS3.
- Select a scan event greater than or equal to 3.
- Specify one or more parent ions in a previous scan event.
- FTMS is selected as the mass analyzer for the current scan event.
- A neutral loss or product ion list is specified for the referenced scan event.
- Either option From neutral loss list or From product list is selected.

- HCD is selected as activation type on the Activation page. See page 5-13.

Select the check box to repeat the previous scan event with HCD activation type. A typical experiment to use this feature would be to trigger an HCD fragmentation experiment with a neutral loss observed in an ion trap MS/MS experiment. See Figure 5-7.

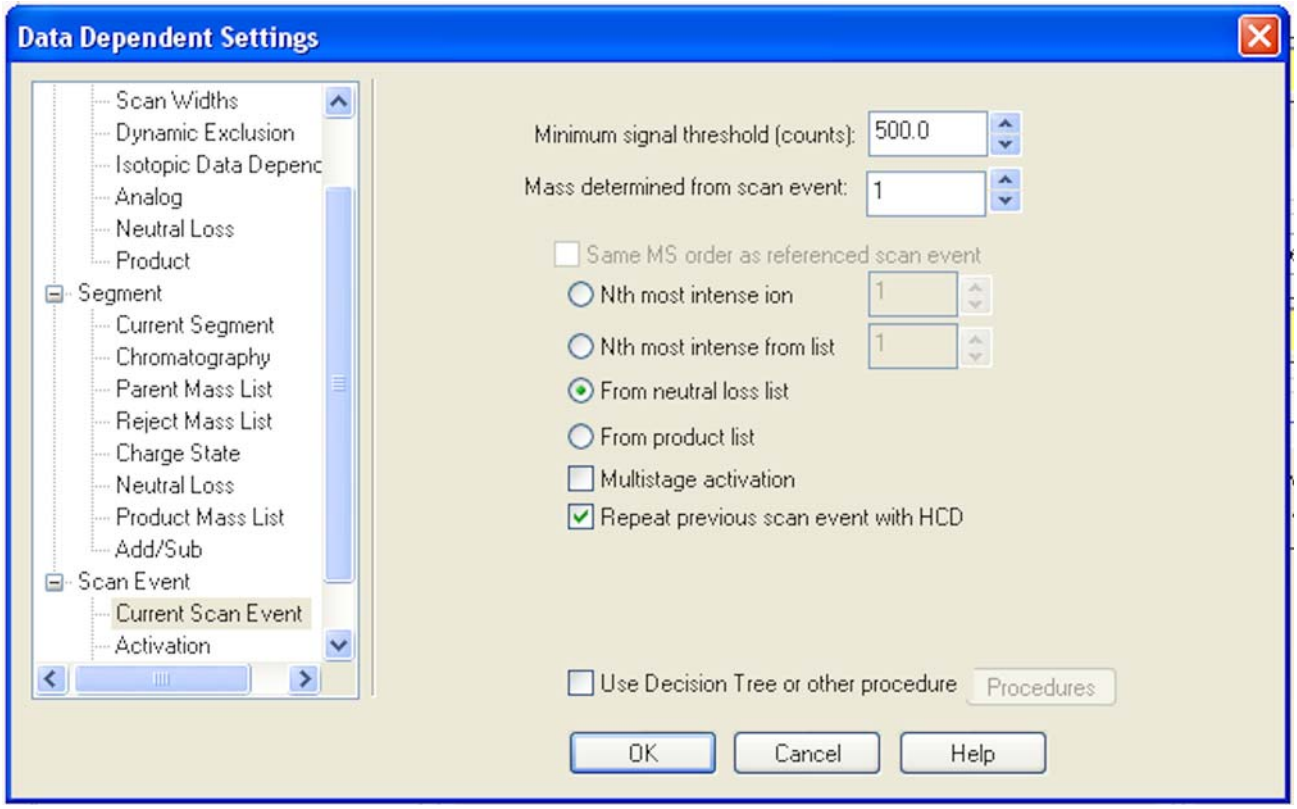


Figure 5-7. Current Scan Event page—Repeat previous scan event with HCD

Repeat previous Scan Event with ETD

The Repeat previous scan event with ETD check box[1] will only be available when all of the following requirements are fulfilled:

- A neutral loss or product ion list is specified for the referenced scan event.
- Either option From neutral loss list or From product list is selected.
- ETD is selected as activation type on the Activation page. See page 5-13.

[1]This feature is available only for the Orbitrap Velos Pro ETD mass spectrometer.

Select the check box to repeat the previous scan event with ETD activation type. A typical experiment to use this feature would be to trigger an ETD fragmentation experiment with a neutral loss observed in an ion trap MS/MS experiment. See Figure 5-8.

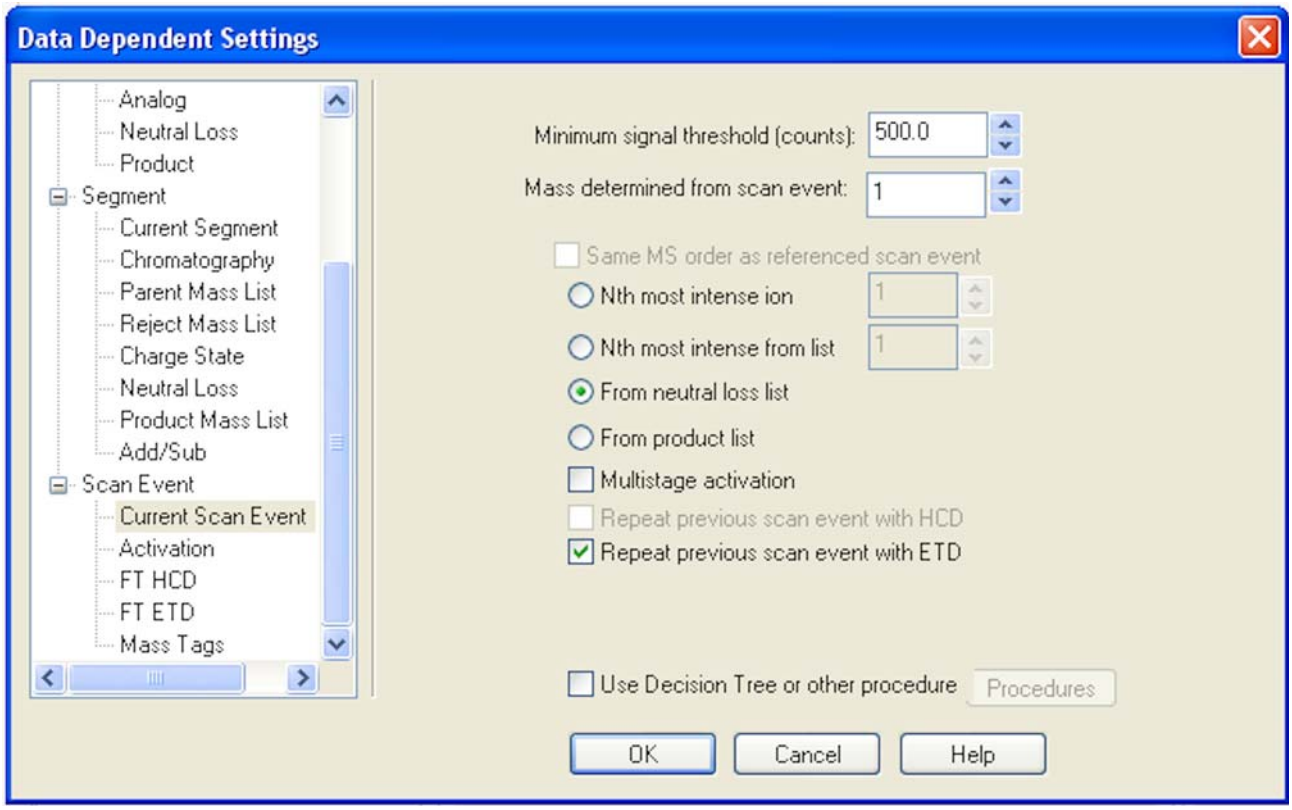


Figure 5-8. Current Scan Event page—Repeat previous scan event with ETD

Scan Width

The scan width of the data dependent FT SIM scan can be selected on the Scan widths page of the Data Dependent Settings dialog box. See Figure 5-9.

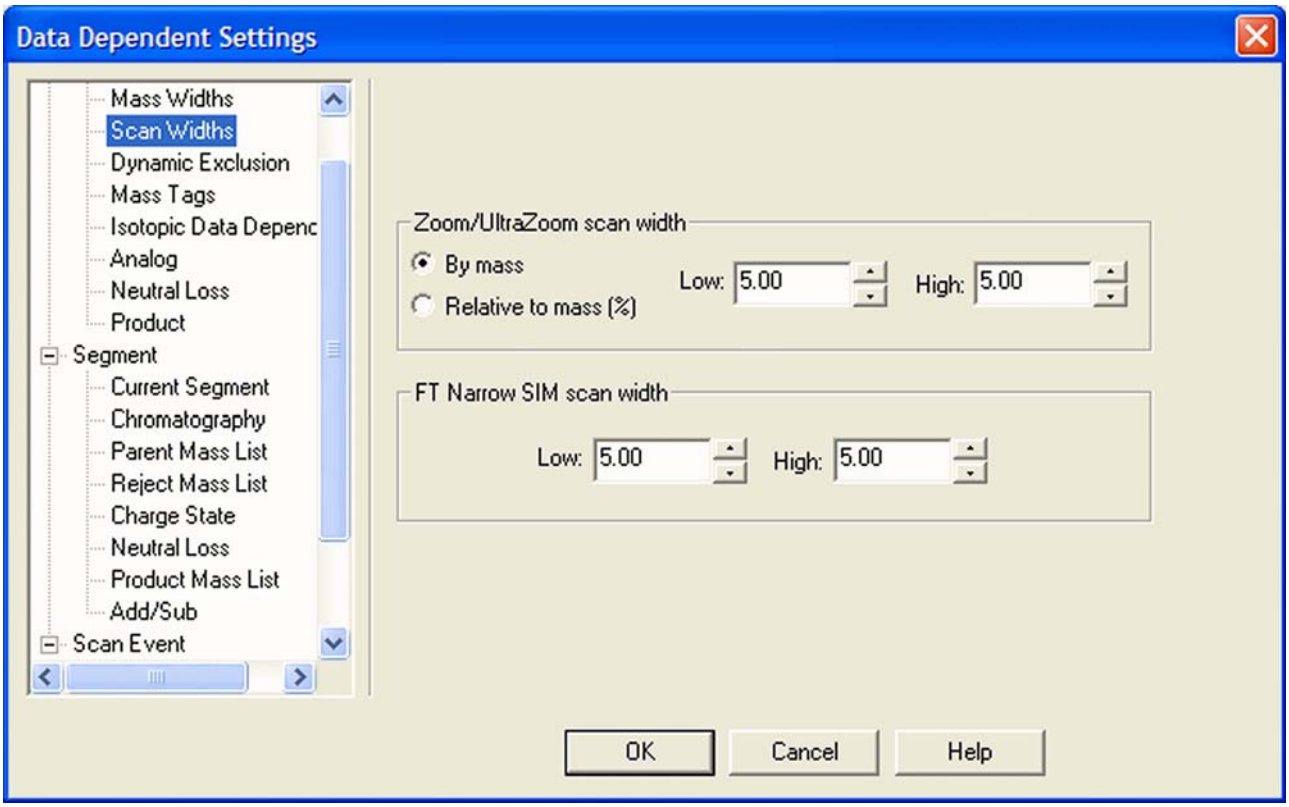


Figure 5-9. Data Dependent Settings dialog box—Scan Widths page

Activation Type

On the Activation page of the Data Dependent Settings dialog box, use the Activation type list box to specify how the ions are activated for fragmentation during a data dependent experiment. See Figure 5-10. It has the following options:

- CID (Collision-induced dissociation)
- PQD (Pulsed-Q dissociation)
- ETD (electron transfer dissociation)[1]

  Use ETD to fragment peptides and proteins. After selecting ETD, Normalized collision energy and Activation Q are not available and Supplemental Activation becomes available. See Figure 7-36 on page 7-39.

- HCD (higher energy CID)

  Use HCD to obtain triple quadrupole-like fragment ion spectra. If you select HCD, the Activation Q spin box becomes unavailable.

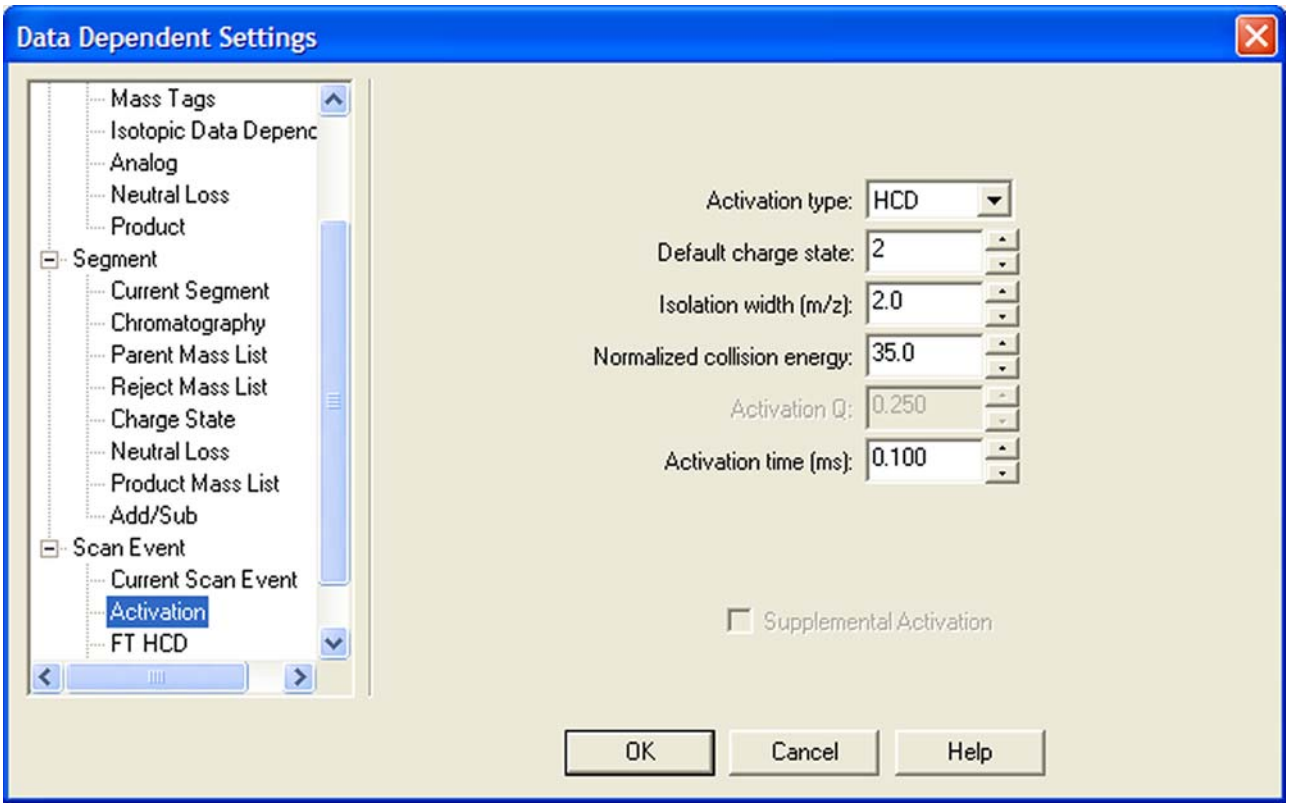


Figure 5-10. Data Dependent Settings dialog box—Activation page

[1]This feature is available only for the Orbitrap Velos Pro ETD mass spectrometer.

FT HCD

The FT HCD page of the Data Dependent Settings dialog box will only be available when FTMS is selected as the mass analyzer for the current scan event. See Figure 5-11.

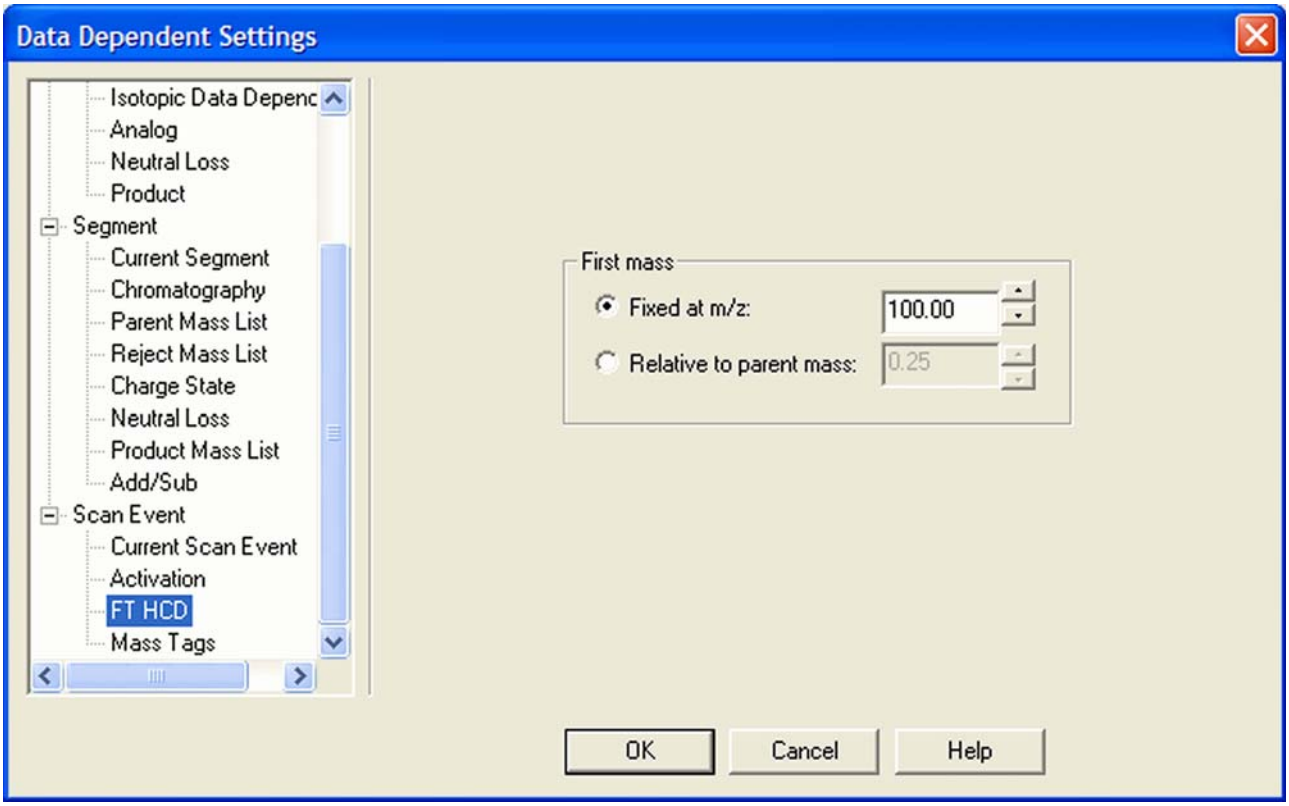


Figure 5-11. Data Dependent Settings dialog box—FT HCD page

The FT HCD page offers two modes for choosing the first mass of the dependent scan:

- a mass with a fixed *m/z* value

  To change the *m/z* value, click the arrows in the spin box to increment [up arrow] or decrement [down arrow] the value. You can set *m/z* to any value from 50 to 4000; default is 100. Alternatively, enter a value in the spin box text field.

- a mass with an *m/z* value that is relative to the precursor mass.

  To change the percentage, click the arrows in the spin box to increment [up arrow] or decrement [down arrow] the value. You can set the percentage to any value from 0 to 4; default is 0.25. Alternatively, enter a value in the spin box text field.

Note The highest *m/z* that the Orbitrap Velos Pro mass spectrometer can analyze is equal to approximately 20 times the *m/z* of the first mass. ▲

FT ETD

The FT ETD page[1] of the Data Dependent Settings dialog box will only be available when FTMS is selected as the mass analyzer for the current scan event. See Figure 5-12.

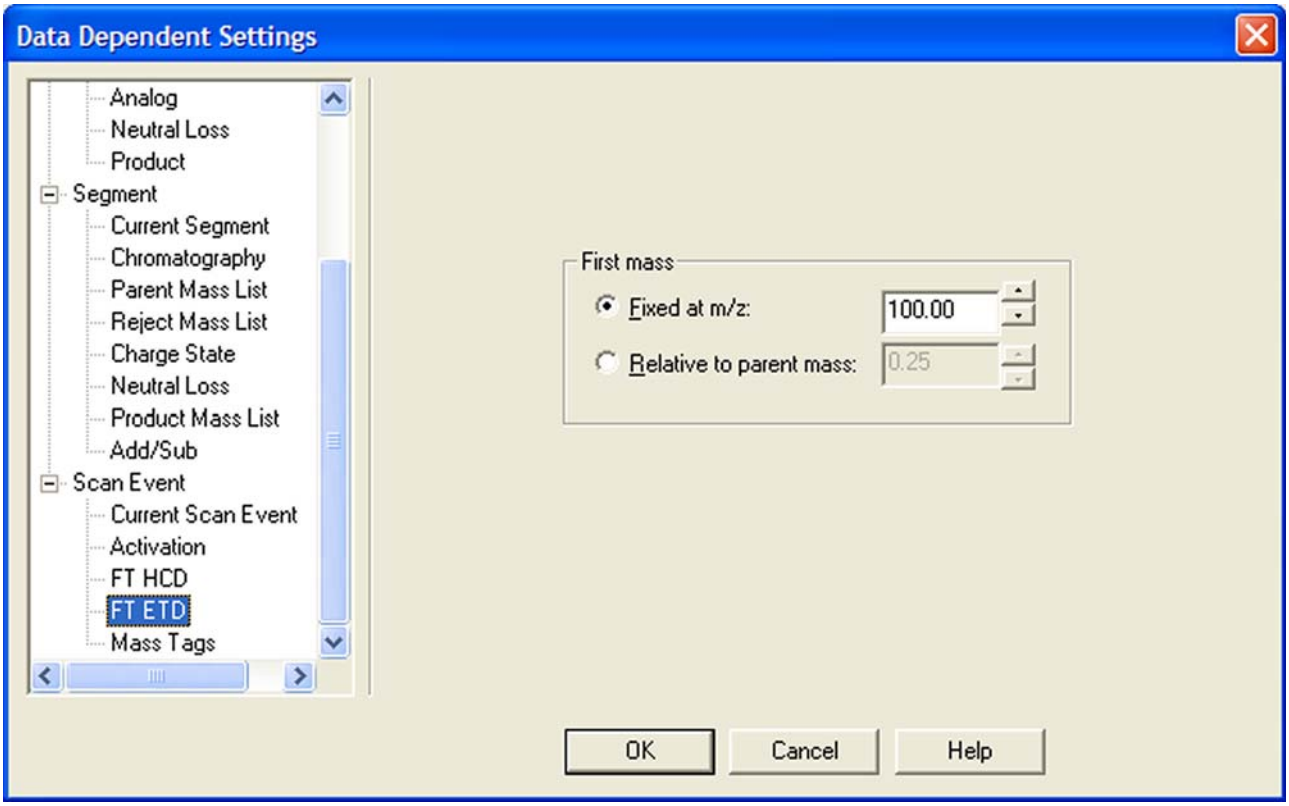


Figure 5-12. Data Dependent Settings dialog box—FT ETD page

Note The highest *m/z* that the Orbitrap Velos Pro mass spectrometer can analyze is equal to approximately 20 times the *m/z* of the first mass. ▲

The FT ETD page offers two modes for choosing the first mass of the dependent scan:

- a mass with a fixed *m/z* value

  To change the *m/z* value, click the arrows in the spin box to increment [up arrow] or decrement [down arrow] the value. You can set *m/z* to any value from 50 to 4000; default is 100. Alternatively, enter a value in the spin box text field.

[1]This feature is available only for the Orbitrap Velos Pro ETD mass spectrometer.

- a mass with an *m/z* value that is relative to the precursor mass.

  To change the percentage, click the arrows in the spin box to increment [up arrow] or decrement [down arrow] the value. You can set the percentage to any value from 0 to 4; default is 0.25. Alternatively, enter a value in the spin box text field.

MS^n Settings for HCD Experiments

Similar to the Define Scan dialog box, use the Instrument Setup to select the Activation type (CID/PQD/HCD). See "MS^n Settings" on page 2-12. Use HCD to obtain triple quadrupole-like fragment ion spectra. If you select HCD as activation type, the HCD charge state spin box becomes available and the Activation Q spin box is disabled. See Figure 5-13.

