

ETD Module

Getting Started Guide

For Velos Pro and LTQ XL Mass Spectrometers
(compatible with Orbitrap Velos Pro, LTQ Orbitrap Velos,
Orbitrap Elite, and LTQ Orbitrap XL)

98000-97003 Revision E September 2015



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Release history: Rev A, March 2008; Rev B, Jan 2009; Rev C, Dec 2010; Rev D, June 2012; Rev E, Sept 2015

Software version: Microsoft Windows 7 Professional (32-bit and 64-bit) SP1—Thermo Foundation 2.0 and later, and Thermo Xcalibur 2.2 and later; Windows XP Workstation SP3—Foundation 1.0.2 SP2 or earlier, and Xcalibur 2.1 SP1 or earlier; Thermo LTQ Tune Plus 2.7.0 and later

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- [Velos Pro/ETD System](#)
- [LTQ XL/ETD System](#)

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

Velos Pro/ETD System

EMC Directive 2004/108/EC

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

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

Low Voltage Safety Compliance

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LTQ XL/ETD System

EMC Directive 2004/108/EC

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

CFR 47, FCC Part 15, Subpart B, Class A: 2013

CISPR 11: 2009 + A1

CISPR 24: 2010

AS/NZS CISPR 22: 2009

ICES-003: 2012

EN 55011: 2009 + A1

EN 55024: 2010

EN 61326-1: 2013

EN 61000-3-2: 2006 + A1+ A2

EN 61000-3-3: 2008

EN 61000-4-2: 2009

EN 61000-4-3: 2006 + A1+ A2

EN 61000-4-4: 2004 + A1

EN 61000-4-5: 2006

EN 61000-4-6: 2009

EN 61000-4-11: 2004

Low Voltage Safety Compliance

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

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



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



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2012/19/EU



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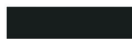
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Preface

The *ETD Module Getting Started Guide* describes how to set up, calibrate, and tune the Thermo Scientific™ MS/ETD system and how to acquire electron transfer dissociation (ETD) data. The mass spectrometer (MS) instruments are the Thermo Scientific Velos Pro™, LTQ™ Velos™, or LTQ XL™.

Note For the LTQ Velos/ETD system, follow the Velos Pro/ETD system information, unless otherwise noted.

Contents

- [Related Documentation](#)
- [Cautions and Special Notices](#)
- [Contacting Us](#)

❖ To suggest changes to the documentation or to the Help

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



Related Documentation

The Velos Pro/ETD and LTQ XL/ETD systems include complete documentation. In addition to this guide, you can also access the following documents as PDF files from the data system computer:

- *LTQ Series Preinstallation Requirements Guide*
- *LTQ Series Getting Connected Guide*
- *LTQ Series Getting Started Guide*
- *LTQ Series Hardware Manual*
- *ETD Module Hardware Manual*
- *Safety and Regulatory Guide*

The ETD module also ships with a printed copy of the *Safety and Regulatory Guide*. This guide contains important safety information about Thermo Scientific liquid chromatography (LC) and mass spectrometry (MS) systems. Make sure that all lab personnel have read and have access to this document.

❖ To view the product manuals

From the Microsoft™ Windows™ taskbar, choose **Start > All Programs > Thermo Instruments > Manuals > *model***, where *model* is your specific LTQ Series model, and then open the PDF file to view it.

Note For Thermo Xcalibur™ version 2.0.7 or earlier (Microsoft Windows XP operating system), choose **Start > All Programs > Xcalibur > Manuals > LTQ > *model***.

❖ To view the data system Help

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

❖ To download user documentation from the Thermo Scientific website

1. Go to www.thermoscientific.com.
2. In the Search box, type the product name and press ENTER.
3. In the left pane, select **Documents & Videos**, and then under Refine By Category, click **Operations and Maintenance**.

4. (Optional) Narrow the search results or modify the display as applicable:
 - For all related user manuals and quick references, click **Operator Manuals**.
 - For installation and preinstallation requirements guides, click **Installation Instructions**.
 - For documents translated into a specific language, use the Refine By Language feature.
 - Use the Sort By options or the Refine Your Search box (above the search results display).
5. Download the document as follows:
 - a. Click the document title or click **Download** to open the file.
 - b. Save the file.

Cautions and Special Notices

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

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



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



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

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.






The *ETD Module Getting Started Guide* contains the following caution-specific symbols (Table 1).



Table 1. Caution-specific symbols and their meanings

Symbol	Meaning
	Chemical hazard: Observe Good Laboratory Practices (GLP) when handling chemicals. Only work with volatile chemicals under a fume or exhaust hood. Wear gloves and other protective equipment, as appropriate, when handling toxic, carcinogenic, mutagenic, corrosive, or irritant chemicals. Use approved containers and proper procedures to dispose of waste oil and when handling wetted parts of the instrument.
	Risk of eye injury: Eye injury could occur from splattered chemicals or airborne particles. Wear safety glasses when handling chemicals or servicing the instrument.

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	(U.S.) 1 (561) 688-8731	(U.S.) 1 (561) 688-8736
	us.customer-support.analyze@thermofisher.com 	us.techsupport.analyze@thermofisher.com 

Contact us	Customer Service and Sales	Technical Support
	❖ To find global contact information or customize your request	
	<ol style="list-style-type: none">1. Go to www.thermoscientific.com.2. Click Contact Us, select the Using/Serviceing a Product option, and then type the product name.3. Use the phone number, email address, or online form.	
	❖ To find product support, knowledge bases, and resources	
	Go to www.thermoscientific.com/support .	
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Introduction

This chapter provides general information about the MS/ETD systems. For additional information, such as procedures for daily operation, maintenance, and system startup and shutdown, refer to the *ETD Module Hardware Manual*.

Note For the LTQ Velos/ETD system, follow the Velos Pro/ETD system information, unless otherwise noted.

Contents

- [MS/ETD System](#)
- [Electron Transfer Dissociation](#)
- [Types of Experiments](#)
- [Tuning and Calibration](#)
- [Diagnostics](#)

MS/ETD System

The Velos Pro/ETD and LTQ XL/ETD systems are members of the Thermo Scientific family of mass spectrometer (MS) detectors, and consist of an electron transfer dissociation (ETD) module installed at the back of a Velos Pro or LTQ XL MS. With the MS/ETD system you can perform ETD mass spectroscopy on analytes.

The Velos Pro and LTQ XL MSs are advanced analytical instruments that include a syringe pump, a divert/inject valve, an atmospheric pressure ionization (API) source, a 2D linear ion trap, and the Xcalibur data system.

The MS/ETD system provides a source of fluoranthene reagent ions that react with analyte molecules in the linear ion trap of the Velos Pro or LTQ XL MS. The ETD module contains two reagent vials, chemical ionization (CI)/carrier gas (nitrogen) handling hardware and flow restrictors, ion volume and filament, ion optics, and heaters for these components.

The nitrogen gas serves two functions in the ETD process:

- As a carrier gas, the nitrogen sweeps the reagent (fluoranthene) from the vial to the ion source where the reagent radical anions form.
- As a CI vehicle, the nitrogen undergoes collisions with 70 eV electrons from the filament in the ion volume. These 70 eV electrons knock electrons off of the nitrogen molecules (creating nitrogen ions). The secondary electrons resulting from these collisions have near thermal-kinetic energies. The fluoranthene molecules capture these thermal electrons to form the reagent radical anions that react with the analyte.

Electron Transfer Dissociation

ETD provides peptide dissociation by transferring electrons to positively charged peptides, leading to a rich ladder of sequence ions derived from cleavage at the amide groups along the peptide backbone. Important posttranslational modifications, such as phosphorylation and glycosylation, are left intact.

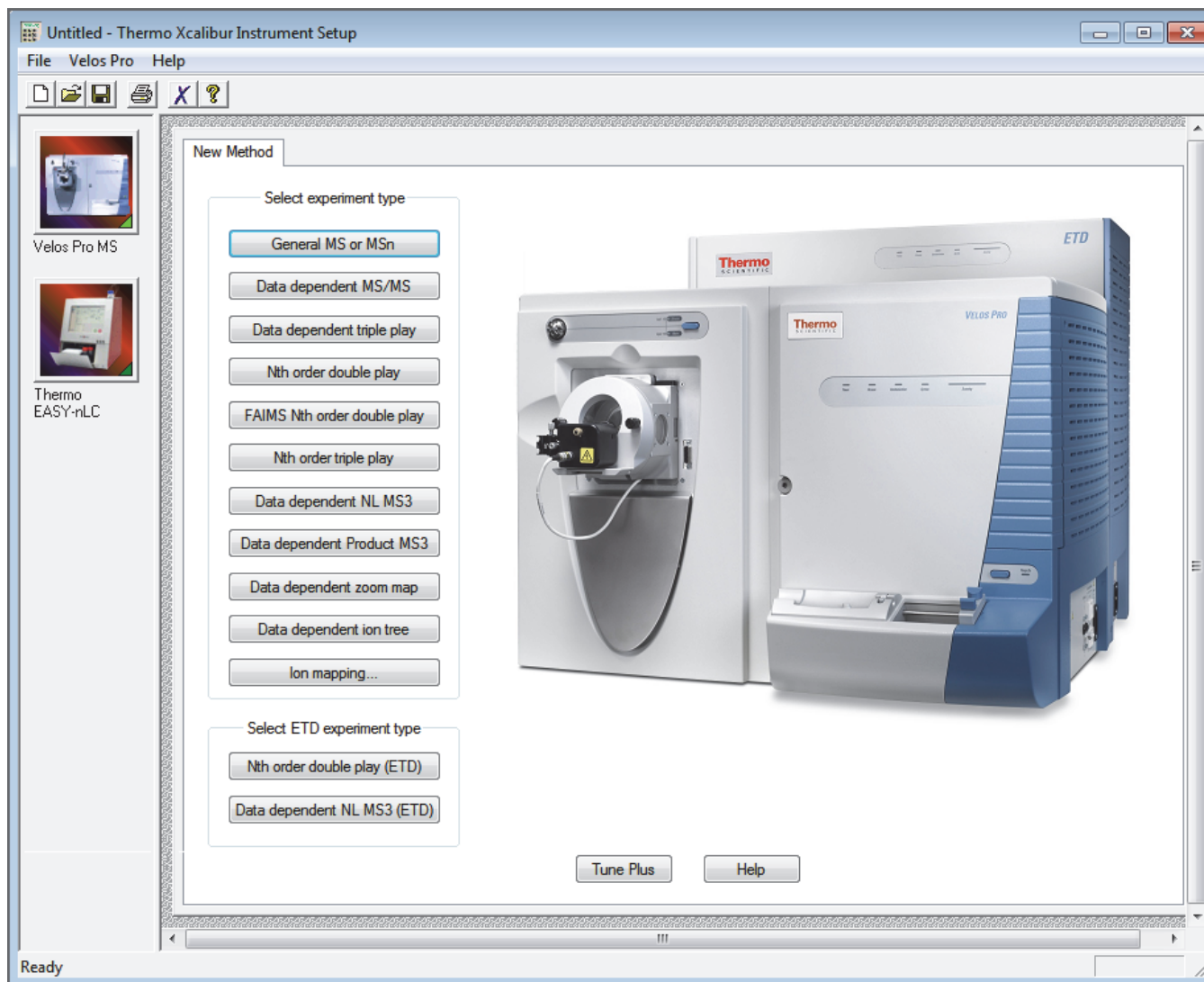
Types of Experiments

The New Method page of the Thermo Xcalibur Instrument Setup window for the Velos Pro or LTQ XL MS ([Figure 1](#)) contains links (buttons) to templates for various types of experiments. To save time entering the parameters for the instrument method, open the template designed for the experiment type that you want to perform, enter the parameters specific to the experiment, and then save the entries as part of an Xcalibur instrument method (.meth file).

The experiments are organized by the following categories:

- [LTQ XL and LTQ Velos Experiment Types](#)
- [ETD Experiment Types](#)

Figure 1. Xcalibur Instrument Setup window showing the New Method page (Velos Pro/ETD system)



LTQ XL and LTQ Velos Experiment Types

As described in the *LTQ Series Getting Started Guide*, the experiment templates available for the Velos Pro and LTQ XL MSs are as follows:

- General MS or MSⁿ experiment
- Data Dependent™ experiments:
 - Data Dependent MS/MS
 - Data Dependent Triple Play
 - Nth Order Double Play
 - FAIMS Nth Order Double Play
 - Nth Order Triple Play
 - Data Dependent NL MS³
 - Data Dependent product MS³
 - Data Dependent Zoom Map
- Data Dependent Ion Tree
- Ion Mapping

ETD Experiment Types

With the addition of the ETD module to the Velos Pro or LTQ XL MS, two other experiment templates are available:

- Nth Order Double Play (ETD)—This experiment is useful as a survey experiment when you cannot determine whether the CID or ETD mode of dissociation works best. For example, CID works best for dissociating doubly-charged ions while ETD works best for ions of a higher charge state.
- Data Dependent NL MS³ (ETD)—Identifying sites of a specific posttranslational modification, indicated by a specific neutral loss, might be easier by using ETD to repeat the MS/MS experiment. ETD is also useful when there are multiple sites of modification.

For additional information, see [Chapter 5, “Running ETD Experiments,”](#) and refer to the Instrument Setup application Help.

Tuning and Calibration

Calibrate the MS to ensure its mass accuracy, resolution, isolation efficiency, and dissociation efficiency. Calibration parameters are instrument parameters whose values do not vary with the type of experiment.

IMPORTANT Thermo Fisher Scientific recommends that you check the calibration once a week and calibrate the MS as needed.

Tune parameters are instrument parameters (for example, ETD optics) whose values can vary with the type of experiment or analyte being mass analyzed. To achieve the highest sensitivity or the lowest limits of detection for an analyte of interest, tune the MS with the analyte.

