

Orbitrap Exploris EFOX

Mass Detector

Software Manual

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About this manual

IMPORTANT

Export classification: not listed / Exportklassifizierung: nicht ausfuhrgenehmigungspflichtig

The Orbitrap Exploris EFOX system is a member of the Thermo Scientific™ family of mass spectrometers that are powered by Orbitrap™ technology.

The Orbitrap Exploris EFOX Software Manual describes software-related operating procedures of the Orbitrap Exploris EFOX mass detector.

To obtain a good understanding of the complete system, we recommend that you study the *Orbitrap Exploris Series Operating Manual*. This manual also contains extensive information that concerns the safety of the personnel that operate the instrument. A basic knowledge of handling computers and of the Xcalibur™ software is assumed for the correct operation of the Orbitrap Exploris EFOX system.

Designed, manufactured 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.

NOTE

Screenshots shown in this document are for demonstration purposes only. The software installed on your system depends on the actual software version and system configuration, and may therefore differ.

Contents

- [Special notices and safety symbols](#)
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Special notices and safety symbols

Make sure you understand the special notices, symbols, and caution labels in this guide. Most of the special notices and cautions appear in boxes; those pertaining to safety also have corresponding symbols.

NOTICE

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

NOTE

Highlights information of general interest.

TIP

Highlights helpful information that can make a task easier.

Typographical conventions

Different typographical conventions have been established for Thermo Fisher Scientific manuals.

Viewpoint orientation

The expressions *left* and *right* used in this manual always refer to the viewpoint of a person that is facing the front side of the instrument.

Data input

Throughout this manual, the following conventions indicate data input and output with 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**.”

Access documentation

You can access product documentation using one of the following ways:

Visit the Help Portal

Full documentation (including release notes, installation instructions, operation manuals, and so on) is maintained on the Thermo Fisher Scientific Chromatography and Mass Spectrometry Help Portal. This documentation portal is regularly updated with the latest content, including video tutorials. Go to docs.thermofisher.com.

With a Thermo Fisher Scientific account, you can use features on the documentation portal, such as bookmarking documents, saving searches, and compiling documents into collections for easy reference.

Procedure

1. Go to docs.thermofisher.com/p/TechDocAccess to display a registration form.
2. Fill out the form to request access as a customer. Among other data, you have to provide the serial number (S/N) of your instrument. You can find the serial number of your instrument on the name plate. Refer to the Operating Manual of your instrument for information about its location.
3. Click **Submit Form**. The form contents will be sent using your default email client. You will receive an email with additional login information.

Result

Upon submitting the form, you will be directed to home page of the Help Portal.

View the product documentation on the data system computer

To view the product manuals, do one of the following:

- From the Windows™ taskbar, select **Start > Thermo Instruments**, and then open the applicable PDF file.

- From the Windows explorer, navigate to the following path:
C:\Program Files\Thermo Scientific\Instruments\instrument mode\version\System\Manuals

Reference documentation

Reference documentation for the Orbitrap Exploris EFOX MS includes the following:

- *Orbitrap Exploris Series Operating Manual*
This manual contains precautionary statements that can prevent personal injury and instrument damage. It also describes the modes of operation and principle hardware components of the instrument. In addition, this manual provides instructions for cleaning and maintaining the instrument.
- *Orbitrap Exploris Series Preinstallation Requirements Guide*
This manual contains information on the necessary environmental conditions in the intended location for the instrument.
- *Orbitrap Exploris Performance Maintenance Manual*
This manual describes the user maintenance for the quadrupole and the bent flatapole.

You can access PDF files of the documents listed above and of this manual from the data system computer. Refer also to the user documentation that is provided by the manufacturers of third-party components:

- Forepump
- Turbomolecular pumps
- Syringe pump
- Data system computer and monitor
- Safety data sheets

Training

Thermo Fisher Scientific offers worldwide training on instruments and software. Experience has shown that maximum results can be obtained from a scientific instrument if the instrument operators receive an adequate training.

We recommend that the key users participate in a basic operator training. For information on training courses and enrollment, contact your local Thermo Fisher Scientific office.

Configure the instrument in Thermo Foundation

To establish control of the mass spectrometer from the data system computer, use the Thermo Foundation™ Instrument Configuration window to configure the instrument.

TIP

The Instrument Configuration window displays all changed set values of given parameters in bold type until you select OK or Apply. To enable the new settings, restart the data system computer.

Procedure

1. From the *Windows* taskbar, select **Start > All Apps > Thermo Foundation x.x > Instrument Configuration**, where x.x. is the installed version.
2. Add the mass spectrometer's icon to the list of Configured Devices, and then configure the mass spectrometer parameters.
3. Select **Done**.

Table 1 Parameters in the Instrument Configuration window

Parameter	Description
System Information	
Model	Displays the mass spectrometer model name.
Serial Number	Display the serial number of the instrument.
Instrument Name	(Optional) The name for the instrument.
Inlet	
Divert Valve	<ul style="list-style-type: none"> • None (for no divert valve) (Default) • A (for a system with one divert valve) • A and B (for a system with two divert valves)
Syringe Pump	Configured: Indicates that the system includes a data-system controlled syringe pump. Default: Not Configured

Parameter	Description
Contact Closure	Select the event type that triggers the contact closure: <ul style="list-style-type: none"> Transition close-to-open (Default) Transition open-to-close
Wait for Gas Flows to Stabilize	When the Wait for Gas Flows to Stabilize checkbox is selected, the instrument remains in Preparing for Run state until the following ion source gas pressures have stabilized depending on the used ion source: <ul style="list-style-type: none"> Sheath Gas (Arb) Aux Gas (Arb) Sweep Gas (Arb) When the ion source gases have reached the values defined in the instrument method, data acquisition starts.
Wait for Temperatures to Stabilize	When the Wait for Temperature to Stabilize checkbox is selected, the instrument remains in Preparing for Run state until the following ion source temperatures have stabilized depending on the used ion source: <ul style="list-style-type: none"> Ions Transfer Tube Temp (°C) Vaporizer Temp (°C) When the ion source temperatures have reached the values defined in the instrument method, the data acquisition starts.
Ion Source	
Default Source	<ul style="list-style-type: none"> NSI (optional) Heated ESI (H-ESI) APCI (atmospheric pressure chemical ionization) (optional) ESI <p>NOTE: Although atmospheric pressure photoionization (APPI) is not a configuration option, the mass spectrometer automatically detects if the ion source contains the APPI vacuum ultraviolet (VUV) lamp through the lamp's USB connection.</p>
Default Source Type	Default Source Type: Heated ESI or APCI <ul style="list-style-type: none"> Dedicated Heated ESI or Dedicated APCI Combination Source
Enable Sweep Gas for NSI Source	Provides sweep gas flow to the NSI source.

Parameter	Description
	IMPORTANT: Sweep gas can help in the desolvation of the nanospray, but excessive sweep gas can deflect the nanospray from the orifice of the ion transfer tube, which decreases sensitivity. You must have the ion sweep cone installed for the sweep gas to work.
Enable Minimum Gas Flow Requirement for Sealed Sources (H-ESI, ESI, APCI, and APPI)	Provides minimum background flow of ion source gases to keep the source housing pressurized. IMPORTANT: When the ion source is in off mode or standby mode, it is pressurized with background nitrogen gas to avoid back-streaming from the drain and introducing contaminants.
Optional Hardware	
Internal Calibration (EASY-IC) Source	Activates or deactivates the EASY-IC™ ion source.
Analog Inputs	
Channel 1 in Use	Reads the 0–10 Vdc analog input signal from an external device and writes the data into the raw data file. For additional information, refer to the instrument manual.
Channel 1 Label	The name you provide for channel 1.
Channel 2 in Use	Reads the 0–10 Vdc analog input signal from an external device and writes the data into the raw data file. For additional information, refer to the instrument manual.
Channel 2 Label	The name you provide for channel 2.
Historical Data Retention	
Once a week on the specified day and time, the data purging process will initiate. This process only occurs if the instrument is in an idle state. In addition to these settings, automatic purging managed by the TNG database service can occur nightly at 11pm if the instrument is in an idle state.	
Day	Use the list box to select the weekday for the data retention. Default: Sunday
Time	Use the list box to select the hour for the data retention. Default: 11 PM
Buttons	
OK	Saves the instrument configuration settings and closes the window.
Cancel	Closes the Instrument Configuration window without saving your changes.
Apply	Applies the changes you made in the Instrument Configuration window.

Tune application

Use the Tune application to control, monitor, tune, and calibrate the Thermo Scientific™ Orbitrap Exploris EFOX mass detector. You can also optimize the instrument for your specific compounds, perform method development, and run real-time mass spectrometry experiments one analysis at a time.

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- [Overview of the Tune application](#)
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Overview of the Tune application

Use the Tune application to operate the Orbitrap Exploris EFOX MS with the data system computer. The Tune application organizes its functions in panes and on pages. To display the panes and pages, select the associated buttons and tabs. If information about setting the parameters for a specific view, page, or dialog box is available, click Help or press the F1 key.

The Tune application has the following main components:


- Three system power icons to set the MS's power mode (on, standby, and off).
- The system readback icon to indicate the various readback states.

- Three panes on the left to define parameters for scanning, ion source, and calibration.
- Four panes on the right to display information about instrument status, history and favorite settings.
- Up to three graphs to show real-time information about the current measurement.

Display the Tune application

Select **Start > All Programs > Thermo Instruments > Orbitrap Exploris Tune**.




TIP

You can set a few preferences for how the Tune application works. Select the Options icon, , and then select **Preferences**.

Buttons and icons

For complete descriptions of features, see the applicable table of the Tune icons and buttons.