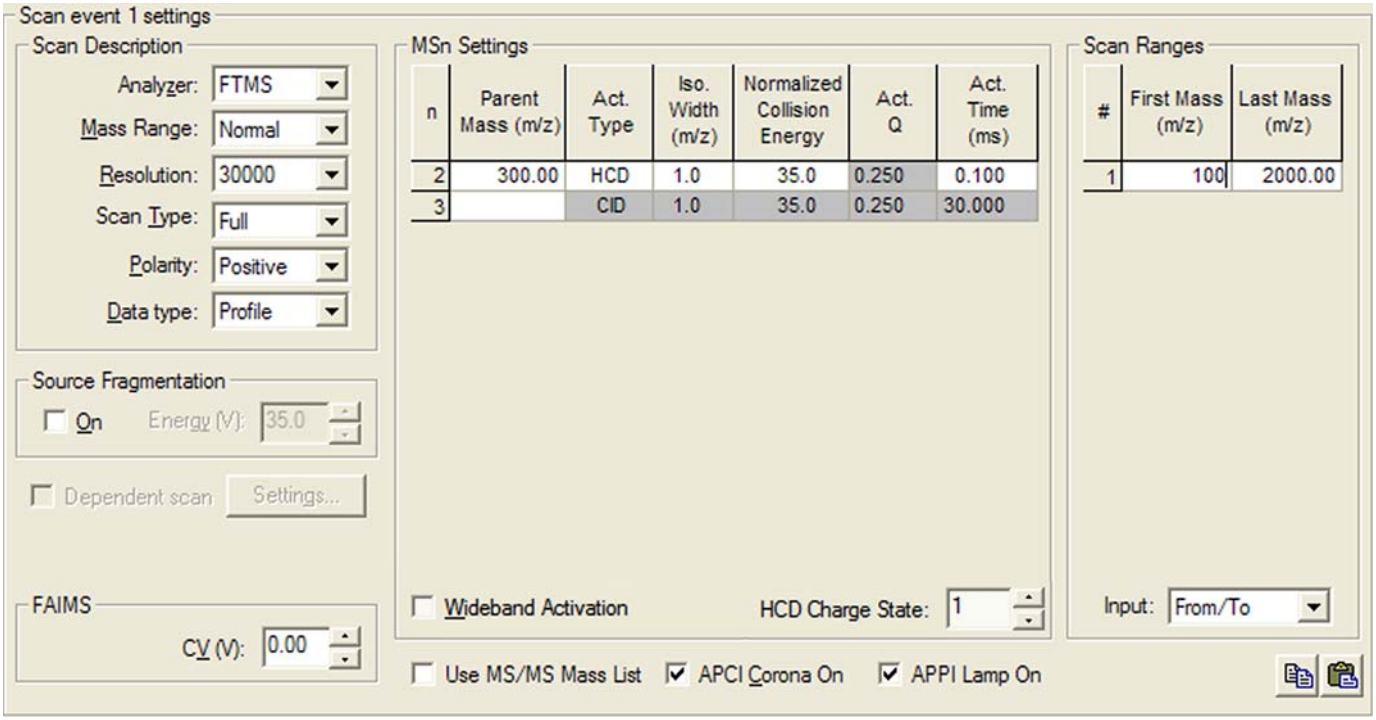


Figure 5-13. MS Detector Setup Page—Scan event settings with HCD experiment

MS^n Settings for ETD Experiments

In the Orbitrap Velos Pro ETD mass spectrometer, use the Instrument Setup to select ETD as the Activation type. See Figure 5-14. Use ETD to fragment peptides and proteins at the amide-N to alpha-C bond to produce c- and z-type fragment ions. Selecting ETD as activation type has the following consequences:

- The Supplemental Activation Check box becomes available and allows entering the corresponding charge state in the spin box.

- The Normalized Collision Energy spin box and the Activation Q spin box are disabled.

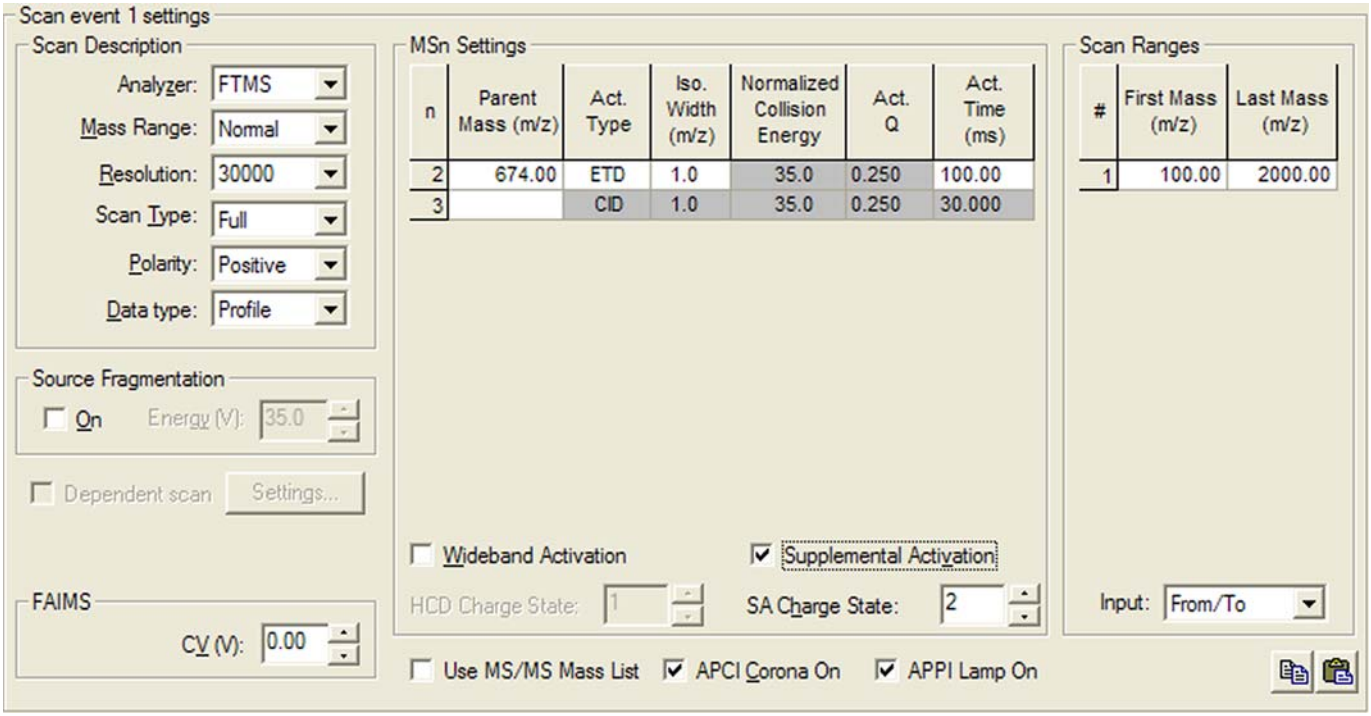


Figure 5-14. MS Detector Setup Page—Scan event settings with ETD experiment

Chapter 6 Instrument Configuration

This chapter gives instructions on how to configure your instrument. It contains the following topics:

Starting Instrument Configuration

Use the Orbitrap Velos Pro Configuration dialog box to enter Orbitrap Velos Pro MS configuration information on several pages, including the FT Settings page and the FT Manual Calibration page.

❖ **To display the Orbitrap Velos Pro Configuration dialog box**

1. Choose **Programs > Thermo Foundation 2.0 > Instrument Configuration** to display the Instrument Configuration dialog box.
2. Click the Orbitrap Velos Pro MS button in the Configured Devices area. See Figure 6-1.

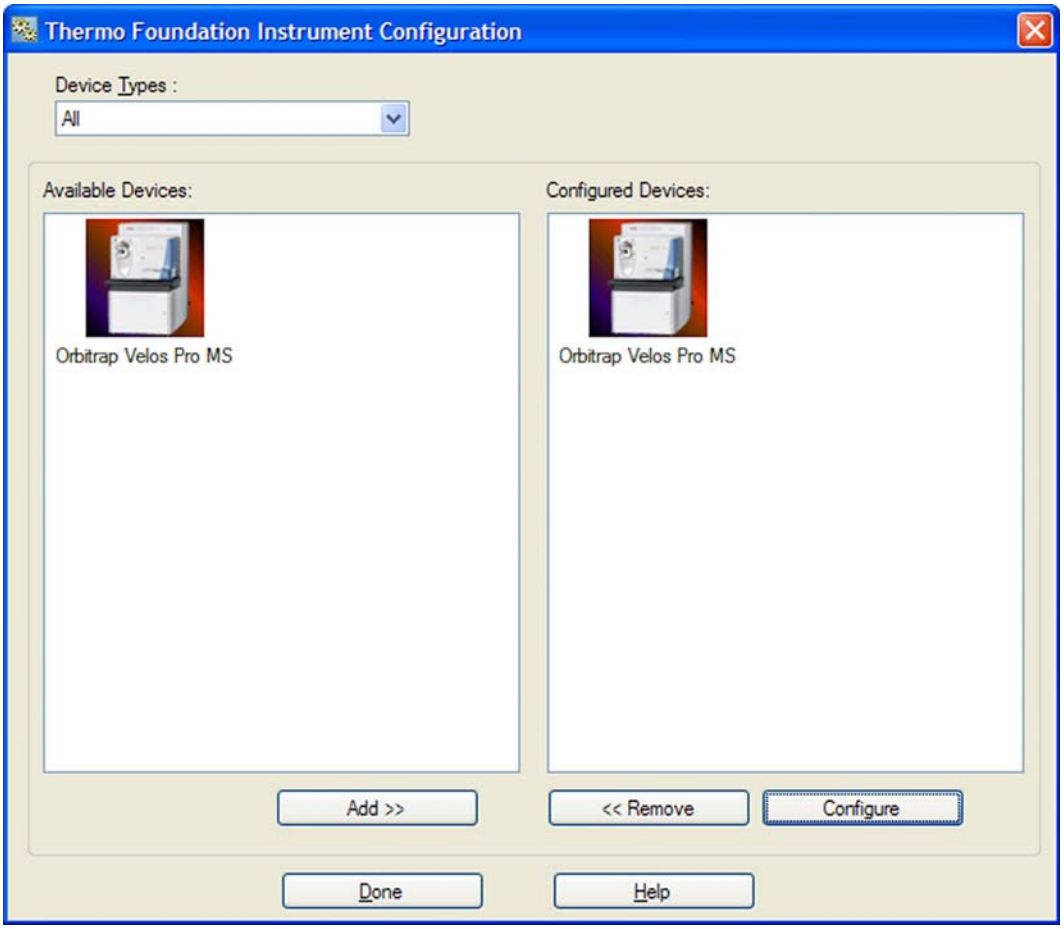


Figure 6-1. Instrument Configuration dialog box

3. Click **Configure** to open the Orbitrap Velos Pro Configuration dialog box.

The elements of the pages are described in the following topics.

See "Configuring the Reagent Ion Source" on page 7-8 for information about the Reagent Ion Source page of the Orbitrap Velos Pro Configuration dialog box, which is available for the Orbitrap Velos Pro ETD mass spectrometer.

FT Settings Page

Use the FT Settings page of the Orbitrap Velos Pro Configuration dialog box to edit the FTMS analyzer temperature setpoint. See Figure 6-2.

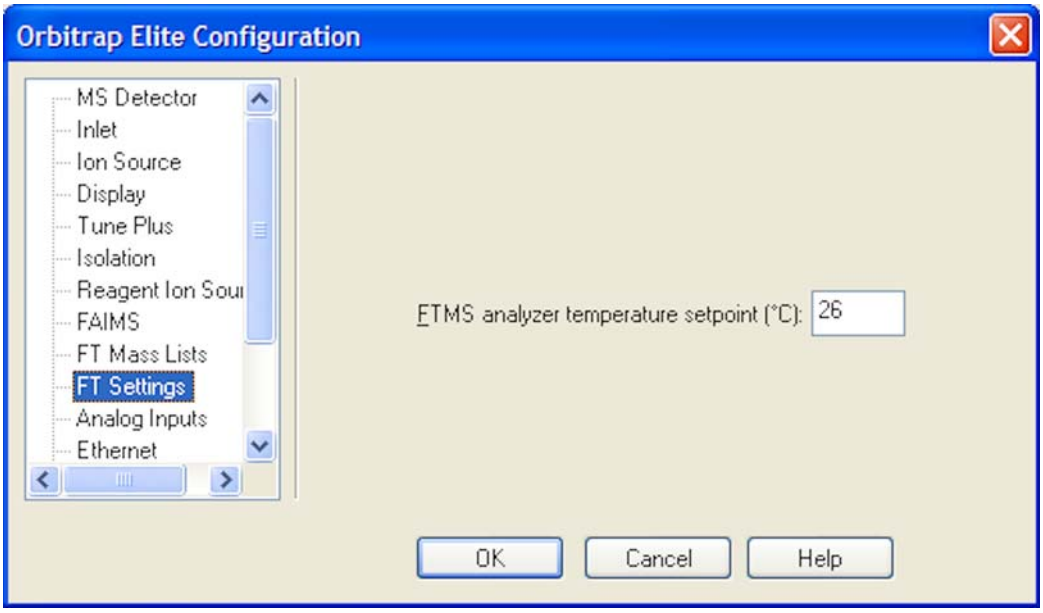


Figure 6-2. Orbitrap Velos Pro Configuration dialog box—FT Settings page

Using the FT Settings page of the Orbitrap Velos Pro Configuration dialog box, you can select the following parameters:

FTMS analyzer temperature setpoint (°C) Enter the desired temperature for the Orbitrap analyzer chamber. The default value is 26.

Note Configuration changes will become effective when you reboot your instrument. ▲

FT Mass Lists Page

Use the FT Mass Lists page of the Orbitrap Velos Pro Configuration dialog box to edit the calibration mass lists, which are displayed in the FT Manual page of the Calibrate dialog box. See Figure 6-3. It is also possible to import or export a mass list as a text file.

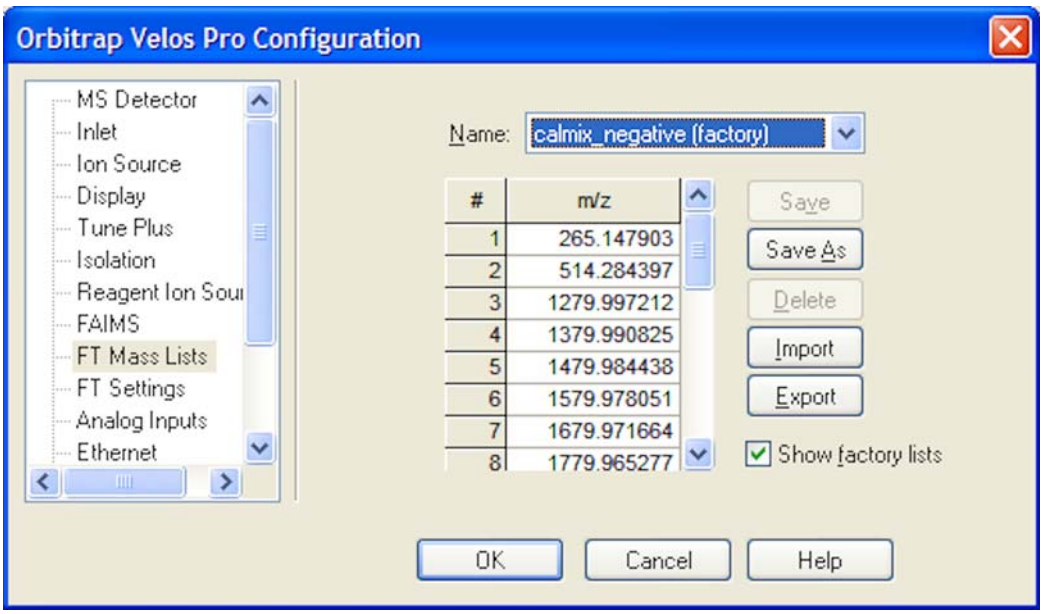


Figure 6-3. Orbitrap Velos Pro Configuration dialog box—FT Mass Lists page

The FT Mass Lists page of the Orbitrap Velos Pro Configuration dialog box has the following parameters:

Name	This list box lists the names of the factory supplied and user created mass lists.
Mass List	This table lists the mass-to-charge ratios of the ions that you are using to calibrate the Orbitrap mass analyzer. You can select an existing mass list in the Name list box, or you can create or modify a mass list by clicking on and editing the entries in the Mass List table.
Save	Click **Save** to save the mass list with the name that is selected in the Name list box.
Save As	Click **Save As** a to save the mass list with a new name.
Delete	Click **Delete** to delete the mass list that is selected in the Name list box.
Import	Click **Import** to import a mass list that is a text file.

Export Click **Export** to export a mass list to a text file.

Show Factory Lists Select this check box to show the factory calibration mass lists in Tune Plus.

Chapter 7 Orbitrap Velos Pro ETD Instruments

This chapter describes only the Orbitrap MS relevant differences in instrument settings and procedures with respect to using an Orbitrap Velos Pro ETD instrument. See also the previous chapters of this guide and the *Orbitrap Velos Pro Hardware Manual*.

This chapter contains the following topics:

For additional information concerning ETD, refer to the *ETD Module Hardware Manual* and the *ETD Module Getting Started Guide*.

Note If your instrument is equipped with a reagent ion source, you need to configure the reagent ion source in Instrument Configuration to get access to all reagent ion source relevant settings of Tune Plus and Instrument Setup. See "Configuring the Reagent Ion Source" on page 7-8. ▲

Tune Plus Window of the Orbitrap Velos Pro ETD Mass Spectrometer

This section describes the differences in the Tune Plus window of an Orbitrap Velos Pro ETD instrument. Spectrum view and Graph view show no differences to the standard instrument. They are described in Chapter 2: "Tune Plus Window".

Status View

The Tune Plus window shows a schematic view of the Orbitrap Velos Pro ETD instrument. See Figure 7-1. Use the instrument control icon in the toolbar to access parameters for the reagent ion source and the reagent ion optics.

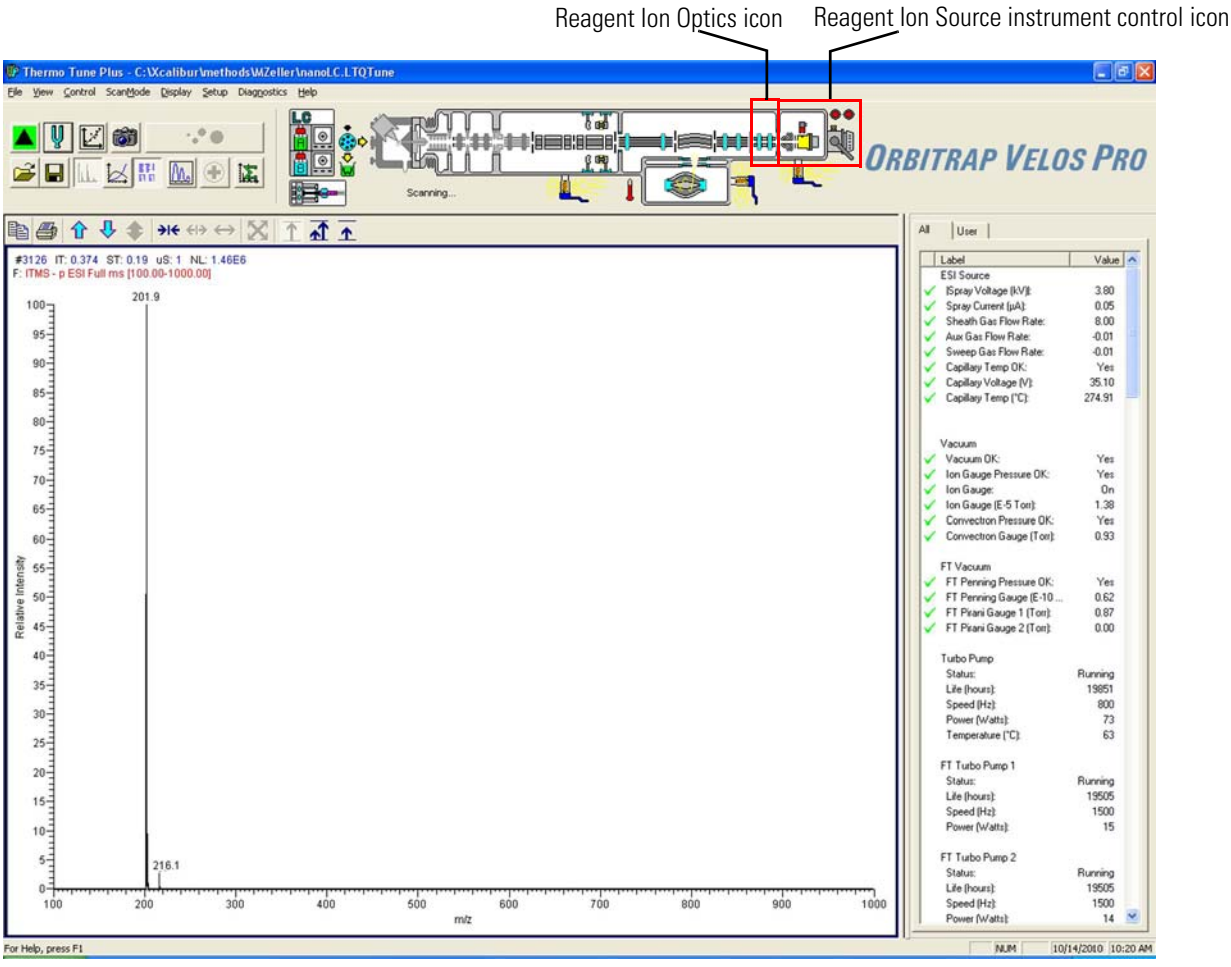


Figure 7-1. Status View for Orbitrap Velos Pro ETD MS

The color of the Reagent Ion Source control icon indicates the status of the reagent ion source:

Reagent ion source off

Reagent ion source on
The status of the filament is shown in the Status view—All page.

The Status view—All page additionally displays information about reagent ion source, reagent ion optics, reagent vacuum, reagent turbopump, and reagent power supplies.

Scan Mode Menu

This section describes the elements of the Scan Mode menu that are different from the standard instrument.

Define Scan Dialog Box

As described in Chapter 2: "Tune Plus Window", you can choose the analyzer type (Ion Trap or FTMS), mass range, resolution, and scan type. See Figure 7-2. You can as well define the scan range (for example, First Mass–Last Mass window).

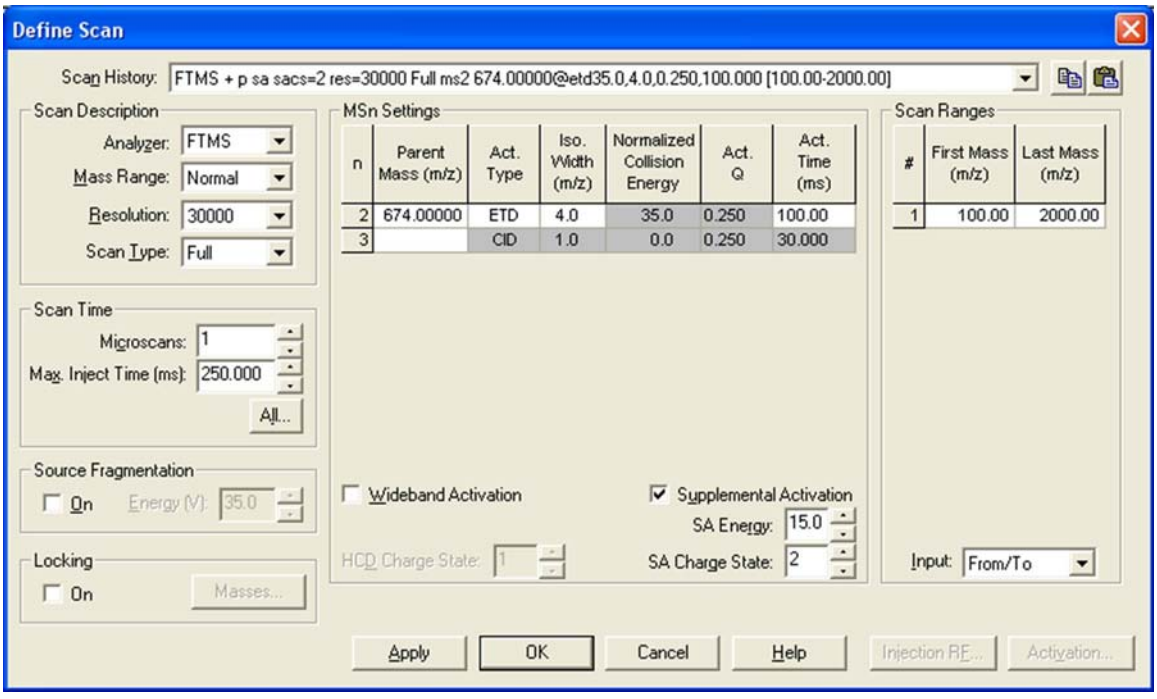


Figure 7-2. Define Scan dialog box for Orbitrap Velos Pro ETD MS

When you select ETD as Activation Type in the MS^n Settings, the Supplemental Activation check box becomes available. Select this check box to enable supplemental activation of ETD MS/MS and MS^n.

Use the SA Energy spin box to enter the percentage of the energy that should be used for supplemental activation. The available range is 0 to 20%.

Use the SA Charge State spin box to enter the charge state of the parent ion for supplemental activation. Available charge states are 2 to 10.

Setup Menu

This section describes the elements of the Setup menu that are different from the standard instrument.

Injection Control Dialog Box—Reagent Page

The number of reagent ions admitted into the linear trap is regulated by the parameters in the Reagent page in the Injection Control dialog box. See Figure 7-3. Open the Reagent page in the Injection Control window by clicking the Injection Control instrument control graphic or click **Setup > Injection Control > Reagent**.

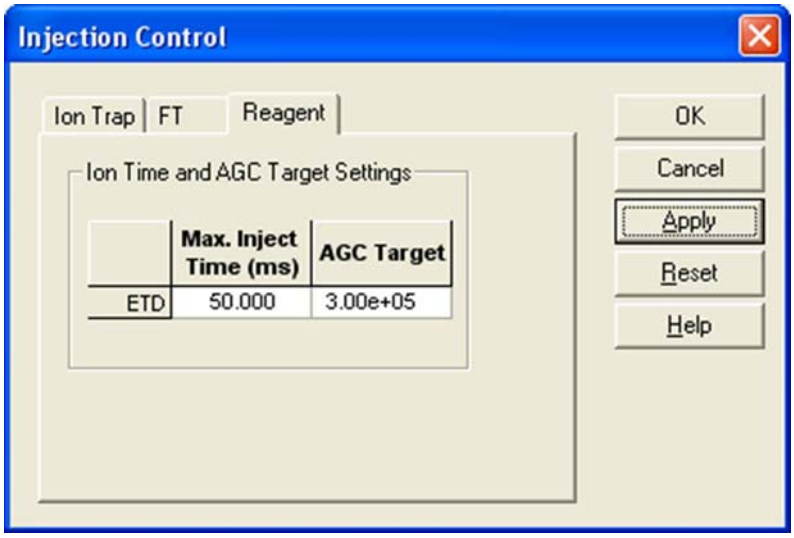


Figure 7-3. Injection Control dialog box—Reagent page

See "Viewing the Injection Reagent Settings" on page 7-27 for information about the parameters available on this dialog box.