To tune and calibrate the MS/ETD system, perform these basic steps:

- Tune and calibrate the MS as described in the *LTQ Series Getting Started Guide*.
- Tune the optics in the ETD module as described in [Chapter 3, “Tuning the ETD Module.”](#)

Diagnostics

To learn more about some of the MS’s diagnostic tools and tests, refer to the *LTQ Series Getting Started Guide*, *LTQ Series Hardware Manual*, and the Tune Plus application Help. To learn more about the ETD module’s diagnostic tools and tests, refer to the *ETD Module Hardware Manual*. (At this time, not all diagnostic tools or tests are discussed in the product guides or Help.)

Setting Up Thermo Foundation Instrument Configuration

Follow these procedures to add the Velos Pro or LTQ XL mass spectrometer and the reagent ion source (ETD module) to Thermo Foundation™ Instrument Configuration and to specify some of their configuration options.

Contents

- [Adding the Mass Spectrometer to the Instrument Configuration](#)
- [Specifying the Reagent Ion Source for the Mass Spectrometer](#)

Adding the Mass Spectrometer to the Instrument Configuration

To control the reagent ion source and the MS from the Xcalibur data system, add these devices to the instrument configuration.

❖ To add the MS to the list of configured devices

1. Close the Xcalibur data system and Tune Plus application.
2. On the Windows taskbar, choose **Start > All Programs > Thermo Foundation *x.x* > Instrument Configuration**, where *x.x* is the installed version of Thermo Foundation, to open the Thermo Foundation Instrument Configuration window.

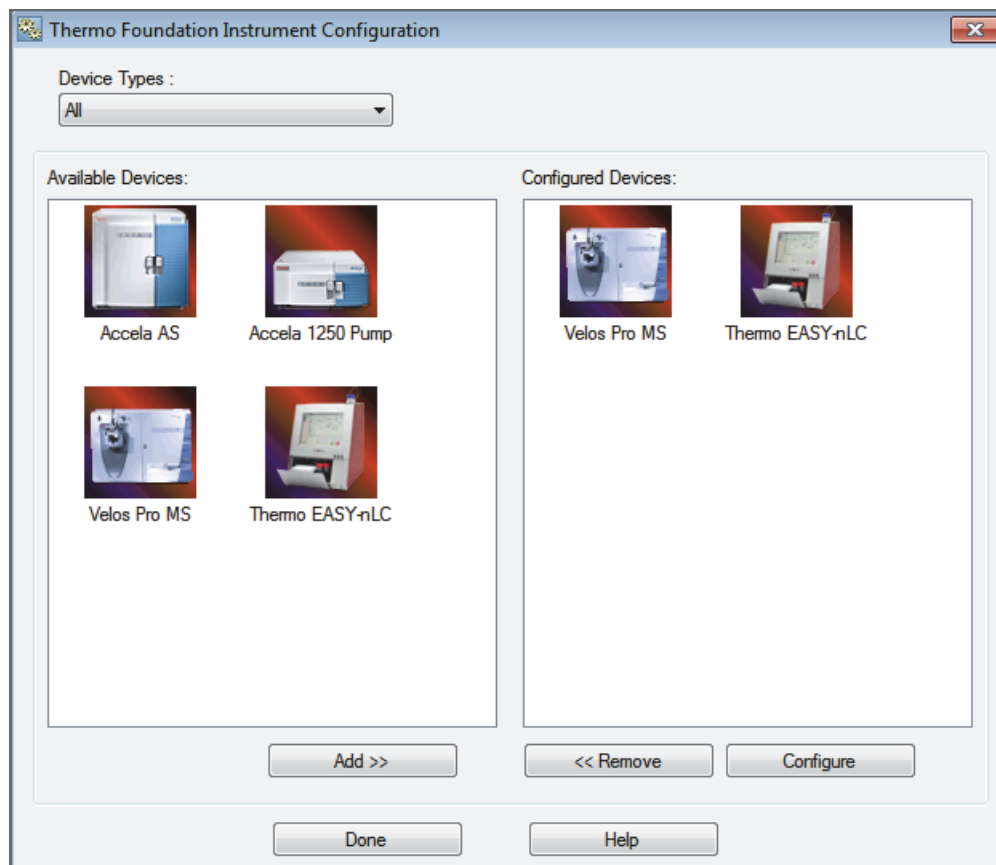
Note For Xcalibur data system version 2.0.7 or earlier, choose **Start > All Programs > Xcalibur > Instrument Configuration**.

3. Select the devices to control from the Xcalibur data system as follows:
 - a. In the Device Types list (Figure 2), select **All**.
 - b. In the Available Devices list, double-click the following:
 - i. The **Velos Pro MS**, **LTQ Velos MS** (not shown), or **LTQ XL MS** (not shown) icon to add it to the Configured Devices list.
 - ii. The appropriate LC device, which for this example is the Thermo EASY-nLC™.

2 Setting Up Thermo Foundation Instrument Configuration

Adding the Mass Spectrometer to the Instrument Configuration

Figure 2. Thermo Foundation Instrument Configuration window with configured devices



Specifying the Reagent Ion Source for the Mass Spectrometer

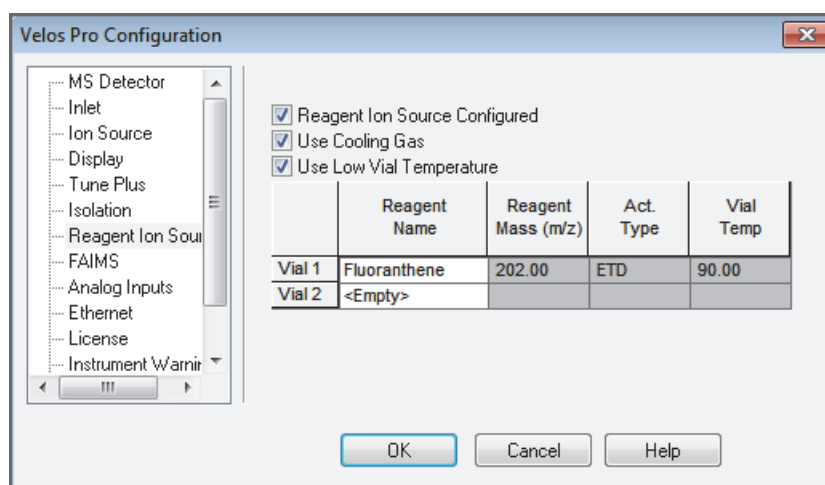
Specify the ETD module as the reagent ion source for the MS.

❖ To specify the reagent ion source



1. In the Foundation Instrument Configuration window, in the Configured Devices list, select the MS icon, and then click **Configure** to open the *Model* Configuration dialog box.
2. In the left pane, select **Reagent Ion Source** to display the reagent ion source configuration page (Figure 3).

Figure 3. Velos Pro Configuration dialog box



3. Select the **Reagent Ion Source Configured** check box.

You must manually select this check box for an MS/ETD system.

4. (Optional) Select the **Use Cooling Gas** check box.

Tip To save your supply of nitrogen cooling gas, clear the Use Cooling Gas check box and allow more time for the reagent vials to cool.

Note The cooling gas only turns on if the vial temperature exceeds 100 °C (212 °F), regardless of the vial temperature setting you select. This document assumes that you enabled the cooling gas feature.

2 Setting Up Thermo Foundation Instrument Configuration

Specifying the Reagent Ion Source for the Mass Spectrometer

5. (Optional; strongly recommended) Select the **Use Low Vial Temperature** check box to heat the vial to 90 °C instead of 108 °C.

IMPORTANT Thermo Fisher Scientific recommends that you select the lower vial temperature setting and have your local field service engineer install the new filament and heater block (available as of October 2011).

Although the lower vial temperature can significantly extend the lifetime of the filament, be aware that it will likely increase the reagent ion injection time, which increases the overall MSⁿ ETD scan times.

The instrument configuration dialog box (Figure 3) also provides information about the contents of the reagent vials.

6. Click **OK**.

A message box opens:

In order for the configuration changes to take effect, you must restart the data system and then the LTQ.

7. Click **OK**.
8. Restart the data system.
9. Restart the MS.

For instructions, refer to the *LTQ Series Hardware Manual*.

Tuning the ETD Module

Follow these procedures to tune the ETD module. Tuning the optics in the reagent ion source provides settings for optimum transmission of the reagent ions (fluoranthene).

Before starting, follow the procedure “Turning On the Reagent Ion Source” in Chapter 4, “Daily Operation,” of the *ETD Module Hardware Manual*.

Note You do not need to tune and calibrate the ETD module as part of your daily routine. However, if you use the MS/ETD system regularly, perform ETD-related calibration at least once a month (Electron Multiplier Gain for the negative ion mode and Reagent Ion Selection on the Check page of the Calibrate dialog box).

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

Contents

- [Opening the Tune Plus Window](#)
- [Tuning the Reagent Ion Optics](#)
- [Viewing the Reagent Ion Optics Settings](#)
- [Saving the ETD Tune Parameters](#)

Opening the Tune Plus Window

There are several ways to open the Tune Plus window (Figure 4).

❖ To open the Tune Plus window

Do one of the following:

- On the Windows taskbar, choose **Start > All Programs > Thermo Instruments > LTQ > *model* Tune**, where *model* is your specific MS/ETD system.

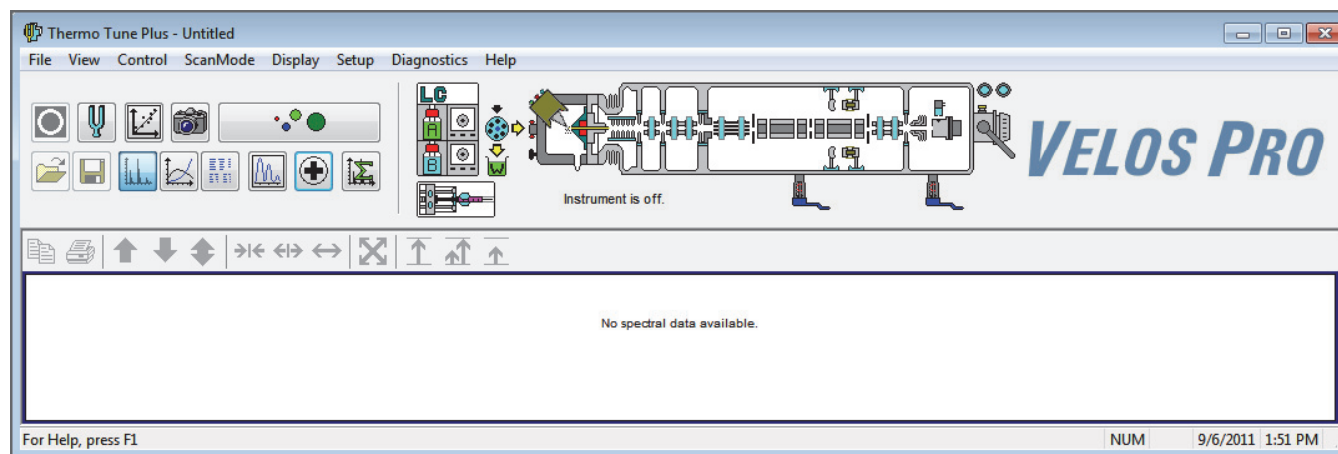
Note For LTQ Series version 2.5.0 or earlier, choose **Start > All Programs > Xcalibur > *model* Tune**.

- In the Xcalibur application, click the **Roadmap View** icon (under the menu bar), the **Instrument Setup** icon, the *model* MS icon, and then **Tune Plus**.

—or—

- In the Xcalibur application, click the **Roadmap View** icon (under the menu bar), the **Instrument Setup** icon, and the *model* MS icon. Then, from the main toolbar choose *model* > **Start Tune Plus**.

Figure 4. Tune Plus window for the Velos Pro/ETD system



Tuning the Reagent Ion Optics

This section describes the three ways to tune the ion optics within the reagent ion source:

- [Automatic Tuning of the Reagent Ion Optics](#)

Automatic tuning of the reagent ion optics is the best method for most situations. Use manual tuning to manually optimize reagent ion optics parameters and reagent ion source parameters that are not automatically tuned, such as CI gas pressure, electron energy, and emission current.

- [Manually Tuning the Reagent Ion Source](#)

With manual tuning you can observe the effects of adjusting these parameters as you change them.

- [Semi-Automatically Tuning the Reagent Ion Optics](#)

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

Note After tuning the reagent ion optics, remember to save the ETD tune parameters. See [“Saving the ETD Tune Parameters.”](#)

Automatic Tuning of the Reagent Ion Optics

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

1. Open the Tune Plus window (see [page 12](#)).
2. Click the **On/Standby** button to select the **On** mode.



On



Standby

Note

When on, the System LED on the MS's front panel turns green and the high voltage to the electron multipliers turns on. (The status LEDs are not provided for the Orbitrap/ETD systems.)

The following conditions can cause the MS/ETD system to remain in standby mode even though you try to turn it on:

- Attempting to turn on the reagent ion source when the restrictor, source, and transfer line heaters are not at their target temperatures.
- When either the MS or the ETD module goes into standby mode. If enabled, the reagent vial nitrogen cooling turns on if the vials are at an elevated temperature.

Exception: If you place the MS/ETD system in standby mode, the cooling nitrogen turns on after a 1-hour delay.

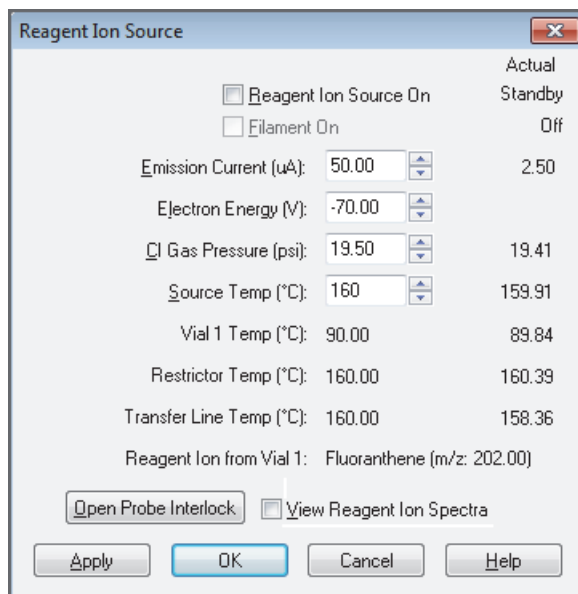
- Whenever the pressure in the MS or the ETD module exceeds its protection limit. If enabled, the reagent vial nitrogen cooling turns on if the vials are at an elevated temperature.
- Whenever the reagent ions' flux intensity becomes insufficient as determined by the Automatic Gain Control™ (AGC) setting. When this occurs, the MS/ETD system completes the current Xcalibur sequence step before entering standby mode. This prevents the loss of analysis results that might be affected by the reduced reagent ion production.