System power icons

Button	Description
<p>A green button indicates the active instrument status.</p> <p>On mode</p> 	<p>In the On state, all voltages, the sheath gas, the auxiliary gas, and the sweep gas are on.</p>
<p>Standby mode</p> 	<p>In the Standby state, the high voltages and the vaporizer heater are off. The RF voltages and the DC offset voltages remain on. The sheath gas and the auxiliary gas remain on at a low flow rate (6 arbitrary units).</p>
<p>Off mode</p> 	<p>In the Off state, the RF and high voltages, ion transfer tube heater, and vaporizer heater are off. In addition, the offset voltages are off. The sheath gas, auxiliary gas, and ion sweep gas remain on at a low flow rate to flush vapors from the ion source.</p>

Instrument and spectrum buttons

Button	Description
<div style="border: 1px solid gray; padding: 2px;"> + Positive </div> <div style="border: 1px solid gray; padding: 2px;"> + Positive </div> <div style="border: 1px solid gray; padding: 2px;"> - Negative </div>	Indicates the ion polarity mode (positive or negative).
<div style="border: 1px solid gray; padding: 2px;"> Profile </div> <div style="border: 1px solid gray; padding: 2px;"> Centroid </div> <div style="border: 1px solid gray; padding: 2px;"> Profile </div>	

Modular valve and syringe pump buttons

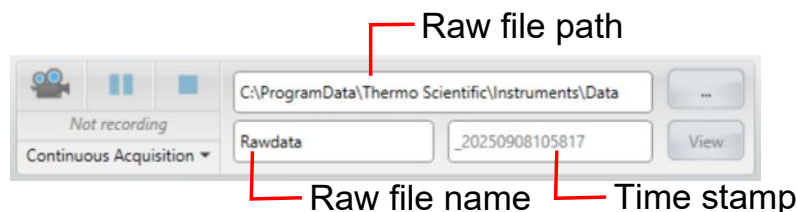
The Tune application displays these buttons for H-ESI, APCI, APPI, and NSI modes.

Button	Description
<div style="border: 1px solid gray; padding: 2px;"> Valve 1-2 </div> <div style="border: 1px solid gray; padding: 2px;"> A </div>	Indicates the position (1-2 or 1-6) for two divert/inject valves (A and B) on the button left side. The down arrow opens two choices: valve A and optional valve B.
<div style="display: flex; justify-content: space-around; align-items: center;"> </div>	
<div style="border: 1px solid gray; padding: 2px;"> Syringe OFF </div> <div style="border: 1px solid gray; padding: 2px;"> Syringe ON (5 $\mu\text{L}/\text{min}$) </div> <div style="border: 1px solid gray; padding: 5px;"> <p>Flow Rate ($\mu\text{L}/\text{min}$) <input style="width: 80px;" type="text" value="5"/></p> <p>Volume (μL) <input style="width: 80px;" type="text" value="100"/></p> <p style="text-align: center;"><input type="button" value="Apply"/></p> <p style="text-align: center;"><input type="button" value="Prime"/></p> </div>	Indicates whether the syringe pump is pumping. The syringe pump settings box defines the pump flow rate and the syringe volume. You can also prime the syringe by pressing and holding Prime.

Data acquisition buttons

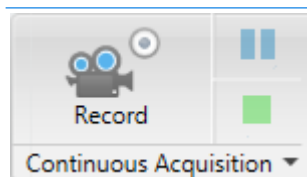
The Tune application saves the acquired data to a raw data file (.raw extension).

Figure 1 Raw file data

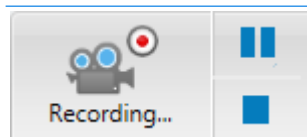


Button	Description
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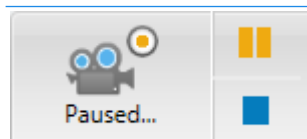
Select the label below the Record button to expand the acquisition settings box.



Select the **Record** toggle button to acquire data to a new raw data file. The name of the file—if not changed—is a combination of the base file name and a time stamp, which consists of the year (YYYY), month (MM), day (DD), and time (HHMMSS).



To stop the data acquisition, select **Stop** (blue square). The color of the square then changes to green.



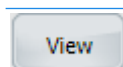
To pause the data acquisition, select **Pause** (two blue bars). The color of the bars then changes to orange. To resume the data acquisition, select **Paused** (shown as an orange circle).

NOTE: If the Instrument Method checkbox in the data acquisition settings box is selected, the Pause feature is not available.



(Optional)

To change the destination folder for the raw data, select the Browse button. The default folder location is in drive: \Thermo\Data.

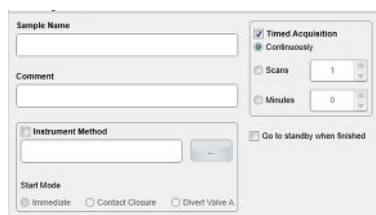


You must manually set FreeStyle as the default application to view the RAW files. (To specify the default application, right-click the RAW file, then navigate to ThermoFisher.FreeStyle.App.exe (C:\Program Files (x86)\Thermo\FreeStyle).

Sample information and data acquisition method




Select the button to the left of the View button to expand the acquisition settings box. As shown in this figure, you can select only one checkbox.








Sample Name	The name of the sample.
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Button	Description
Comment	A comment that describes the acquisition.
Instrument Method	Indicates to load an instrument method and start the data acquisition with one of these options: <ul style="list-style-type: none"> • Immediate: Starts the acquisition immediately when you select Record. • Contact Closure: Starts the acquisition when the mass spectrometer receives a contact closure signal from the connected analog autosampler. • Divert Valve: Starts the acquisition when the position of the divert/inject valve changes from its current position.
Timed Acquisition	Indicates how long the mass spectrometer acquires data as follows: <ul style="list-style-type: none"> • Continuously: The mass spectrometer acquires data until you select Stop. • Scans: The number of scans acquired by the mass spectrometer before the data acquisition stops. • Minutes: The time (in minutes) that the mass spectrometer acquires data.
Go to Standby When Finished	The mass spectrometer goes in standby mode when the current acquisition is completed.
Enable FAIMS CV Scan	This parameter is available only when the system has detected an FAIMS source. Indicates the use and configuration of the FAIMS compensation voltage (CV) scan tool. The Start and Stop values defining the FAIMS CV range. The Step value defines the step size in volts within the range. The Estimated Duration read-only value counts down the estimated completion time (in seconds) for the CV scan. Range (start/stop): -300 to 300; default: -100 to 100 Range (step): 0.1-2; default: 1




Instrument status icons

Icon	Description
NOTE When you start data acquisition, the instrument status icon does not change.	
Normal 	The system parameters are within tolerance.
Initializing	The system is initializing.

Icon	Description
	Changing Settings
	One or more settings are changing.
	Source Off
	The ion source is off.
	Disconnected
	There is no communication between the mass spectrometer and the data system.
	Error
	An error has occurred.
	





Miscellaneous icons

Icon	Description
	This icon appears at the far right side of the Tune window, below the Instrument Status icon.
	Displays the Options menu.
	The Copy and Paste icons appear at the bottom of the Ion Source page.
Copy	(Ion Source page)
	Copies the ion source parameters to the clipboard.
Paste	(Ion Source page)
	Pastes the ion source parameters from the clipboard.
	TIP: You can use the clipboard to transfer the ion source parameters between the Tune application and the Method Editor application.
Diagnostics	Displays the Diagnostics pane.
	
Monitor Mass Accuracy	Displays the Monitor Mass Accuracy dialog box.
	Plots the difference between the measured peak position and the theoretical peak positions in the mass list. To start or stop monitoring the mass accuracy, select the icon.
Plot Chromatogram	Displays the Plot Chromatogram dialog box.

Icon	Description
	Plots a chromatogram for the TIC/Base Peak or User Defined m/z . Additionally, the Spray Stability as %RSD of the ion current can be plotted. The %RSD must be less than 15% for the spray stability to pass the evaluation in the beginning of a calibration procedure. To start or stop the ion current display, select the icon.
Rotate Plots 	Rotates the displayed graphics.
Graph mode 	Divides the screen into up to three areas.

OptiSpray cartridge controls

(Available when the OptiSpray ion source is connected to the MS.)

Button	Description
 Infusion	Cartridge type
 InActive	Select this button to display the OptiSpray cartridge details.
 Infusion	Cartridge status
 InActive	Indicates the OptiSpray cartridge's current status. Select this button to switch the OptiSpray cartridge between the Inactive and Active positions. This button is unavailable during emitter optimization. <ul style="list-style-type: none"> • Default active: Emitter is positioned in front of the ion transfer tube (default: 44.50 mm x, 5.66 mm y, 4.80 mm z) NOTE: After an emitter optimization (quick, full, or manual) has been performed, the optimized position is saved as the new active position. • Inactive: Emitter is positioned away from the ion transfer tube (30.00 mm x, 5.66 mm y, 4.80 mm z) NOTE: The inactive emitter position cannot be changed.

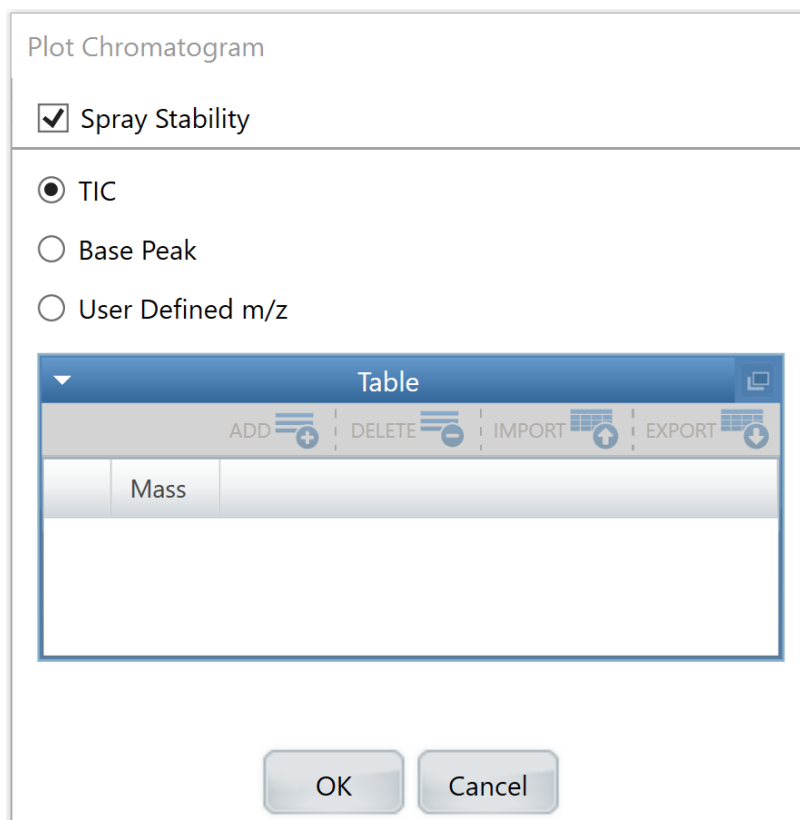
Display real-time chromatograms and spectra

Use the Plot Chromatogram dialog box to plot the chromatogram and the Monitor Mass Accuracy dialog box to plot the difference between the measured and theoretical peak positions.