Reagent Ion Optics Dialog Box

Use the Reagent Ion Optics dialog box to specify voltages for the reagent ion source lenses, the back lens, and the transfer multipole. Usually, the reagent ion optics parameters are optimized by the automatic tune procedure to maximize the transmission of reagent ions from the reagent ion source to the linear ion trap.

You may obtain small improvement in the signal by manual tuning and changing the settings. You can change the settings in this dialog box only after selecting the View Reagent Ion Spectra check box in the Reagent Ion Source dialog box. See Figure 7-4. See "Tuning the Reagent Ion Optics" on page 7-12 for more information.

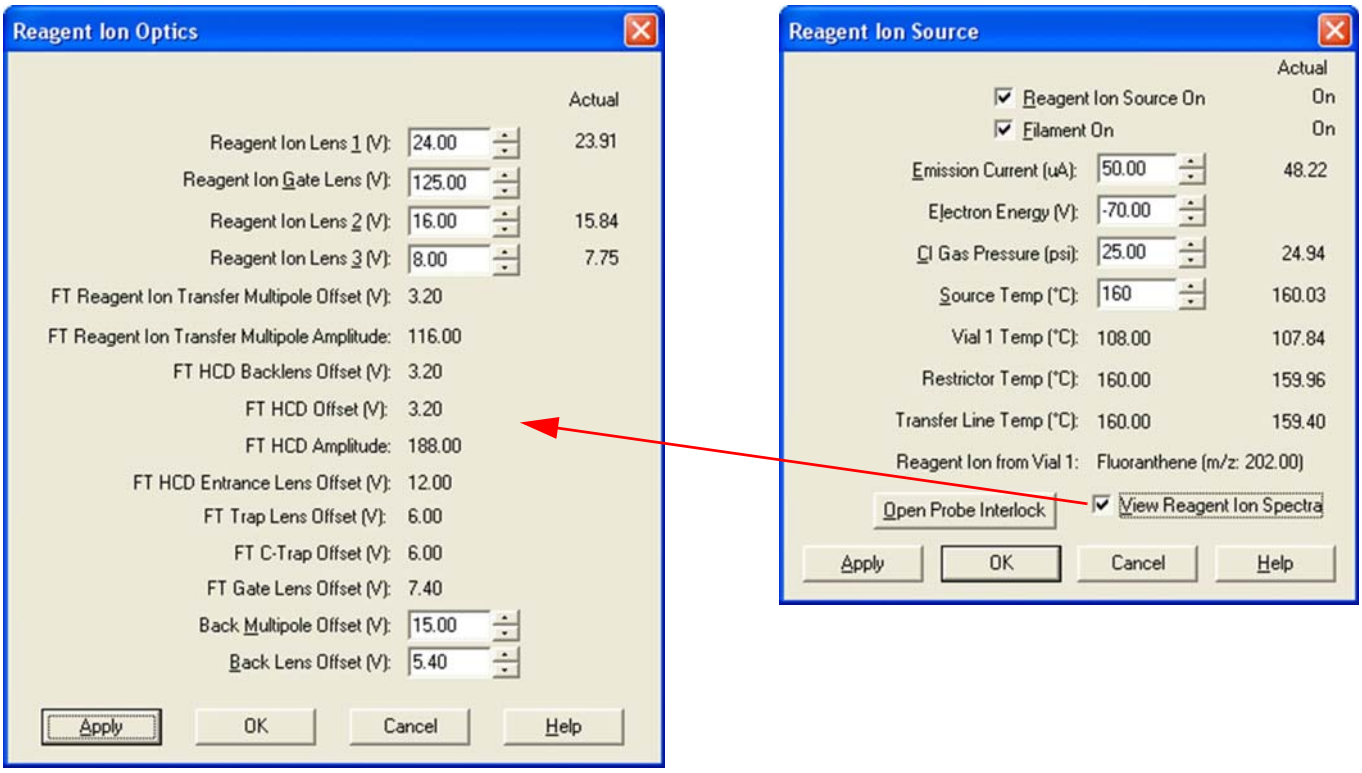


Figure 7-4. Activating Reagent Ion Optics dialog box

Note The reagent ion optics lenses have broad optimums—except the back lens. Increasing or decreasing the back lens offset by 3 or 4 V can significantly reduce the signal. ▲

Reagent Ion Source Dialog Box

Use the Reagent Ion Source dialog box to set selected ETD reagent ion source parameters. See Figure 7-5 on page 7-6. The actual parameter values are shown on the right side of the dialog box.

Use the Reagent Ion Source On check box to turn on or off the reagent ion source, including the vial heaters. If the reagent vials are not at their target temperatures, a “Please Wait” message window appears. Typically, heating takes 5 to 10 minutes. Turn off the reagent ion source if you do not plan to use it for an extended period, overnight for example.

Use the Filament On check box to turn on or off the filament, which produces electrons. When the reagent vials reach their target temperatures, the filament automatically turns on and the Filament On check box automatically shows a check mark. (You can force the filament to turn on before the vials reach their target temperatures by selecting the check box.) To prolong the lifetime of the filament, turn it off if the reagent ion source will not be in use for an hour or more.

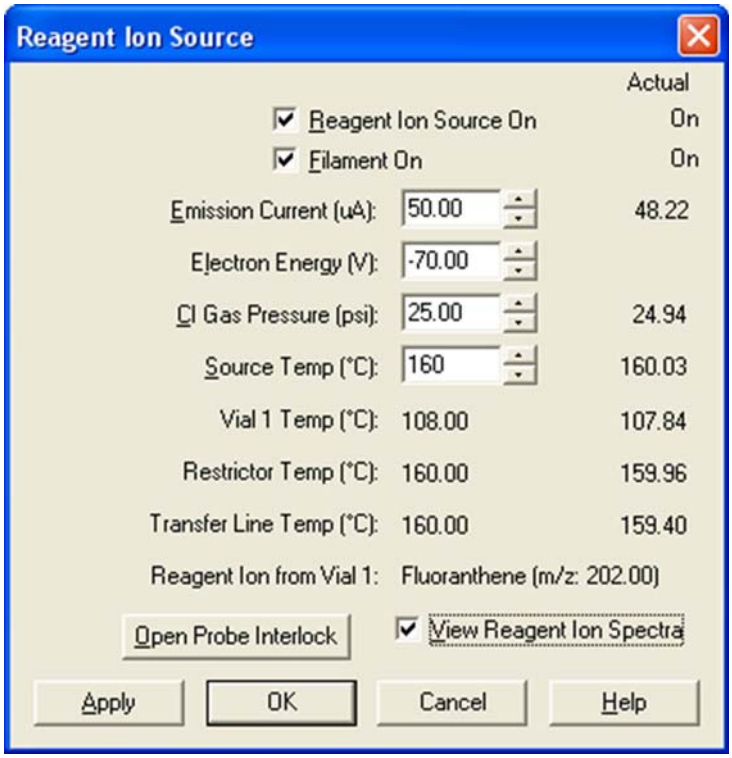


Figure 7-5. Reagent Ion Source dialog box

Click **Open Probe Interlock** to evacuate the inlet valve block to a target pressure of less than 0.1 mTorr. When the target pressure is achieved, a message states that you can open the ball valve. Refer to the *Orbitrap Velos Pro Hardware Manual* for maintenance instructions for the reagent ion source.

When you select the View Reagent Spectra check box, the following actions become possible:

- The injection of analyte ions, but not reagent ions, into the mass analyzer for mass analysis is stopped. This allows measuring reagent ion intensity and also checking for reagent contamination by observing the mass spectrum.

- You can change the parameter values in the Reagent Ion Optics dialog box. See page 7-5.
- You can autotune the reagent ion source. See page 7-12.

Vacuum Dialog Box—Reagent Page

Use the Reagent page of the Vacuum dialog box to switch on the ion gauge in the ETD Module. It also displays the pressure readings of the ion gauge and the Convectron gauge in the ETD Module. See Figure 7-6.

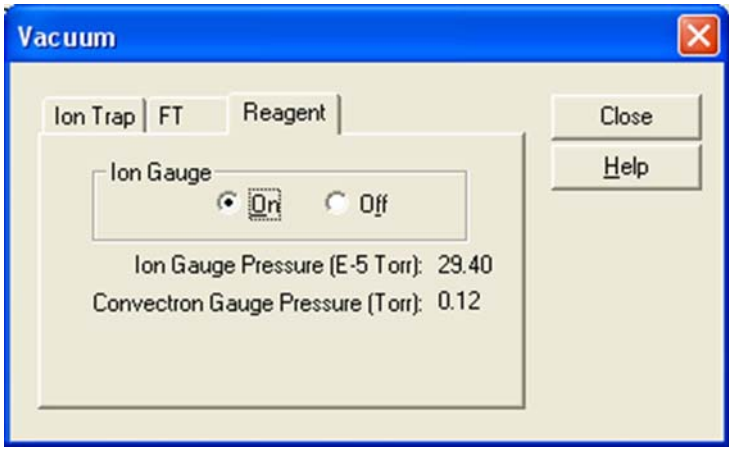


Figure 7-6. Reagent page of the Vacuum dialog box

Configuring the Reagent Ion Source

After you have installed the reagent vials for the ETD Module as described in the *Orbitrap Velos Pro Hardware Manual*, you have to configure the reagent ion source of the Orbitrap Velos Pro ETD mass spectrometer.

❖ **To configure the reagent ion source**

1. From the Instrument Configuration window, click **Orbitrap Velos Pro MS** in the Configured Devices area.
2. Click **Configure** to open the Orbitrap Velos Pro Configuration dialog box.
3. Click **Reagent Ion Source** in the left hand section of the Orbitrap Velos Pro Configuration window. Use the Reagent Ion Source page of the Configuration dialog box to configure the reagent ion source and activating the cooling gas. See Figure 7-7.
4. Select the Reagent Ion Source Configured check box as shown in Figure 7-7.

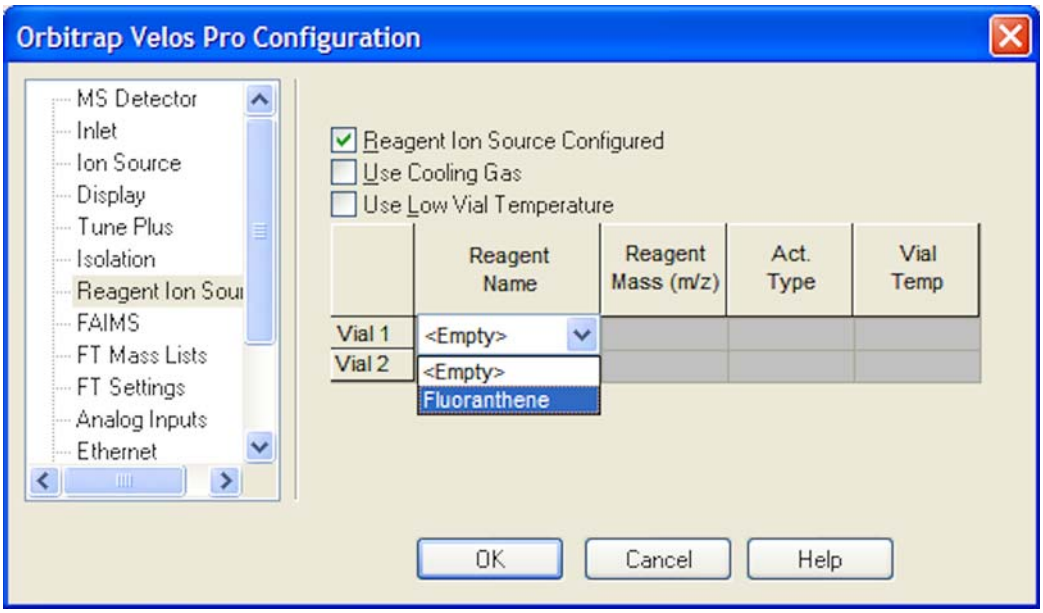


Figure 7-7. Enabling the reagent ion source

5. In the Vial 1 row, click the Reagent Name list box and select Fluoranthene. The Fluoranthene Vial 1 Reagent Mass, Activation Type, and default Vial Temperature will appear in the table.

Caution The empty vial in the Vial 2 heater is an integral part of the carrier/CI gas system. It is necessary to keep the carrier/CI gas system closed to the laboratory. If no vial is placed in the Vial 2 heater:

- The carrier/CI gas containing the reagent may escape to the laboratory causing a safety problem.
- The ETD Module will not operate correctly and the filament will burn out. ▲

6. Select the Use Cooling Gas check box as shown in Figure 7-8.

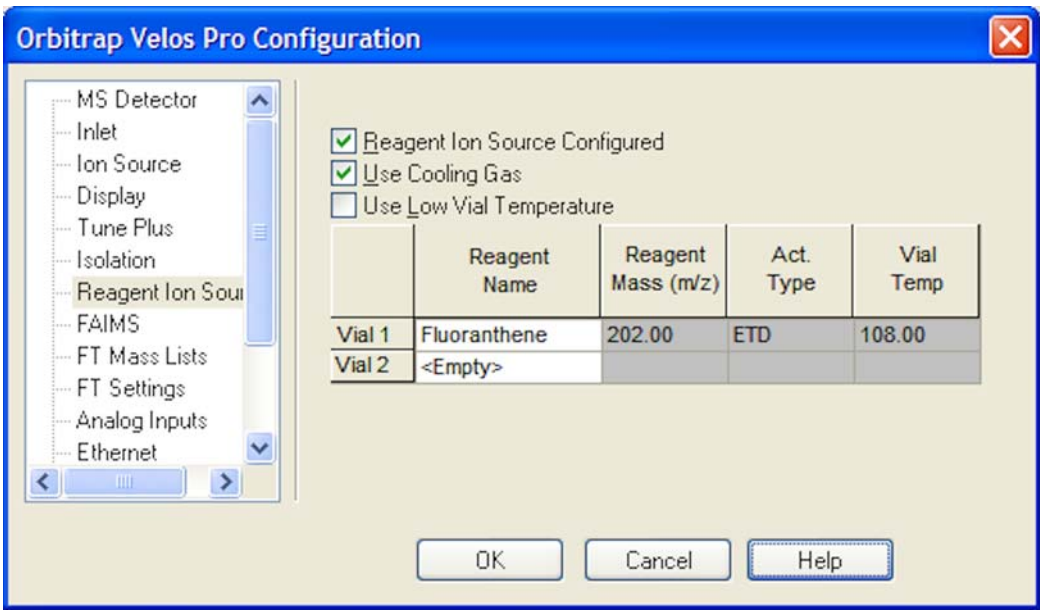


Figure 7-8. Orbitrap Velos Pro Configuration dialog box—Reagent Ion Source page

Note Select the **Use Low Vial Temperature** check box to heat the vial to 90 °C, rather than 108 °C. Operating at the lower vial temperature will reduce the rate of reagent consumption and may extend the lifetime of the filament. However, it might increase the reagent injection time. ▲

7. Click **OK** in the Orbitrap Velos Pro Configuration dialog box.

   A message box informs you that for the configuration changes to take effect, you will need to reboot the data system and then the mass spectrometer.

8. Click **Done** in the Instrument Configuration window.
9. Reboot the data system.
10. Reboot the Orbitrap Velos Pro ETD mass spectrometer.

The system software is now configured for using the reagent ion source of the Orbitrap Velos Pro ETD system.

Powering On the ETD Module and Viewing Reagent Ion Spectra

After the reagent vials have been installed as described in the *Orbitrap Velos Pro Hardware Manual*, power on the ETD Module by placing the Orbitrap Velos Pro ETD mass spectrometer in On mode. Turn on the reagent ion source to view the reagent ion spectra.

Powering On the ETD Module

❖ **To power on the ETD Module**

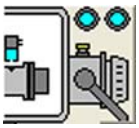
1. Toggle the ETD Module service switch to the Operating Mode (ON) position if it is not already in this position.
2. Toggle the Orbitrap Velos Pro MS FT Electronics switch to the On position. This turns the ETD Module on if the ETD Module service switch is already in the Operating Mode (ON) position.

For detailed instructions about starting up and shutting down the instrument, refer to the *Orbitrap Velos Pro Hardware Manual*.

Turning On the Reagent Ion Source and Viewing Reagent Ion Spectra

Even when the ETD module is turned on, the reagent ion source within it is not turned on until you turn it on.

❖ **To turn on the reagent ion source**



1. Click the **Reagent Ion Source** instrument control icon in the toolbar of the Tune Plus window. (See icon in margin and Figure 7-1 on page 7-2.) The Reagent Ion Source dialog box appears. (See Figure 7-5 on page 7-6.)
2. In the Reagent Ion Source dialog box, click the **Reagent Ion Source On** check box. (See Figure 7-5.) If the reagent vials are not at their target temperature, a message box appears. See Figure 7-9. When the reagent vials reach their target temperature, voltage is applied to the ETD Module ion optics. The filament automatically turns on; the Filament On check box automatically shows a check mark and its actual condition switches from Off to On.

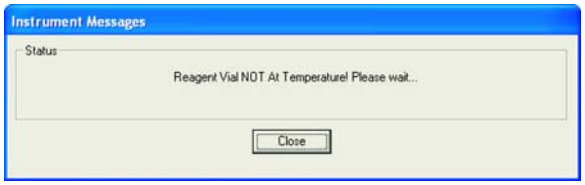


Figure 7-9. Message box: Reagent Vial NOT At Temperature!

❖ **To view the reagent ion spectra**

1. Select the **View Reagent Ion Spectra** check box in the Reagent Ion Source dialog box. See Figure 7-10.

2. Reagent ion peaks appear in the Spectrum view. See Figure 7-10.

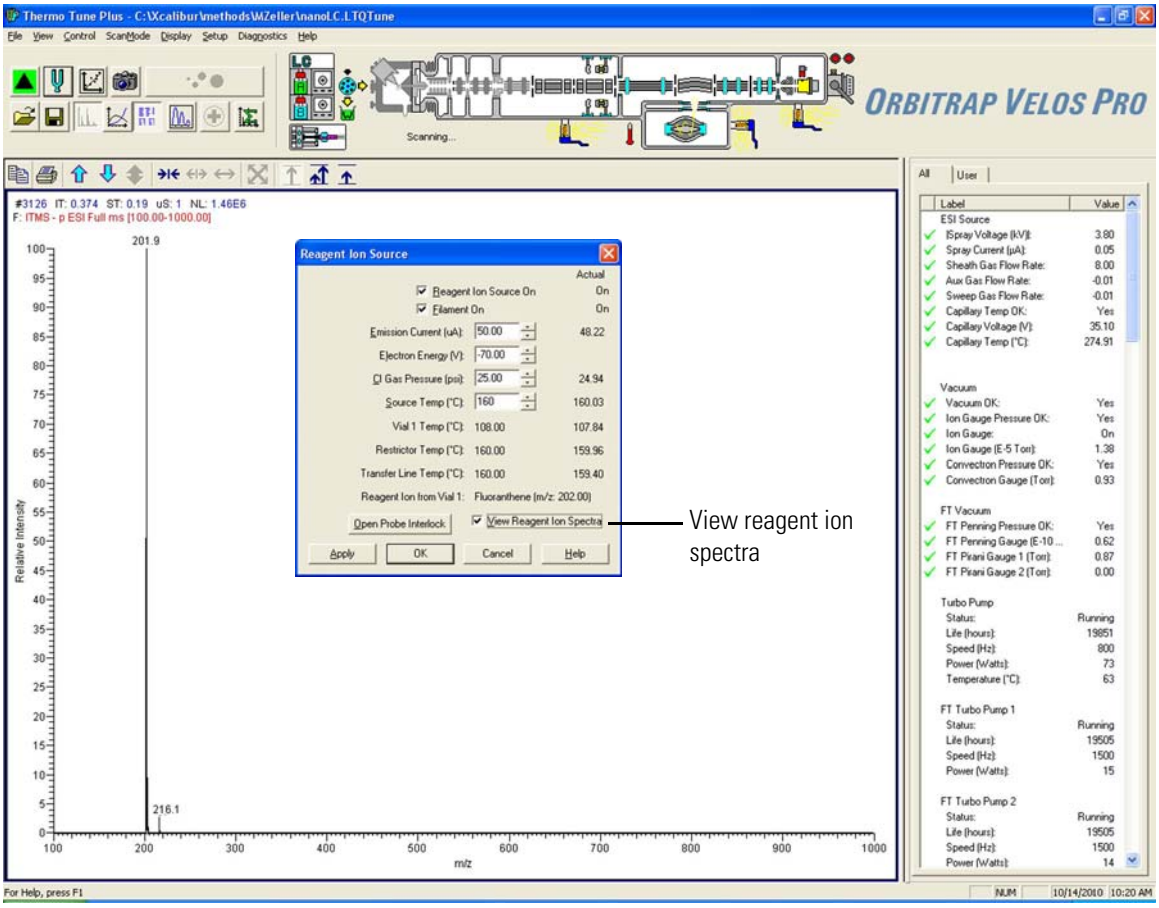


Figure 7-10. Tune Plus window showing the fluoranthene radical anion mass spectrum

If the spectrum is satisfactory, proceed to the next step. If the spectrum is not satisfactory, tune the reagent ion optics as described in "Tuning the Reagent Ion Optics" on page 7-12.

3. Clear the View Reagent Ion Spectra check box.

The Tune Plus Spectrum view from the ETD module is cleared.

Tuning the Reagent Ion Optics

This section describes how to tune the ETD Reagent Ion Optics settings to obtain optimized reagent ion transmission.

There are three ways to tune the ion optics within the reagent ion source: automatically, semi-automatically, and manually.

- "Automatically Tuning the Reagent Ion Source", next section
- "Manually Tuning the Reagent Ion Source" on page 7-14
- "Semi-Automatically Tuning the Reagent Ion Optics" on page 7-17

Automatically tuning the reagent ion source is the best method for most situations. In some cases it may be appropriate to perform manual tuning. Choose manual tuning to manually optimize reagent ion optics parameters and reagent ion source parameters that are not automatically tuned such as Emission Current, Electron Energy, and CI gas pressure. Manual tuning is done by observing the effects of adjusting these parameters on the reagent ion signal intensity.

Note Tune Plus provides an evaluation procedure for CI gas pressure under **Diagnostics > Diagnostics > Tools > System evaluation > Reagent CI gas pressure evaluation**. Thermo Fisher Scientific recommends performing this procedure after replacing the filament and/or the ion volume. ▲

Use semi-automatic tuning to optimize each lens setting individually within an optimization range and according to the step size you select.

Automatically Tuning the Reagent Ion Source

Automatically tuning the reagent ion source assures the best ion optics settings for optimum transmission of reagent ions (fluoranthene).

❖ **To automatically tune the reagent ion source**

1. On the Windows taskbar, choose **Start > All Programs > Thermo Instruments > LTQ > LTQ Tune**.

   The Tune Plus window opens. (See Figure 7-1 on page 7-2.)

2. Click the **On/Off/Standby** button to take the Orbitrap Velos Pro ETD mass spectrometer out of Standby mode and turn it on.
3. Click the **Display Graph View** button.
4. If the reagent ion source is not on, turn it on as described in "Turning On the Reagent Ion Source and Viewing Reagent Ion Spectra" on page 7-10.

5. In the Reagent Ion Source dialog box, select the **View Reagent Ion Spectra** check box. (See Figure 7-11.)

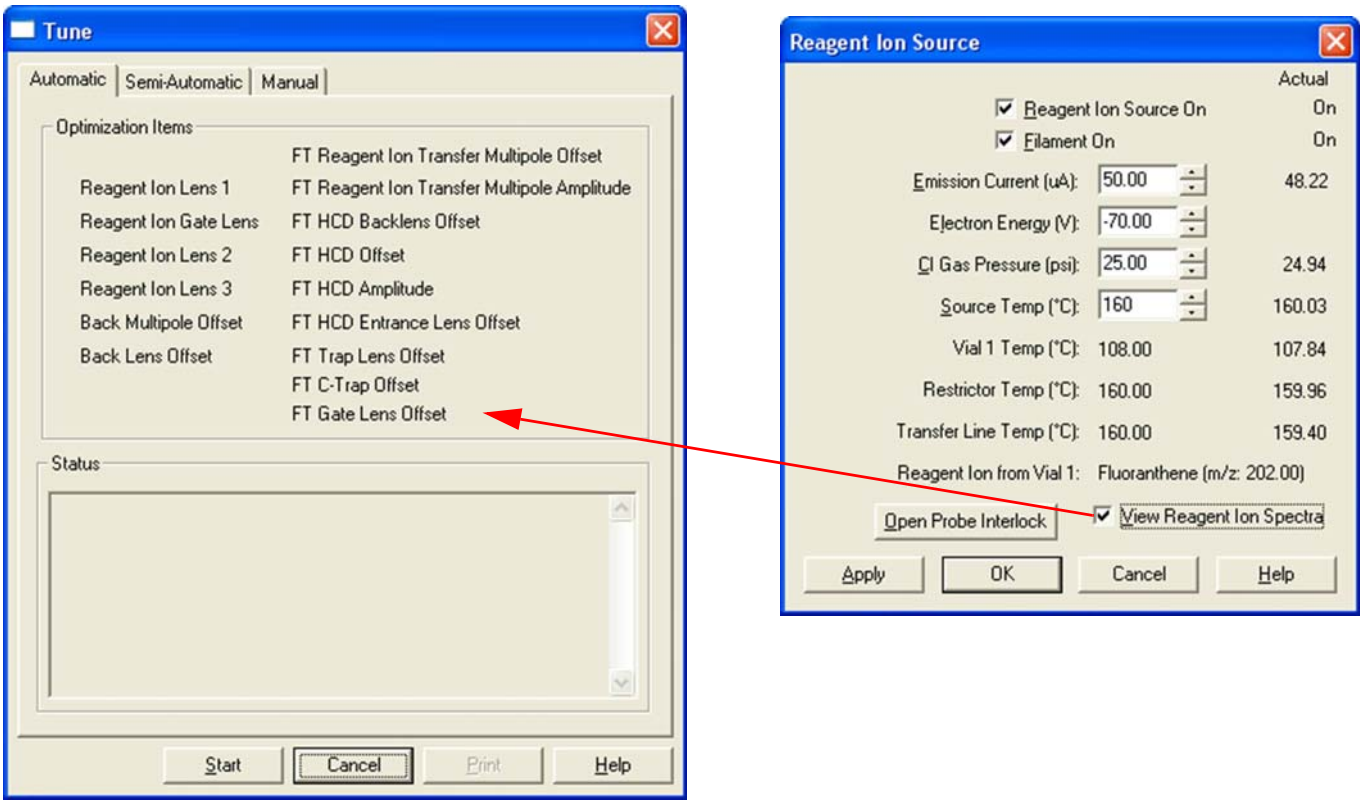


Figure 7-11. Activating Reagent Ion Source tuning

6. Click the **Tune** button. (See icon in margin.) The Tune dialog box appears.

7. The left side of the underlying view shows the reagent ion spectra (fluoranthene). See Figure 7-12 on page 7-14. The center view shows one of the parameters that are automatically tuned (back lens potential tuned to maximize the signal intensity at *m/z* 202).