3. Click the **Display Graph View** button.

4. Set the reagent ion source as follows:
 - a. Choose **Setup > Reagent Ion Source** to open the Reagent Ion Source dialog box (Figure 5).

Figure 5. Reagent Ion Source dialog box (default view)



- b. Select the following check boxes:
 - **Reagent Ion Source On**
 - **Filament On**
 - **View Reagent Ion Spectra**

For tuning, you must manually select the Filament On check box.

- c. Click **OK**.

Note

If the reagent vials are not at their target temperature, a message appears:

Reagent Vial NOT At Temperature! Please wait ...

The System LED on the ETD module flashes green to indicate that the reagent vial heaters are turned on but are not at their target temperatures (the other heaters are at their target temperatures).

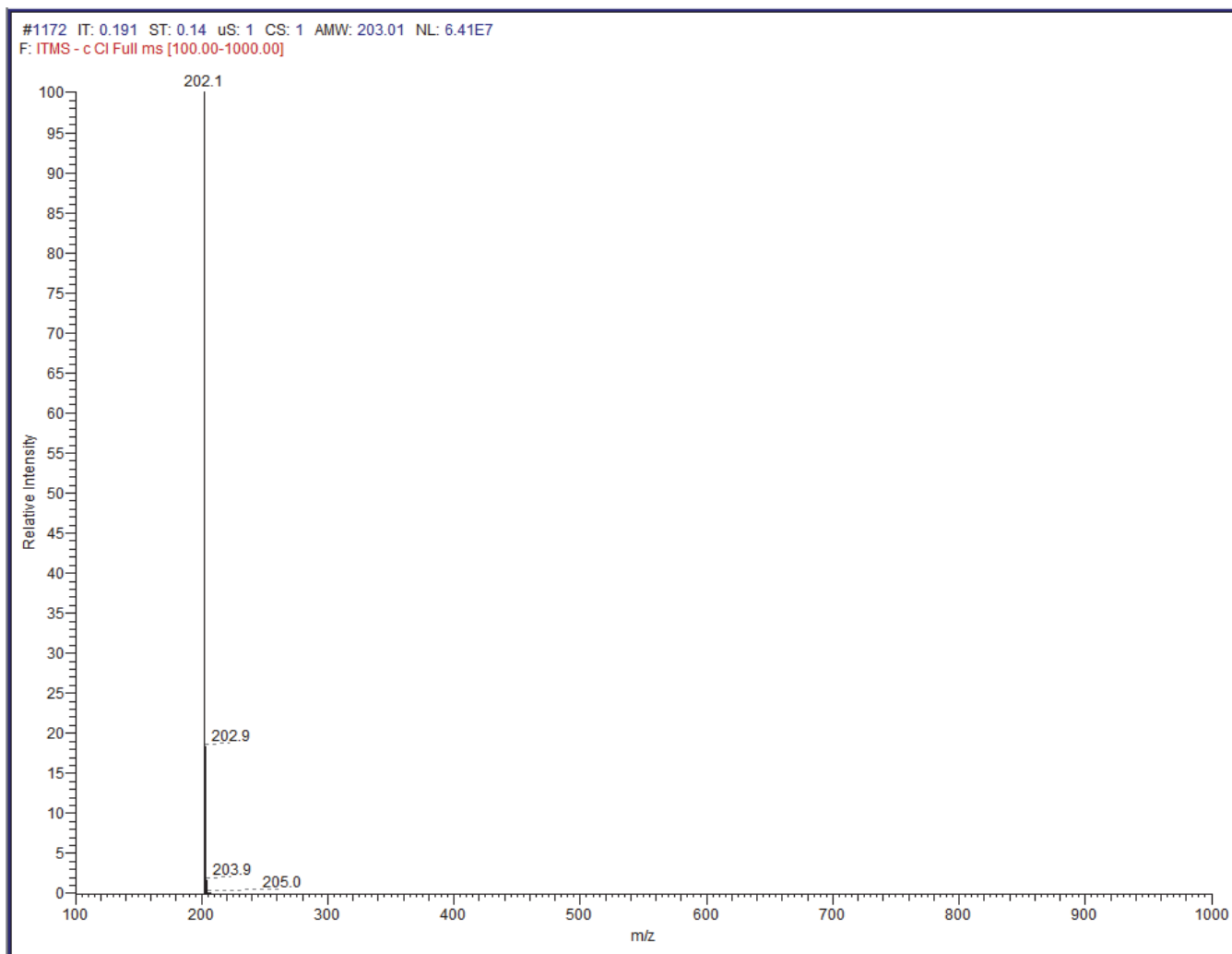
When the reagent vial heaters reach their target temperatures, which can take 20 minutes or more, the System LED turns solid green.

When the reagent vials reach their target temperature, the system applies voltage to the ETD module ion optics. By selecting the Filament On check box, you ensure that the filament turns on and the Filament LED turns solid green.

(The status LEDs are not provided for the Orbitrap/ETD systems.)

Figure 6 shows the reagent ion spectrum at peak m/z 202.

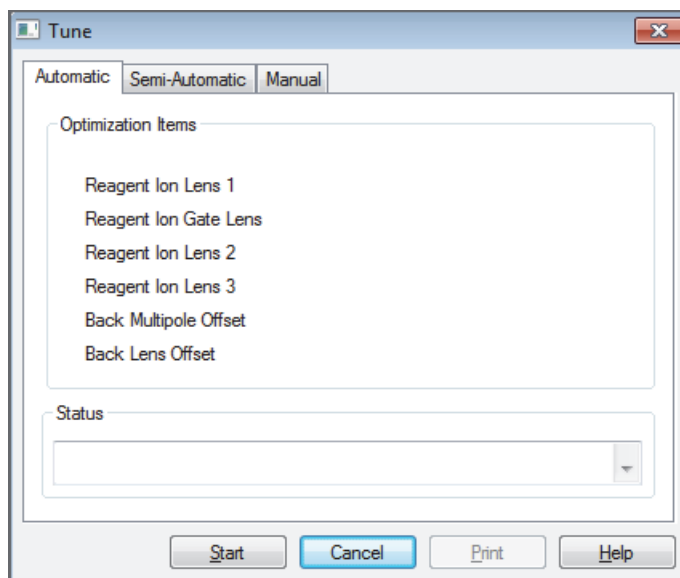
Figure 6. Reagent ion spectrum for the Velos Pro/ETD system (centroid mode)





5. Click the **Tune** button to open the Tune dialog box (Figure 7).

Figure 7. Tune dialog box showing the Automatic page



6. Click **Start**.

The system begins automatically tuning the reagent ion optics in the ion source. The Status area displays the message “Optimization Complete” after completing automatic tuning. This message also states the percentage change in the reagent ion signal (m/z 202) intensity relative to the prior value.

Table 2 lists the expected reagent signal intensity in profile (not centroid) mode for the MS/ETD systems, including Orbitrap™ systems. All values assume that the reagent ion isolation is on; and the use of a clean reagent ion source, clean ion volume and holder, good reagent ion optics tune, and good and recent detector gain calibration in negative ion mode.

Table 2. Expected profile intensities of m/z 202

ETD system	Expected profile intensities of m/z 202	
	Reagent vial 108 °C with the original filament and heater block	Reagent vial 90 °C with the new filament and heater block ^a (recommended)
Standalone MS with ETD		
Velos Pro/ETD	$\sim 4 \times 10^7$ or greater	$\sim 1 \times 10^7$ or greater
LTQ XL/ETD	$\sim 1 \times 10^7$ or greater	$\sim 2.5 \times 10^6$ or greater
Orbitrap MS with ETD		
LTQ Orbitrap Velos Pro/ETD	$\sim 1 \times 10^7$ or greater	$\sim 3 \times 10^6$ or greater
LTQ Orbitrap Elite/ETD	$\sim 1 \times 10^7$ or greater	$\sim 3 \times 10^6$ or greater
LTQ Orbitrap XL/ETD	$\sim 3 \times 10^6$ or greater	$\sim 1 \times 10^6$ or greater

^a As of October 2011

- Repeat the Automatic Tune procedure if the percentage change is greater than 20%.

Note This is an iterative process. At some point, the signal intensity does not improve.

Manually Tuning the Reagent Ion Source

❖ To manually tune the reagent ion source

- Open the Tune Plus window (see [page 12](#)).
- Click the **Display Graph View** button.
- Set the reagent ion source as follows:
 - Choose **Setup > Reagent Ion Source** to open the Reagent Ion Source dialog box.
 - Select the following check boxes if they are not already selected:
 - **Reagent Ion Source On**
 - **Filament On**
 - **View Reagent Ion Spectra**
 - Click **OK**.

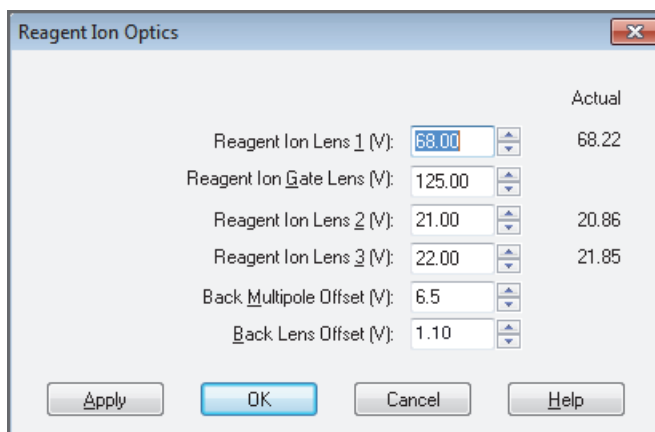


Note For information about the reagent vial temperatures, see [page 14](#).

4. Choose **Setup > Reagent Ion Optics** to open the Reagent Ion Optics dialog box (Figure 8).

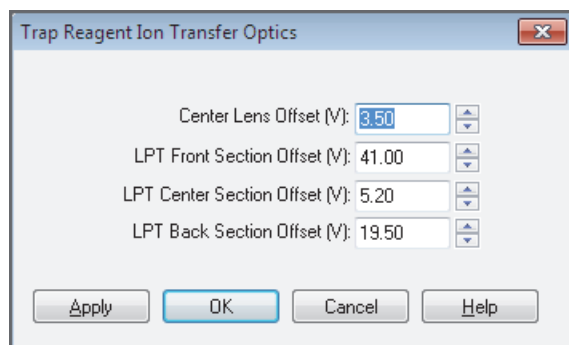
Observe these parameters during the tuning process.

Figure 8. Reagent Ion Optics dialog box



5. (For Velos Pro/ETD only) Choose **Setup > Trap Ion Transfer** to open the Trap Reagent Ion Transfer Optics dialog box (Figure 9).

Figure 9. Trap Reagent Ion Transfer Optics dialog box

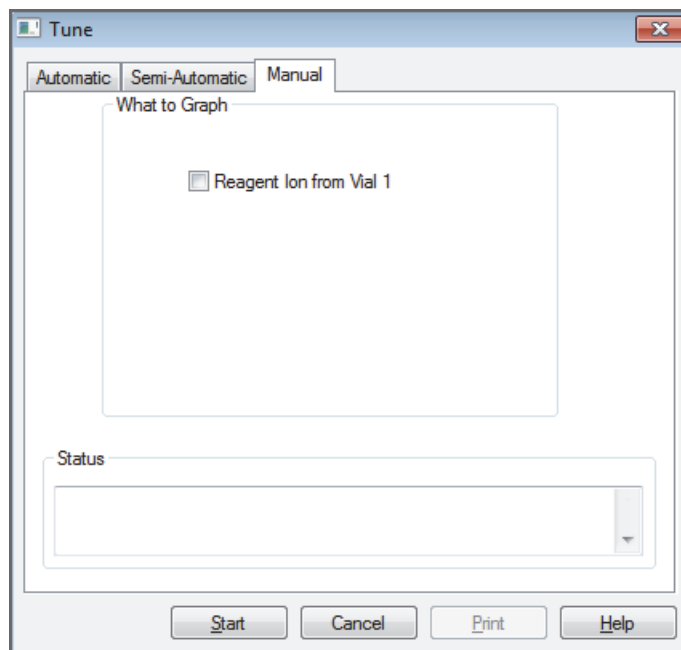


After automatic tuning is complete, the values in this dialog box are optimally set. Changing these values changes the reagent ion abundance in the view reagent ion spectra mode.



- Click the **Tune** button to open the Tune dialog box, and then click the **Manual** tab (Figure 10).

Figure 10. Tune dialog box showing the Manual page



- Select the **Reagent Ion from Vial *Number*** check box.

The vial number corresponds to the Instrument Configuration setting (see [page 9](#)).

- Click **Start**.

A plot of the reagent ion signal intensity appears in the Tune Plus window ([Figure 11](#)).