Control the chromatogram view

Use the Plot Chromatogram dialog box to plot the chromatogram from the TIC, the base peak, or one or more user-defined m/z values. For the user-defined mass table, you can import the settings from (or export them to) a CSV, a TXT, or an XML file.

Figure 2 Plot Chromatogram dialog box




NOTE

The Tune application always displays the Chromatogram view. Observe the chromatogram trace to see how system parameter changes affect the ion signal intensity so that you can optimize the parameter settings as needed.

Display the TIC or the ion current for a select mass as a function of time

Procedure

1. Select the Plot Chromatogram icon, , at the bottom of the Tune window.
2. Select the chromatogram type, and then select **OK**.

Chromatogram data and spray stability data (if selected) are shown in separate windows during the monitoring.

3. To stop the plot, select the Plot Chromatogram icon again.

Parameters in the Plot Chromatogram dialog box

Parameter	Description
Spray Stability	Plots the spray stability. Plots a chromatogram for the TIC/Base Peak or the User Defined <i>m/z</i> . Additionally, the Spray Stability as %RSD of the ion current can be plotted. The %RSD must be less than 15% for the spray stability to pass the evaluation in the beginning of a calibration procedure.
TIC	Plots the TIC chromatogram.
Base Peak	Plots the base peak chromatogram.
User Defined <i>m/z</i>	Plots the signal intensities of the ions you enter in the mass table as a function of time.
Mass table	

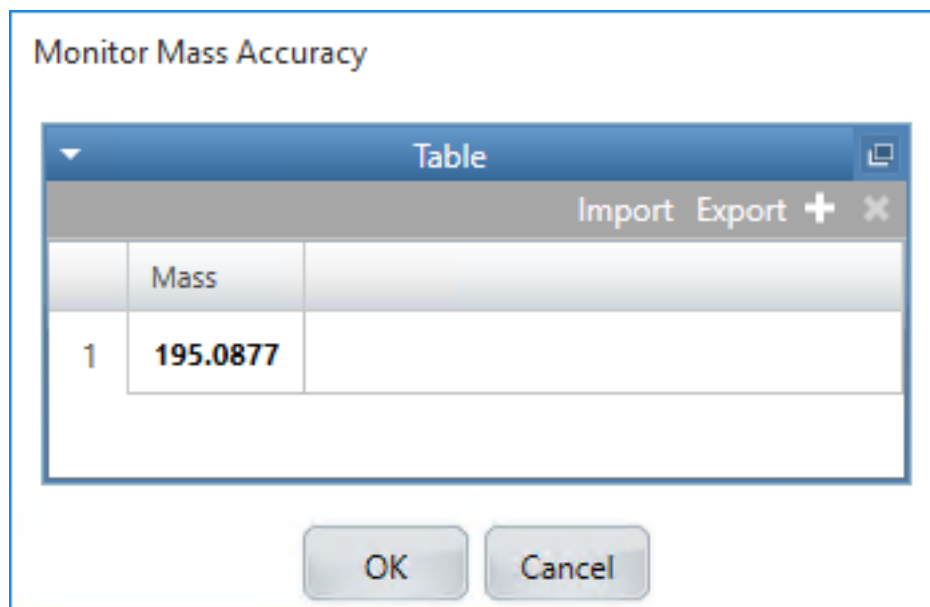
Shortcut Menu Commands in the Chromatogram View

Command	Description
Copy	Copies the chromatogram to the Clipboard.
Print	Sends a copy of the chromatogram to the printer.


Monitor the mass accuracy and signal stability

Use the Monitor Mass Accuracy dialog box to plot the difference between the measured and theoretical peak positions in the mass list. For the mass table, you can import the settings for the mass table from (or export them to) a CSV, a TXT, or an XML file.

Figure 3 Monitor Mass Accuracy dialog box



Procedure

1. Select the **Monitor Mass Accuracy** icon, , at the bottom of the Tune window.
2. Enter one or more m/z values with up to four decimal places accuracy within the allowed scan range. Then select **OK**.

NOTE

When you enter masses in the table, include at least two decimal places.

A plot “Monitoring Mass Accuracy” starts in the upper view panel: mass list entries are identified within a mass tolerance of ± 25 ppm. Mass accuracy (ppm) is plotted against the scan number. The monitored mass traces are shown in the legend of the plot.

3. To stop the plot, select the **Monitor Mass Accuracy** icon again.

Parameters in the Monitor Mass Accuracy Dialog Box

Zoom in on a spectrum

In the Spectrum view, you can zoom in on one or both axes as needed.

Procedure

1. To zoom in on both axes, drag the cursor diagonally across the spectrum to make a rectangle where the corners define the displayed axes ranges.
2. To zoom in on one axis, drag the cursor horizontally or vertically along the axis or the spectrum to define the displayed range.
3. To go back to the default view, select **Reset Scaling** in the shortcut menu.

Shortcut menu commands in the Spectrum View

Command	Description
Reset Scaling	Resets the axes to the default intensity and mass ranges.
Copy to Clipboard	Copies a screen capture of the mass spectrum to the Clipboard.
Pan	To use this tool, you must first zoom in on the spectrum. Then you can select this tool to slide the spectrum back and forth along the x axis by holding down the left mouse button and moving the mouse cursor.
Print	Sends a copy of the mass spectrum to the printer.

Command	Description
Show Frequency	Displays the frequency spectrum (in kHz) in blue. Clear this option to display the m/z spectrum.
Show Absolutes	Changes the y -axis scale from percentages to ion flux (ions/s).
Display	<ul style="list-style-type: none"> Normalized: The y-axis scale of the mass spectrum normalizes so that the most intense peak equals 100. Fixed: The y-axis scale of the mass spectrum does not change. Creep: The y-axis scale of the mass spectrum automatically increases if the peak intensity increases, but does not decrease if the peak intensity decreases.
Profile Label	Adds the resolution (R) and charge state (z) values next to the peak labels.
Profile Label Setting	<ul style="list-style-type: none"> Resolution: Calculates the resolution (R) and labels the peaks with it. Charge: Labels the peaks with the charge state (z).

Header information in the spectrum view

This table defines the information at the top of the real-time spectrum.

Header information	Description
Scan number (#)	The scan count, which only resets to zero when you start data acquisition.
RT	The retention time (in minutes), which also resets to zero when you start a data acquisition.
NL	The normalization level, which is a measure of the ion signal intensity.
Injection time	The injection time (in milliseconds) per scan.
Scan settings (filter)	The detector type, polarity mode (+ or -), data type (profile or centroid), source type, scan type, and other settings.

Control the ion source

The Ion Source *pane* uses the following real-time indicators to identify the readback status for each parameter:


- Green square (■)—Indicates that the parameter has reached the specified value.
- Orange square (■)—Indicates that the parameter is trending toward the specified value.

- Red square (■)—Indicates that the parameter could not reach the specified value.

NOTE

A red border around a parameter box indicates that the value is out of range.

TIP

After setting the parameters on the Ion Source pane, you can use the  button to copy the parameters settings for pasting into an instrument method.

Parameters on the Ion Source page

Use the Ion Source page to set the ion source parameters.

Figure 4 Tune application—Ion Source page

ION SOURCE	DEFINE SCAN	CALIBRATION
Ion Source	Optimization	
Current LC Flow (µL/min)	0	Get Defaults
Ion Source Type	Heated ESI	
Pos Ion Spray Voltage (V)	3400	0
Neg Ion Spray Voltage (V)	2000	
Sheath Gas (Arb)	0	0.00
Aux Gas (Arb)	7	0.00
Sweep Gas (Arb)	0	0.00
Ion Transfer Tube Temp (°C)	320	0.0
Vaporizer Temp (°C)	0	0.0

Parameter	Description
Current LC Flow (µL/min)	(Ion Source Type: H-ESI or APCI) The LC flow rate (µL/min).

Parameter	Description
	<p>Select Get Defaults, and then select Apply to update the ion source parameters. The Tune application sets the applicable ion source parameters to the appropriate default values for the specified LC flow rate.</p> <p>Range: 0–3000; default: 0</p> <hr/> <p>NOTE:</p> <ul style="list-style-type: none"> • Selecting Apply does not start the LC. • When the ion source is in Off mode or Standby mode, it is pressurized with background nitrogen gas to avoid back-streaming from the drain and introducing contaminants.
Ion Source Type	<p>The configuration for the ion source housing. The instrument automatically recognizes the source type and displays the corresponding parameters for the detected source type.</p> <p>In case of a OptaMaxNG ion source (Heated ESI and APCI), you can select the source type that you want to use.</p> <p>Although atmospheric pressure photoionization (APPI) is not a configuration option, the mass spectrometer automatically detects if the ion source contains the APPI vacuum ultraviolet (VUV) lamp through the lamp's USB connection.</p> <hr/> <p>NOTE: For some ion source types (MALDI, for example), you can specify additional settings in the Define Scan subtree of the Diagnostics pane.</p>
Pos Ion Spray Voltage (V)	<p>(Ion Source Type: ESI, H-ESI, NSI, or OptiSpray)</p> <p>Specify the positive source spray voltage (absolute value, in volts).</p> <p>Range: 0–6,000 (ESI and H-ESI)</p> <p>Range: 0–3,000 (NSI and OptiSpray)</p>
Neg Ion Spray Voltage (V)	<p>(Ion Source Type: ESI, H-ESI, NSI, or OptiSpray)</p> <p>Specify the negative source spray voltage (absolute value, in volts).</p> <p>Range: 0–5,500 (ESI and H-ESI)</p> <p>Range: 0–2,500 (NSI and OptiSpray)</p>
Sheath Gas (Arb)	<p>(Ion Source Type: H-ESI, APCI, or OptiSpray)</p> <p>The sheath gas flow rate (arbitrary units).</p> <p>Range: 0–80 (H-ESI, APCI); default: 0 (or determined by the Tune application based on the LC flow rate)</p> <p>Range: 0–25 (OptiSpray); default: 0 (or determined by the Tune application based on the LC flow rate)</p>
Aux Gas (Arb)	<p>(Ion Source Type: H-ESI or APCI)</p>