8. Click the Automatic tab in the Tune dialog box if it is not already the active tab. The Automatic page of this window appears.

9. Click **Start** in the Automatic page of the Tune dialog box. The system will begin automatically tuning the ion optics of the reagent ion source. The Status box of the dialog box indicates that automatic tuning is completed by displaying the message, “Optimization Complete”. This message will also indicate the percentage change in the reagent ion signal intensity at *m/z* 202 relative to the prior value. A typical reagent signal intensity is about 1–2E7 in centroid mode when the system has been cleaned and the ion volume is new.

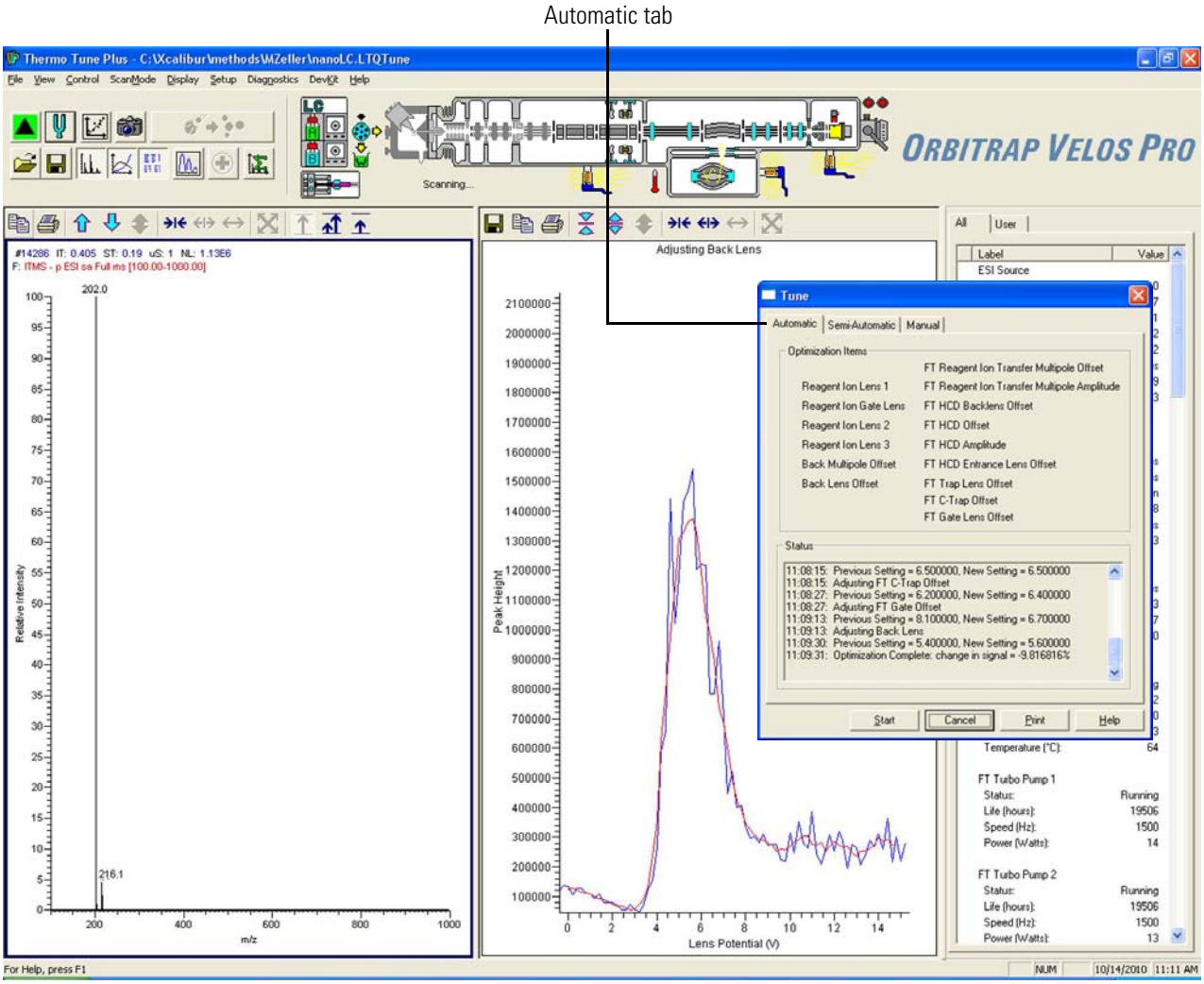


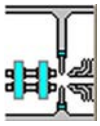
Figure 7-12. Tune Plus window with Automatic page of Tune dialog box displayed

10. Rerun Automatic Tune if the percentage change is greater than 20%. This is an iterative process. At some point there will be no more improvements in signal intensity.

Manually Tuning the Reagent Ion Source

❖ **To manually tune the reagent ion source**

1. Click the **Display Graph View** icon in Tune Plus.
2. If the reagent ion source is not on, open the Reagent Ion Source dialog box and turn on the reagent ion source as described in "Turning On the Reagent Ion Source and Viewing Reagent Ion Spectra" on page 7-10.
3. In the Reagent Ion Source dialog box, select the **View Reagent Ion Spectra** check box. See Figure 7-10 on page 7-11.



4. Click the **Reagent Ion Optics** icon at the top of the Tune Plus window. (See icon in margin and Figure 7-13.) The Reagent Ion Optics dialog box appears.

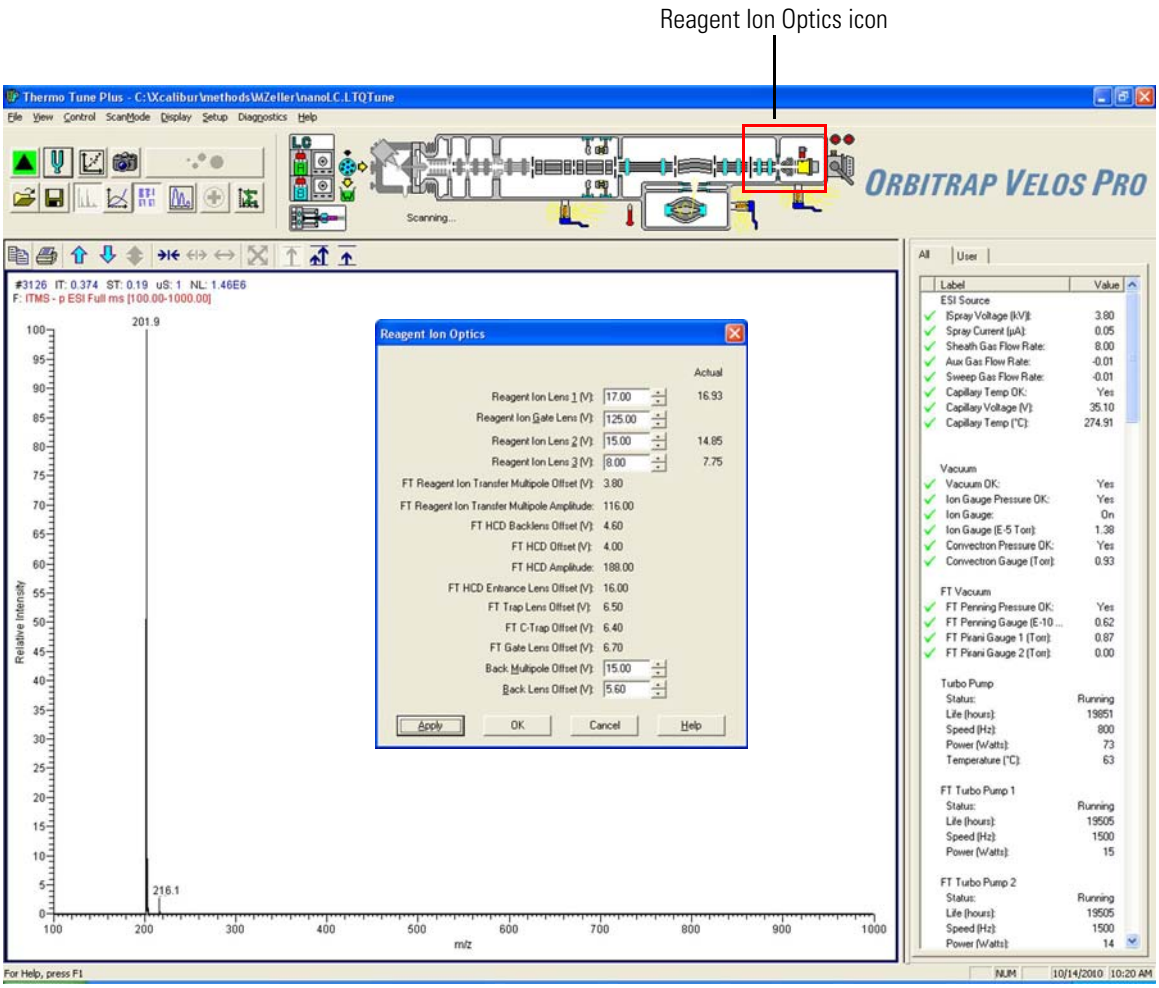


Figure 7-13. Tune Plus window with Reagent Ion Optics dialog box

5. Click the **Tune** button in Tune Plus. The Tune dialog box appears.

   You can also tune the reagent ion source. When the Reagent Ion Source dialog box is open (See Figure 7-5 on page 7-6.), click the **Tune** button in Tune Plus.

6. Click the **Manual** tab in the Tune dialog box if the Manual page is not already visible. (See Figure 7-14 on page 7-16.)

7. Select the **Reagent Ion from Vial 1** check box.

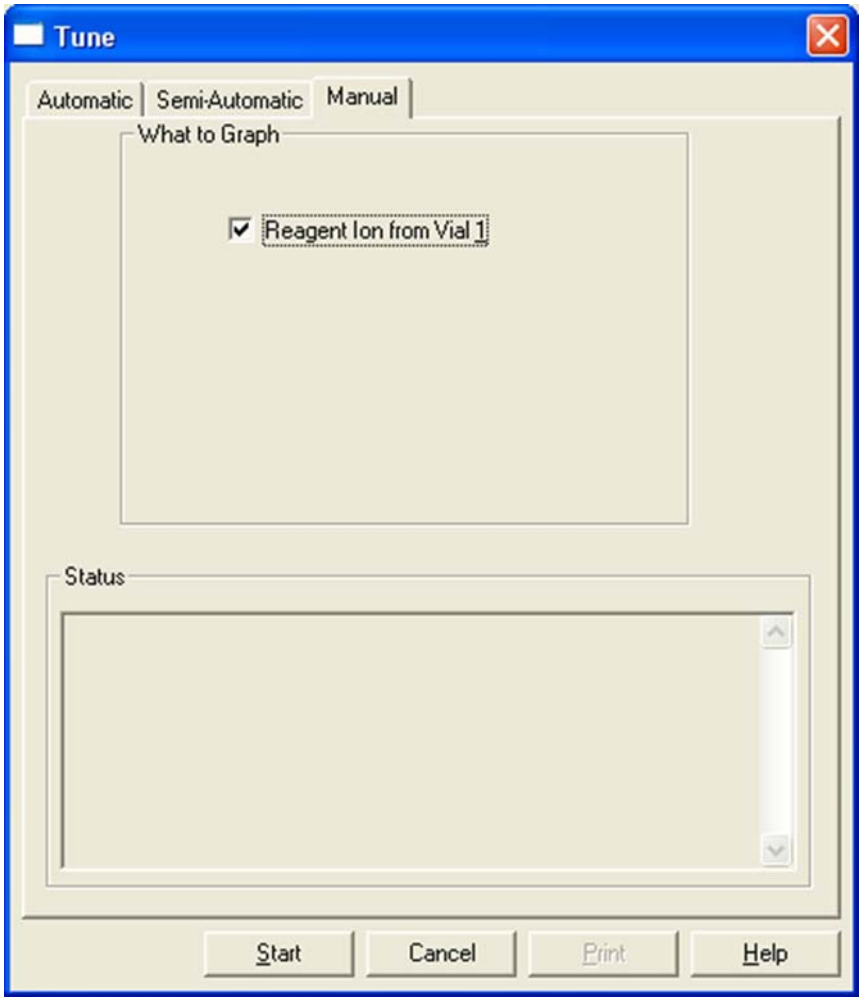


Figure 7-14. Reagent Ion Source Tune dialog box—Manual page

8. Click **Start**.

   The Graph view displays a plot of the reagent ion intensity. See Figure 7-15 on page 7-17. You can observe the response of the reagent ion intensity to changes in the lens parameters (Reagent Ion Optics dialog box) and Emission Current, CI gas pressure, and Electron Energy (Reagent Ion Source dialog box). Adjust these parameters to achieve the maximum reagent ion signal intensity.

Note Increasing the emission current might shorten the filament life time. Therefore, Thermo Fisher Scientific recommends readjusting the emission current only in exceptional circumstances. ▲

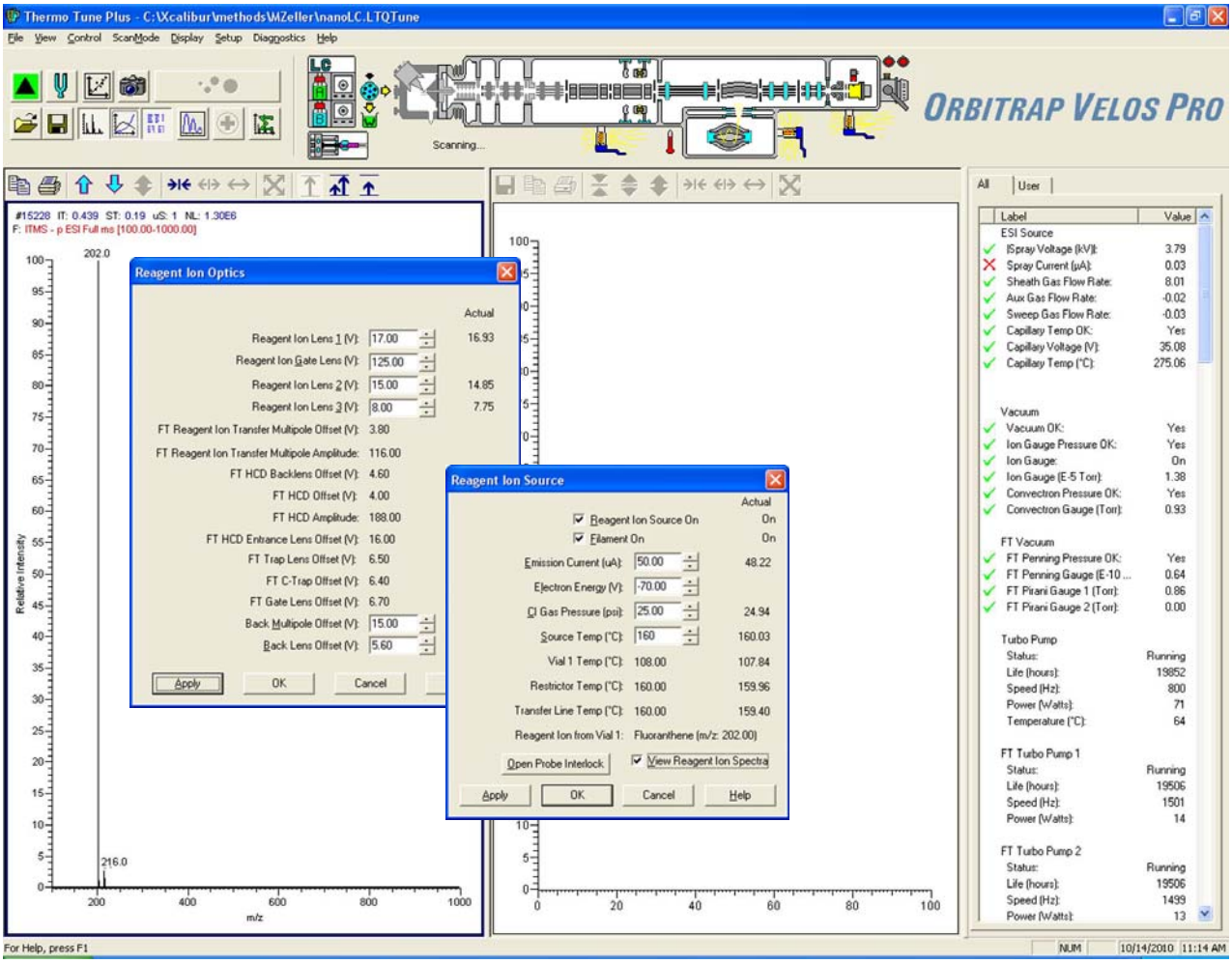


Figure 7-15. Tune Plus window showing the Display Graph view for manual tuning of the Reagent Ion Source

Semi-Automatically Tuning the Reagent Ion Optics

Use the semi-automatic tuning method to fine-tune the lens parameters to a range of settings and in step increments.

❖ **To semi-automatically tune the reagent ion optics**

1. On the Windows taskbar, choose **Start > All Programs > Thermo Instruments > LTQ > LTQ Tune**.

   The Tune Plus window opens. (See Figure 7-1 on page 7-2.)

2. Click the **Display Graph View** button.
3. If the Reagent Ion Source is not on, turn it on:

   a. In the Tune Plus window, choose **Setup > Reagent Ion Source**. The Reagent Ion Source dialog box opens. (See Figure 7-5 on page 7-6.)

b. Select the **Reagent Ion Source On** check box.
If the reagent vials are not at their target temperature, a message appears:

Reagent Vial NOT At Temperature! Please wait . . .

When the reagent vials reach their target temperature, voltage is applied to the Orbitrap Velos Pro ETD system's ion optics. The filament automatically turns on (the Filament On check box automatically shows a check mark and its actual condition switches from Off to On).

4. In the Reagent Ion Source dialog box, select the **View Reagent Ion Spectra** check box.

5. In the Control/Scan Mode toolbar, click the **Tune** button.

The Tune dialog box opens with the Automatic page shown by default (Figure 7-11 on page 7-13).

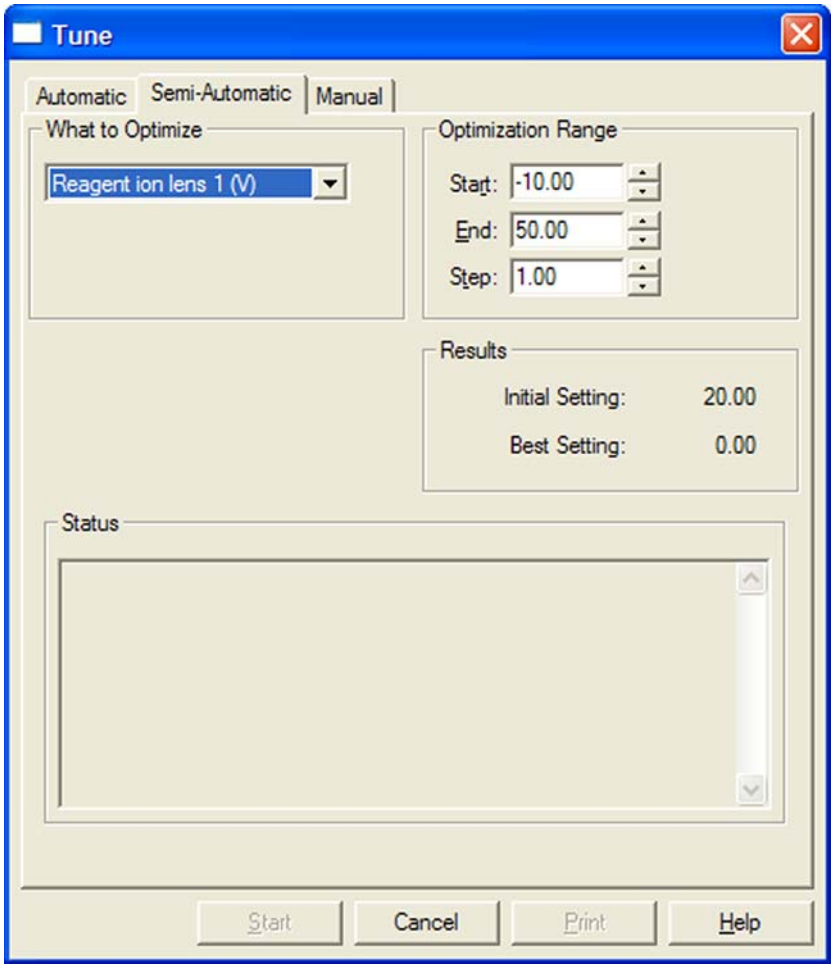


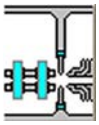
Figure 7-16. Semi-Automatic page of the Tune dialog box

6. Click the Semi-Automatic tab. The Semi-Automatic page opens. See Figure 7-16 on page 7-18.
7. From the What to Optimize list, select the item you want to tune:
   - Reagent ion lens 1 (V)
   - Reagent ion gate lens (V)
   - Reagent ion lens 2 (V)
   - Reagent ion lens 3 (V)
   - Back Multipole Offset (V); see also "Tuning the Quadrupole Mass Filter" on page 7-21
   - Back lens (V)
8. Adjust the settings in the Optimization Range area:
   - Start
   - End
   - Step
9. Click **Start**.

The results of your settings are shown in the Results area.

Viewing the Current Reagent Ion Optics Settings

❖ **To view the current Reagent Ion Optics settings**



1. Click the **Reagent Ion Optics** instrument control icon in the Tune Plus window to open the Reagent Ion Optics dialog box. The parameters in the Reagent Ion Optics dialog box have been optimized by the Auto Tune process. See Figure 7-17.

Note When the View Reagent Ion Spectra check box in the Reagent Ion Source dialog box is not selected, the parameters in the Reagent Ion Optics dialog box cannot be changed. ▲

2. Click **OK** to close the Reagent Ion Optics dialog box.

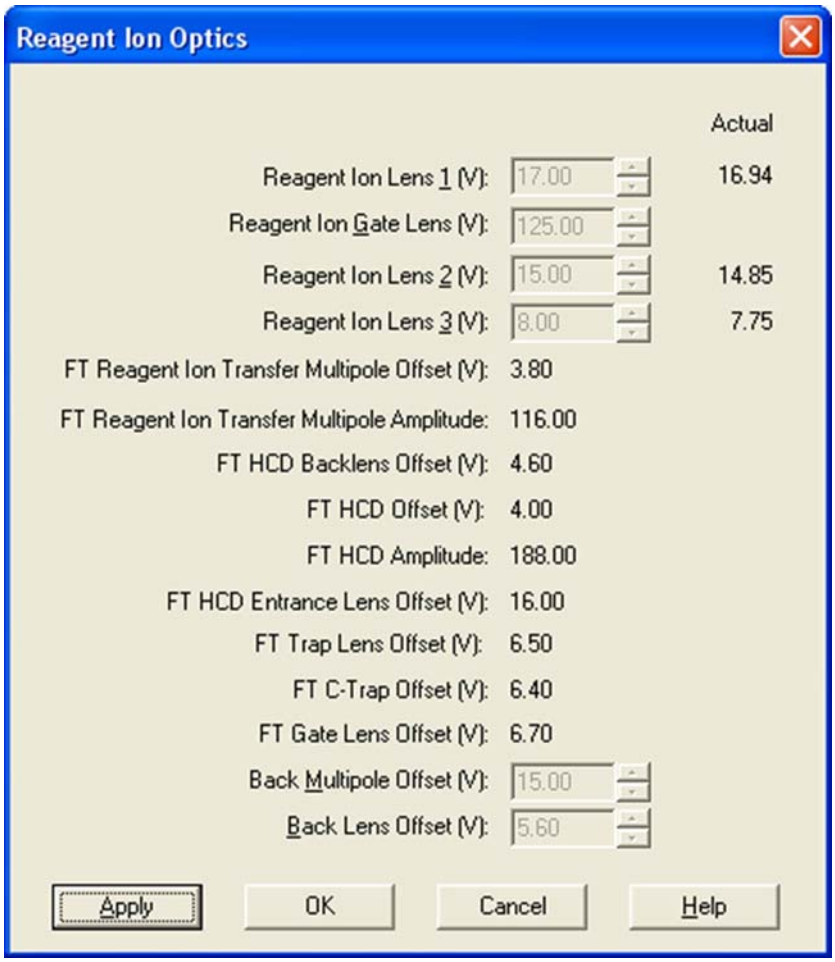


Figure 7-17. Reagent Ion Optics dialog box

Saving Your ETD Tune Method

After tuning is complete, save the ETD Tune parameters in a tune method.