Observe:

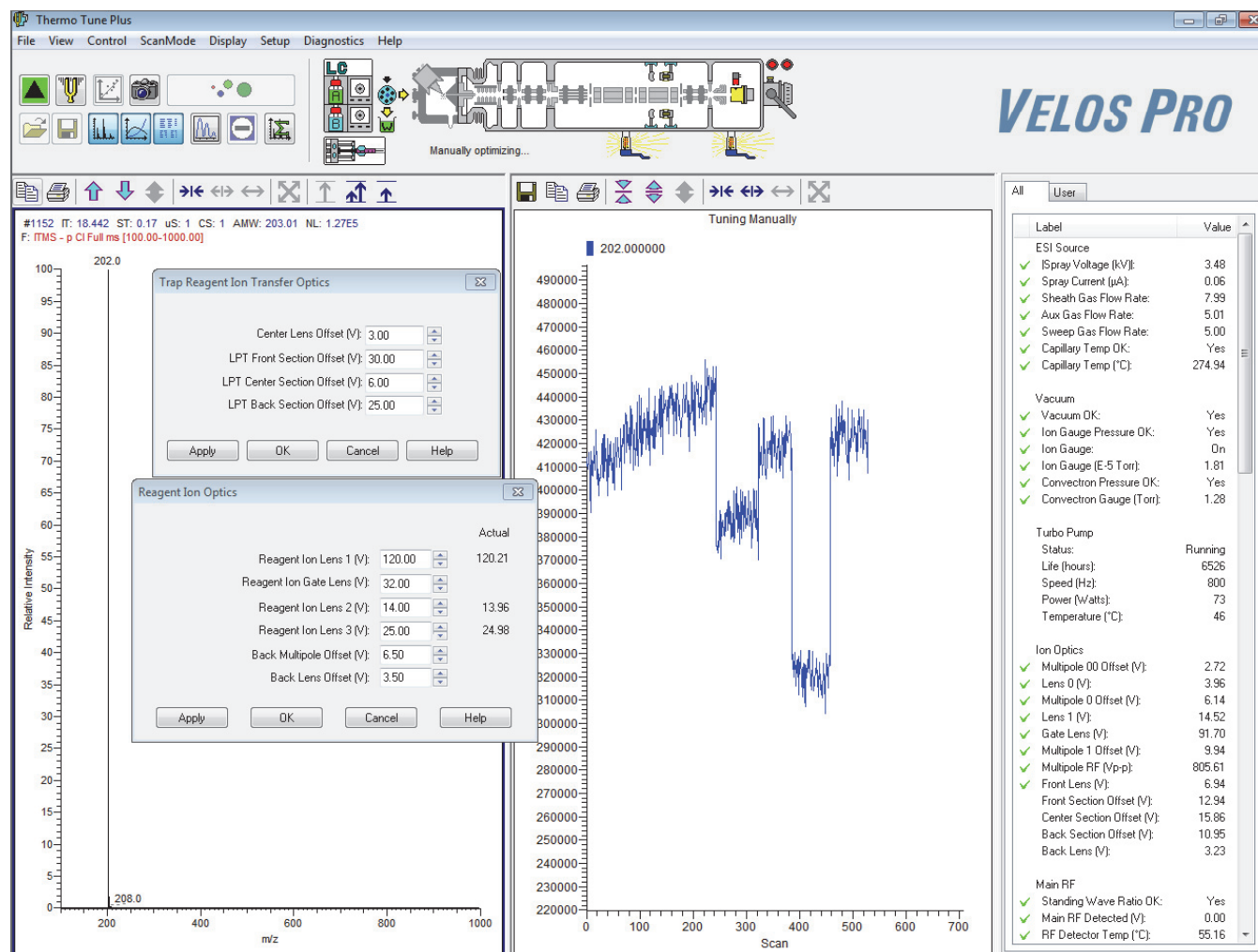
- In the Reagent Ion Optics dialog box, the response of the reagent ion signal intensity to changes in the lens parameters
- In the Reagent Ion Source dialog box, the emission current, electron energy, and CI gas pressure

You can adjust these parameters to achieve the maximum reagent ion signal intensity.

3 Tuning the ETD Module

Tuning the Reagent Ion Optics

Figure 11. Graph view of the reagent ion source tuning in Tune Plus (Velos Pro/ETD system)



9. When you are finished, click **OK** in the open dialog boxes.

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. Open the Tune Plus window (see [page 12](#)).
2. Click the **Display Graph View** button.
3. Set the reagent ion source as follows:
 - a. Choose **Setup > Reagent Ion Source** to open the Reagent Ion Source dialog box.
 - b. Select the following check boxes if they are not already selected:
 - **Reagent Ion Source On**
 - **Filament On**
 - **View Reagent Ion Spectra**
 - c. Click **OK**.

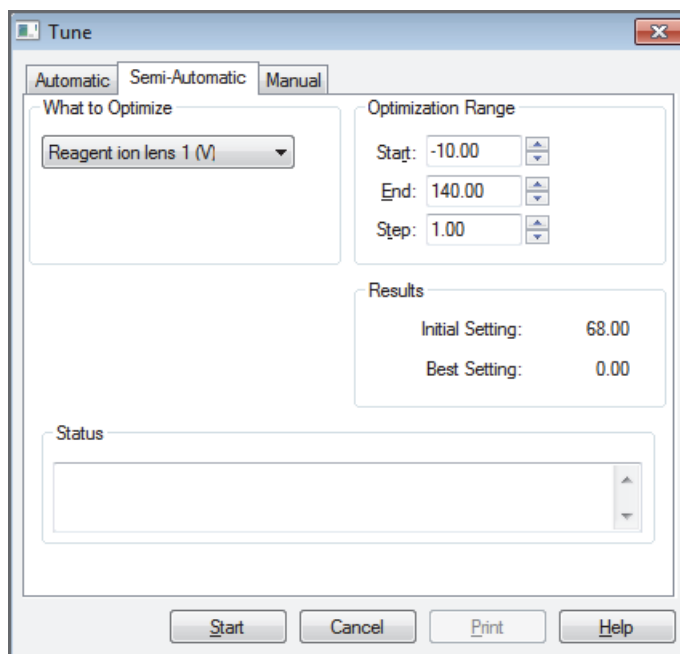


Note For information about the reagent vial temperatures, see [page 14](#).



4. Click the **Tune** button to open the Tune dialog box, and then click the **Semi-Automatic** tab ([Figure 12](#)).

Figure 12. Tune dialog box showing the Semi-Automatic page



3 Tuning the ETD Module

Viewing the Reagent Ion Optics Settings

5. In the What to Optimize list, select one of the following items to tune, as applicable:
 - Reagent Ion Lens 1 (V)
 - Reagent Ion Gate Lens (V)
 - Reagent Ion Lens 2 (V)
 - Reagent Ion Lens 3 (V)
 - Back Multipole Offset (V)
 - Front Section LPT Offset (V) (Velos Pro/ETD only)
 - Center Section LPT Offset (V) (Velos Pro/ETD only)
 - Back Section LPT Offset (V) (Velos Pro/ETD only)
 - Center Lens Offset (V) (Velos Pro/ETD only)
 - Back Lens (V)
6. Under Optimization Range, adjust the settings in the Start, End, and Step boxes.
7. Click **Start**.

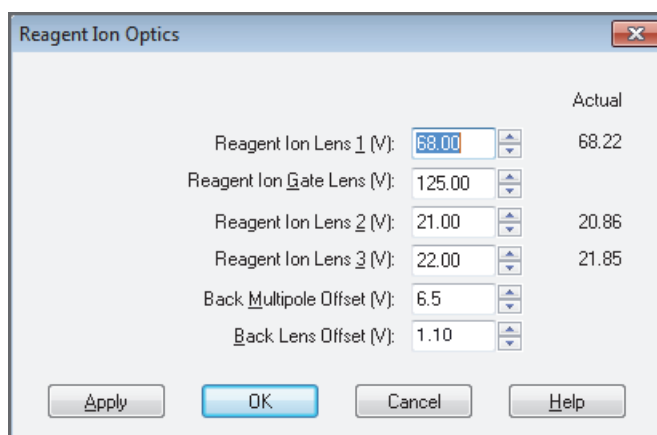
Viewing the Reagent Ion Optics Settings

❖ To view the current reagent ion optics settings

1. Open the Tune Plus window (see [page 12](#)).
2. Choose **Setup > Reagent Ion Optics** to open the Reagent Ion Optics dialog box ([Figure 13](#)).

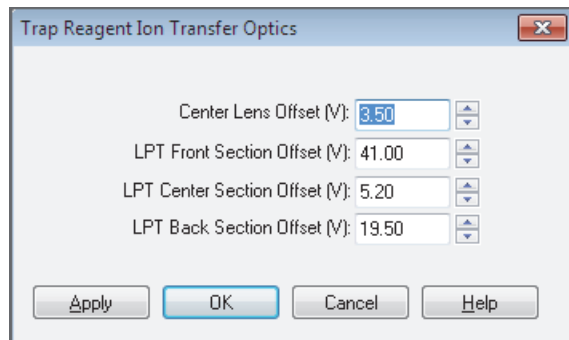
The Reagent Ion Optics dialog box ([Figure 13](#)) shows the optimized settings from an example tuning. Your actual optimized values depend on the level of cleanliness of the ion source and ion volume/holder. Ensure that you reoptimize the ion source voltages and CI gas pressure flow after 5–10 days of use, after changing the ion volume, and after cleaning the ion source.

Figure 13. Reagent Ion Optics dialog box



3. (For Velos Pro/ETD only) Choose **Setup > Trap Ion Transfer** to open the Trap Reagent Ion Transfer Optics dialog box (Figure 14).

Figure 14. Trap Reagent Ion Transfer Optics dialog box



4. When you are finished viewing the settings in these dialog boxes, click **OK** in each dialog box.

Saving the ETD Tune Parameters

Tip You must save the tune parameters while the MS/ETD system is on.

For LTQ version 2.6 and earlier, after completing the tuning, you can save the ETD Tune parameters in a tune method file.

❖ To save the ETD tune parameters (LTQ version 2.6 and earlier)



1. In the Tune Plus window, click the **Save** button.
2. Browse to a location, and then specify a file name.
3. Click **Save**.

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

3 Tuning the ETD Module

Saving the ETD Tune Parameters

Performing an ETD Infusion Experiment

Follow these procedures to perform an ETD infusion experiment using angiotensin I.

Contents

- [Setting the Reagent Injection Settings](#)
- [Troubleshooting an AGC Target Error](#)
- [Obtaining an ETD Spectrum for Angiotensin I](#)
- [Optimizing the Reagent Ion Reaction Time](#)

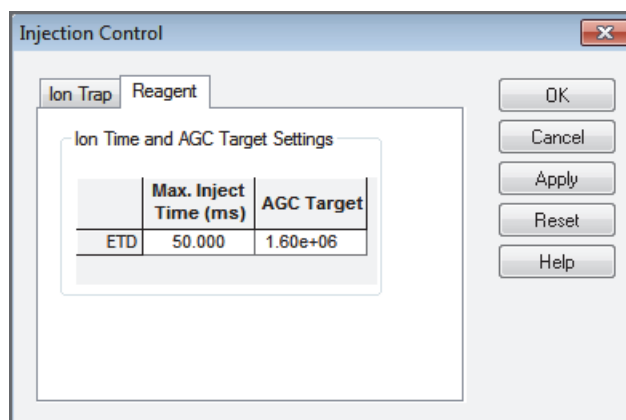
Setting the Reagent Injection Settings

The parameters on the Reagent page in the Injection Control dialog box ([Figure 15](#)) regulate the number of reagent ions admitted into the ion trap of the MS.

❖ To set the reagent ion injection settings

1. Open the Tune Plus window (see [page 12](#)).
2. Choose **Setup > Injection Control** to open the Injection Control dialog box.
3. Click the **Reagent** tab ([Figure 15](#)).

Figure 15. Injection Control dialog box showing the Reagent page



The ETD reagent injection control consists of two parameters:

- Max. Inject Time (ms)—Specifies the maximum time that the system allows for anions to be injected into the trap. The default value is 50 ms.
 - AGC Target—Sets the target number of reagent anions to be injected into the trap to perform ETD. Table 3 lists the typical AGC target ranges and the recommended initial settings. If the reagent source and filament are on, changing the reagent target value triggers a reagent AGC event. Normally, reagent AGC events occur when a method starts and every 30 minutes during an acquisition.
4. Set the AGC Target value to the recommended setting specified in the following table.

Table 3. AGC target values

MS/ETD system	Typical AGC target range	Recommended setting
Velos Pro/ETD	6×10^5 to 1.6×10^6	6×10^5
LTQ XL/ETD	$2\text{--}4 \times 10^5$	4×10^5

5. Click **OK**.

The reagent ion source injects reagent anions into the trap until the ETD AGC target is reached. The time allowed to reach the ETD AGC target cannot exceed the maximum injection ion time setting; the maximum injection ion time takes precedence over the AGC target.

Troubleshooting an AGC Target Error

Two reagent parameters—maximum injection time and AGC target (Figure 15)—influence the ETD reaction results. The ETD (ion/ion) reaction rate increases with the reagent AGC target setting until the trap reaches the maximum storage density for the ions. As the trapped reagent population (proportional to the reagent AGC target setting) reaches this level, the reagent ion cloud size grows instead of becoming more dense. This generally occurs at above 6×10^5 for the Velos Pro/ETD system and above 4×10^5 for the LTQ XL/ETD system.

If the maximum ion injection time for the reagent stays within the set limit, the ion injection time automatically adjusts (through AGC) to provide the appropriate number of reagent ions for the ETD reaction as defined by the reagent target. The adjustment of the ion injection time occurs regardless of the reagent ion abundance or of the optimal reaction time that you set. The ETD product ion yield or spectra do not vary; low reagent ion abundance only extends the overall scan time but does not alter the quality of the product ion spectra.

The reaction time required to yield the maximum number of product ions decreases approximately reciprocally with the reagent target setting, while the ion injection time required to achieve the reagent AGC target setting grows proportionally.

For 2+, 3+, and 4+ charge state precursor ions and reagent ion profile intensities in the neighborhood of 1×10^7 for the Velos Pro MS and 2.5×10^6 for the LTQ XL MS (see [Table 2](#)), the reaction time is longer than the reagent injection time for all reasonable reagent target settings. Therefore, you can minimize the overall scan time by using a sufficiently high reagent target to yield the shortest optimal ETD reaction time. If the ETD reagent abundance (flux) is relatively low, the reagent injection time can exceed the ETD reaction time. Use of a lower reagent target and extending the reaction times might reduce the overall scan cycle time and, therefore, increase the number of ETD spectra acquired per minute. At the earliest opportunity, take the appropriate steps to restore the reagent ion abundance (see the list on this page).