Parameter	Description
	<p>The auxiliary gas flow rate (arbitrary units).</p> <p>Range: 0–25; default: 2 (or determined by the Tune application based on the LC flow rate)</p> <p>NOTE: If the vaporizer temperature is set to values above 100 °C, the auxiliary gas is automatically set to 5 unless it is not set higher manually.</p>
Sweep Gas (Arb)	<p>The sweep gas flow rate.</p> <p>Range: 0–20; default: 0 (or determined by the Tune application based on the LC flow rate)</p> <p>In the Instrument Configuration, you can switch on the sweep gas for the NSI source.</p>
Ion Transfer Tube Temp (°C)	<p>The temperature of the ion transfer tube (capillary) in °C.</p> <p>Range: 0–400; default: 320 (or determined by the Tune application based on the LC flow rate)</p>
Vaporizer Temp (°C)	<p>(Ion Source Type: H-ESI or APCI)</p> <p>The temperature (in °C) of the APCI vaporizer, which vaporizes sample and solvent, or of the H-ESI source heater, which heats the auxiliary gas.</p> <p>Range: 0–550; default: 0 (off) (or determined by the Tune application based on the LC flow rate)</p> <p>NOTE: You must set the auxiliary gas to 5 or greater for vaporizer temperatures above 100 °C.</p>
Pos Ion Discharge Current (µA)	(Ion Source Type: APCI)
Neg Ion Discharge Current (µA)	<p>The APCI source discharge current (microamperes).</p> <p>Range: 0–100; default: 4 (positive polarity mode) and 10 (negative polarity mode)</p> <p>IMPORTANT: The actual possible maximum value of the discharge current is limited by the maximum value of the spray voltage of 5000 V.</p>
APPI Lamp	<p>(Ion Source Type: H-ESI or APCI)</p> <p>Switches on or off the optional APPI lamp.</p> <p>Tune displays the readback value for the current lamp state next to the list box, where 0 is off and 1 is on.</p> <p>Default: Off</p> <p>NOTE: The Tune application displays this parameter only when the ion source contains the optional APPI lamp. The lamp state shown in the application might not match the lamp state shown in Method Editor if an instrument method uses APPI mode.</p>

Parameters for the FAIMS Pro System

For additional information, refer to the *FAIMS Pro System User Guide*.

Parameter	Description
Total Carrier Gas Flow (L/min)	The flow rate for the FAIMS and carrier nitrogen gas. Range: 3.5–7.7; default: 4.6
FAIMS Mode	The temperatures for the FAIMS electrodes are as follows: <ul style="list-style-type: none"> Standard Resolution: Provides the best transmission mode by setting the inner and outer electrodes to 100 °C. High Resolution: Provides twice the resolution of the standard resolution mode by setting the inner electrode to 80 °C and the outer electrode to 100 °C. User Defined: Displays the fields to enter the electrode temperatures. <p>NOTE: The electrode temperatures affect ion separation. Although ion transmission might be lower in FAIMS high resolution mode, the peaks are narrower due to the electrodes' temperature differential.</p>
FAIMS Inner Electrode Temp (°C)	(FAIMS Mode: User defined) The maximum temperature for the inner electrode in the FAIMS Pro system (in °C). Range: 70–100; default: 100
FAIMS Outer Electrode Temp (°C)	(FAIMS Mode: User defined) The maximum temperature for the outer electrode in the FAIMS Pro system (in °C). Range: 70–100; default: 100

Emitter Optimization tab

When the OptiSpray ion source is connected to the MS, the Emitter Optimization tab becomes available in the Ion Source pane.

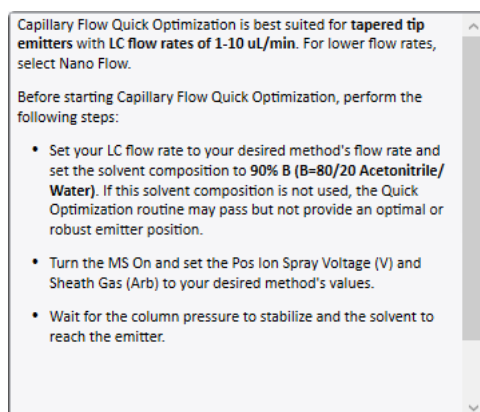
The following table describes the different optimization methods that you can use to set to emitter's active position. For additional information, refer to the *OptiSpray Ion Source User Guide*.

Parameter	Description
Optimization Mode	Select the mode for optimizing the emitter position: <ul style="list-style-type: none"> Quick: Applies sheath gas and scans a m/z range (positive mode) containing acetonitrile solvent solution and cluster ions to set the emitter position. If FAIMS is installed, the FAIMS voltages are set to Off for this procedure.

Parameter	Description
	<ul style="list-style-type: none"> • Full: Averages the sum of up to 10 entered ions in SIM mode with an isolation of 1 m/z (range: 50–2,000 m/z) and uses the result as the emitter position. Can be performed in positive or negative mode. If FAIMS is installed, the FAIMS voltage status that was set prior to full optimization is maintained throughout the procedure. • Manual: Uses manual movement commands to move the emitter in increments of 0.1 mm to 0.5 mm on the x (left/right) axis and along the user's choice of the y (up/down) axis, z (toward, away) axis, or combined yz (diagonal) axis. This mode is independent of ion signal. NOTE: For manual optimization, the cartridge must be in the Active position.
Optimization Flow (Quick optimization mode)	<p>Select the flow mode to use for the quick emitter optimization routine:</p> <ul style="list-style-type: none"> • Nano Flow: Flow rate less than 1 $\mu\text{L}/\text{min}$. Requires 20% B (80/20 ACN/Water) solvent. • Capillary Flow: Flow rate between 1-10 $\mu\text{L}/\text{min}$. Requires 90% B (80/20 ACN/Water) solvent.

The Emitter Optimization tab also contains an Instructions area that provides a quick set of instructions for the capillary flow and nano flow optimization routines.

Figure 5 Instructions area in the Emitter Optimization tab



Optimize the ion source parameters

Follow this procedure for the H-ESI and APCI source types to maximize the ion signal for your application.

NOTE

A red border around a parameter box indicates that the value is out of range.

Figure 6 Tune application—Optimization page

Ion Source	Optimization		
Select source condition to optimize			
<input type="checkbox"/>	Pos Ion Spray Voltage (V)	4100	4076
<input checked="" type="checkbox"/>	Sheath Gas (Arb)	9	9.51
<input type="checkbox"/>	Aux Gas (Arb)	0	4.41
<input type="checkbox"/>	Sweep Gas (Arb)	0	0.00
OPTIONS			
	Start Value	0	
	Stop Value	15	
	Step Size	3	
	Signal Type	TIC	
Select Accept or Reject to continue. User clicked Accept. Optimization In Progress...			
000			
Abort			

During the optimization, the Tune application displays the progress. While the optimization procedure is running, the upper right graph window plots Intensity RSD values per optimizing condition (10 scans).

After the optimization procedure is finished, the upper left graph window displays a smoothed optimization curve, the optimized parameter value in the legend box, and highlights this value with a vertical line in the graph.

You can abort the optimization. When the optimization completes, you can select to generate a report. Then, an Ion Source Optimization Report is generated and presented to you.

Procedure

1. On the Ion Source - Optimization page, select the parameter that you want to optimize (for example, start value, stop value, step size, and signal type options).
2. To enter the value of a parameter, select the arrow in the spin box to increase [up arrow] or to decrease [down arrow] the value. Or, enter the value in the spin box text field.
3. If applicable, select the required option in the list box.
4. Select **Optimize**.

Parameters for the ion source - Optimization page

Parameter	Description
Spray Voltage (V)	(H-ESI mode) Optimizes the source electrospray voltage (absolute value, in volts).
Sheath Gas (Arbitrary)	(H-ESI and APCI modes) Optimizes the sheath gas flow rate (in arbitrary units).
Aux Gas (Arbitrary)	(H-ESI and APCI modes) Optimizes the auxiliary gas flow rate (in arbitrary units).
Sweep Gas (Arbitrary)	Optimizes the sweep gas flow rate (in arbitrary units).
Discharge Current (μA)	(APCI mode) Optimizes the APCI source discharge current (in microamperes).
Options	
Start Value	The initial value of the parameter being optimized.
Stop Value	The final value of the parameter being optimized.
Step Size	The step size of the parameter being optimized.
Signal Type	<ul style="list-style-type: none"> • TIC (default): Maximizes the total ion current (TIC) signal. • Base Peak: Maximizes the base peak signal. • m/z: Maximizes the signal of the ion with the m/z value that you specify in the m/z box.
m/z	(Signal Type: m/z)

Parameter	Description
	The m/z of the ion to optimize on.
Buttons	
Optimize	Starts the optimization based on the ion source parameters.
Abort	Stops the optimization based on the ion source parameters.
Accept	Accepts the optimized ion source parameter.
Reject	Rejects the optimized ion source parameter.

Set up the mass spectrometer for data acquisition

Set up the mass spectrometer for data acquisition by using the Define Scan pane. For the mass table, you can import the settings from (or export them to) a CSV, TXT, or XML file.

Specify the scan type and the precursor settings

To specify a scan type and the precursor settings, select from the following parameters.