❖ **To save the tune method**

1. On the File/Display toolbar, click the **Save** button.

   The Save As dialog box opens.

2. Browse to choose a location and specify a file name.

3. Click **Save**.

Tuning the Quadrupole Mass Filter

Stable calibration of the quadrupole mass filter between the linear ion trap and the C-Trap is achieved by manually adjusting the Back Multipole DC Offset voltage.

❖ **To adjust the Back Multipole DC Offset voltage**

1. Open the Toggles page in the Diagnostics dialog box.

2. Set **Isolate reagent ion** to On. See Figure 7-18.

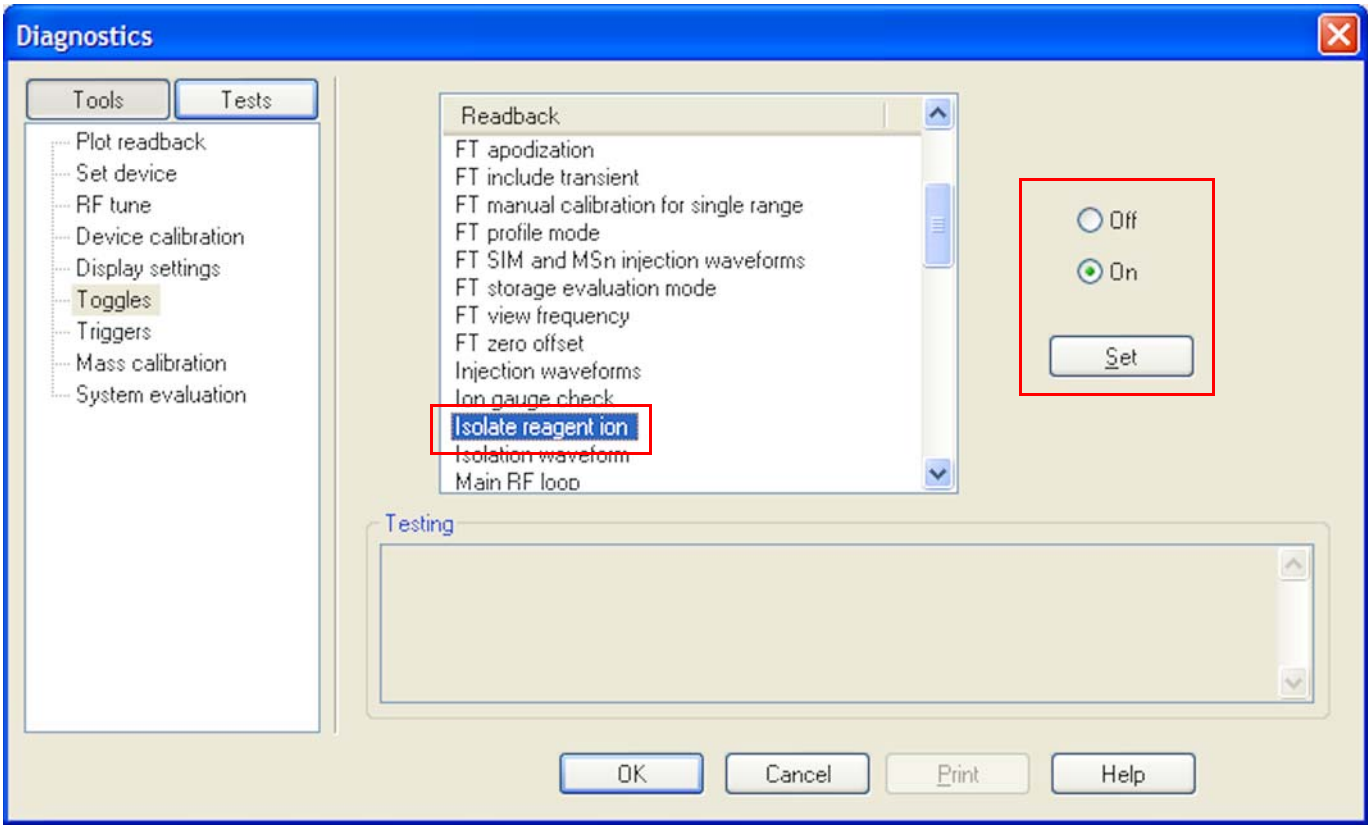


Figure 7-18. Activating Reagent Ion Isolation

3. In the Reagent Ion Source dialog box, select the View Reagent Ion Spectra check box.

4. Open the Tune dialog box and perform a semi-automatic tune of the Back Multipole Offset with the settings shown in Figure 7-19 on page 7-22 (Start 0, End 40, Step 0.35).

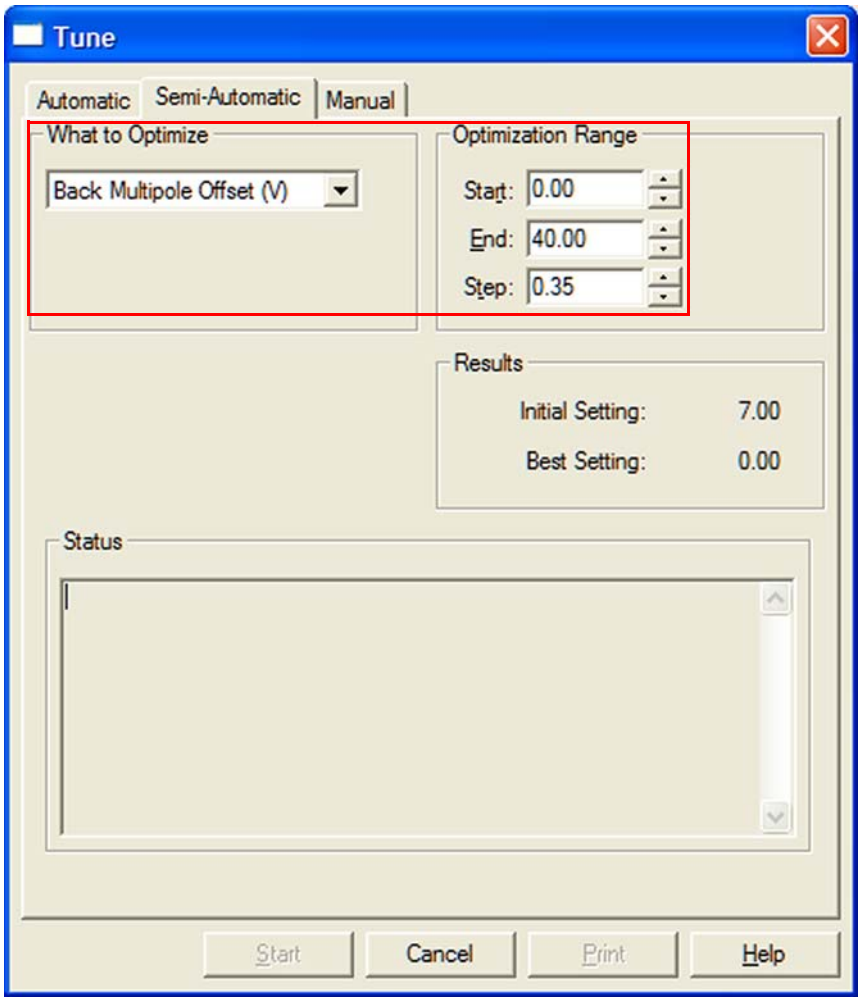


Figure 7-19. Semi-automatic tune of the Back Multipole Offset DC voltage

5. Search for a local maximum just before the oscillations on the signal start. (In the example shown in Figure 7-21, that would be at about 17 V).

6. Open the Reagent Ion Optics dialog box. Enter the new value for the Back Multipole Offset voltage into the respective spin box and click **Apply**.

7. Open the Calibrate dialog box and perform a semi-automatic calibration of the Reagent Ion Selection. See Figure 7-20 on page 7-23 and Figure 7-23 on page 7-25.

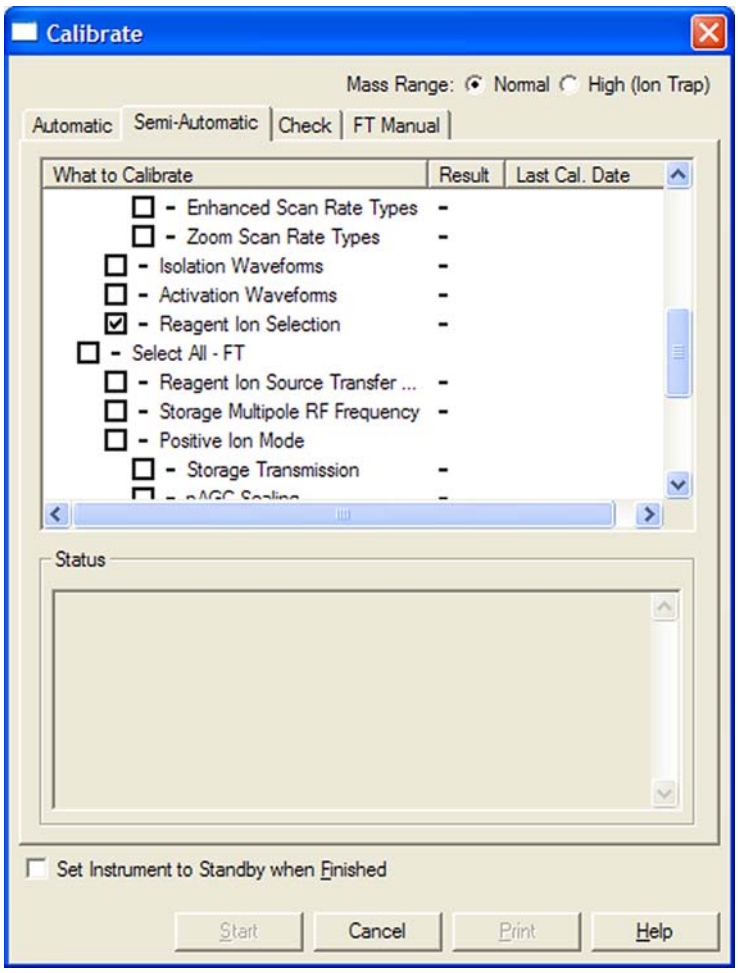


Figure 7-20. Calibrating the Reagent Ion Selection

Choosing the Operating Point for the Quadrupole Mass Filter

The performance of the quadrupole mass filter strongly depends on the kinetic energy (that is, DC Offset) of the ions. If the kinetic energy is too low it causes a poor transmission; if the kinetic energy is too high it leads to oscillations (noding) of the signal.

In the example shown in Figure 7-21, noding starts at above 20 V. In this region, the signal is fragile. Depending on the DC Offset (for comparison: 10 V, 17 V, and 32 V; red points) the calibration curves look differently.

The best operating point for the filter is the immediate region before the oscillations start. In the example, that would be the range between 15 V and 20 V.

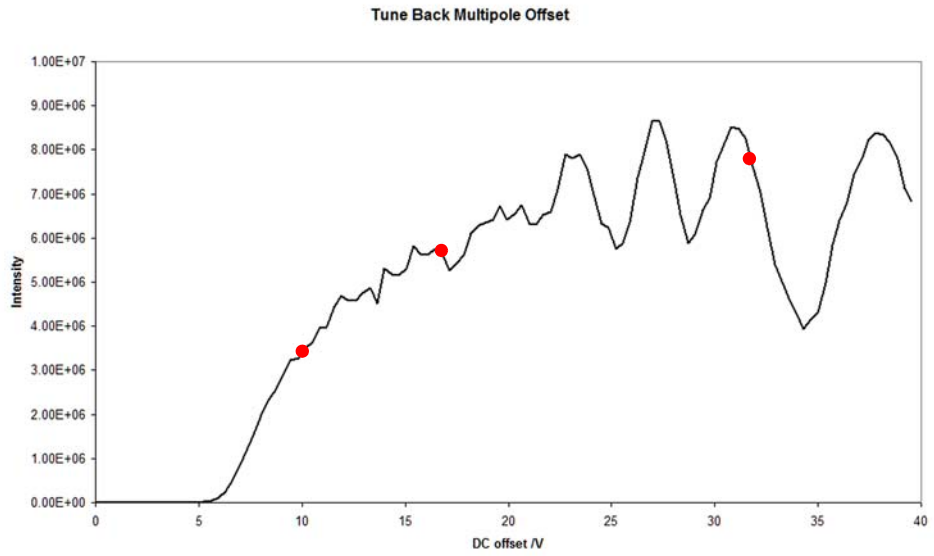


Figure 7-21. Dependence of the signal from the DC Offset

If the DC Offset is chosen too low, intensity losses in the filter are too large. During the calibration, the signal still increases after the stable region is reached. See Figure 7-22. As a consequence, the transmission at the operating point of the filter is poor.

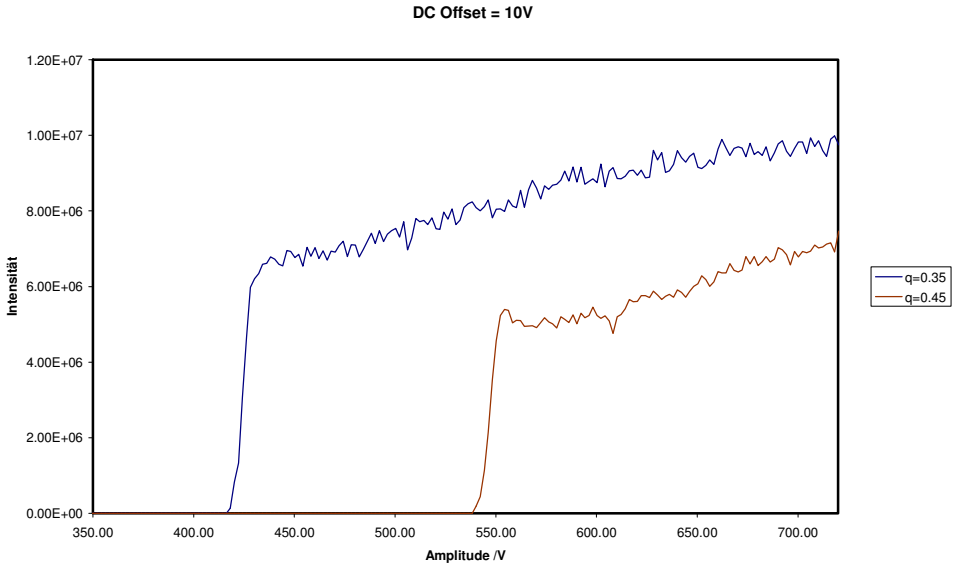


Figure 7-22. Chosen DC Offset (10 V) is too low for calibration

With offset voltages between 15 V and 20 V, no noding is visible. See Figure 7-23. The transmission reaches a maximum shortly after the start of the stable region, resulting in a high transmission at optimum filter efficiency. The calibration results in a broad plateau with constant intensity.

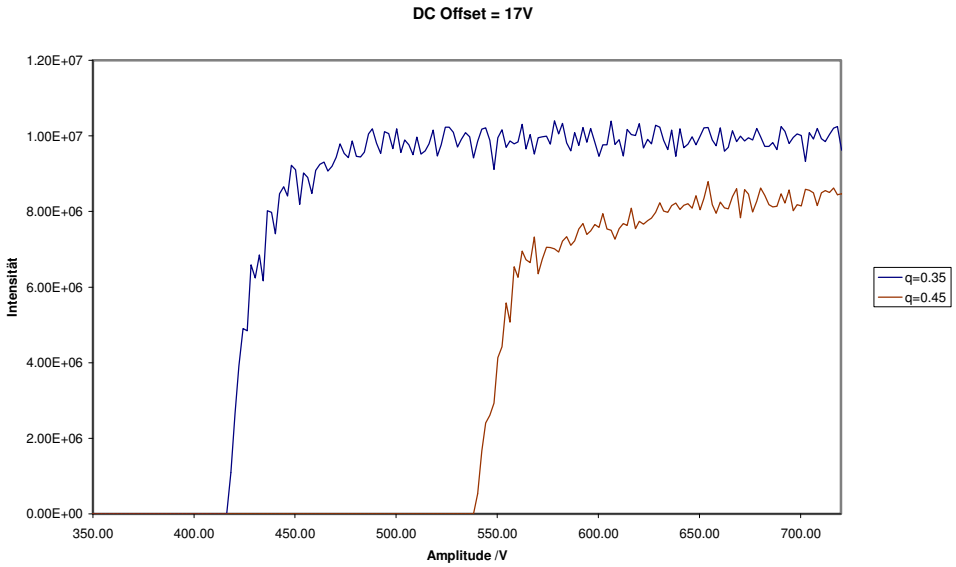


Figure 7-23. Point of optimum performance

If the DC Offset is chosen too low, strong oscillation on the signal occur. See Figure 7-24. This creates problems when calibrating the filter. Because the maxima are very narrow, the anion signal is susceptible to changes of the ion optics (thermal drift of voltages, deterioration of the reagent source, etc.) Therefore, this region is not suitable for stable operation of the ETD system even though it may include the maximum of the anion intensity.

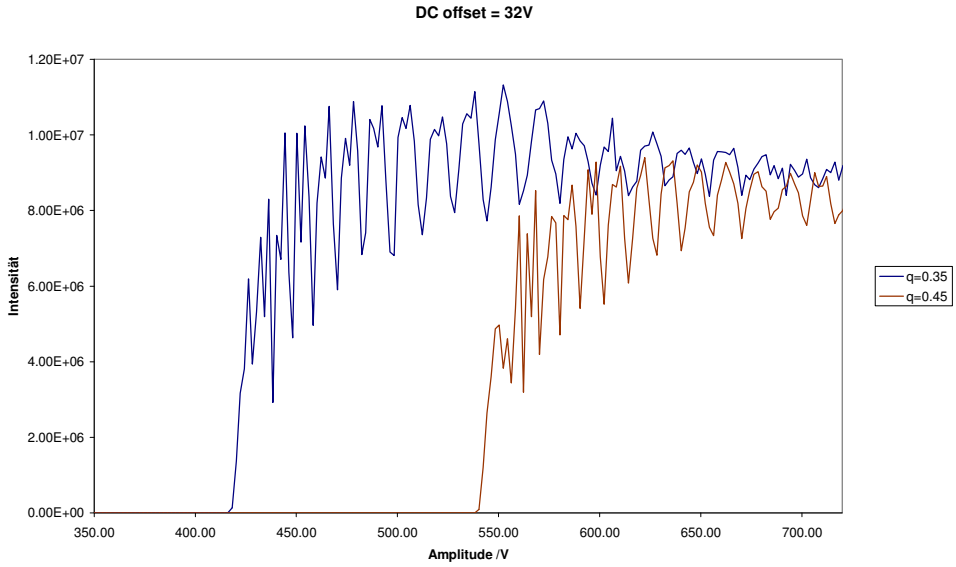
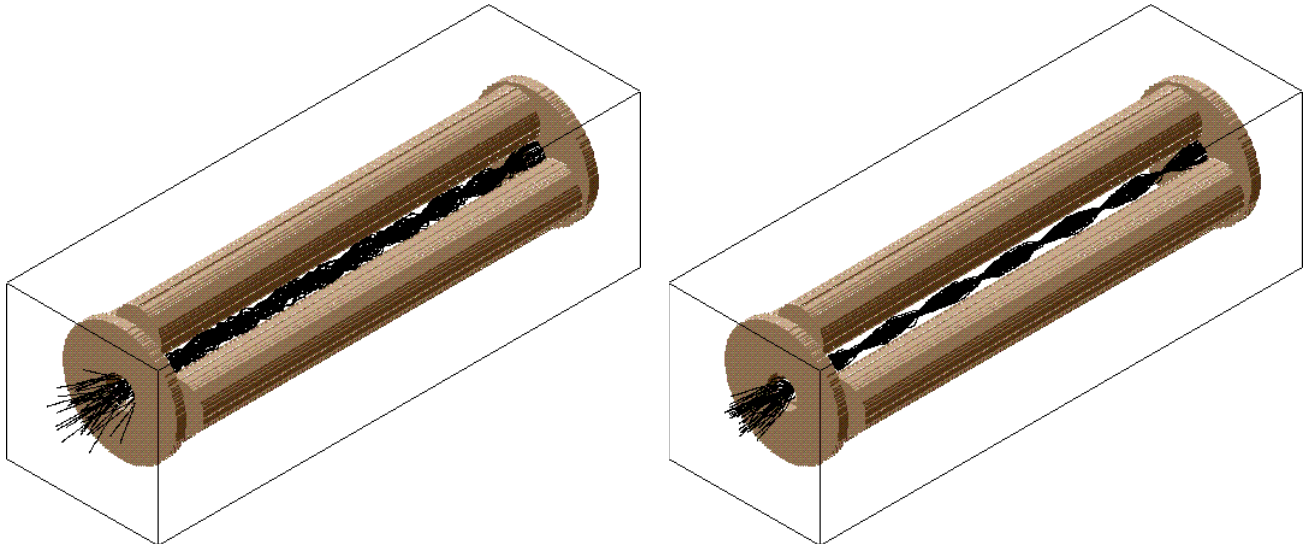


Figure 7-24. Chosen DC Offset (32 V) is too high for calibration

„Noding“ in Quadrupoles

In quadrupole filters, the intensity of the nodes increases with the kinetic energy of the ions. See Figure 7-25.



Low kinetic energy: weakly developed nodes

High kinetic energy: strongly developed nodes

Figure 7-25. “Noding” in quadrupoles

Depending on the location of the nodes, the ions leave the quadrupole as an either convergent beam (good transmission) or divergent beam (bad transmission). Tuning the voltages moves the place of the nodes, leading to oscillations of the signal.

Performing an ETD Infusion Experiment

Procedures for performing an ETD infusion experiment are described in the following sections:

- Viewing the Injection Reagent Settings
- Troubleshooting an AGC Target Error
- Obtaining an ETD Spectrum for Angiotensin I
- Optimizing the Reagent Ion Reaction Time

Viewing the Injection Reagent Settings

The number of reagent ions admitted into the linear ion trap is regulated by the parameters in the Reagent page of the Injection Control dialog box.

❖ **To view the injection reagent ion settings**

1. On the Windows taskbar, choose **Start > All Programs > Thermo Instruments > LTQ > LTQ Tune**.

   The Tune Plus window opens. (See Figure 7-1 on page 7-2.)

2. Click the Injection Control instrument control graphic or click **Setup > Injection Control** to display the Injection control dialog box.

3. Click the Reagent tab to display the Reagent page. See Figure 7-26.

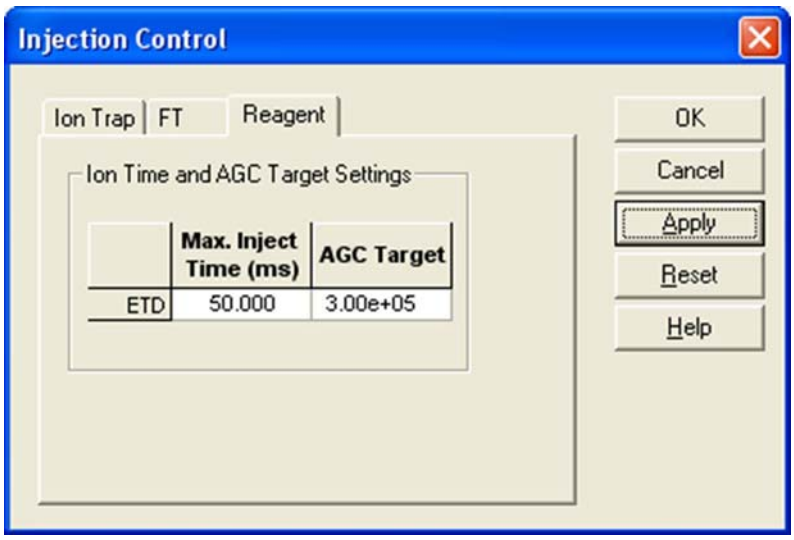


Figure 7-26. Injection Control dialog box—Reagent page

The ETD Reagent Injection control consists of two parameters:

- The AGC Target parameter sets the target number of reagent anions to be injected into the linear ion trap to perform ETD. The default value for this parameter is 3E5.
- The Max. Inject Time (ms) parameter specifies the maximum amount of time that the system allows for anions to be injected into the trap. The default value for this time is 50 ms.

The reagent ion source injects reagent anions into the linear ion trap until the ETD AGC target is reached. The time allowed to reach the ETD AGC target cannot exceed the Maximum Injection time (the Maximum Injection time takes precedence over the AGC target).

Troubleshooting an AGC Target Error

If the AGC target is not reached due to the Maximum Injection time limit, the system displays an error message advising you that the AGC target has not been reached within the specified time limit (Maximum Injection time limit exceeded). This implies that the sensitivity of the reagent ion source is too low. To deal with this error, try the following procedures:

1. To increase the sensitivity of the source, run automatic tuning of the Reagent Ion Source. (See "Automatically Tuning the Reagent Ion Source" on page 7-12.)
2. The sensitivity decrease might be due to a dirty ion volume. A sufficiently contaminated ion volume causes the Maximum Injection time limit to be exceeded. Clean or change the ion volume. Refer to the *Orbitrap Velos Pro Hardware Manual* for the procedure to do this.
3. The decrease of the signal might be due to a deformed filament. Change the filament. Refer to the *Orbitrap Velos Pro Hardware Manual* for the procedure to do this.
4. The sensitivity decrease might be due to a dirty reagent ion source and its optics. A sufficiently contaminated reagent ion source and its optics causes the Maximum Injection time limit to be exceeded. Clean or change the reagent ion source and its optics. Refer to the *Orbitrap Velos Pro Hardware Manual* for the procedure to do this.
5. Increase the emission current. However, doing this might shorten the filament life time.
6. Increase the Maximum Injection time limit. This is a temporary way to eliminate the error message. The Maximum Injection time limit can be increased up to the limits imposed by the overall scan cycle time.