If the AGC target is not reached because of the maximum injection time limit, the system displays an error message:

Maximum Injection time limit exceeded.

The intensity of the reagent ion source is likely to be too low. You can resolve this error several ways:

- Increase the reagent ion transmission of the reagent ion source to the ion trap by running the automatic tuning of the reagent ion source; see [“Tuning the Reagent Ion Optics.”](#)
- Reoptimize the reagent carrier/CI gas flow. As the ion volume and holder become dirty with use, the optimal pressure for the reagent ion production increases.
- Increase the emission current in the Reagent Ion Source dialog box ([Figure 5](#)). However, an increase in emission current might decrease the filament life.
- Increase the maximum injection time limit. This is a temporary way to eliminate the error message. You can increase the maximum injection time limit up to the limits imposed by the overall scan cycle time.
- Clean or change the ion volume; refer to Chapter 5, “Maintenance,” in the *ETD Module Hardware Manual*. The decrease in the ion intensity might be due to a dirty ion volume. A sufficiently contaminated ion volume causes the maximum injection time limit to be exceeded. (After you clean or change the part, retune the reagent ion source and reoptimize the reagent carrier/CI gas flow.)
- Clean the reagent ion source and its optics; refer to Chapter 5, “Maintenance,” in the *ETD Module Hardware Manual*. The sensitivity decrease might be due to a dirty reagent ion source, its optics, or both. A contaminated reagent ion source or its optics can also cause the maximum injection time limit to be exceeded. (After you clean the parts, retune the reagent ion source and reoptimize the reagent carrier/CI gas flow.)

4 Performing an ETD Infusion Experiment

Obtaining an ETD Spectrum for Angiotensin I

Obtaining an ETD Spectrum for Angiotensin I

This section assumes that you are infusing angiotensin I into the Velos Pro/ETD or LTQ XL/ETD system according to the procedures in the *LTQ Series Getting Started Guide*. For instructions about preparing the angiotensin I solutions, see [Appendix B, “Angiotensin I Solutions.”](#)

❖ To obtain an ETD spectrum of angiotensin I

1. Open the Tune Plus window (see [page 12](#)).
2. Click the **On/Standby** button to select the **On** mode.
3. Set the reagent ion source as follows:
 - a. Choose **Setup > Reagent Ion Source** to open the Reagent Ion Source dialog box ([Figure 16](#)).

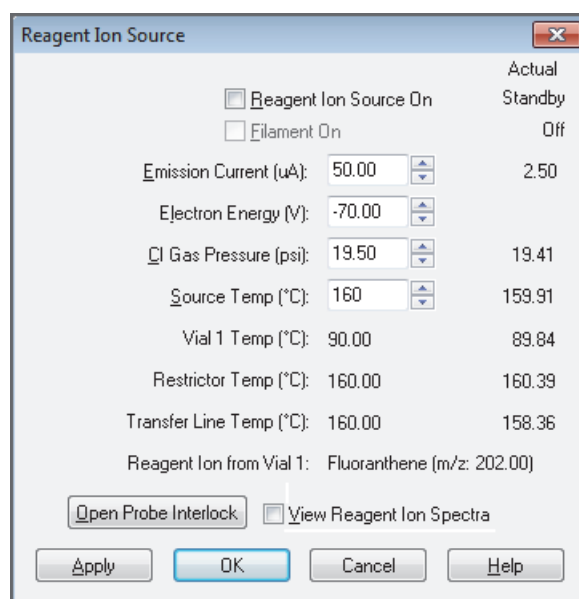


On



Standby

Figure 16. Reagent Ion Source dialog box (default view)



- b. Select the following check boxes:
 - **Reagent Ion Source On**
 - **Filament On**
- c. Click **OK**.

Note

If the reagent vials are not at their target temperature, a message appears:

Reagent Vial NOT At Temperature! Please wait ...

The System LED on the ETD module flashes green to indicate that the reagent vial heaters are turned on but are not at their target temperatures (the other heaters are at their target temperatures).

When the reagent vial heaters reach their target temperatures, which can take 20 minutes or more, the System LED turns solid green.

When the reagent vials reach their target temperature, the system applies voltage to the ETD module ion optics. By selecting the Filament On check box, you ensure that the filament turns on and the Filament LED turns solid green.

(The status LEDs are not provided for the Orbitrap/ETD systems.)



4. In the Tune Plus window, click the **Define Scan** button to open the Define Scan dialog box (Figure 17), and then do the following:

- a. Under MSn Settings, in the $n = 2$ row, enter the Parent Mass (m/z) of the 3+ charge state of angiotensin I.

The molecular weight of angiotensin I (acetate hydrate) is 1296 Da. The mass-to-charge ratio of the parent ion $[M + 3H]^{3+}$ is 433.3.

- b. In the Act. Type list, select **ETD**.
- c. In the Act. Time (ms) list, enter **50.0**.
- d. (Optional) To identify more dissociated ions of the precursor ions, do the following:
 - i. Select the **Supplemental Activation** check box.
 - ii. For this example, in the SA Charge State box, enter **3**, which is the charge state of m/z 433.3 from the angiotensin I precursor peak.

The default range is 2–6.

For additional information, refer to the Tune Plus application Help.

4 Performing an ETD Infusion Experiment

Obtaining an ETD Spectrum for Angiotensin I

Figure 17. Define Scan dialog box configured for ETD on angiotensin I

Scan History: ITMS + c cv=0.0 Full ms [150.00-2000.00]

Scan Description

Mass Range: Normal

Scan Rate: Normal

Scan Type: Full

Scan Time

Microscans: 1

Max. Inject Time (ms): 100.000

All...

Source Fragmentation

On Energy (V): 35.0

MSn Settings

n	Parent Mass (m/z)	Act. Type	Iso. Width (m/z)	Normalized Collision Energy	Act. Q	Act. Time (ms)
2	433.30	ETD	1.0	35.0	0.250	50.00
3		CID	1.0	0.0	0.250	10.00

Supplemental Activation

SA Energy: 15.0

SA Charge State: 2

Wideband Activation

Scan Ranges

#	First Mass (m/z)	Last Mass (m/z)
1	115.00	2000.00

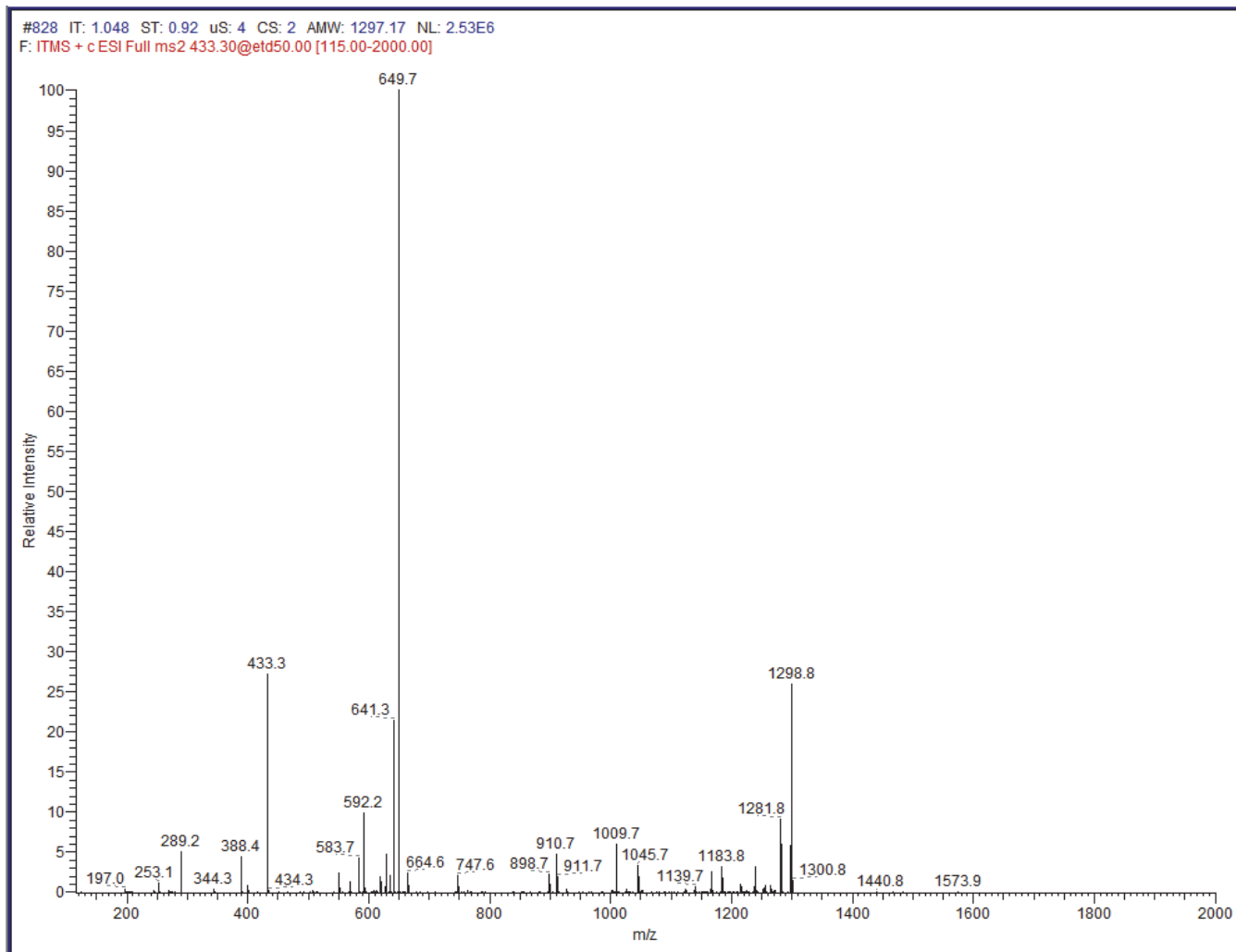
Input: From/To

Apply OK Cancel Help Injection RF... Activation...

5. Click **OK**.

The Define Scan dialog box closes and the Tune Plus window displays the ETD MS/MS spectrum of angiotensin I (Figure 18).

Figure 18. ETD MS/MS spectrum of angiotensin I (Velos Pro/ETD system)



Optimizing the Reagent Ion Reaction Time

In some cases obtaining an optimized reagent ion reaction time is helpful for a specific analyte. For 2+ peptides, the optimal reaction time is approximately 100 ms, and for 3+ ions it is approximately 50 ms. To determine a more accurate value, follow the procedure in this section. This section assumes that the system generates the reagent ions as described in “Turning On the Reagent Ion Source” in the *ETD Module Hardware Manual*.

Note When setting up a data-dependent experiment, you can shorten the ETD reaction time for charge states that are higher than two by selecting the Enable Charge State Dependent ETD Time feature on the Charge State page of the Data Dependent Settings dialog box. For additional information, refer to the Tune Plus application Help.

4 Performing an ETD Infusion Experiment

Optimizing the Reagent Ion Reaction Time

❖ To optimize the reagent ion reaction time

1. Turn on ETD activation for the analyte (angiotensin I, in this case).
2. Open the Tune Plus window (see [page 12](#)).
3. Choose **Setup > Reagent Ion Source** to open the Reagent Ion Source dialog box.
4. Clear the **View Reagent Ion Spectra** check box, and then click **OK**.
5. Click the **Define Scan** button to open the Define Scan dialog box, and then do the following:
 - a. Under MSn Settings, in the Parent Mass (m/z) box, enter the mass for the analyte.
 - b. In the Act. Type list, select **ETD**.

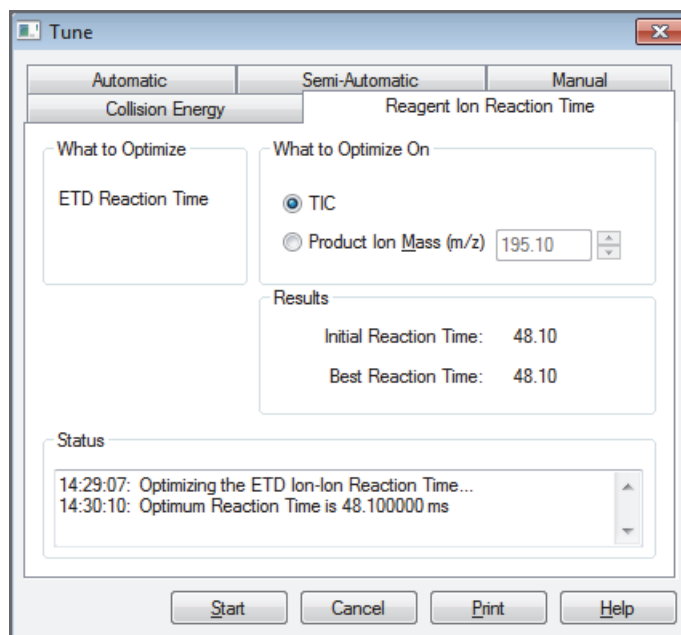


6. In the Tune Plus window, click the **Tune** button to open the Tune dialog box.

For ETD tuning, the Tune dialog box has two additional tabs, Collision Energy and Reagent Ion Reaction Time.