Parameter	Description
Scan Type	<ul style="list-style-type: none"> Full Scan: One stage of mass analysis. EASY-IC™ Calibrant: Displays the reagent ion spectrum for m/z 202 for the internal EASY-IC™ ion source. <p>IMPORTANT: When you select EASY-IC™ Calibrant, the isolation width and all other m/z scan parameters are automatically set to allow for the detection of the fluoranthene reference mass.</p>
Orbitrap Resolution	<p>The mass resolution of the Orbitrap analyzer, which is proportional to $1/\sqrt{m/z}$. Mass resolution is defined as the observed m/z value divided by the smallest difference $\Delta m/z$ for two ions that can be separated: $m/z / \Delta m/z$. Spectra acquired at higher Orbitrap Resolution allow greater resolution in m/z but take longer to acquire. The selected resolution is specified for a peak at m/z 200.</p> <p>The available options are: 15 000, 22 500, 30 000, 45 000, 60 000, and 90 000.</p>
Scan Range (m/z)	<p>(Scan Range Mode: Define m/z Range)</p> <p>The range (in m/z) over which the mass analyzer detects peaks. Range: 40–1500; default: 70–1000</p> <p>TIP: Because the system optimizes the transfer of ions for the defined mass range, we recommend that you restrict the scan range to the region of interest.</p>


Parameter	Description
RF Lens (%)	<p>Specify the value to control the RF amplitude applied to the S-lens. This value is a scaling factor applied to the nominal mass-to-voltage relationship.</p> <p>Range: 0–200; default: 70</p> <p>Decreasing the RF level decreases the transmission of high m/z ions through the S-lens, increases the transmission of the low m/z ions through the S-lens, and potentially decreases the amount of fragmentation of fragile ions in the S-lens. Increasing the RF level has the opposite effects.</p> <p>TIP: For stable ions, use the default value as a starting point. A fine tuning of the MS is recommended, however.</p>
AGC Target	<p>Indicates Standard (default) or Custom for the Automatic Gain Control™ (AGC) target value, which controls the number of ions that are injected into the mass analyzer.</p> <ul style="list-style-type: none"> Standard (default): The system sets the recommended target (good starting point) in an automated fashion per scan type. Custom: You can set the AGC Target in percent.
Normalized AGC Target (%)	<p>(AGC Target: Custom)</p> <p>Specifies the Automatic Gain Control (AGC) target. This is a percentage representing the maximum number of charges to accumulate for a given analysis.</p> <p>Range: 1–1000; default: 100</p> <p>This is the normalized AGC Target value, it is represented as a percentage to aid in calculating the desired AGC Target. The base normalized value is different for scan types. The values are as follows:</p> <p>Scan Type = Full Scan; Normalized Base (100%), IRM = 1e6</p> <p>The IRM fills with ions until it reaches the AGC Target or the maximum injection time. The MS then transfers the ions to the Orbitrap analyzer.</p>
Maximum Injection Time Mode	<p>(Infusion Mode: Liquid Chromatography)</p> <p>Specifies the maximum injection time (max IT) that is allowed to reach the AGC Target. The IRM collects ions until it reaches the AGC Target or the max IT. The mass spectrometer then transfers the ions to the Orbitrap analyzer. Some scan types allow multiplexing. When multiplexing is used, the AGC Target is applied for each target individually.</p> <ul style="list-style-type: none"> Auto: The system calculates the maximum injection time available (according to the transient length of the previous scan) to balance between sensitivity and scan speed (in

Parameter	Description
	<p>parallel acquisition). In data dependent experiments, the maximum injection time for the first MS² is set according to the transient length of the master scan. Therefore, the first MS² scan following the master scan (typically) may receive a longer injection time than the subsequent MS² in the same cycle.</p> <ul style="list-style-type: none"> • Custom: You can define the maximum injection time for the scan type. In contrast to “Auto,” in data dependent MS² scans the maximum injection time will be the same for each scan. <p>When using “Custom,” you can extend the max. IT beyond the transient length of the Orbitrap scan. Please be aware that the instrument may slow down if the total max. IT is utilized. When extending the max. IT to double the transient length (for example, 55 ms for 15k resolution), you should consider using a higher resolution (in this example 30k) because this also increases the S/N by a factor of ca. 1.4. Longer max. ITs may lead to better spectral quality in MS² scans in data dependent scans.</p> <p>In targeted (quantitative) experiments, longer injection times (when allowed by longer max ITs) may lead to higher sensitivity.</p>
Maximum Injection Time (ms)	<p>(Maximum Injection Time Mode: Custom)</p> <p>Maximum time in milliseconds that is allowed to accumulate ions in the IRM until the AGC Target is reached.</p> <p>Range: 1–1000; default: 100</p>
Microscans	<p>Number of scans to average in a given spectrum.</p> <p>Range: 1–10; default: 1</p> <p>A microscan is one ion injection followed by ion detection. The MS sums microscans to produce one scan, which improves the signal-to-noise ratio of the mass spectral data.</p> <p>TIP: The overall scan time increases linearly with the number of microscans, significantly slowing the rate of spectral acquisition.</p>
Source Fragmentation	<p>Select the checkbox to switch on ion source CID.</p> <p>An offset voltage in the ion source accelerates the ions into the background gas. Collisions with the background gas might help in the desolvation of the ions and might increase sensitivity.</p>
Energy (V)	<p>(Source Fragmentation: On)</p> <p>The collision energy (in electron volts) for ion source fragmentation.</p> <p>Range: 1–135; default: 35</p>

Parameter	Description
	If you set the source fragmentation too high, fragmentation of ions might occur.
Use EASY-IC™	(Lock Mass Correction: EASY-IC™, Mode ≠ RunStart) If On is selected, it provides an internal reference mass that is used for mass correction during a run.
FAIMS Voltages	(Ion Source Type: NSI, H-ESI, APCI, ESI, OptiSpray; with FAIMS Mode enabled) Switches On (default) or Off the FAIMS voltages.
FAIMS CV (V)	(FAIMS Voltages: On) The optimized FAIMS compensation voltage. Range: -300 to 300; default: 0 The observed CV values on the MS are aligned with those of the other mass spectrometer platforms. This means the transmission for one m/z should be the same for the same CV setting on all platforms. For targeted CV experiments, it is recommended to use the value that was determined by a CV scan (performed in the Tune window) or optimized with single CV experiments in an HPLC run.

Acquire a data file with the Tune application

Procedure

1. Open the Data Acquisition pane, and then do the following:
2. (Optional) To change the destination folder for the raw data, select the  button.

The default folder location is in C:\Thermo\Data.


3. In the File Name box, type the name of the analyte (for example, reserpine).

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

4. In the Sample Name box, type the name of the analyte (or another suitable label).
5. In the Comment box, type a comment about the experiment.

For example, describe the ionization mode, scan type, scan rate, sample amount, or method of sample introduction. The data system includes the comment in the header information for the raw data file.

6. Under Timed Acquisition, select the **Continuously** option (acquires data until you stop the acquisition).
7. Select **Record** to start data acquisition.

After the Tune parameters reach their specified settings, the data acquisition process begins and the small circle on the Record button changes to red (.

8. When you are finished, select **Record** again to stop the acquisition.

The small circle on the Record button changes to gray (not recording).

Tune, calibrate, or check the mass spectrometer calibration

You can tune, calibrate, or check the mass spectrometer calibration with the special calibrant probe (Ion Source Type: ESI).

Use the FlexMix™ calibration solution (Pierce™ P/N A39239) to calibrate the Orbitrap Exploris EFOX instrument:

- It is designed for both positive and negative mass calibration in the range from m/z 40–3000.
- It is stable at room temperature. Do not expose it to light or heat.

IMPORTANT

After a bakeout, the system must be re-calibrated. The instrument recognizes that it has been vented and adds certain calibrations steps to the system calibration that only need to be run after a bakeout.

NOTE

You can set a few preferences for how the Tune application works. Select



the Options icon, , and then select **Preferences**.

If a System Calibration fails, restarting the calibration applies the values from the previously successful sequence and continues from that previous point.

Calibration procedures

The Calibration pane shows the last time you calibrated the mass spectrometer. Generally, you should calibrate the mass spectrometer every month of operation for optimum performance over the entire mass range.

NOTE

We recommend to use the calibrant probe (Ion Source Type: ESI) for all calibrations. In the course of the ion source optimization, monitor the Spray Current in the Status pane (Ion Source > Spray Current). Make sure that the Spray Current is not higher than 0.5 μA in positive mode and not higher than 0.3 μA in negative mode.

IMPORTANT

While running the calibration procedures, you cannot run methods in the Xcalibur data system.

Calibration parameters are instrument parameters that affect the efficiency, sensitivity, mass accuracy, and resolution of the ion accumulation and detection process.

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

- Mass-dependent—Affects the RF voltage of the ion funnel and the DC offset voltages of the lenses and multipoles. Use the Calibration Options pane to optimize the mass-dependent tune parameters.
- Compound-dependent—Affects the ion source parameters, such as the spray voltage or spray current, and the sheath, auxiliary, and sweep gas flow rates. See [Optimize the ion source parameters](#) to optimize the compound-dependent tune parameters.

The Calibration pane has the following parameters:

Parameter	Description
Status	
Polarity	Select the polarity for which you want to see the calibration data.
Recommended Calibrations	This is the recommended date for recalibration. The recommendations are based on the dates of the last successful calibrations and predetermined time intervals. Dates displayed in bold and italic font indicate that the recommended date has been exceeded. If the calibration failed, the recommended date is the current date.
Last Successful Calibration	This is the date of the last successful calibration.