Maximum Injection Time limit and AGC Target influence the scan duration when obtaining an ETD spectrum.

Obtaining an ETD Spectrum for Angiotensin I

This section assumes that you are infusing Angiotensin I into the Orbitrap Velos Pro ETD mass spectrometer according to the procedures described in the *LTQ Series Getting Started* manual. The recipe for this solution is given in "Angiotensin I Solutions" on page 7-41.

❖ **To obtain an ETD spectrum of Angiotensin I**

1. Open the Tune Plus application. The Tune Plus window appears.
2. Click **On/Off/Standby** to On. The mass spectrometer scans the infused analyte and produces a mass spectrum. See Figure 7-27.

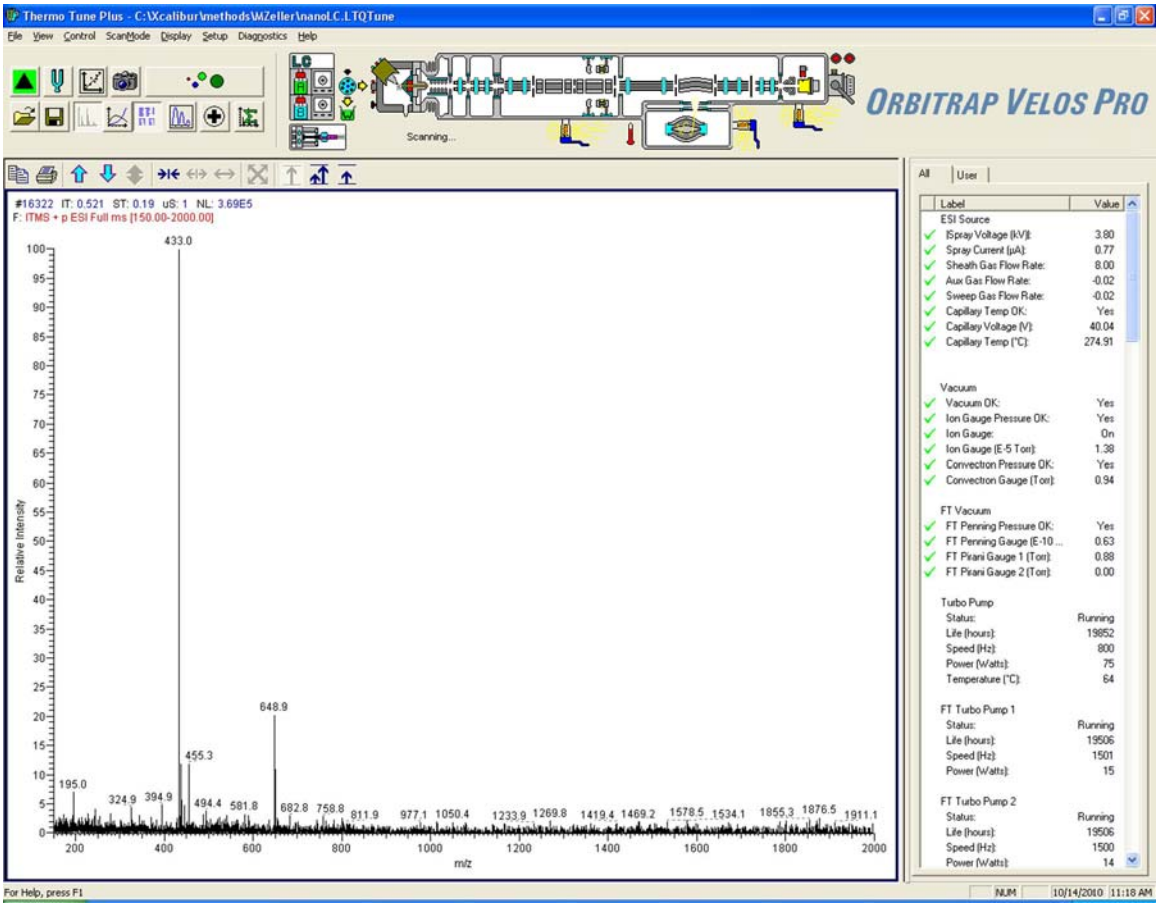


Figure 7-27. Tune Plus window showing a mass scan of infused Angiotensin I

3. Turn on the reagent ion source as explained in "Turning On the Reagent Ion Source and Viewing Reagent Ion Spectra" on page 7-10.

4. Click **Define Scan** to display the Define Scan dialog box. See Figure 7-28 on page 7-30.

5. Enter the parent ion *m/z* of the 3+ charge state of Angiotensin I in the n=2 line of the Define Scan dialog box.

Note The molecular weight of Angiotensin I (acetate hydrate) is 1296 u and the $(M + 3H)^{3+}$ parent is giving rise of a signal at *m/z* 433.0. ▲

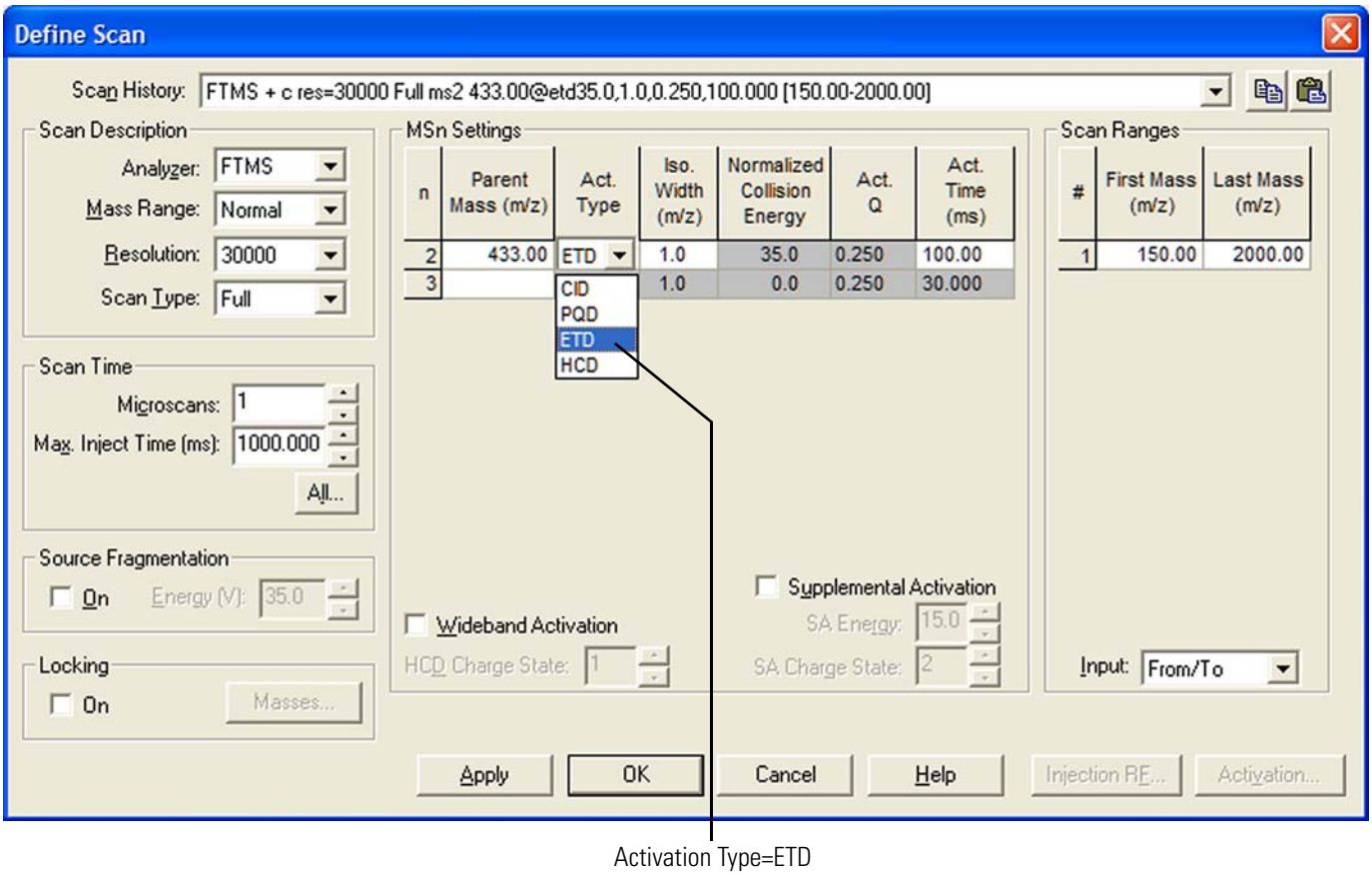


Figure 7-28. Define Scan window with the Activation Type ETD

6. Select **ETD** from the Activation Type list box in the Define Scan dialog box.

7. Click **Apply** in the Define Scan dialog box.

8. Click **OK** in the Define Scan dialog box. The Define Scan dialog box closes and the ETD MS/MS spectrum of Angiotensin I appears in the Tune Plus window. See Figure 7-29 on page 7-31.

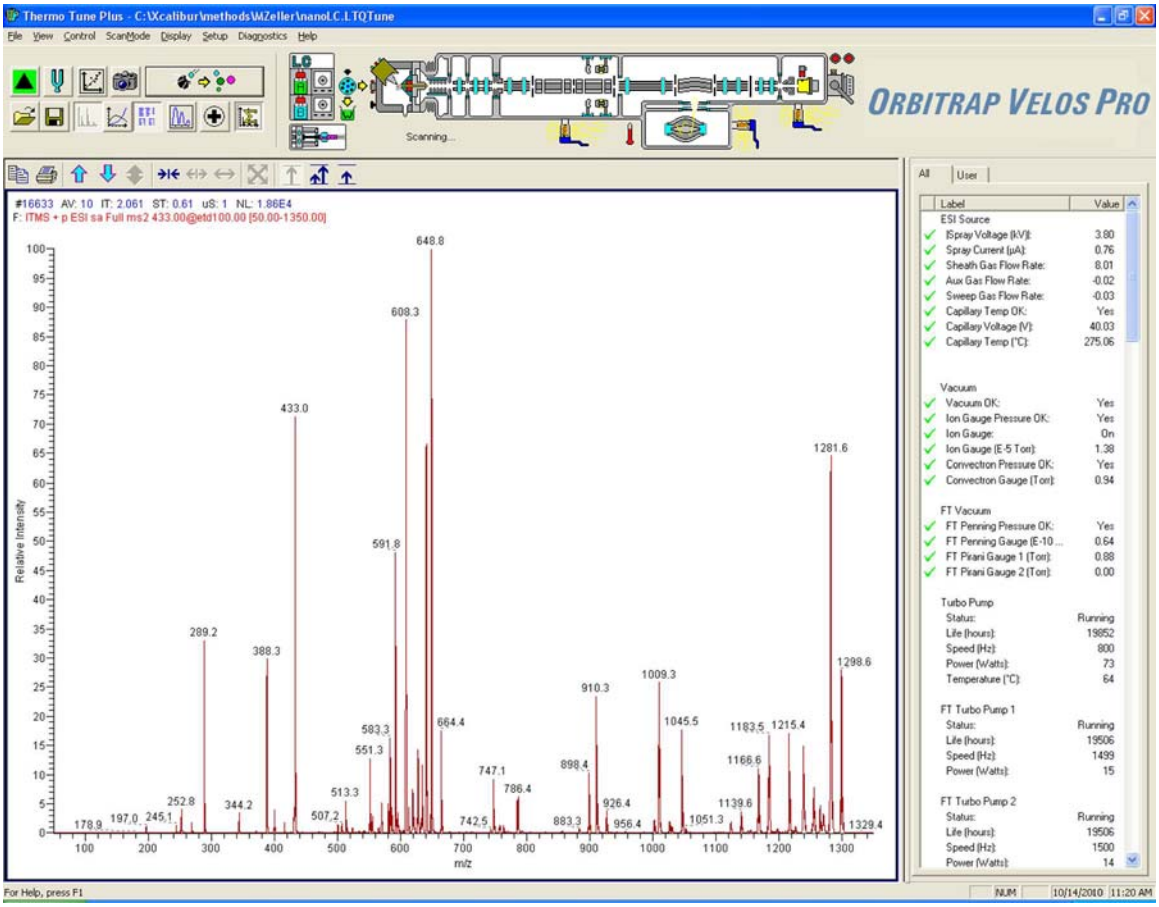


Figure 7-29. ETD MS/MS spectrum of Angiotensin I

Optimizing the Reagent Ion Reaction Time

Typically, the system default Reagent Ion Reaction Time of 100 ms is appropriate for doubly charged ions. In some cases it is helpful to obtain an optimized Reagent Ion Reaction Time for your specific analyte, especially for ions with higher charge states. The procedures presented in this topic assume that your system is generating the reagent ions as described in "Turning On the Reagent Ion Source and Viewing Reagent Ion Spectra" on page 7-10.

❖ **To obtain an optimized reagent ion reaction time**

1. Turn on ETD activation for the analyte of interest (Angiotensin I in this case).

2. On the Control/Scan Mode toolbar, click the **Define Scan** button. to display the Define Scan dialog box.
3. Enter the parent ion mass for the analyte of interest.
4. From the Act. Type list, select ETD. (See Figure 7-28 on page 7-30.)

5. Click **Tune**. The Tune dialog box opens with two additional tabs displayed for tuning ETD. See Figure 7-30 on page 7-32.

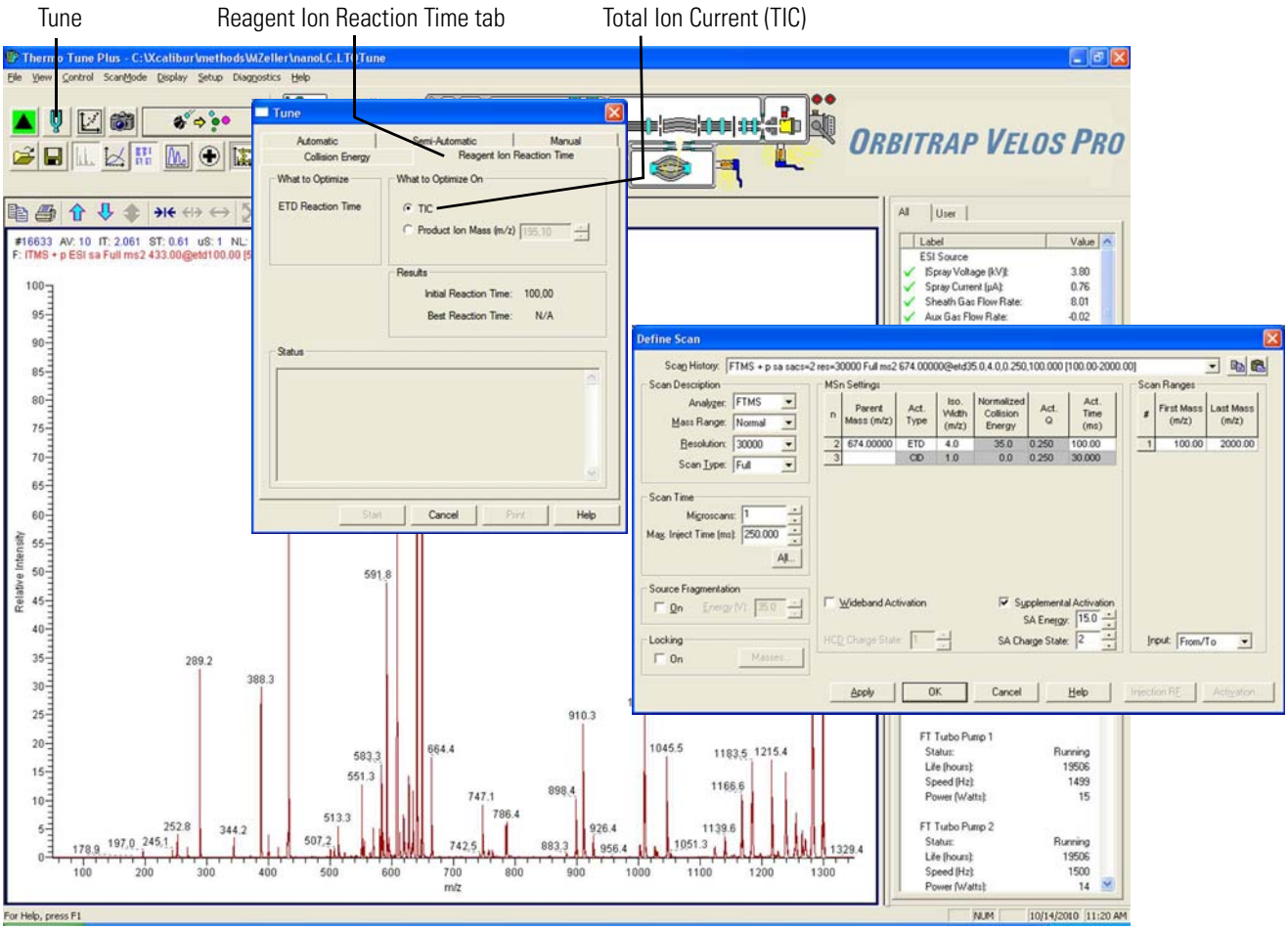


Figure 7-30. Tune and Define Scan windows open in Tune Plus

6. Click the **Reagent Ion Reaction Time** tab in the Tune dialog box. See Figure 7-30.

7. Optimize on either the total ion current of the product ions (TIC) or the *m/z* of particular product ions.

   - Optimize on the total ion current (TIC) of the product ions:

     i. Click **TIC** in the What To Optimize On section of the Tune dialog box. (See Figure 7-30.)

     ii. Click **Start**. The software generates a graph of the product ion TIC versus reaction time. The Status box of the Tune dialog box shows the optimized reagent ion reaction time after the Tune process is completed.

     iii. A pop up dialog asks if you want to accept the optimized value. If you accept the optimized value, the reagent ion reaction time is set to this optimized value in the Define

Scan dialog box. Otherwise, it is restored to its previous value.

The reagent ion reaction time is now optimized based on the total ion current.

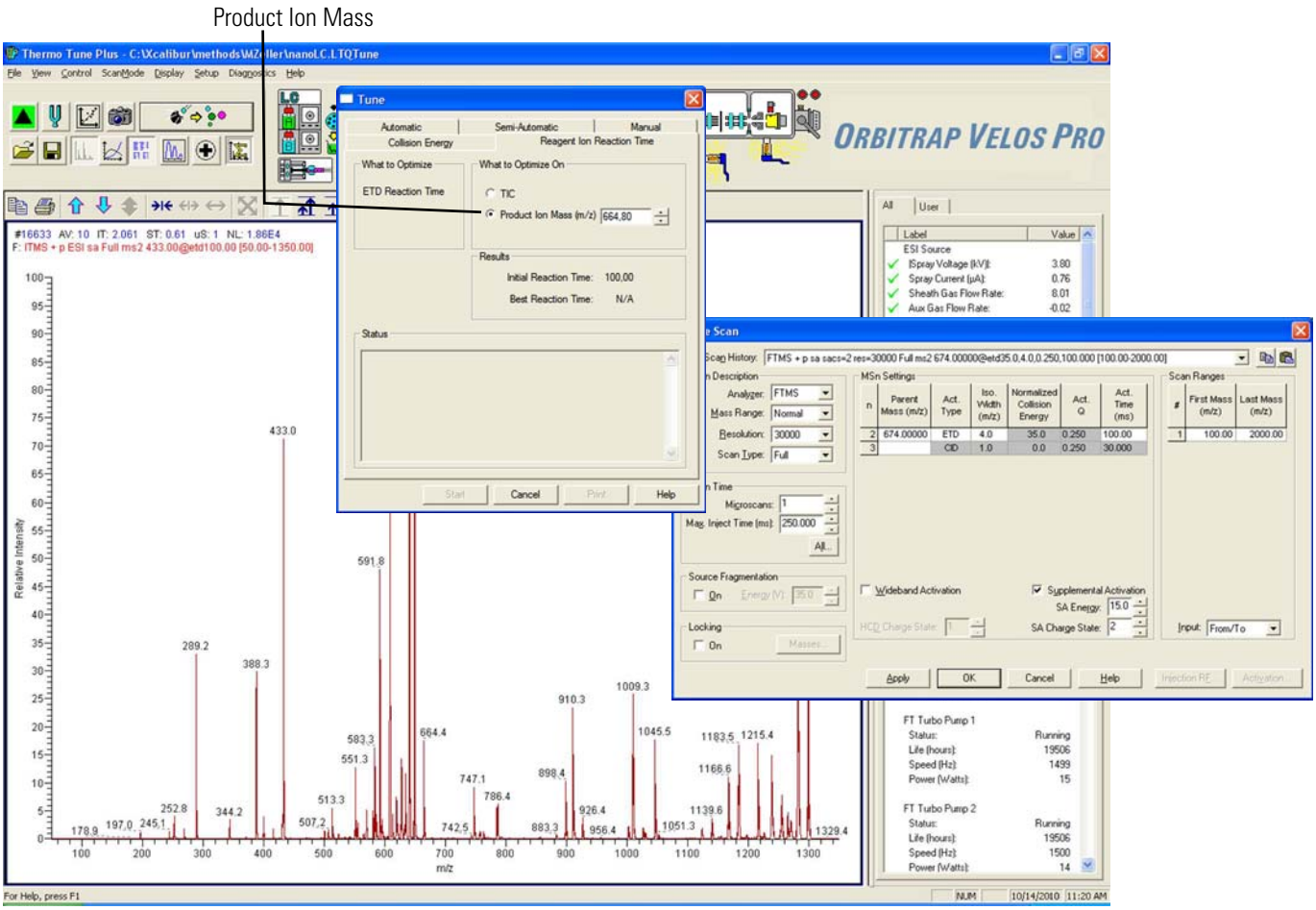


Figure 7-31. Tune window showing Product Ion Mass selected for Reagent Ion Reaction Time Optimization

- Optimize on the *m/z* of product ions:

  i. Click **Product Ion Mass** in the What To Optimize On section of the Tune dialog box. See Figure 7-31. The spin box adjacent to Product Ion Mass becomes active.

  ii. Enter the *m/z* of the fragment of interest into the spin box.

  iii. Click **Start**. The software generates a graph of intensity of the *m/z* of interest versus reaction time. The Status box of the Tune dialog box shows a reagent ion reaction time after the Tune process is completed.

  iv. A pop up dialog asks if you want to accept the optimized value. If you accept the optimized value, the reagent ion reaction time is set to this optimized value in the Define

Scan dialog box. Otherwise, it is restored to its previous value.

The reagent ion reaction time is now optimized based on a particular *m/z* of product ions.

Creating an Xcalibur Instrument Method That Uses ETD Activation

❖ **To create an Xcalibur instrument method that uses ETD activation**

1. Open the Xcalibur application (for example, from the Microsoft Windows desktop). The Roadmap Home Page appears. See Figure 7-32.

2. Click the **Instrument Setup** icon in the Roadmap Home Page.

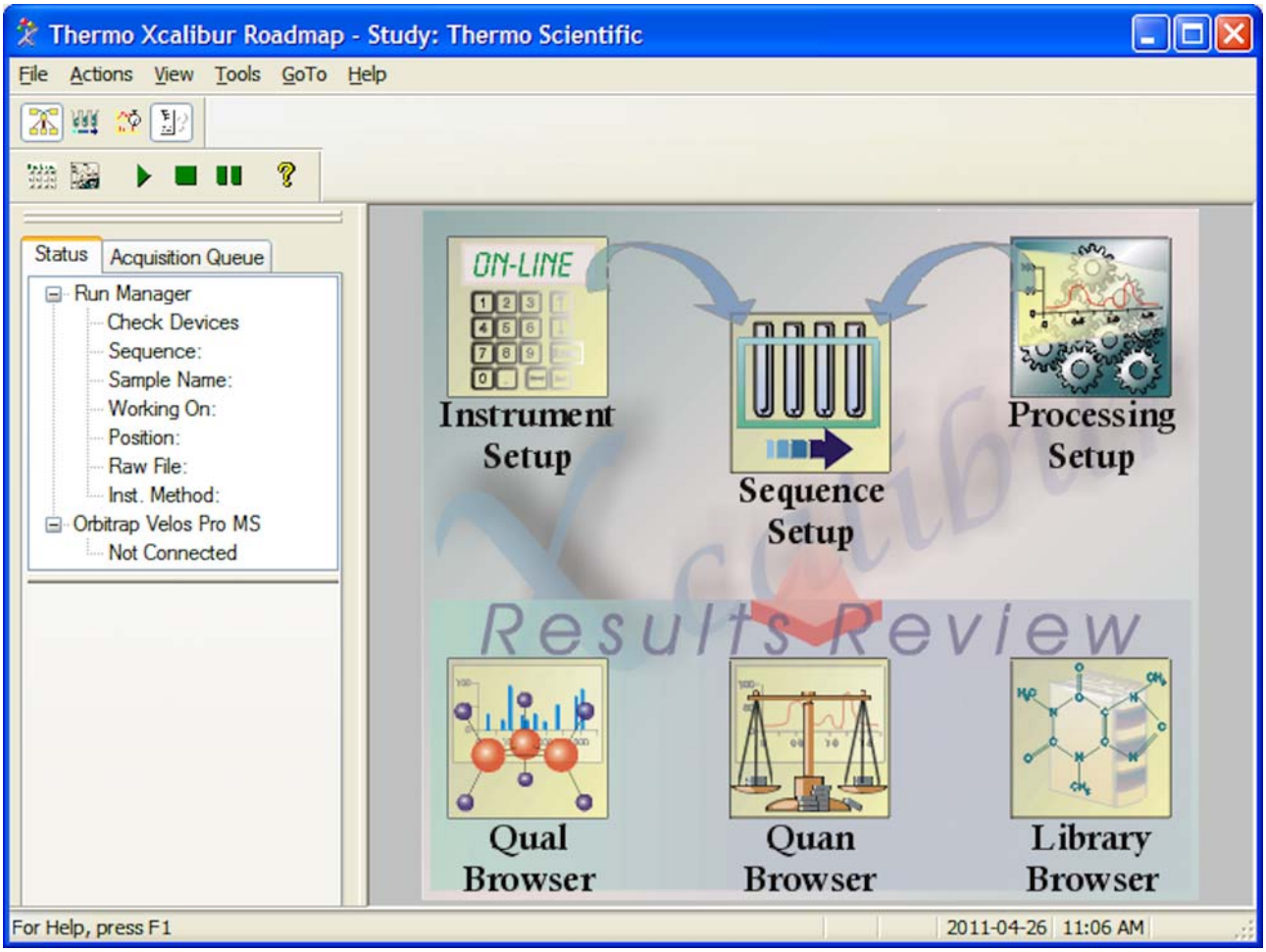


Figure 7-32. Xcalibur Roadmap Home Page

3. Click **General MS or MSn** in the Select Experiment Type section of the New Method view. See Figure 7-33 on page 7-36.