7. Click the **Reagent Ion Reaction Time** tab ([Figure 19](#)).

Figure 19. Tune dialog box showing the Reagent Ion Reaction Time page



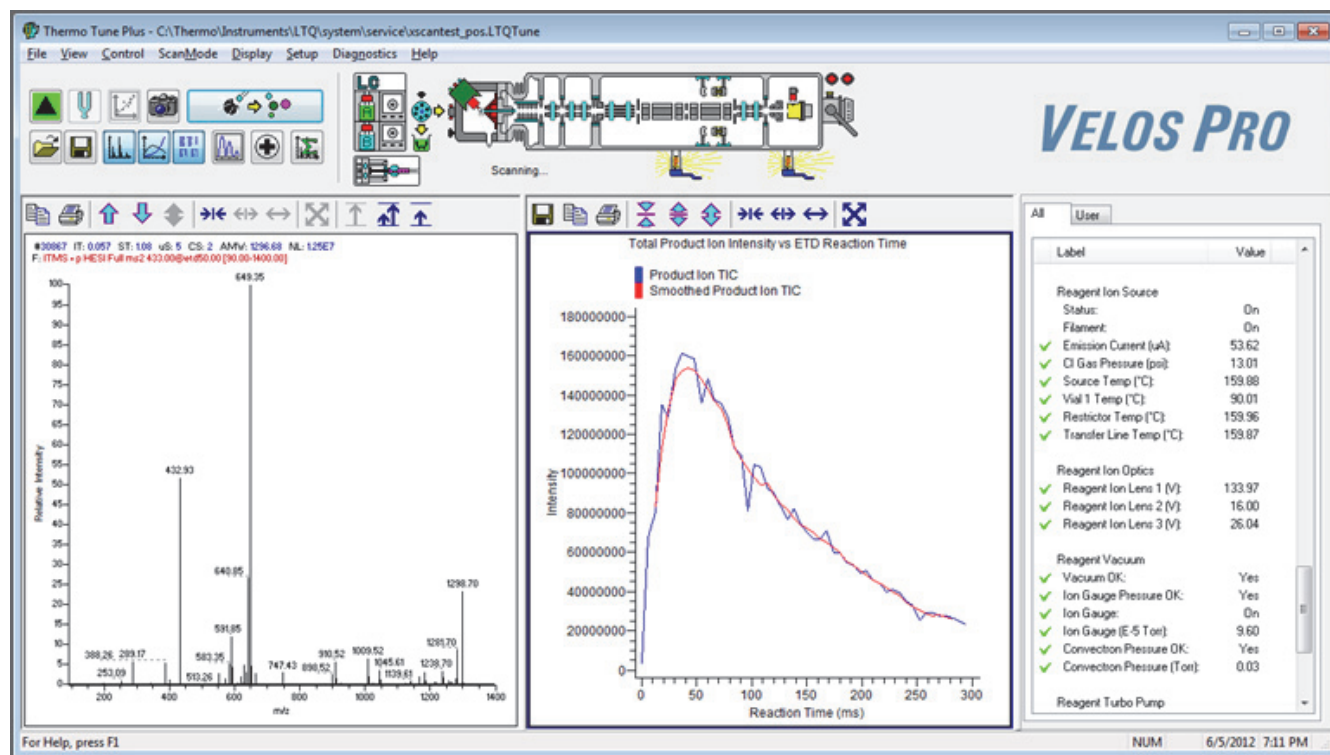
8. Select the **TIC** option.
9. Click **Start**.

The Tune Plus application generates a graph showing the intensity of your choice of either the mass-to-charge ratio of the ion of interest or the product ion's total ion current (TIC), excluding the precursor, versus the reaction time. The Status area of the page shows a reagent ion reaction time after the tune process is complete.

A message then prompts you to accept the optimized value. If you accept the optimized value, the reaction time parameter for the reagent ion sets to this optimized value in the Define Scan dialog box. Otherwise, the parameter returns to its previous value.

For this example, the reagent ion reaction time is now optimized based on the TIC (Figure 20).

Figure 20. ETD MS/MS spectrum of m/z 433.3 and the optimization graph displayed in Tune Plus (Velos Pro/ETD system)



To optimize on a particular product ion, in the Tune dialog box under What to Optimize On, select the **Product Ion Mass (m/z)** option (Figure 21), enter the mass-to-charge ratio of the selected product ion, and then repeat step 7.

4 Performing an ETD Infusion Experiment

Optimizing the Reagent Ion Reaction Time

Figure 21. Tune dialog box showing the Reagent Ion Reaction Time page (m/z 388.30)

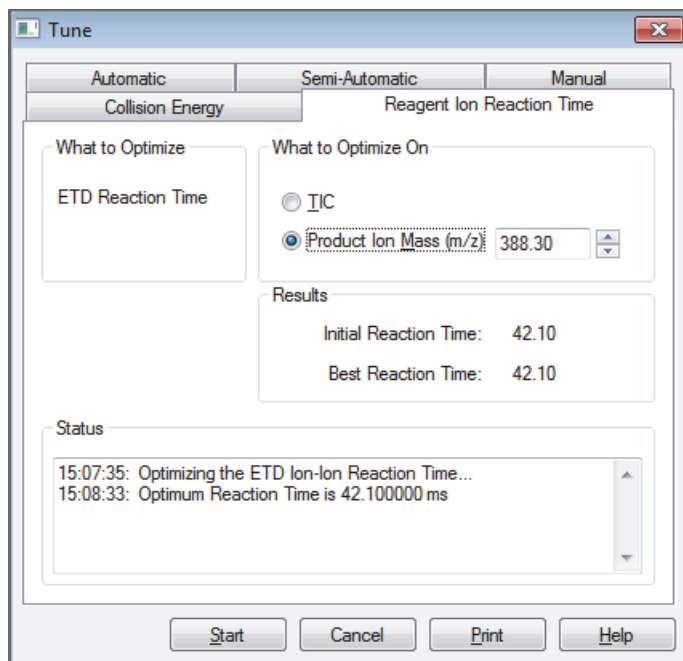
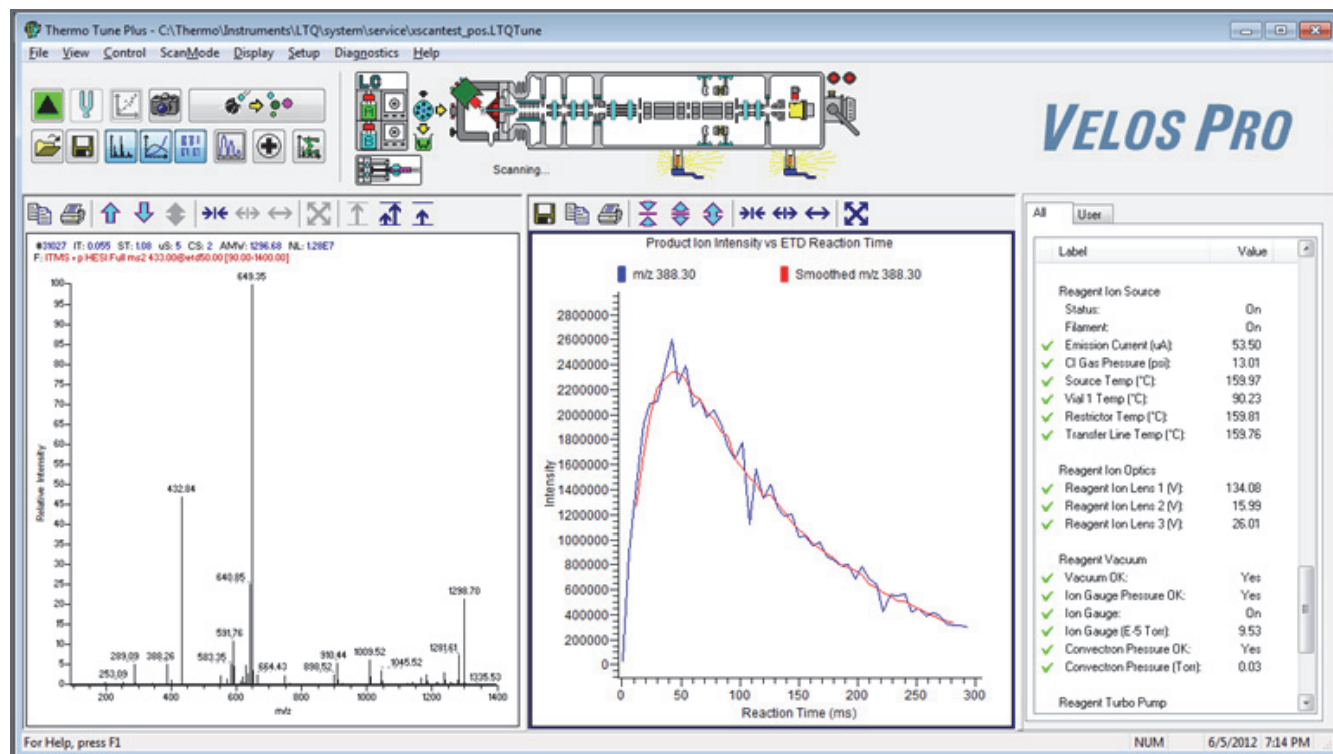


Figure 22 shows the ion spectra and optimization graphs for m/z 388.30.

Figure 22. ETD MS/MS spectrum of m/z 433.3 and the optimization graph displayed in Tune Plus (Velos Pro/ETD system)



Running ETD Experiments

This chapter describes the two ETD-specific experiment templates provided by the Xcalibur data system. For additional information, refer to the *LTQ Series Getting Started Guide*. Also included is an example for using the data-dependent decision tree (DDDT) logic procedure.

Contents

- [Nth Order Double Play \(ETD\) Experiment](#)
- [Data Dependent NL MS³ \(ETD\) Experiment](#)
- [Data Dependent Decision Tree Procedure](#)

Nth Order Double Play (ETD) Experiment

❖ To create an Nth Order Double Play (ETD) method

1. Choose **Start > All Programs > Thermo Xcalibur > Xcalibur**. to open the Xcalibur application.

Note For Xcalibur data system version 2.0.7 or earlier, choose **Start > All Programs > Xcalibur > Xcalibur**.

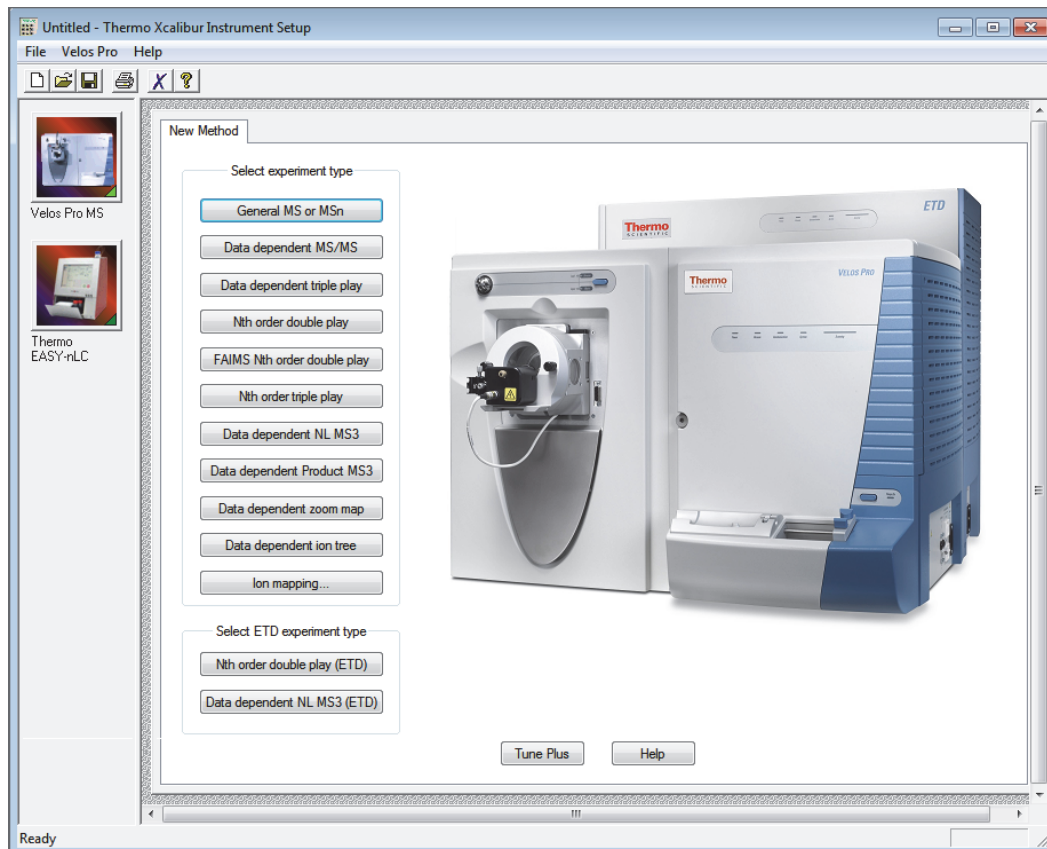
2. Choose **GoTo > Instrument Setup** to display the Instrument Setup window.

5 Running ETD Experiments

Nth Order Double Play (ETD) Experiment

3. In the left Available Devices pane, click the **Velos Pro MS** (shown in [Figure 23](#)) or **LTQ XL MS** icon to open the New Method page for the device.

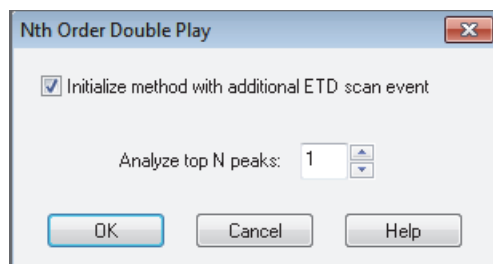
Figure 23. New Method page for the Velos Pro/ETD system



4. At the bottom, under Select ETD Experiment Type, click **Nth Order Double Play (ETD)** to open the Nth Order Double Play dialog box ([Figure 24](#)).

The Initialize Method with Additional ETD Scan Event check box is selected as the default setting.

Figure 24. Nth Order Double Play dialog box



5. In the Analyze Top N Peaks box, enter the number of top peaks.
6. Click **OK** to open the Nth Order Double Play with ETD template.

- Toward the bottom, clear the **APCI Corona On** and **APPI Lamp On** check boxes (Figure 25).