Parameter	Description
Calibration	
During the calibration, the Tune application displays event messages in the gray window:	
<ul style="list-style-type: none"> Started/finished subprocedures with results Reached/resumed checkpoints Calibration result 	
The system reports all results in the gray window. The result is either “pass” or “failed.” If the result is “failed,” the system displays a recommended action based on the failure type.	
Mode	<ul style="list-style-type: none"> Calibrate The system runs all selected mass calibration or system calibration procedures with selected polarity in the correct order. Check The system runs all selected mass check or system check procedures with the selected polarity.
IMPORTANT: The check procedures do not update the recommended date for next full system calibration or update the calibration parameters.	
Polarity	Select the polarity for which you want to perform the calibration or check.
Type	<ul style="list-style-type: none"> Mass: Uses the masses of a stable FlexMix spray to calibrate or check the masses. Mass & System: Performs a whole system calibration or check. This calibration/check requires FlexMix solution. One-Point Mass: Uses the internal calibration source with fluoranthene to calibrate the mass with one point. This calibration type can be performed anytime between runs without disconnecting the HPLC. You may subsequently evaluate the mass range for your analytes, for example with a system suitability test. IMPORTANT: This option is available only for calibration and not for checking. The one-point mass calibration runs in both polarities—starting with the negative ion mode. Customized Mass: Uses external masses from a list for the calibration. Select Load user-defined mass lists to get access to a directory where you can <ul style="list-style-type: none"> recall/load available mass list files,

Parameter	Description
	<ul style="list-style-type: none"> – save new mass list files, – save updated mass list files, – delete user-defined mass list files. To do so, delete the mass list file manually in the Windows Explorer. <p>You can select provided mass lists and user-defined mass lists. A mass list can contain both positive and negative m/z values.</p> <p>For each polarity, you can specify up to ten theoretical masses. The Tune application shows only the reference masses whose polarity matches the selected polarity. If you want to use a mass in a list, select the corresponding checkbox.</p> <ul style="list-style-type: none"> • IC Source: Performs a calibration of the internal calibration source. <hr/> <p>IMPORTANT: One-Point Mass calibration and IC Source calibration are available only when the Internal Calibration (EASY-IC) Source checkbox is selected in the Thermo Foundation™ Instrument Configuration window.</p>

Instructions

This field displays appropriate notifications about various routine states, for example spray stability, calibration mixture evaluation, contamination, or degradation. It gives recommendations about next steps to solve issues.

Parameter	Description
	<div style="border: 1px solid #ccc; padding: 10px;"> <p style="text-align: center; margin: 0;">Instructions</p> <p>Spray is unstable. Please perform following tasks and retry:</p> <ul style="list-style-type: none"> • Adjust HESI probe position (typically near position 1 with syringe flow rate at 3-5 $\mu\text{l}/\text{min}$) • Adjust the HESI needle height • Optimize spray voltage (starting with 3.5 kV; increasing or decreasing it while keeping spray current $\leq 0.5 \mu\text{A}$) • Change sheath and aux gas settings (typically 3 or lower, no sweep gas, with syringe flow rate at 3-5 $\mu\text{l}/\text{min}$) • If there are repeated spikes in the spray stability graph <ul style="list-style-type: none"> • Check if it is hard to push the syringe, if so, back flush the tubing and HESI needle insert with methanol • Snip off a small piece at both ends of the PEEK tubing • Inspect the tip of the needle insert (preferably under a microscope) for dirt or damage. Replace the HESI needle insert if necessary </div>

IMPORTANT

To minimize the possibility of cross-contamination, do not use the same syringe and PEEK tubing for the calibration and the sample measurement. Before you start using your analyte, flush the inlet components again.

After the calibration, you see either a green check (✓) adjacent to the calibration name to indicate a successful calibration or a red X mark (✗) to indicate a failed calibration. A date appears in the Last Calibrated column for each successful calibration test. A date does not appear for failed calibrations.

Calibration checkpoints

When a calibration fails, a regular report is generated. When you start the calibration again, the instrument re-applies the calibration values from the parts of the sequence that already succeeded, and then continues/retries from the point where it failed. Checkpoints from where the sequence can resume are valid for one day or until instrument reset. After four unsuccessful restarts of the calibration, the calibration starts from the beginning.


After each calibration, you can choose to generate a report. A report generated after the finished calibration contains all successful parts of the entire calibration sequence, even those that came before the checkpoint.

IMPORTANT

An unsuccessful or partially successful sequence does not change the instrument calibration. The calibration results are stored only when the whole system calibration has passed.

Use the panes at the right side

Four panes on the right of the Tune application window display information about instrument status, history and favorite settings. To expand or collapse

the panes, select the   buttons.

Favorites pane

The parameter panel of the Favorites pane shows the active ion source and several scan parameters.

The System Settings pane provides default Ion Source and Define Scan settings for the ESI source recommended for calibrating the MS.

The User Settings pane allows you to save user-defined Ion Source and Define Scan settings.

NOTE

You cannot rename or delete the System Settings.

Save a favorite state

You can save the current key parameters of the mass spectrometer by using the Favorites pane. A validation procedure checks if the Ion Source settings and the Define Scan settings were applied before saving these parameters as Favorite settings. If this condition is not fulfilled, an error message appears that reminds you to apply the active settings.

Procedure

1. Modify parameters in one of the Ion Sources or Define Scan panes.
2. Select **Apply**.
3. Select the Favorites tab to display the Favorites pane.
4. In the Favorites pane, select **Save Current State**.

5. Type a unique name in the box that appears, and then select **Save Current State** .

Result

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

Load a favorite state

You can load the current or previously saved key mass spectrometer parameters by using the Favorites pane. When you *load* a favorite state, its key parameters appear in the Ion Source and Define Scan panes, but the Tune application does not automatically submit them to the mass spectrometer. Changed properties are shown in bold in the parameter panel.

Procedure

- Select the name in the Favorites list, or right-click it and select **Load**.

Result

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

Apply a favorite state

You can apply previously saved key mass spectrometer parameters by using the Favorites pane. When you *apply* a favorite state, the Tune application submits the parameters to the mass spectrometer and also displays them in the parameters panel.

Procedure

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

Delete a favorite state

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

Procedure

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

Rename a favorite state

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

Procedure

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

Status pane and Control pane

Use the Status pane and the Control pane to monitor the working condition of your instrument.

View the Status pane

The Status pane displays real-time status information in a tree view for many mass spectrometer parameters and components. The status readback indicators update every few seconds and are as follows:

- Green square (■)—Indicates that the parameter is within its expected range
- Orange square (■)—Indicates that the parameter is trending toward the expected range
- Red square (■)—Indicates that the parameter is outside of its expected range

Figure 7 Status pane—By Function

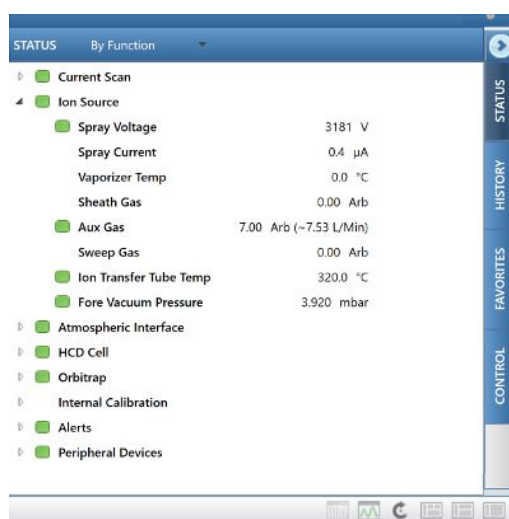
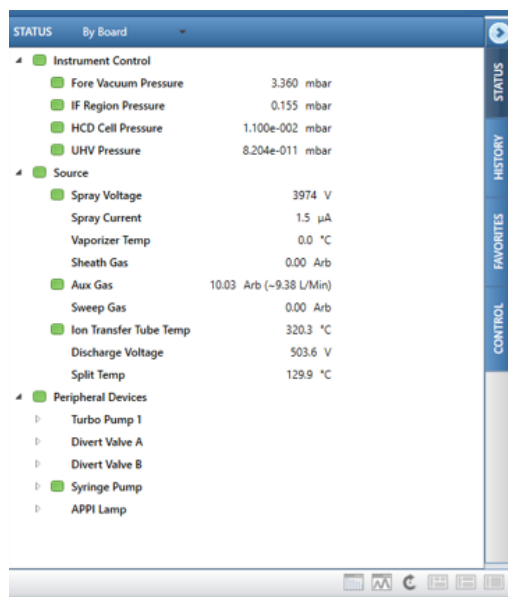


Figure 8 Status pane—By Board



View the Status pane options

Procedure

1. Select the **Status** button at the right side of the Tune window.
2. In the header of the Status pane, select the downward arrow, and then select **By Function** (default) or **By Board**.
3. Select the arrow next to the category that you want to expand.

View the Control pane

The Control pane displays real-time status information in a tree view for many mass spectrometer parameters and components. The status readback indicators update every few seconds.

Procedure

1. Select the **Control** button at the right side of the Tune window.
2. Select the symbol next to the category that you want to expand.

History pane

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

When you select a given time, the pane indicates what changes were made (shown in bold black) compared to current Ions Source settings and Define Scan Settings.

By right-clicking, you can either apply the settings directly or just load the settings into the parameter panel where you can then make further changes and apply them. By double-clicking on the change record, settings are directly applied into the parameter panel for further changes.