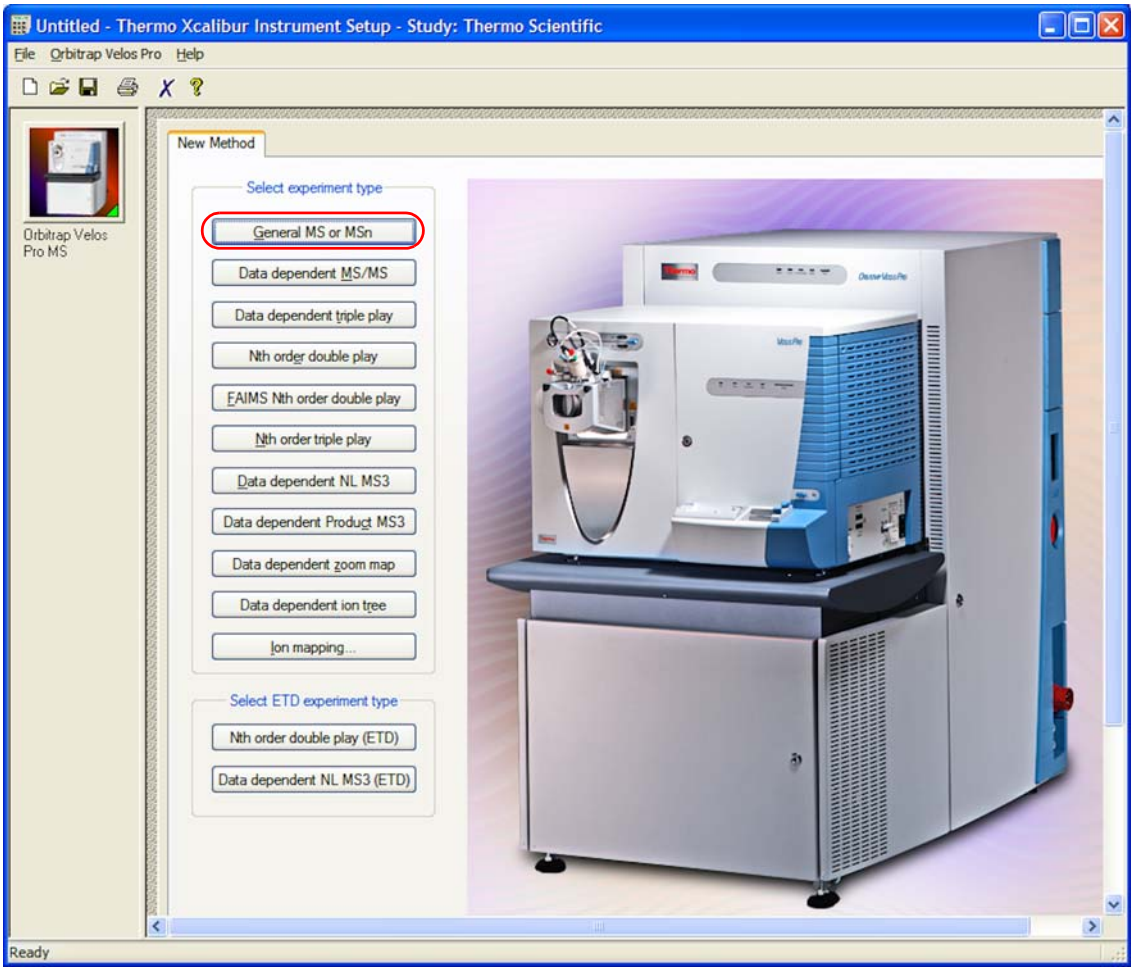


Figure 7-33. New Method view in Xcalibur Instrument Setup

4. Click the **MS Detector Setup** tab if this is not already the selected tab in the Untitled-Instrument Setup window. See Figure 7-34.

5. Load the appropriate Tune Method (for example, a method saved as described in "Saving Your ETD Tune Method" on page 7-21).

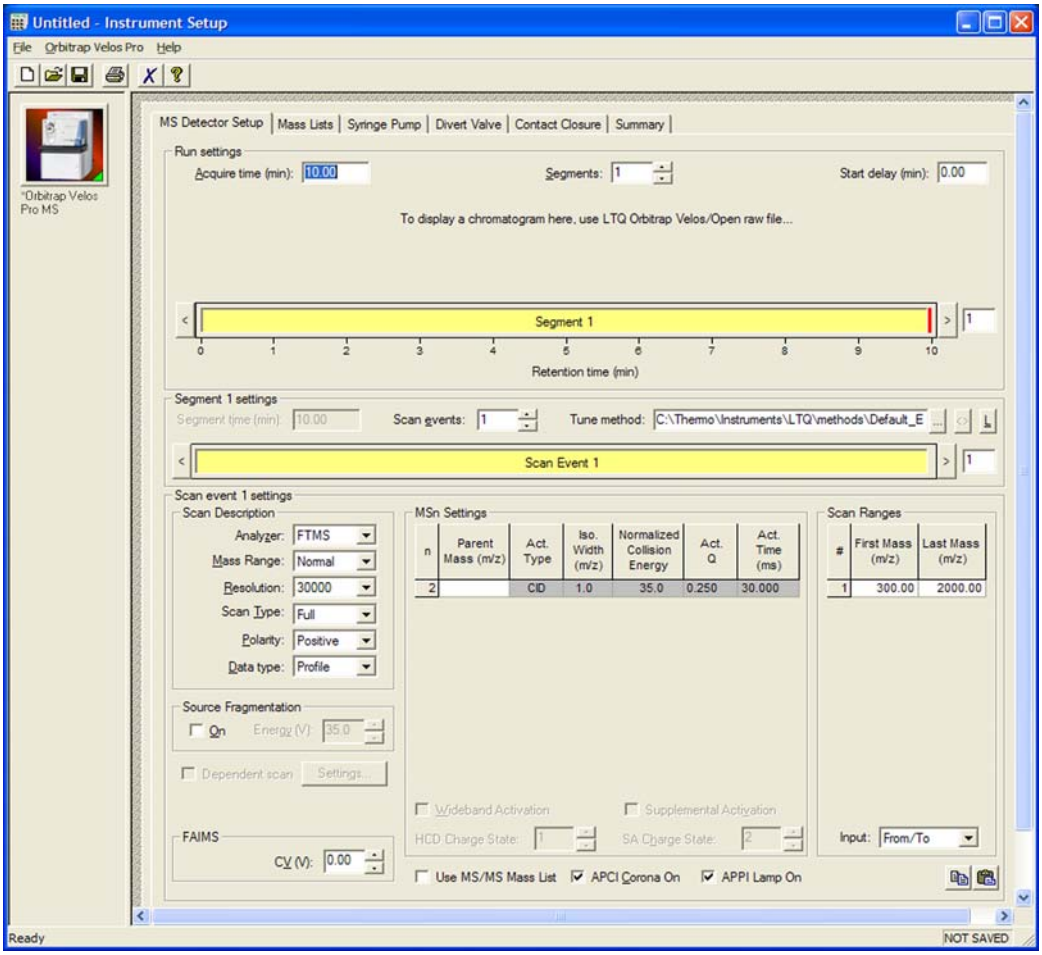


Figure 7-34. Xcalibur MS Detector Setup view

6. Choose Scan Events to be 2 or more in the Segment 1 Settings portion of the MS Detector Setup view. See Figure 7-35 on page 7-38.

7. Select the Scan Event 2 bar (or the bar for a Scan Event >2). See Figure 7-35.

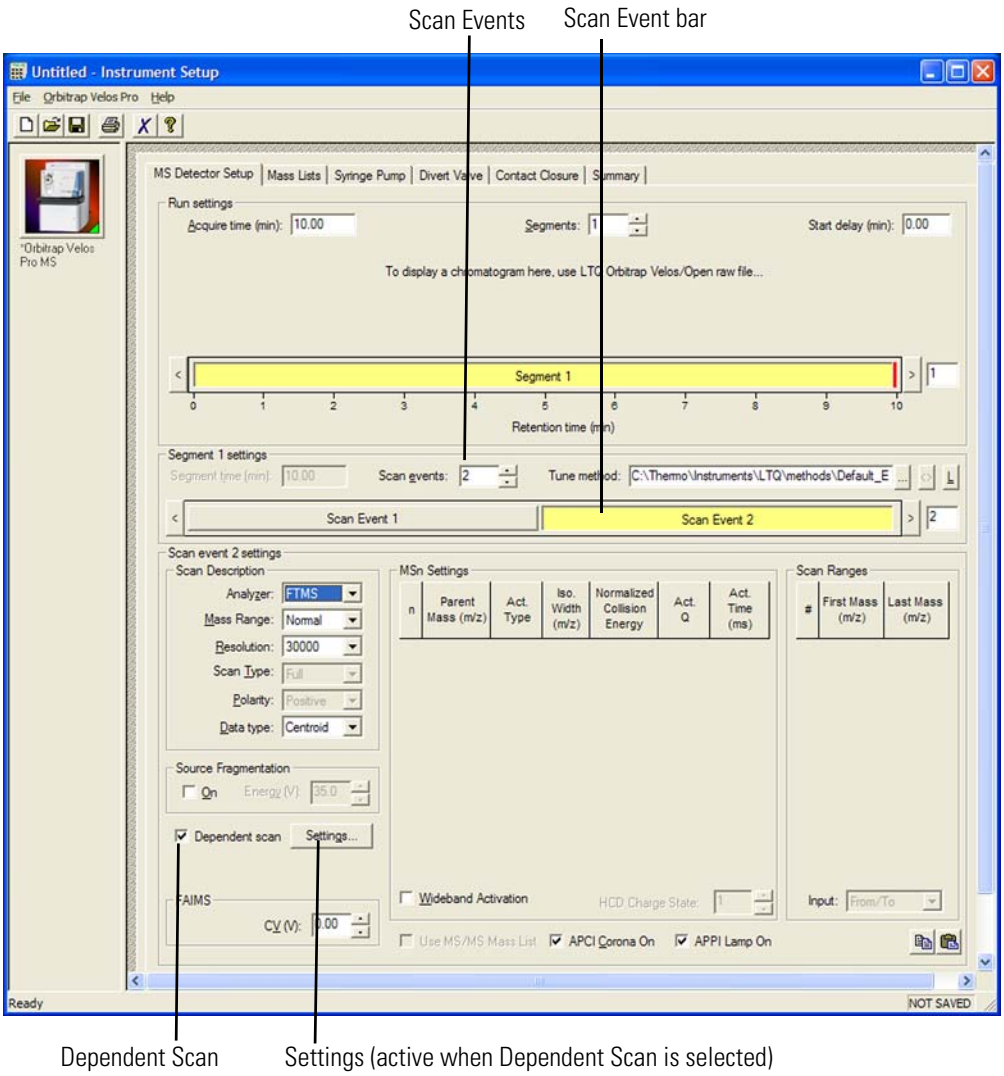


Figure 7-35. Xcalibur Instrument Setup

8. Select the **Dependent Scan** check box at the lower left corner of the MS Detector Setup view. (See Figure 7-35.) The adjacent **Settings** button becomes active.

9. Click **Settings**. A Data Dependent Settings dialog box appears. See Figure 7-36 on page 7-39.

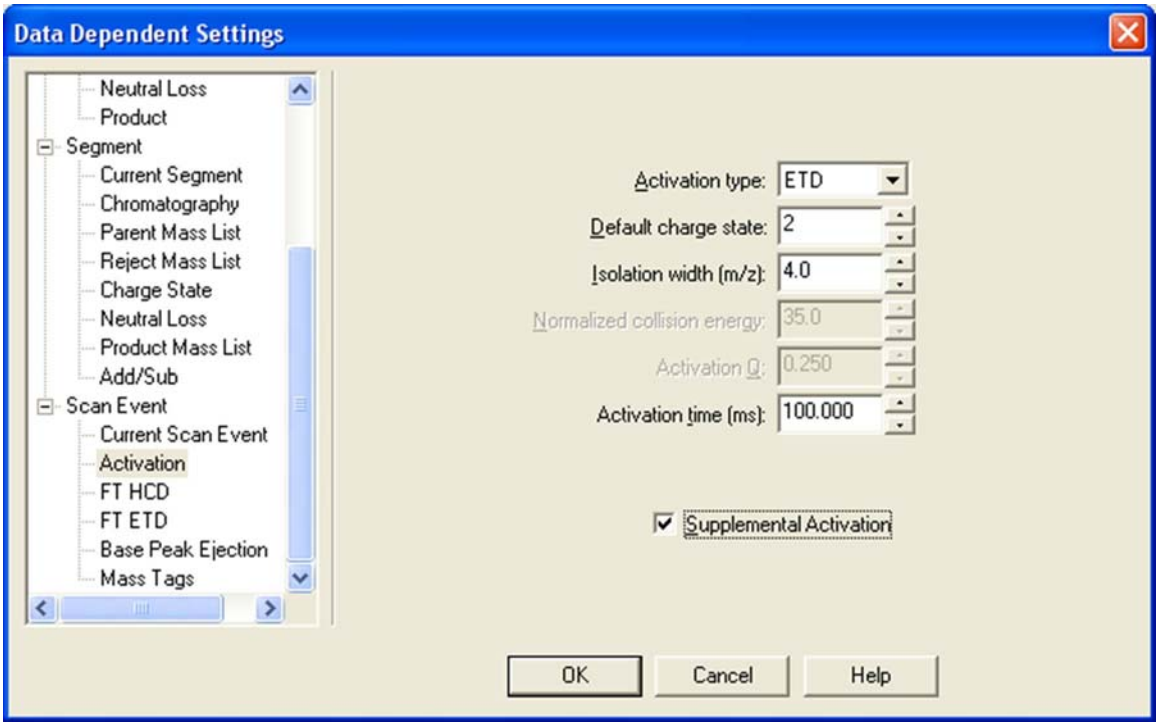


Figure 7-36. Data Dependent Settings dialog box in MS Detector Setup—Activation page

10. In the Data Dependent Settings dialog box, do the following:

    a. Choose **Scan Event > Activation** in the menu on the left side of the window.

    b. Select an Activation Type: **ETD**, **CID**, or **PQD**. The choice of Activation Type may be different for each Scan Event.

    c. For **Default Charge State** use values of 2 or more.

    d. For **Isolation Width** use values between 2 and 4.

    e. The Activation Time is either left at its default value or chosen as discussed in "Optimizing the Reagent Ion Reaction Time" on page 7-31.

    f. Click **OK** to close the Data Dependent Settings dialog box.

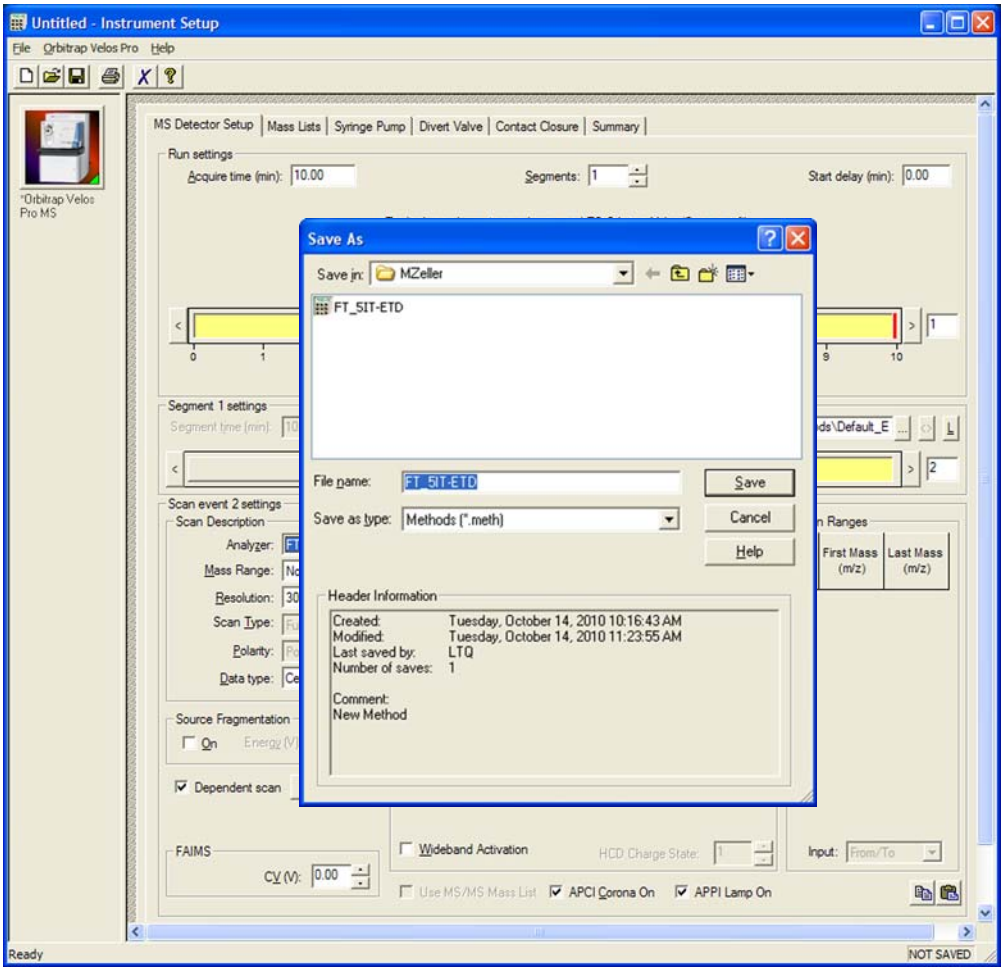


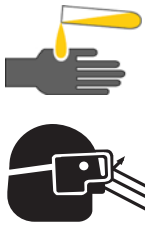
Figure 7-37. Save As window in Xcalibur Instrument Setup, MS Detector Setup view

11. Click **File > Save As** to save your Xcalibur method under the file name of your choice. (See Figure 7-37.) This method can be chosen and run when Sequence Setup is chosen in the Xcalibur Roadmap Home Page. (See Figure 7-32 on page 7-35.) Refer to your Xcalibur Help and the previous chapters of this manual for information about the other Data Dependent settings.

Angiotensin I Solutions

This section provides instructions for the preparation of solutions containing Angiotensin I (acetate hydrate). A stock solution is diluted to make a test solution. The test solution is used to demonstrate the application of the Orbitrap Velos Pro ETD mass spectrometer and to optimize the reagent ion reaction time.

Handle Angiotensin I in accordance with its Material Safety Data Sheet (MSDS).



Warning **Avoid exposure to potentially harmful materials.** Always wear protective gloves and safety glasses when you handle solvents or corrosives. Also contain waste streams and use proper ventilation. Refer to your supplier's Material Safety Data Sheet (MSDS) for proper handling of a particular solvent. ▲

Note Store and handle all chemicals in accordance with standard safety procedures. The Material Safety Data Sheet (MSDS) describing the chemicals being used should be freely available to laboratory personnel for them to examine at any time. Material Safety Data Sheets provide summarized information on the hazard and toxicity of specific chemical compounds. MSDSs also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures for cleaning spills or dealing with leaks. ▲

Producers and suppliers of chemical compounds are required by law to provide their customers with the most current health and safety information in the form of an MSDS. Read the MSDS for each chemical you use. Dispose of all laboratory reagents in the appropriate way. (Refer to the MSDS.)

The Angiotensin I in your ETD Reagent Kit (Thermo Reagent Kit P/N 98000-62008, Thermo Angiotensin P/N 00301-15517) is Sigma/Aldrich #A9650.

The Angiotensin I MSDS is obtained by clicking the MSDS link at:

www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/A9650

Other potentially hazardous chemicals used in the procedures in this section include:

- Glacial acetic acid
- Methanol

Handle these chemicals in accord with their MSDS documents.

Preparing the Angiotensin I Stock Solution

❖ **To prepare an Angiotensin I stock solution**

1. Obtain the 1 mg vial of Angiotensin I in your accessory kit.
2. Add 382 µL of water, 382 µL of methanol, and 8 µL of glacial acetic acid to the 1 mg of Angiotensin I.
3. Ensure that the Angiotensin I is thoroughly dissolved.
4. Label the vial *Angiotensin I stock solution* and store it in a freezer until it is needed.

Preparing the Angiotensin I Test Solution

❖ **To prepare an Angiotensin I sample solution**

1. Pipet 100 µL of the stock solution (1nmol/µL) of Angiotensin I into a clean polypropylene microcentrifuge tube.
2. Add 900 µL of 50:50 methanol/water (0.1% acetic acid) to the tube.
3. Mix this solution (100 pmol/µL) thoroughly.
4. Pipet 19.8 mL of 0.1% acetic acid—50:50 methanol/water into a clean 20 mL glass scintillation vial.
5. Add 200 µL of the 100 pmol/µL solution into the scintillation vial to bring the final volume to 20 mL.
6. Mix this 1 pmol/µL solution thoroughly.
7. Label the vial *Angiotensin I test solution* and store it in a freezer until it is needed.

Appendix A Miscellaneous Information

This appendix contains supplemental information for the previous chapters. It contains the following topics:

FT Analyzer Information in Scan Header

Use the Qual Browser window to open a raw file and to display scan header information for a selected scan in any of the cells. Choose **View > Scan Header** to display the Scan Header of the current scan in the active cell.

FT Analyzer Settings

The scan header information of an FTMS scan includes information about the FT Analyzer Settings that is not available in the usual Reports (Tune method, Instrument method, Status log, or Error log):

T=1e5	AGC Target for this scan (here: 1e+05)
PsIT=0.65	Prescan Inject Time (here: 0.65 ms)
Tog=(…)	Manual diagnostic toggles are set different from their default values. See Table A-1 below for detailed information.
iWf	Inject waveform on for this scan.
PvR=2e4	Preview analysis active for this scan
DiagManualSettings	Calibration parameters were manually changed under Diagnostics.

Table A-1. Actual settings of manual toggles

Tog = (…)	Relevant Toggle	Current setting	Default setting
ApoOff	FT apodization	Off	On
TrExp	FT include transient	On	Off
FullP	FT profile mode	Full	Reduced
IWFoff	FT SIM and MS^n injection waveforms	Off	On
Freq	FT view frequency	On	Off
Offset	FT zero offset	On	Off

FT Analyzer Messages

The scan header of an FT scan includes also so-called FT Analyzer Messages:

RF=1535V	RF amplitude value (here: 1535 V)
HCD=148eV	HCD collision energy in eV (here: 148 eV)
Ufill=0.45	Maximum ion time reached. Here: the real number of ions is only ~45% of the target value.
MCal=4d	Last mass calibration for this scan range is several days old (here: 4 days)
Est=0x24	Machine-readable result message for post-processing tools
DAC=0.98	FT transient measurement near saturation, this might result in spectral harmonics (typically target value too high)
TCal=[195..]	This is a hint that the current scan range settings for the FT analyzer are outside the calibrated storage/transfer mass range. Transfer parameters are extrapolated.
Lock=(inj524.3,1/1,+3ppm)	Information about lock mass settings, extra SIM injection of lock mass ions, number of identified lock masses in the spectrum, and deviation of corrected (locked) masses compared to the external mass calibration.
Stable=15min	Shows the elapsed stabilization time of the FTMS analyzer high voltage electronics after last off state or polarity switch. For best external mass accuracies, it is required to let the FTMS analyzer high voltage electronics stabilize before performing an acquisition or mass calibration.

TempDiff=1	There is a temperature difference in the FTMS analyzer temperature between mass calibration time and current state. This may be caused by setting a different analyzer temperature setpoint in instrument configuration, by rapid significant changes in the ambient temperature, or by not waiting for temperature stabilization after instrument (temperature regulation) was off.
PkOvf	Internal Peak detection overflow in the FT spectrum analysis

Note The actual FT Analyzer Messages can also be displayed in the Tune Spectrum view, see "Spectrum View" on page 2-3. ▲

Data Size of FT Raw Files

The data size of a raw file with FT data depends on many parameters: for example, on the number of scans, the resolution setting, and the data format.

Table A-2 below displays typical data sizes (per scan) of an FT spectrum (negative mode calibration solution, scan range *m/z* 120–1 200, AGC target 5E5, 1 microscan, resolution setting 60 000) at different FT data formats.

Table A-2. Typical data sizes (per scan) of an FT spectrum

FT Data Format	Typical data size / scan
Centroid	ca. 10 kB
Reduced Profile	ca. 20 kB
Full Profile	ca. 2800 kB

Glossary

This section lists and defines terms used in this guide. It also includes acronyms, metric prefixes, symbols, and abbreviations.

A

A ampere

AC alternating current

ADC analog-to-digital converter; a device that converts data from analog to digital form.

adduct ion An ion formed by the joining together of two species, usually an ion and a molecule, and often within the ion source, to form an ion containing all the constituent atoms of both species.