Figure 25. Nth Order Double Play with ETD template

The screenshot displays the software interface for an Nth Order Double Play experiment with ETD. The interface is organized into several sections:

- Run settings:** Includes 'Acquire time (min): 10.00', 'Segments: 1', and 'Start delay (min): 0.00'. A note states: 'To display a chromatogram here, use Velos Pro/Open raw file...'. A retention time scale from 0 to 10 minutes is shown.
- Segment 1 settings:** Includes 'Segment time (min): 10.00', 'Scan events: 3', and 'Tune method: C:\Thermo\Instruments\LTQ\methods\Default_ESIL.T'. A bar shows 'Scan Event 1' selected.
- Scan event 1 settings:**
 - Scan Description:** Mass Range: Normal, Scan Rate: Normal, Scan Type: Full, Polarity: Positive, Data type: Centroid.
 - Source Fragmentation:** On, Energy (V): 35.0, Dependent scan, Settings...
 - FAIMS:** C_v (V): 0.00
- MSn Settings:** A table with columns: n, Parent Mass (m/z), Act. Type, Iso. Width (m/z), Normalized Collision Energy, Act. Q, Act. Time (ms).

n	Parent Mass (m/z)	Act. Type	Iso. Width (m/z)	Normalized Collision Energy	Act. Q	Act. Time (ms)
2		CID	1.0	35.0	0.250	10.00

 - Supplemental Activation
 - Wideband Activation
 - APCI Corona On
 - APPI Lamp On
 - SA Charge State: 2
- Scan Ranges:** A table with columns: #, First Mass (m/z), Last Mass (m/z).

#	First Mass (m/z)	Last Mass (m/z)
1	300	2000.00

At the bottom, there are buttons for 'New method', 'Tune Plus', and 'Help'. A blue dashed box highlights the 'APCI Corona On' and 'APPI Lamp On' checkboxes, with a blue arrow pointing to them from the text 'Clear these check boxes.' below.

- Enter the scan event settings by clicking each **Scan Event Number** bar.

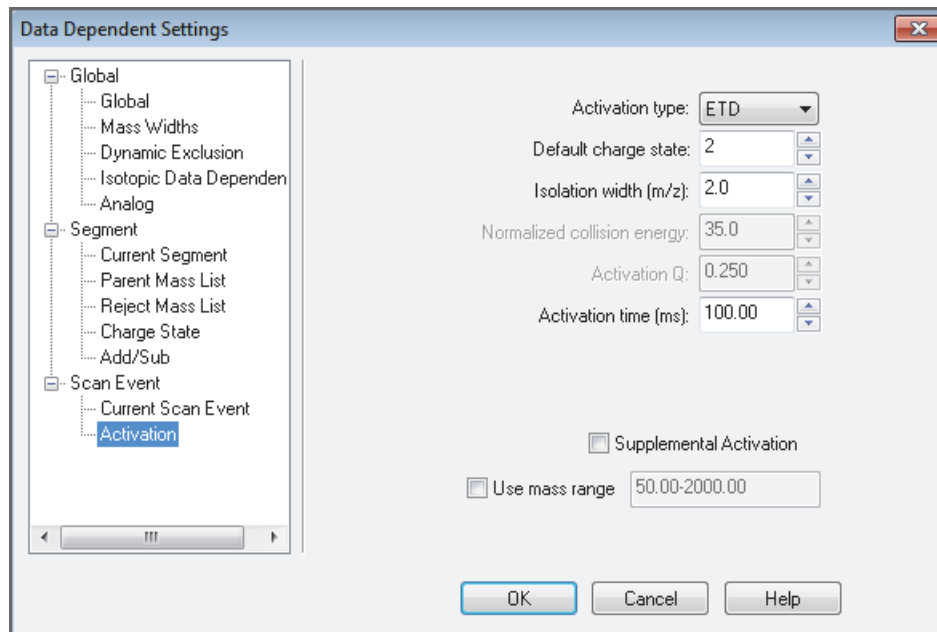
For Scan Event 2 and Scan Event 3, the Dependent Scan check box (near the lower left corner) and its adjacent Settings button become active.

- (For Scan Event 2 and Scan Event 3 only) Click **Settings** to open the Data Dependent Settings dialog box.

For the ETD experiment type templates, the Data Dependent Settings dialog box has fewer pages.

10. In the Data Dependent Settings dialog box, in the Scan Event list, click **Activation** to display the Activation page (Figure 26).

Figure 26. Data Dependent Settings dialog box showing the Activation parameters



11. Do the following:
 - a. In the Activation Type box, select **ETD**.
 - b. In the Default Charge State box, enter the default value **2**.
For information about the default charge state, refer to the Instrument Setup application Help.
 - c. In the Isolation Width (m/z) box, enter **2**, **3**, or a value between **2** and **3**.
The Normalized Collision Energy and Activation Q parameters are unavailable because they are not applicable to ETD.
 - d. In the Activation Time (ms) box, leave the default value or enter a different value as described in [“Optimizing the Reagent Ion Reaction Time.”](#)
 - e. Select the **Use Mass Range** check box, and use the default range of 110.00-2000.00.
 - f. Click **OK**.



12. In the Tune Plus window, click the **Save** button.
13. Browse to the *drive:\Xcalibur\methods* folder, and specify a file name.
14. Click **Save**.

Data Dependent NL MS³ (ETD) Experiment

With the Data Dependent NL MS³ (ETD) experiment, the MS performs an ETD-activated MS/MS full scan on the precursor ion if the CID-activated MS/MS scan indicates a predefined neutral loss (NL). This experiment type can identify and characterize metabolites and post-translational modifications.

For additional information, refer to the Instrument Setup application Help.

❖ To run a Data Dependent NL MS³ (ETD) experiment

1. Choose **Start > All Programs > Thermo Xcalibur > Xcalibur.** to open the Xcalibur application.

Note For Xcalibur data system version 2.0.7 or earlier, choose **Start > All Programs > Xcalibur > Xcalibur.**

2. Choose **GoTo > Instrument Setup** to display the Instrument Setup window.
3. In the Available Devices pane, click the **Velos Pro MS** or **LTQ XL MS** icon to open the New Method page (Figure 23).
4. At the bottom, under Select ETD Experiment Type, click **Data Dependent NL MS³ (ETD)** to open the Data Dependent NL MS³ dialog box (Figure 27).

The Initialize Method to Repeat the MS₂ Event Using ETD check box is selected as the default setting.

Figure 27. Data Dependent NL MS³ dialog box

#	Mass	Name
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

5. In the Analyze Top N Peaks box, enter the number of top peaks.

5 Running ETD Experiments

Data Dependent NL MS³ (ETD) Experiment

6. In the Neutral Loss Masses table, do one of the following:

- Type the mass and name for the neutral loss masses to identify.

–or–

- Click **Import** to import a list of neutral loss masses from a tab-delimited text file or from an .xml file.

Figure 28 shows an example of a populated neutral loss table for a single phosphorylation modification where the ion of interest might have a charge state of 1+, 2+, or 3+.

Figure 28. Example data in the Neutral Loss Masses table

#	Mass	Name
1	98.00	Phosphorylation (charge 1+
2	49.00	Phosphorylation (charge 2+
3	32.67	Phosphorylation (charge 3+
4		
5		
6		
7		
8		
9		
10		

7. Click **OK** to open the Data Dependent NL MS³ template.

- Toward the bottom, clear the **APCI Corona On** and **APPI Lamp On** check boxes (Figure 29).

Figure 29. Data Dependent NL MS³ template

The screenshot shows the 'Data Dependent NL MS3' software interface. The 'Run settings' section includes 'Acquire time (min): 10.00', 'Segments: 1', and 'Start delay (min): 0.00'. The 'Segment 1 settings' section includes 'Segment time (min): 10.00', 'Scan events: 3', and 'Tune method: C:\Thermo\Instruments\LTQ\methods\Default_ESI.LT'. The 'Scan Event 1 settings' section includes 'Scan Description' with 'Mass Range: Normal', 'Scan Rate: Normal', 'Scan Type: Full', 'Polarity: Positive', and 'Data type: Centroid'. The 'Source Fragmentation' section includes 'On' checked, 'Energy (V): 35.0', and 'Dependent scan' unchecked. The 'FAIMS' section includes 'CY (V): 0.00'. The 'MSn Settings' section includes a table with columns 'n', 'Parent Mass (m/z)', 'Act. Type', 'Iso. Width (m/z)', 'Normalized Collision Energy', 'Act. Q', and 'Act. Time (ms)'. The table has one row with values: 2, CID, 1.0, 35.0, 0.250, 10.00. The 'Scan Ranges' section includes a table with columns '#', 'First Mass (m/z)', and 'Last Mass (m/z)'. The table has one row with values: 1, 110.00, 2000.00. The 'APCI Corona On' and 'APPI Lamp On' checkboxes are highlighted with a blue dashed box. Below the screenshot, a blue arrow points to the text 'Clear these check boxes.'

- Enter the scan event settings by clicking each **Scan Event 1 Number** bar.
For Scan Event 2 and Scan Event 3, the Dependent Scan check box (near the lower left corner) and its adjacent Settings button become active.
- (For Scan Event 2 and Scan Event 3 only) Click **Settings** to open the Data Dependent Settings dialog box (Figure 10).
- In the Data Dependent Settings dialog box, in the Scan Event list, click **Activation** to display the Activation page (Figure 26).

12. Do the following:
 - a. In the Activation Type box, select **ETD**.
 - b. In the Default Charge State box, enter the default value **2**.

For information about the default charge state, refer to the Instrument Setup application Help.
 - c. In the Isolation Width (m/z) box, enter a value **2**, **3**, or a value between 2 and 3.
 - d. The Normalized Collision Energy and Activation Q parameters are unavailable because they are not applicable to ETD.
 - e. In the Activation Time (ms) box, leave the default value or enter a different value as described in “[Optimizing the Reagent Ion Reaction Time](#).”
 - f. Select the **Use Mass Range** check box, and use the default range of 110.00-2000.00.
 - g. Click **OK**.



13. In the Tune Plus window, click the **Save** button.
14. Browse to the *drive:\Xcalibur\methods* folder, and specify a file name.
15. Click **Save**.

Data Dependent Decision Tree Procedure

The data dependent decision tree (DDDT) procedure uses real-time logic to select the most effective dissociation method, ETD or CID, depending on the peptide's mass-to-charge ratio and charge state. ETD is more effective for peptides with three or more charges, and CID is more effective for those with one or two charges.

❖ To configure the data dependent decision tree procedure

1. Choose **Start > All Programs > Thermo Xcalibur > Xcalibur** to open the Xcalibur application.

Note For Xcalibur data system version 2.0.7 or earlier, choose **Start > All Programs > Xcalibur > Xcalibur**.

2. Choose **GoTo > Instrument Setup** to display the Instrument Setup window.
3. In the Available Devices pane, click the **Velos Pro MS** or **LTQ XL MS** icon to open the New Method page ([Figure 23](#)).

4. Click one of the following data-dependent experiment type icons, as applicable:

- **General MS or MSn**
- **Data Dependent MS/MS**
- **Data Dependent Triple Play**
- **Nth Order Double Play**
- **FAIMS Nth Order Double Play**
- **Nth Order Triple Play**
- **Nth Order Double Play (ETD)**

The Instrument Setup window opens showing the selected experiment template.

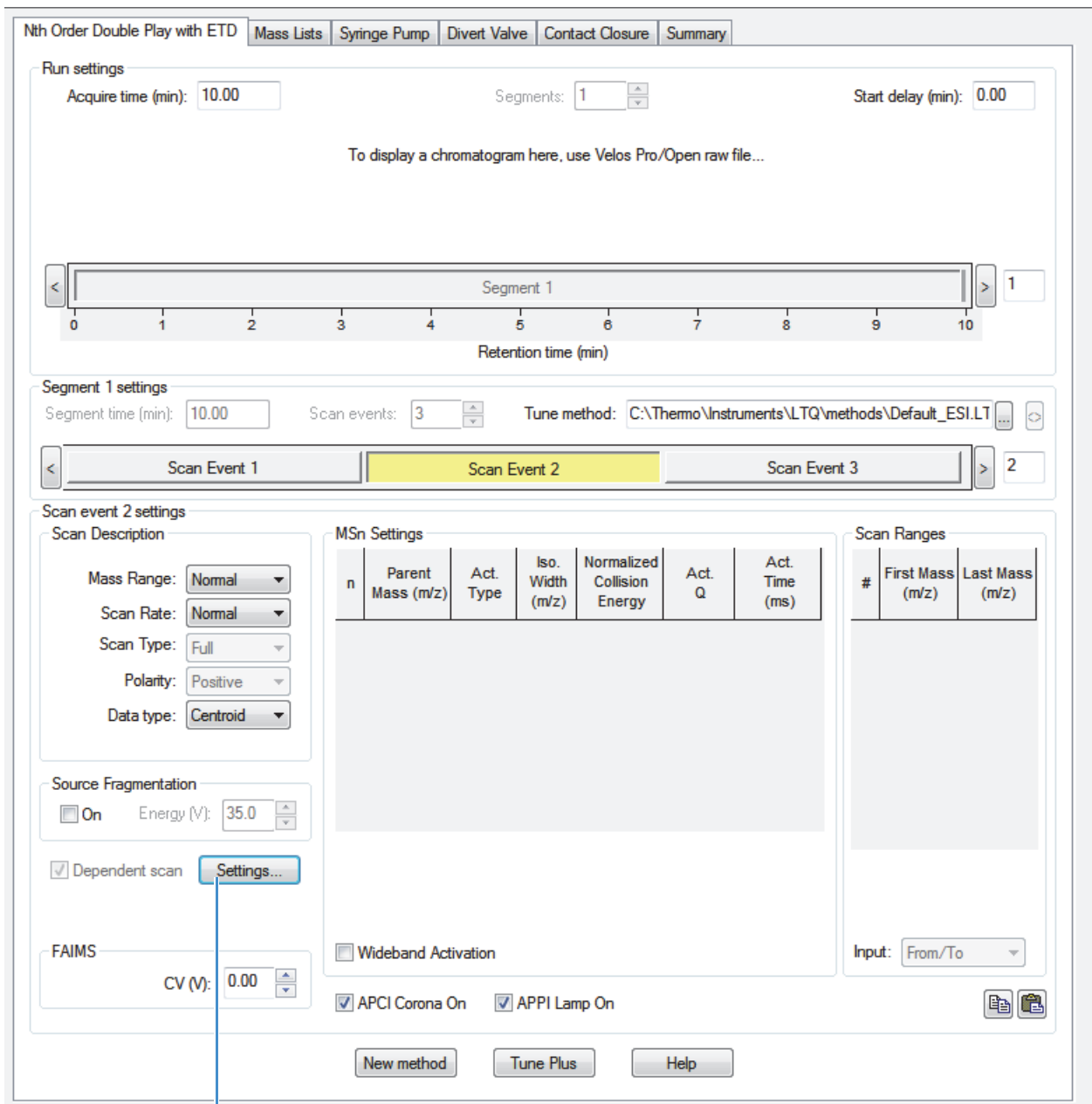
5. Enter the scan event settings by clicking each **Scan Event *Number*** bar.