View or modify a change record

Procedure

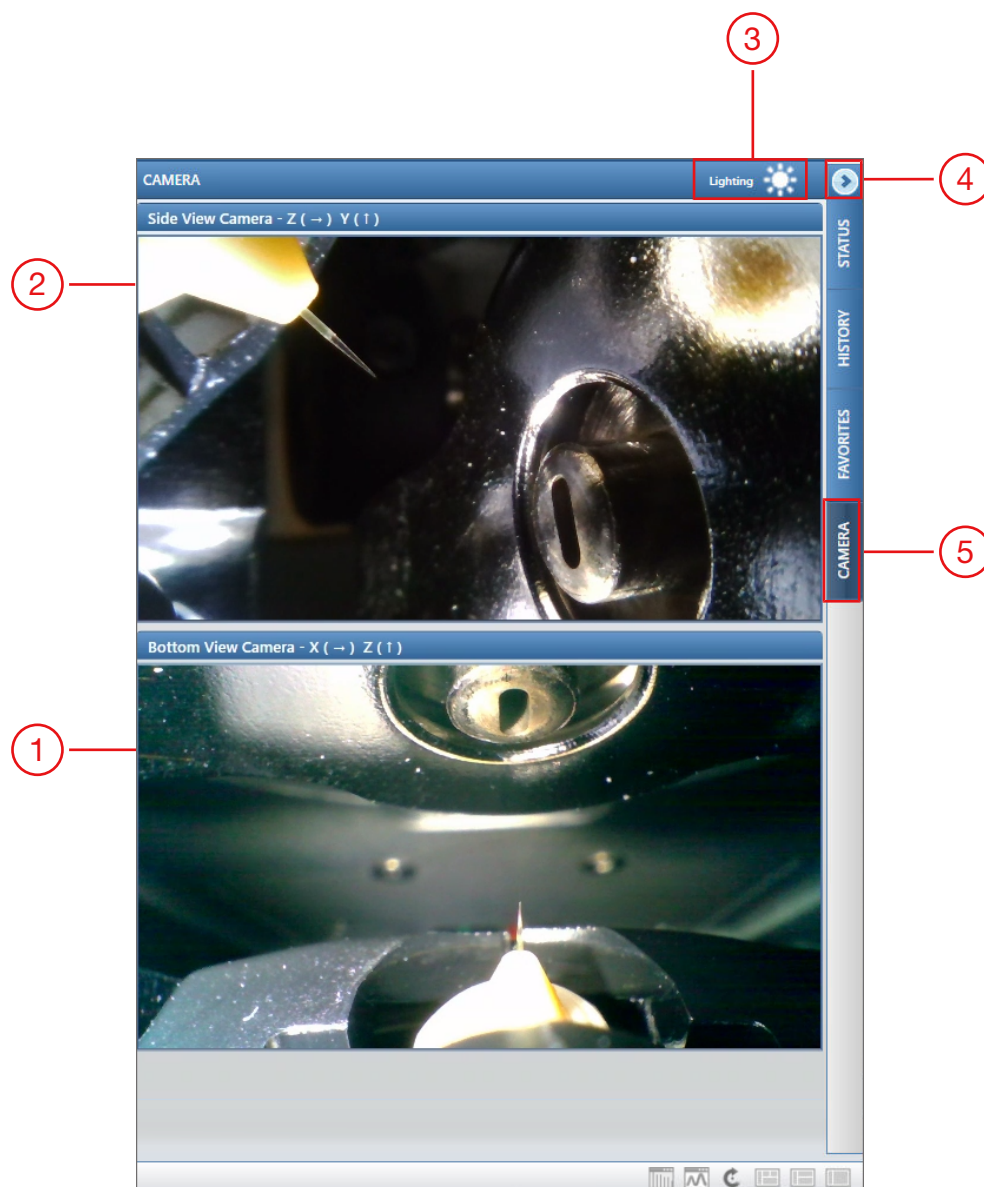
1. Select from the following:
 - Select a change record to display its parameters, or right-click it and select **Load**. Parameters shown in bold differ from their default values.
 - Double-click a change record, and then select **Apply** in the Ion Source pane or Define Scan pane to submit its parameters to the mass spectrometer. You can also right-click the change record and select **Apply** in the shortcut menu.

Camera pane

The Camera pane is available in the Tune application whenever the OptiSpray ion source is installed and powered on. There are two camera views that allow you to view the emitter from the bottom and the side, as well as two lighting modes for visualizing the emitter and the spray. These lighting modes function when the mass spectrometer is in the On or Standby mode.

The following figure shows the camera controls.

Figure 9 Camera pane







No.	Description	No.	Description
1	Bottom-view of the emitter	4	Expand/collapse the Camera pane
2	Side-view of the emitter	5	Access the camera views
3	Change between camera lighting modes		

Procedure

- Perform any of the procedures in the following table to control the OptiSpray camera.

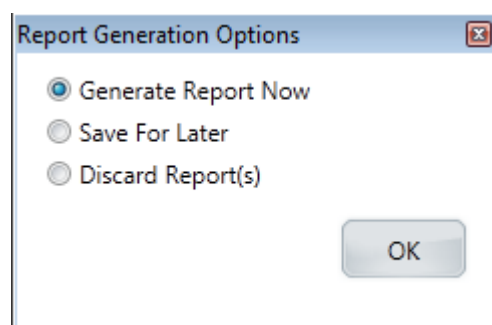
Table 2 Tasks for controlling the OptiSpray camera

Task	Procedure
Open the camera pane	From the right side of the Tune window, select Camera .
Adjust the lighting mode	From the header bar of the Camera pane, select Lighting to change the lighting mode to one of the following:  : Emitter visualization lighting (white) is active  : Spray visualization lighting (green) is active
Expand the camera window	Select  .
Collapse the camera window	Select  . The camera lighting turns off.
Resize the camera window	Select and hold the left-side corner or the middle bar of the camera window, then drag to the size you want.

Generate reports

You can generate a report after running the procedures on the Calibration and Diagnostics panes. Depending on the settings in the Tune Preferences dialog box, no report is created, the report is created automatically, or the Report Generation Options dialog box is displayed.

Figure 10 Report Generation Options dialog box



Select one of the following options:

- **Generate Report Now:** The PDF report is generated and saved in the following default folder: C:\Thermo\Instruments\Reports.
- **Save For Later:** You can specify a folder to save the PDF report.
- **Discard Report(s):** No PDF report is saved.

The PDF report has the following content:

- The title distinguishes between Calibration Reports and Calibration Check Reports.
- The header contains Date & Time, Instrument Model name, Instrument Serial, and Software Version.

- The PDF report comprises a section header for every (sub-)calibration run.
- The table summarizes all calibration results or calibration check results:
 - The column “Name” includes name of Calibration; sub-steps are indented. A unit is shown if required.
 - The column “Result” comprises test result of a calibration/check shown as “Passed” highlighted in green or “Failed” highlighted in red. Sub-step results are shown as “Passed” or “Failed,” too.
 - The columns “Value”, “Minimum” and “Maximum” are restricted to calibration/check values. If no relevant value is available, the field will be filled with “-”.
 - The column “Comment” comprises further important information about passed results or reasons for failure.
- The mid section summarizes calibration/check plots and additional information for each Calibration/Check procedure; each procedure is highlighted in bold letters and a larger font.
- The PDF report is finalized by a general “System Configuration” report containing graphs of some of the sub-procedures.

Procedure

1. In the Report Generation Options dialog box, select **Generate Report Now** and select **OK**.

NOTE

If you save the report for later, a Report button appears next to the Start button until you view the report. You cannot run methods in the Xcalibur data system until you view or cancel the saved report.

2. Save the report as a PDF file.

Result

After completing the procedures, the Tune application prompts you to generate a report with the results.

Specify options for the Tune application

The Options menu of the Tune application has the following commands:

Command	Description
Preferences	Displays the Tune Preferences dialog box
Load standard diagnostics	Displays the Diagnostics pane (left pane).

Command	Description
View calibration reports	Opens the folder that contains the calibration reports.
View other reports	Opens the folder that contains the other reports.
View Instrument	Displays a window that shows a schematic drawing of the instrument ion optics and analyzer.
Tune Help	Displays the Tune Help.
Instrument Manuals	Opens the folder that contains the instrument manuals that are installed with the Tune application.
Instrument Web page	Displays the website of the instrument.
About Tune	Displays the About dialog box with information about the instrument, the current Tune version, and the active licenses.

Procedure

- Select the Options icon, .

Set preferences for the Tune application

Use the Tune Preferences dialog box to specify the behavior of the Tune application.

The Tune Preferences dialog box has the following parameters:


Parameter	Description
General	<ul style="list-style-type: none"> • Clear Calibration checkboxes when complete • Clear Diagnostics checkboxes when complete • Enable Hotlink for Define Scan and Ion Source Panel

Parameter	Description
Report Options	<p>Select the Hotlink checkbox to allow the Tune application to send any changes immediately to the instrument.</p> <p>You can select different options for calibration reports and other reports:</p> <ul style="list-style-type: none"> • Automatically generate reports • To change the destination folder for the reports, select the Browse button. The default folder location is in C:\ProgramData\Thermo\Exploris\Instrument\Reports\. • Show Report Generation Options dialog box • Do not generate reports
Report Content Options	<ul style="list-style-type: none"> • Show Console • Show graph • Show spectrum • Show system configuration • Show embedded system configuration
Alerts Console Options	<p>Use these parameters to specify what is displayed in the alerts console. The log files are stored in C:\ProgramData\Thermo\Exploris\Log.</p> <ul style="list-style-type: none"> • Show warnings • Show recovered errors and warnings • Show information • Minutes included in the log file before error detected • Minutes included in the log file after error detected
Mass Self-Calibration Options	<p>Select Run One-Point Mass Self-Calibration to enable the periodic unattended calibration procedure. Use this procedure to ensure that the mass accuracy remains in the specifications for at least four weeks (when run daily).</p> <p>Use the parameters of the Schedule Self-Calibration area to specify the interval or the weekdays and the hours for the self-calibration.</p> <p>Whether the instrument actually performs the self-calibration depends on its power mode:</p> <ul style="list-style-type: none"> • Instrument in Standby: procedure runs • Instrument On without raw data acquisition: procedure runs

Parameter	Description
	<ul style="list-style-type: none"> Instrument On with raw data acquisition: procedure remains on hold. If a sequence is active, the procedure runs when the sequence is finished. If multiple sequences (batches) are queued, the procedure runs between the active sequence and the next sequence in the queue. The next sequence will recommence when the calibration has passed. Instrument Off: procedure is inactive <p>The procedure generates a PDF report and stores the file on the data system computer. When the instrument passes the self-calibration, the system updates the master.cal file and the mass calibration performance status on the Calibration Status panel.</p> <p>IMPORTANT: This parameter is available only when the Internal Calibration (EASY-IC) Source checkbox is selected in the Thermo Foundation Instrument Configuration window.</p>

Display the Tune Preferences dialog box

Procedure

1. Select the Options icon, .
2. Select **Preferences**.

Use the alerts console


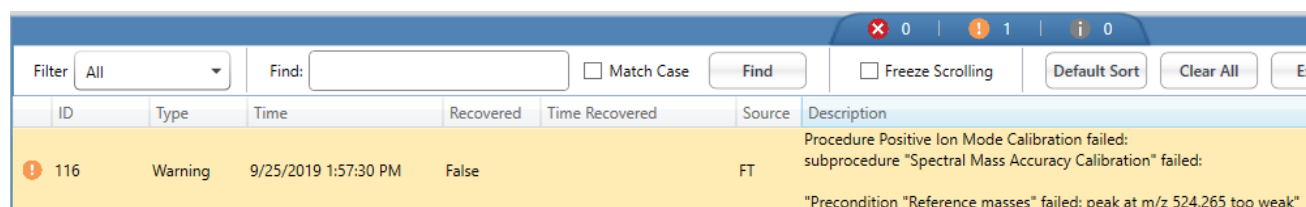
The alerts tab appears at the bottom of the screen only when the Tune application generates an alert message (for example, , which indicates one [1] error). The application shows one or more icons that indicate the types of messages. You can filter the messages by type and sort their order by selecting any of the column headings.