AGC™ See Automatic Gain Control™ (AGC).

APCI See atmospheric pressure chemical ionization (APCI).

APCI corona discharge current The ion current carried by the charged particles in the APCI source. The voltage on the APCI corona discharge needle supplies the potential required to ionize the particles. The APCI corona discharge current is set; the APCI corona discharge voltage varies, as required, to maintain the set discharge current.

See also corona discharge and APCI corona discharge voltage.

APCI corona discharge voltage The high voltage that is applied to the corona discharge needle in the APCI source to produce the APCI corona discharge. The corona discharge voltage varies, as required, to maintain the set APCI spray current.

See also APCI spray current.

APCI manifold The manifold that houses the APCI sample tube and nozzle, and contains the plumbing for the sheath and auxiliary gas.

APCI needle, corona discharge A needle to which a sufficiently high voltage (typically ±3 to ±5 kV) is applied to produce a chemical ionization plasma by the corona discharge mechanism.

See also chemical ionization (CI), chemical ionization (CI) plasma, atmospheric pressure chemical ionization (APCI), and corona discharge.

APCI nozzle The nozzle in the APCI probe that sprays the sample solution into a fine mist.

See also atmospheric pressure chemical ionization (APCI).

APCI sample tube A fused silica tube that delivers sample solution to the APCI nozzle. The APCI sample tube extends from the sample inlet to the APCI nozzle.

See also atmospheric pressure chemical ionization (APCI), and API stack.

APCI source Contains the APCI probe assembly, APCI manifold, and API stack.

See also atmospheric pressure chemical ionization (APCI), APCI manifold, and API stack.

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

APCI vaporizer A heated tube that vaporizes the sample solution as the solution exits the sample tube and enters the atmospheric pressure region of the APCI source.

See also atmospheric pressure chemical ionization (APCI).

API See atmospheric pressure ionization (API).

API atmospheric pressure region The first of two chambers in the API source. Also referred to as the spray chamber.

API capillary-skimmer region The area between the capillary and the skimmer, which is surrounded by the tube lens. It is also the area of first-stage evacuation in the API source.

API heated capillary A tube assembly that assists in desolvating ions that are produced by the ESI or APCI probe.

See also API heated capillary voltage.

API heated capillary voltage The DC voltage applied to the heated capillary. The voltage is positive for positive ions and negative for negative ions.

See also API source and API heated capillary.

API ion transfer capillary A tube assembly that assists in desolvating ions that are produced by the ESI, NSI, or APCI probe.

See also API ion transfer capillary offset voltage and API ion transfer capillary temperature.

API ion transfer capillary offset voltage A DC voltage applied to the ion transfer capillary. The voltage is positive for positive ions and negative for negative ions.

See also API source and API ion transfer capillary.

API ion transfer capillary temperature The temperature of the ion transfer capillary, which should be adjusted for different flow rates.

See also API source and API ion transfer capillary.

API source The sample interface between the LC and the mass spectrometer. It consists of the API probe (ESI or APCI) and API stack.

See also atmospheric pressure ionization (API), ESI source, APCI source, ESI probe, and API stack.

API spray chamber The first of two chambers in the API source. In this chamber the sample liquid exits the probe and is sprayed into a fine mist (ESI or NSI) or is vaporized (APCI) as it is transported to the entrance end of the ion transfer capillary.

API spray shield A stainless steel, cylindrical vessel that, in combination with the ESI or APCI flange, forms the atmospheric pressure region of the API source.

See also atmospheric pressure ionization (API).

API stack Consists of the components of the API source that are held under vacuum and includes the API spray shield, API ion transfer capillary, API tube lens, skimmer, the ion transfer capillary mount, and the tube lens and skimmer mount.

See also atmospheric pressure ionization (API) and API source.

API tube lens A lens in the API source that separates ions from neutral particles as they leave the ion transfer capillary. A potential applied to the tube lens focuses the ions toward the opening of the skimmer and helps to dissociate adduct ions.

See also API tube lens offset voltage, API source, API ion transfer capillary, and adduct ion.

API tube lens and skimmer mount A mount that attaches to the heated capillary mount. The tube lens and skimmer attach to the tube lens and skimmer mount.

API tube lens offset voltage A DC voltage applied to the tube lens. The value is normally tuned for a specific compound.

See also API tube lens, adduct ion, and source CID.

AP-MALDI See atmospheric pressure matrix-assisted laser desorption/ionization (AP-MALDI).

APPI See Atmospheric Pressure Photoionization (APPI).

ASCII American Standard Code for Information Interchange

atmospheric pressure chemical ionization (APCI) A soft ionization technique done in an ion source operating at atmospheric pressure. Electrons from a corona discharge initiate the process by ionizing the mobile phase vapor molecules. A reagent gas forms, which efficiently produces positive and negative ions of the analyte through a complex series of chemical reactions.

See also electrospray ionization (ESI).

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

atmospheric pressure matrix-assisted laser desorption/ionization (AP-MALDI) Matrix-assisted laser desorption/ionization in which the sample target is at atmospheric pressure.

See also matrix-assisted laser desorption/ionization (MALDI).

Atmospheric Pressure Photoionization (APPI) A soft ionization technique in which an ion is generated from a molecule when it interacts with a photon from a light source.

atomic mass unit Atomic Mass Unit (u) defined by taking the mass of one atom of carbon12 as being 12u; unit of mass for expressing masses of atoms or molecules.

Automatic Gain Control™ (AGC) Sets the ion injection time to maintain the optimum quantity of ions for each scan. With AGC on, the scan function consists of a prescan and an analytical scan.

See also ion injection time.

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

auxiliary gas flow rate The relative rate of flow of auxiliary gas (nitrogen) into the API source reported in arbitrary units.

auxiliary gas inlet An inlet in the API probe where auxiliary gas is introduced into the probe.

See also auxiliary gas and atmospheric pressure ionization (API).

auxiliary gas plumbing The gas plumbing that delivers outer coaxial nitrogen gas to the ESI or APCI nozzle.

auxiliary gas valve A valve that controls the flow of auxiliary gas into the API source.

B

b bit

B byte (8 b)

baud rate data transmission speed in events per second

BTU British thermal unit, a unit of energy

C

°C degrees Celsius

CE central electrode (of the Orbitrap analyzer);

European conformity. Mandatory European marking for certain product groups to indicate conformity with essential health and safety requirements set out in European Directives.

cfm cubic feet per minute

chemical ionization (CI) The formation of new ionized species when gaseous molecules interact with ions. The process can involve transfer of an electron, proton, or other charged species between the reactants.

chemical ionization (CI) plasma The collection of ions, electrons, and neutral species formed in the ion source during chemical ionization.

See also chemical ionization (CI).

CI See chemical ionization (CI).

CID See collision-induced dissociation (CID).

cm centimeter

cm^3 cubic centimeter

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

collision-induced dissociation (CID) An ion/neutral process in which an ion is dissociated as a result of interaction with a neutral target species.

consecutive reaction monitoring (CRM) scan type A scan type with three or more stages of mass analysis and in which a particular multi-step reaction path is monitored.

Convectron™ gauge A thermocouple bridge gauge that is sensitive to the pressure as well as the thermal conductivity of the gas used to measure pressures between X and Y.

corona discharge In the APCI source, an electrical discharge in the region around the corona discharge needle that ionizes gas molecules to form a chemical ionization (CI) plasma, which contains CI reagent ions.

See also chemical ionization (CI) plasma and atmospheric pressure chemical ionization (APCI).

CPU central processing unit (of a computer)

CRM See consecutive reaction monitoring (CRM) scan type.

C-Trap curved linear trap

<Ctrl> control key on the terminal keyboard

D

d depth

Da dalton

DAC digital-to-analog converter

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

data-dependent scan A scan mode that uses specified criteria to select one or more ions of interest on which to perform subsequent scans, such as MS/MS or ZoomScan.

DC direct current

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

DS data system

DSP digital signal processor

E

ECD See electron capture dissociation (ECD).

EI electron ionization

electron capture dissociation (ECD) A method of fragmenting gas phase ions for tandem mass spectrometric analysis. ECD involves the direct introduction of low energy electrons to trapped gas phase ions.

See also electron transfer dissociation (ETD) and infrared multiphoton dissociation (IRMPD).

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

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

See also fluoranthene.

electrospray ionization (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.

EMBL European Molecular Biology Laboratory

<Enter> Enter key on the terminal keyboard

ESD ElectroStatic Discharge. Discharge of stored static electricity that can damage electronic equipment and impair electrical circuitry, resulting in complete or intermittent failures.

ESI See electrospray ionization (ESI).

ESI flange A flange that holds the ESI probe in position next to the entrance of the heated capillary, which is part of the API stack. The ESI flange also seals the atmospheric pressure region of the API source and, when it is in the engaged position against the spray shield, compresses the high-voltage safety-interlock switch.

ESI probe A probe that produces charged aerosol droplets that contain sample ions. The ESI probe is typically operated at liquid flows of 1 µL/min to 1 mL/min without splitting. The ESI probe includes the ESI manifold, sample tube, nozzle, and needle.

ESI source Contains the ESI probe and the API stack.

See also electrospray ionization (ESI), ESI probe, and API stack.

ESI spray current The flow of charged particles in the ESI source. The voltage on the ESI spray needle supplies the potential required to ionize the particles.

ESI spray voltage The high voltage that is applied to the spray needle in the ESI source to produce the ESI spray current. In ESI, the voltage is applied to the spray liquid as it emerges from the nozzle.

See also ESI spray current.

ETD See electron transfer dissociation (ETD).

eV Electron Volt. The energy gained by an electron that accelerates through a potential difference of one volt.

Extensible Markup Language See XML (Extensible Markup Language).

external lock mass A lock that is analyzed in a separate MS experiment from your sample. If you need to run a large number of samples, or if accurate mass samples will be intermingled with standard samples, you might want to use external lock masses. These allow more rapid data acquisition by eliminating the need to scan lock masses during each scan.

See also internal lock mass.

F

f femto (10^{-15})

°F degrees Fahrenheit

***.fasta* file** extension of a SEQUEST™ search database file

ft foot

Fast Fourier Transform (FFT) An algorithm that performs a Fourier transformation on data. A Fourier transform is the set of mathematical formulae by which a time function is converted into a frequency-domain function and the converse.

FFT See Fast Fourier Transform (FFT).

fluoranthene A reagent anion that is used in an electron transfer dissociation (ETD) experiment.

firmware Software routines stored in read-only memory. Startup routines and low-level input/output instructions are stored in firmware.

forepump The pump that evacuates the foreline. A rotary-vane pump is a type of forepump.

Fourier Transform - Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) A technique that determines the mass-to-charge ratio of an ion by measuring its cyclotron frequency in a strong magnetic field.

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

fragmentation The dissociation of a molecule or ion to form fragments, either ionic or neutral. When a molecule or ion interacts with a particle (electron, ion, or neutral species) the molecule or ion absorbs energy and can subsequently fall apart into a series of charged or neutral fragments. The mass spectrum of the fragment ions is unique for the molecule or ion.

FT Fourier Transformation

FT-ICR MS See Fourier Transform - Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS).

FTMS Fourier Transformation Mass Spectroscopy

full-scan type Provides a full mass spectrum of each analyte or parent ion. With the full-scan type, the mass analyzer is scanned from the first mass to the last mass without interruption. Also known as single-stage full-scan type.

FWHM Full Width at Half Maximum

G

g gram

G Gauss; giga (10^9)

GC gas chromatograph; gas chromatography

GC/MS gas chromatography / mass spectrometer

GUI graphical user interface

H

h hour

h height

handshake A signal that acknowledges that communication can take place.

HCD See higher energy collision-induced dissociation (HCD).

header information Data stored in each data file that summarizes the information contained in the file.

H-ESI probe Heated-electrospray ionization (H-ESI) converts ions in solution into ions in the gas phase by using electrospray ionization (ESI) in combination with heated auxiliary gas.

higher energy collision-induced dissociation (HCD)

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

high performance liquid chromatography (HPLC)

Liquid chromatography in which the liquid is driven through the column at high pressure. Also known as high pressure liquid chromatography.

HPLC See high performance liquid chromatography (HPLC).

HV high voltage

Hz hertz (cycles per second)

I

ICR ion cyclotron resonance

ID inside diameter

IEC International Electrotechnical Commission

IEEE Institute of Electrical and Electronics Engineers

in. inch

infrared multiphoton dissociation (IRMPD) In infrared multiphoton dissociation (IRMPD), multiply charged ions consecutively absorb photons emitted by a infrared laser until the vibrational excitation is sufficient for their fragmentation. The fragments continue to pick up energy from the laser pulse and fall apart further to ions of lower mass.

See also electron capture dissociation (ECD).

instrument method A set of experiment parameters that define Xcalibur operating settings for the autosampler, liquid chromatograph (LC), mass spectrometer, divert valve, syringe pump, and so on. Instrument methods are saved as file type .meth.

internal lock mass A lock that is analyzed during the same MS experiment as your sample and is contained within the sample solution or infused into the LC flow during the experiment. Internal lock masses provide the most accurate corrections to the data.

See also external lock mass.

I/O input/output

ion gauge Measures the pressure in the mass analyzer region (high vacuum region) of the vacuum manifold.

ion injection time The amount of time that ions are allowed to accumulate in the ion trap mass analyzer when AGC is off. With AGC on, the ion injection time is set automatically (up to the set maximum ion injection time) based on the AGC target value.

See also: Automatic Gain Control™ (AGC).

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

ion source A device that converts samples to gas-phase ions.

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

ion sweep gas Extra nitrogen gas that flows along the axis of the API ion transfer capillary (between the ion sweep cone and the capillary block) towards the API spray. The sweep gas flow is thus countercurrent to the flow of the ions.

See also ion sweep gas pressure.

ion sweep gas pressure The rate of flow of the sweep gas (nitrogen) into the API source. A measurement of the relative flow rate (in arbitrary units) to provide the required flow of nitrogen gas out from the Ion Sweep cone towards the API spray.

See also ion sweep gas.

IRMPD See infrared multiphoton dissociation (IRMPD).

K

k kilo (10^3, 1000)

K kilo (2^{10}, 1024)

KEGG Kyoto Encyclopedia of Genes and Genomes

kg kilogram

L

l length

L liter

LAN local area network

lb pound

LC See liquid chromatography (LC).

LC/MS See liquid chromatography / mass spectrometry (LC/MS).

LED light-emitting diode

LHe liquid helium

liquid chromatography (LC) A form of elution chromatography in which a sample partitions between a stationary phase of large surface area and a liquid mobile phase that percolates over the stationary phase.

liquid chromatography / mass spectrometry (LC/MS) An analytical technique in which a high-performance liquid chromatograph (LC) and a mass spectrometer (MS) are combined.

LN2 liquid nitrogen

lock mass A known reference mass in the sample that is used to correct the mass spectral data in an accurate mass experiment and used to perform a real-time secondary mass calibration that corrects the masses of other peaks in a scan. Lock masses with well-defined, symmetrical peaks work best. You can choose to use internal lock mass or external lock mass.

log file A text file, with a .log file extension, that is used to store lists of information.

M

µ micro (10^{-6})

m meter; milli (10^{-3})

M mega (10^6)

M^+ molecular ion

MALDI See matrix-assisted laser desorption/ionization (MALDI).

matrix-assisted laser desorption/ionization (MALDI) A method of ionizing proteins where a direct laser beam is used to facilitate vaporization and ionization while a matrix protects the biomolecule from being destroyed by the laser.

MB Megabyte (1 048 576 bytes)

MH^+ protonated molecular ion

microscan One mass analysis (ion injection and storage or scan-out of ions) followed by ion detection. Microscans are summed, to produce one scan, to improve the signal-to-noise ratio of the mass spectral data. The number of microscans per scan is an important factor in determining the overall scan time.

min minute

mL milliliter

mm millimeter

MRFA A peptide with the amino acid sequence methionine–arginine–phenylalanine–alanine.

MS mass spectrometer; mass spectrometry

MS MS^n power: where n = 1

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

MSDS Material Safety Data Sheet

MS/MS Mass spectrometry/mass spectrometry, or tandem mass spectrometry is an analytical technique that involves two stages of mass analysis. In the first stage, ions formed in the ion source are analyzed by an initial analyzer. In the second stage, the mass-selected ions are fragmented and the resultant ionic fragments are mass analyzed.

MS^n scan mode The scan power equal to 1 to 10, where the scan power is the power *n* in the expression MS^n. MS^n is the most general expression for the scan mode, which can include the following:

- The scan mode corresponding to the one stage of mass analysis in a single-stage full-scan experiment or a selected ion monitoring (SIM) experiment
- The scan mode corresponding to the two stages of mass analysis in a two-stage full-scan experiment or a selected reaction monitoring (SRM) experiment
- The scan mode corresponding to the three to ten stages of mass analysis (n = 3 to n = 10) in a multi-stage full-scan experiment or a consecutive reaction monitoring (CRM) experiment

See also MS scan modes and MS/MS.

multipole A symmetrical, parallel array of (usually) four, six, or eight cylindrical rods that acts as an ion transmission device. An RF voltage and DC offset voltage are applied to the rods to create an electrostatic field that efficiently transmits ions along the axis of the multipole rods.

m/z Mass-to-charge ratio. An abbreviation used to denote the quantity formed by dividing the mass of an ion (in u) by the number of charges carried by the ion. For example, for the ion $C_7H_7^{2+}$, m/z=45.5.

N

n nano (10^{-9})

nanospray ionization (NSI) A type of electrospray ionization (ESI) that accommodates very low flow rates of sample and solvent on the order of 1 to 20 nL/min (for static nanospray) or 100 to 1000 nL/min (for dynamic nanospray).

NCBI National Center for Biotechnology Information (USA)

NIST National Institute of Standards and Technology (USA)

NMR Normal Mass Range

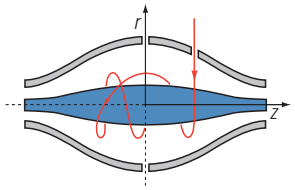
NSI See nanospray ionization (NSI).

O

octapole An octagonal array of cylindrical rods that acts as an ion transmission device. An RF voltage and DC offset voltage applied to the rods create an electrostatic field that transmits the ions along the axis of the octapole rods.

OD outside diameter

Orbitrap mass analyzer The Orbitrap™ mass analyzer consists of a spindle-shape central electrode surrounded by a pair of bell-shaped outer electrodes. Ions inside the mass analyzer orbit in stable trajectories around the central electrode with harmonic oscillations along it.



Two detection electrodes record an image current of the ions as they undergo harmonic oscillations. A Fourier transformation extracts different harmonic frequencies from the image current. An ion's

mass-to-charge ratio *m/z* is related to the frequency *f* of its harmonic oscillations and to the instrumental constant *k* by:
$m/z = k/f^2$

OT Orbitrap

See Orbitrap mass analyzer.

OVC outer vacuum case

Ω ohm

P

p pico (10^{-12})

Pa pascal

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

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

See also: parent ion.

PCB printed circuit board

PDA detector Photodiode Array detector is a linear array of discrete photodiodes on an integrated circuit chip. It is placed at the image plane of a spectrometer to allow a range of wavelengths to be detected simultaneously.

PE protective earth

PID proportional / integral / differential

P/N part number

p-p peak-to-peak voltage

ppm parts per million

PQD pulsed-Q dissociation

precursor ion An electrically charged molecular species that can dissociate to form fragments. The fragments can be electrically charged or neutral species. A precursor ion (PR) can be a molecular ion or an electrically charged fragment of a molecular ion. Also known as parent ion.

precursor mass Mass of the corresponding precursor (or parent) ion or molecule.

psig pounds per square inch, gauge

PTM posttranslational modification

pulsed Q dissociation (PQD) Collision-induced dissociation that involves precursor ion activation at high Q, a time delay to allow the precursor to fragment, and then a rapid pulse to low Q where all fragment ions are trapped. The fragment ions can then be scanned out of the ion trap mass analyzer and detected. PQD eliminates the “1/3 Rule” low mass cut-off for MS/MS data.

Q

quadrupole A symmetrical, parallel array of four hyperbolic rods that acts as a mass analyzer or an ion transmission device. As a mass analyzer, one pair of opposing rods has an oscillating radio frequency (RF) voltage superimposed on a positive direct current (DC) voltage. The other pair has a negative DC voltage and an RF voltage that is 180 degrees out of phase with the first pair of rods. This creates an electrical field (the quadrupole field) that efficiently transmits ions of selected mass-to-charge ratios along the axis of the quadrupole rods.

R

RAM random access memory

raw data Uncorrected liquid chromatograph and mass spectrometer data obtained during an acquisition. Xcalibur and Xcalibur-based software store this data in a file that has a .raw file extension.

resolution The ability to distinguish between two points on the wavelength or mass axis.

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

RF radio frequency

RF lens A multipole rod assembly that is operated with only radio frequency (RF) voltage on the rods. In this type of device, virtually all ions have stable trajectories and pass through the assembly.

RF voltage An AC voltage of constant frequency and variable amplitude that is applied to the ring electrode or endcaps of the mass analyzer or to the rods of a multipole. Because the frequency of this AC voltage is in the radio frequency (RF) range, it is referred to as RF voltage.

RMS root mean square

ROM read-only memory

rotary-vane pump A mechanical vacuum pump that establishes the vacuum necessary for the proper operation of the turbomolecular pump. (Also called a roughing pump or forepump.)

RS-232 An accepted industry standard for serial communication connections. This Recommended Standard (RS) defines the specific lines and signal characteristics used by serial communications controllers to standardize the transmission of serial data between devices.

RT An abbreviated form of the phrase *retention time (RT)*. This shortened form is used to save space when the retention time (in minutes) is displayed in a header, for example, RT: 0.00-3.75.

S

s second

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

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

See also selected reaction monitoring (SRM) scan type.

selected reaction monitoring (SRM) scan type A scan type with two stages of mass analysis and in which a particular reaction or set of reactions, such as the fragmentation of an ion or the loss of a neutral moiety, is monitored. In SRM a limited number of product ions is monitored.

SEM secondary electron multiplier

Serial Peripheral Interface (SPI) hardware and firmware communications protocol

serial port An input/output location (channel) for serial data transmission.

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

sheath gas flow rate The rate of flow of sheath gas into the API source. A measurement of the relative flow rate (in arbitrary units) that needs to be provided at the sheath gas inlet to provide the required flow of sheath gas to the ESI or APCI nozzle.

sheath gas inlet An inlet in the API probe where sheath gas is introduced into the probe.

sheath gas plumbing The gas plumbing that delivers sheath gas to the ESI or APCI nozzle.

sheath gas pressure The rate of flow of sheath gas (nitrogen) into the API source. A measurement of the relative flow rate (in arbitrary units) that needs to be provided at the sheath gas inlet to provide the required flow of inner coaxial nitrogen gas to the ESI or APCI nozzle. A software-controlled proportional valve regulates the flow rate.

See also sheath gas.

sheath gas valve A valve that controls the flow of sheath gas into the API source. The sheath gas valve is controlled by the data system.

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

SIM See selected ion monitoring (SIM) scan type.

skimmer A vacuum baffle between the higher pressure capillary-skimmer region and the lower pressure region. The aperture of the skimmer is offset with respect to the bore of the ion transfer capillary.

source CID A technique for fragmenting ions in an atmospheric pressure ionization (API) source. Collisions occur between the ion and the background gas, which increase the internal energy of the ion and stimulate its dissociation.

SPI See Serial Peripheral Interface (SPI).

SRM See selected reaction monitoring (SRM) scan type.

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

See also sweep gas flow rate.

sweep gas flow rate The rate of flow of sweep gas into the API source. A measurement of the relative flow rate (in arbitrary units) to provide the required flow of nitrogen gas to the sweep cone of the API source.

See also sweep gas.

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

T

T Tesla

target compound A compound that you want to identify or quantitate or that a specific protocol (for example, an EPA method) requires that you look for. Target compounds are also called analytes, or target analytes.

TIC See total ion current (TIC).

TMP See turbomolecular pump.

Torr A unit of pressure, equal to 1 mm of mercury and 133.32 Pa.

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

tube lens offset The voltage offset from ground that is applied to the tube lens to focus ions toward the opening of the skimmer.

See also source CID.

Tune Method A defined set of mass spectrometer tune parameters for the ion source and mass analyzer. Tune methods are defined by using the instrument software's tune window and saved as tune file.

A tune method stores tune parameters only. (Calibration parameters are stored separately, not with the tune method.)

tune parameters Instrument parameters whose values vary with the type of experiment.

turbomolecular pump A vacuum pump that provides a high vacuum for the mass spectrometer and detector system.

TWA time weighted average

U

u atomic mass unit

UHV ultra high vacuum

ultra-high performance liquid chromatography (U-HPLC) See high performance liquid chromatography (HPLC).

Ultramark 1621 A mixture of perfluoroalkoxycyclotriphosphazenes used for ion trap calibration and tuning. It provides ESI singly charged peaks at *m/z* 1022.0, 1122.0, 1222.0, 1322.0, 1422.0, 1522.0, 1622.0, 1722.0, 1822.0, and 1921.9.

UMR Universal Mass Range

V

V volt

V AC volts alternating current

V DC volts direct current

vacuum manifold A thick-walled, aluminum chamber with machined flanges on the front and sides and various electrical feedthroughs and gas inlets that encloses the API stack, ion optics, mass analyzer, and ion detection system.

vacuum system Components associated with lowering the pressure within the mass spectrometer. A vacuum system includes the vacuum manifold, pumps, pressure gauges, and associated electronics.

vent valve A valve that allows the vacuum manifold to be vented to air or other gases. A solenoid-operated valve.

vol volume

W

w width

W watt

WEEE European Union Waste Electrical and Electronic Equipment Directive. Provides guidelines for disposal of electronic waste.

X

XML (Extensible Markup Language) A general-purpose markup language that is used to facilitate the sharing of data across different information systems, particularly via the Internet.

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