For Scan Event 2 and higher, the Dependent Scan check box (near the lower left corner) is selected and its adjacent Settings button becomes active. [Figure 30](#) shows the Nth Order Double Play (ETD) template with Scan Event 2 selected.

5 Running ETD Experiments

Data Dependent Decision Tree Procedure

Figure 30. Nth Order Double Play with ETD template (Scan Event 2 selected)

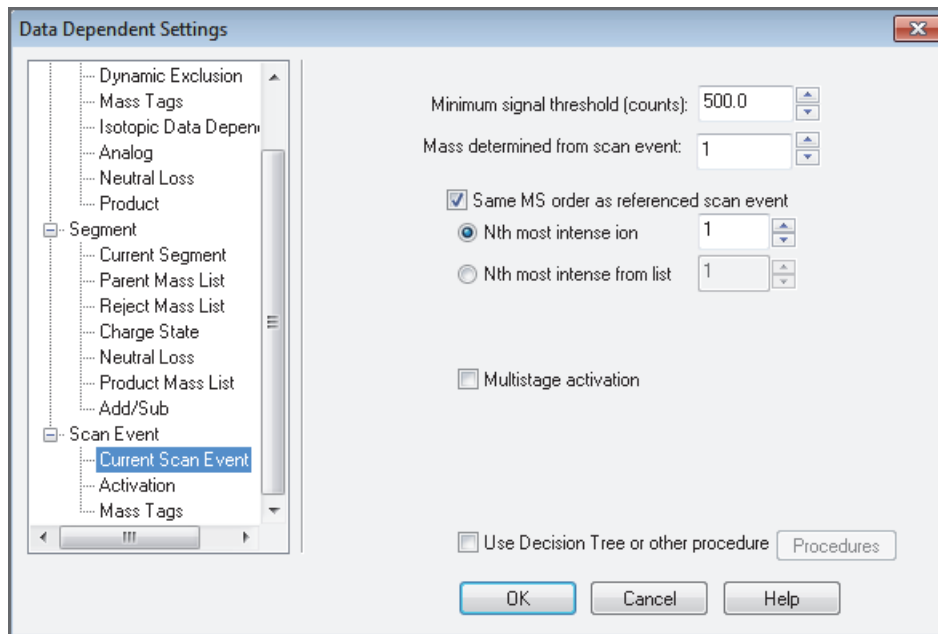


Dependent Scan Settings button

6. Select the scan event, other than Scan Event 1, to implement the DDDT procedure.
The DDDT procedure does not work with the first “independent” scan event.
7. Below the Source Fragmentation area, click **Settings** (Figure 30) to open the Data Dependent Settings dialog box.

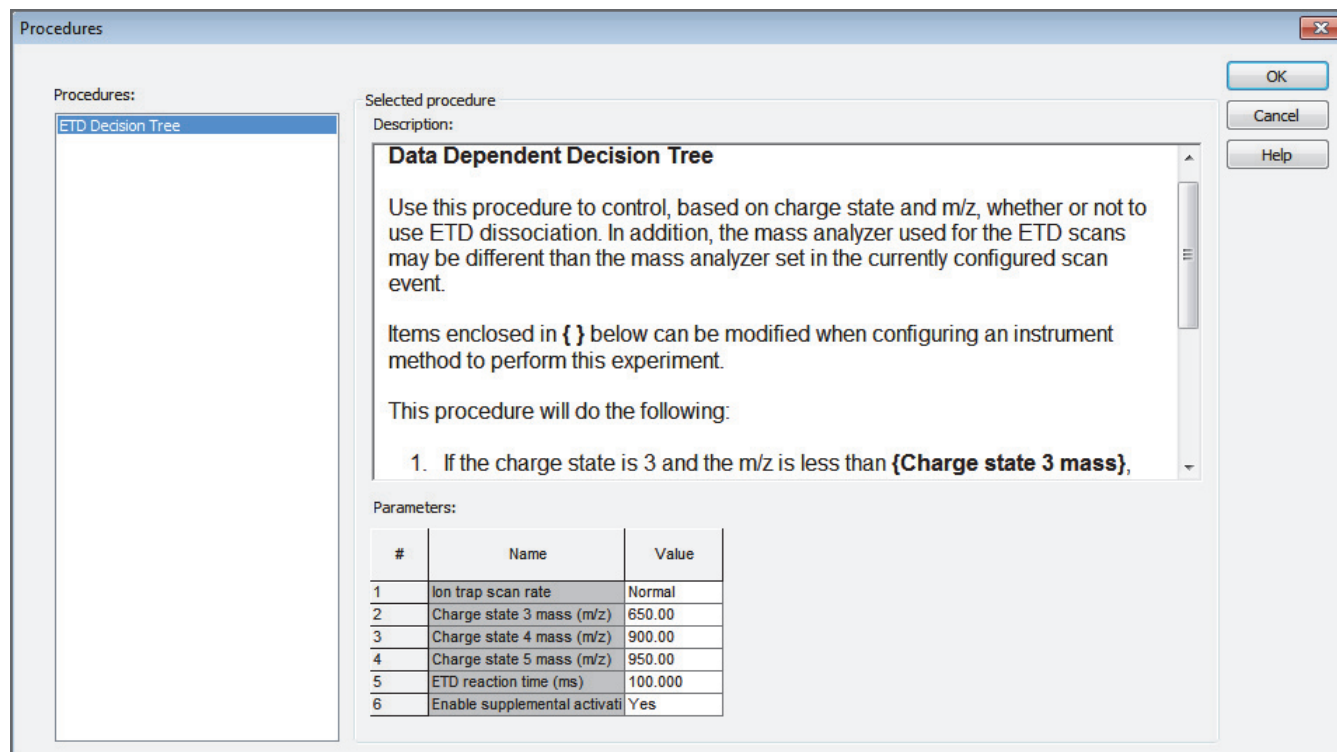
- In the Data Dependent Settings dialog box, in the Scan Event list, click **Current Scan Event** to display the Current Scan Event page (Figure 31).

Figure 31. Current Scan Event page in the Data Dependent Settings dialog box



- Select the **Use Decision Tree or Other Procedure** check box to open the Procedures dialog box (Figure 32).

Figure 32. Procedures dialog box for the Data Dependent Decision Tree (Velos Pro/ETD)



5 Running ETD Experiments

Data Dependent Decision Tree Procedure

10. In the Parameters table, select or enter a value in each row.

For information about these parameters, refer to the Instrument Setup application Help.

11. Click **OK**.

12. In the Data Dependent Settings dialog box, click **OK**.



13. In the Tune Plus window, click the **Save** button.

14. Browse to the *drive:\Xcalibur\methods* folder, and specify a file name.

15. Click **Save**.

Troubleshooting

The MS/ETD system uses the following consumables:

- Fluoranthene
- Filament
- Ion volume

After a period of time, you might need to replenish the ETD fluoranthene reagent, replace the filament, and clean or replace the ion volume. As the fluoranthene is depleted, the ETD reagent mass-to-charge peak intensity diminishes by more than 10 times within a few hours of operation ($m/z = 202$ in view reagent ion spectra mode).

Periodically checking this peak intensity is a good way to monitor the ETD module consumables. To view the ETD reagent m/z signal, follow the procedure “Turning On the Reagent Ion Source” in the *ETD Module Hardware Manual*.

Tip Using the 90 °C vial temperature setting, expect the filament and ion volume to last for at least 1000 hours of operation, and a vial of the fluoranthene reagent to last for approximately 6 months of continuous use.

[Table 3](#) lists some ETD module problems, their causes, and their possible solutions.

Table 3. ETD module problems, causes, and solutions (Sheet 1 of 2)

Problem	Cause	Solution
With the emission current at the correct level, no ions appear at m/z 202.	The m/z 202 is outside of the displayed mass range.	Change the displayed mass range to include m/z 202.
	The system is displaying the front source spectra instead of the reagent ion spectra. After automatically tuning the reagent ion source, the spectrum view redisplay the spectra of ions from the API source, and the View Reagent Ion Spectra check box in the Reagent Ion Source dialog box is clear.	In the Reagent Ion Source dialog box (Figure 5), select the View Reagent Ion Spectra check box.
With the emission current at the correct level, the m/z 202 signal intensity drops slowly over several days.	The filament is sagging.	Replace the filament as described in the <i>ETD Module Hardware Manual</i> .
	The ion volume is contaminated.	Clean or replace the ion volume, as described in the <i>ETD Module Hardware Manual</i> , when the ETD reagent ion injection time becomes excessive (excessively lengthening scan times), typically 50–100 ms.
With the emission current at the correct level, the m/z 202 signal significantly drops within one hour.	The reagent vial might be empty.	Replace the fluoranthene vial as described in the <i>ETD Module Hardware Manual</i> .
With a low emission current, there is a sudden and complete drop of the m/z 202 signal.	The filament might have just blown out.	Check the filament. Replace it if necessary as described in the <i>ETD Module Hardware Manual</i> .

Table 3. ETD module problems, causes, and solutions (Sheet 2 of 2)

Problem	Cause	Solution
The m/z 202 signal is less than $\sim 2 \times 10^5$ in profile mode.	The reagent ion source needs retuning. See Table 2 for the typical profile mode m/z 202 signal intensities.	Retune according to the instructions in Chapter 3, “Tuning the ETD Module.”
	The ion volume slowly becomes contaminated. Reoptimize the reagent carrier/CI gas flow.	For details, see “Optimizing the Reagent Ion Reaction Time.”
A system error message advises that the maximum injection time has been reached for the ETD AGC.	The AGC target has not been reached within the specified time limit. The ion volume is contaminated from use.	<ol style="list-style-type: none"> 1. Clean the ion volume as described in the <i>ETD Module Hardware Manual</i>. 2. Increase the maximum injection time limit, which is typically set to 50 ms. <p>For details, see “Setting the Reagent Injection Settings.”</p>

Fluoranthene



CAUTION Avoid exposure to potentially harmful materials.

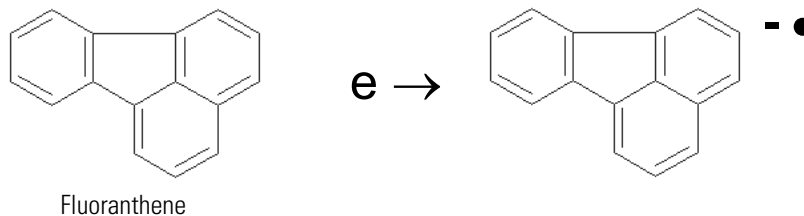
By law, producers and suppliers of chemical compounds are required to provide their customers with the most current health and safety information in the form of Material Safety Data Sheets (MSDSs) or Safety Data Sheets (SDSs). The MSDSs and SDSs must be freely available to lab personnel to examine at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

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

Fluoranthene is used as the ETD reagent in the ETD module portion of the LTQ XL/ETD and Velos Pro/ETD systems. Fluoranthene is potentially hazardous. Use it in accordance with its MSDS.

The fluoranthene radical anion is generated according to the reaction shown in [Figure 33](#).

Figure 33. ETD reagent (fluoranthene radical anion) generation from fluoranthene



Thermo Fisher Scientific supplies fluoranthene as a two-vial kit. One vial contains 0.15 g of fluoranthene and the other is the required empty vial. The fluoranthene vial in the Reagent Kit for the Velos Pro/ETD or LTQ XL/ETD system is Sigma-Aldrich™ Supelco™ #48535. To obtain another fluoranthene vial, click the MSDS link from the Sigma-Aldrich website:

www.sigmaaldrich.com/catalog/search/ProductDetail/SUPELCO/48535

A Fluoranthene

Angiotensin I Solutions

This appendix describes how to prepare solutions containing angiotensin I (human acetate hydrate). Dilute a stock solution to make a test solution, which you use to demonstrate the application of the Velos Pro/ETD or LTQ XL/ETD system and to optimize the reagent ion reaction time.



CAUTION Avoid exposure to potentially harmful materials.

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

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

Angiotensin I (human acetate hydrate) is potentially hazardous. Use it in accordance with its MSDS.

The 1 mg vial of angiotensin I in the Reagent Kit for the Velos Pro/ETD or LTQ XL/ETD system is Sigma-Aldrich Sigma #A9650. To obtain another angiotensin I vial, click the MSDS link from the Sigma-Aldrich website:

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

The procedures in this section include other potentially hazardous chemicals:

- Glacial acetic acid
- Methanol

Handle these chemicals according to their MSDS guidelines.

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

1. Remove the 1 mg vial of angiotensin I from the accessory kit.
2. Add the following to the 1 mg of angiotensin I:
 - 382 μL of water
 - 382 μL of methanol
 - 8 μL of glacial acetic acid
3. Mix the solution thoroughly.

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

1. Pipet 100 μL of the stock solution (1 nmol/ 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 the 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 the solution (1 pmol/ μL) thoroughly.
7. Store this sample solution (1 pmol/ μL) in a refrigerator until it is needed.



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