Figure 11 Alerts console







ID	Type	Time	Recovered	Time Recovered	Source	Description
116	Warning	9/25/2019 1:57:30 PM	False		FT	Procedure Positive Ion Mode Calibration failed: subprocedure "Spectral Mass Accuracy Calibration" failed: "Precondition "Reference masses" failed: peak at m/z 524.265 too weak"

Open or close the alerts console


Procedure

- When the alerts tab appears at the bottom of the screen, select it.

Table 3 Parameters in the alerts console

Parameter	Description
Alert type icons	<ul style="list-style-type: none">  Errors  Warnings  Recovered errors and warnings  Information <p>The number after the icon indicates the total number of messages for this type.</p>
Filter	Select the type of message to show.
Find	Searches the displayed messages by entering one or more keywords. To search all messages, set the Filter to All.
Match case	Makes the search case sensitive.
Freeze Scrolling	Prevents the list of messages from scrolling as new messages appear.
Buttons	
Default Sort	Resets the order of the alert types to show the errors first, then the warnings, and then the remaining messages sorted by time.
Clear All	Removes all of the messages, except for the active error and warning messages.
Export	Exports all alert messages to a CSV file.

About dialog box


Use the About dialog box to display information about the instrument, the current Tune version, and the active licenses. To copy the instrument identification to the clipboard, select the  button.

The About dialog box has the following parameters:

Parameter	Description
End User License Agreement	Display various dialog boxes with legal information and information about third party licenses used by the Tune application.
Add license	Displays the License dialog box.
Close	Saves your changes and closes the dialog box.

Display the About dialog box


Procedure

1. Select the Options icon, .
2. Select **About Tune**.


Run diagnostics

Only Thermo Fisher Scientific field service engineers can run the complete diagnostics on the mass spectrometer subsystems. As a customer, your access to the diagnostics is limited.

TIP

You can set a few preferences for how the Tune application works. Select the Options icon, , and then select **Preferences**.

Procedure

1. Select the Diagnostics icon, , at the bottom of the Tune window.
2. Select the arrow next to the category that you want to expand.
3. Select the components that you want to test.
4. If necessary, enter new values for the selected parameters.
5. Select **Start**.

Category	Description
The descriptions for the individual parameters are built into the application. To see a tooltip description, point to the parameter name, and for additional information, select Learn More.	
System	A manual bake-out can be started, different diagnostic checks can be performed. Also, you can evaluate the purity of the FlexMix calibration solution and burn in the internal calibration source.
Mass Calibration	
Partial Calibration	When you know that certain subsystems might be out of specs, you can calibrate the healthy subsystems from here.
Optional Calibration	Procedures that provide additional calibrations to prepare the instrument for specific applications.

Category	Description
	<p>For example, a dedicated low m/z mass calibration can be performed. It improves the mass accuracy for low m/z applications. However, the standard m/z mass accuracy (150–2000 m/z) may shift in a sub-ppm level after running this procedure.</p>
FAIMS	<p>Contains procedures for the FAIMS source performance.</p> <p>The checkboxes in this area are available only when the system has detected an FAIMS source.</p>
OptiSpray	<p>(Available when the OptiSpray ion source is installed.)</p> <p>Evaluates the hardware interlocks, internal electrical boards, and the motor function of the OptiSpray ion source. It also tests the heater, sheath gas, and spray current of the OptiSpray cartridges.</p>
Tools	<p>The Tools section contains tools for different functions. Not all are described here.</p>
Calibration > Skip Spray Stability Evaluation for Mass Calibration	<p>This function allows to skip the spray stability check, which is run before the mass calibration. This allows to run the mass calibration with a less stable spray, for example when a mass calibration needs to be performed with the HESI sprayer.</p> <p>However, we strongly recommend the low-flow setup with the calibrant sprayer.</p> <p>Generally, the spray stability evaluation generates real-time graphs of the TIC and of the relative standard deviation (RSD) of the TIC. The %RSD must be less than 15% for the spray stability evaluation to pass. For the system calibration, the %RSD should be less than 10%.</p> <p>TIP: If the spray stability test fails, see Optimize the ion source parameters. For instructions, refer to the instrument manual.</p>
Calibration > Change Mass Calibration Due Time	<p>By default, the recommended calibration time is 25 hours after the last successful mass calibration. Here, the due time setting (“Recommended Date”) for the next mass calibration can be customized. You may consider a longer time period between two succeeding mass calibrations.</p> <p>Mass accuracy is ≤ 3 ppm within 25 hours (specification). Please evaluate any change in the due time setting. A change in the due time will adjust the recommended date and thus the time point when a warning message appears in the resulting raw data files.</p> <p>Procedure</p> <ol style="list-style-type: none"> 1. In the Diagnostics pane, select Change Mass Calibration Due Time.

Category	Description
	<ol style="list-style-type: none"> 2. In the table below, the parameter value becomes visible and shows the current setting. 3. Enter a new validity period (for example, 72—for 3 days). 4. Select Start to store the new setting. <p>Result</p> <p>A green check will appear next to the Parameter in the tree.</p>
Define Scan > Disable AGC mode	<p>Per default, the MS is running with the Automatic Gain Control (AGC) switched on, that is the ion flux from the ion source is constantly monitored and a forecast of the ions to come is made. The forecast can be erroneous in case of a non-continuous ion-flux ion source, such as a MALDI source. Therefore, you can select between AGC on or off:</p> <ul style="list-style-type: none"> • 1: Prescan (default, electrometer is enabled, AGC is on) • 0: Fixed (electrometer is switched off, AGC is off). The MS will always use the maximum injection time set in the Tune application. <p>The settings are applied by selecting the Start button.</p>
Define Scan > Set Multi RF Injection Threshold Ratio	<p>This parameters allows to widen the mass range injected through the ion funnel (interface) by one single injection. The factor describes the mass range width (between first mass and last mass) that is transferred through the interface by a single injection. With a factor of 4 (default), this allows to transfer from m/z 100 to m/z 400 or from m/z 250 to m/z 1000, for example.</p> <p>Range: 1.2–1000</p> <p>You can access a wider mass range with one single injection by entering a larger value. For example, type “6” and press <Enter>. You can now access from m/z 250 to m/z 1500 (factor of 6 from first mass setting) with one injection, for example.</p> <p>The settings are applied by selecting the Start button.</p> <p>TIP: The system is optimized for a factor of 4—and it s a reasonable value when using MALDI in non-imaging applications. Keep in mind that the change of the factor to larger values will (usually) sacrifice ion transfer through the interface, particularly for the higher m/z values in the interrogated mass ranges transferred by this factor.</p>
Define Scan > External Handshake	<p>Three parameters are available for the external handshake. You can set specific values in the Parameter Value column.</p> <ul style="list-style-type: none"> • Scan Handshake mode <ul style="list-style-type: none"> 0: Off (default)

Category	Description
	<p>1: Handshake</p> <p>2: Handshake (MS only)</p> <p>Select Handshake to insert a hardware handshake mechanism between scans. In this mode, trigger lines are used to establish a mechanism of scan synchronization with an external device. There is a Ready output signal from the MS and a Trigger input signal to the MS. The external device can start an MS scan (acquisition of a single spectrum) self-timed, but it needs to adhere to the Ready output signal coming from the MS, to not overrun the mass spectrometer timing.</p> <ul style="list-style-type: none"> • Handshake N-th Counter Input range: 1–1000000, default 1 This parameter is available only when Scan Handshake mode is active. A set value of greater than 1 will insert a handshake only every N-th scan. A set value of, for example, 10 will enable for 10 scans to be subsequently acquired for a single trigger input. The trigger input needs to be set for the first scan of a series only. The other scans (in this example, 9 scans) will also run in absence of the trigger input. This is applied in a continuous MS application like DESI or MALDI in a line scan. • Handshake min. Trigger Delay (msec) Input range: 0–500, default 10 This parameter is available only when Scan Handshake mode is active. It provides an option to reduce the jitter between an external trigger input and the actual injection of ions for the MS scan, in case exact timing between the external trigger input and the actual ion injection event is needed. <p>The settings are applied by selecting the Start button.</p>
Define Scan > Diagnostic Trigger out	<p>To synchronize external events (for example, laser pulsing) with scan events that are not individually triggered by a trigger input signal, various states of a scan event can be reported to the external device.</p> <ul style="list-style-type: none"> • 0: Off (default) • 1: Inject Event • 2: HCD Event; syncs with HCD trapping time, the contact is closed when ions are in the HCD cell • 3: Acquisition

Category	Description
	<ul style="list-style-type: none"><li data-bbox="608 297 1442 488">• 4: PreTrigger; a contact closure is registered on the peripheral panel Digital Out 1B. The pretrigger signal can be measured with an oscilloscope using a circuit with a pull up resistor. The cycle duration of the measured signals fits to the cycle time of the chosen resolution. <p data-bbox="587 499 1442 651">For a typical MALDI application, select the value “1” to synchronize laser pulse, desorption/ionization, and ion injection. This output signal can be especially helpful in combination with a Handshake N-th Counter selected larger than 1.</p> <p data-bbox="587 663 1442 698">The settings are applied by selecting the Start button.</p>

Method Editor application

With the Method Editor application, you create an MS instrument method (combined with the optional autosampler and liquid chromatograph instrument methods) by defining the experiment type and setting various parameters. These include settings for the MS, syringe pump, and divert valve; and the mass ranges and fragmentation transitions for the experiments.

NOTE

This topic describes the parameters of the Method Editor standalone application. Additional parameters are displayed when you start the Instrument Setup application from within the Xcalibur™ mass spectrometry data system.

TIP

The parameter descriptions for the scan types are built into the application. To see a tooltip description, point to the parameter name, and for additional information, select **Learn More**.

Contents

- [Menus](#)
- [Global system parameters](#)
- [Method Editor overview](#)
- [Qualitative view](#)
- [Use the experiment templates](#)
- [Summary page](#)

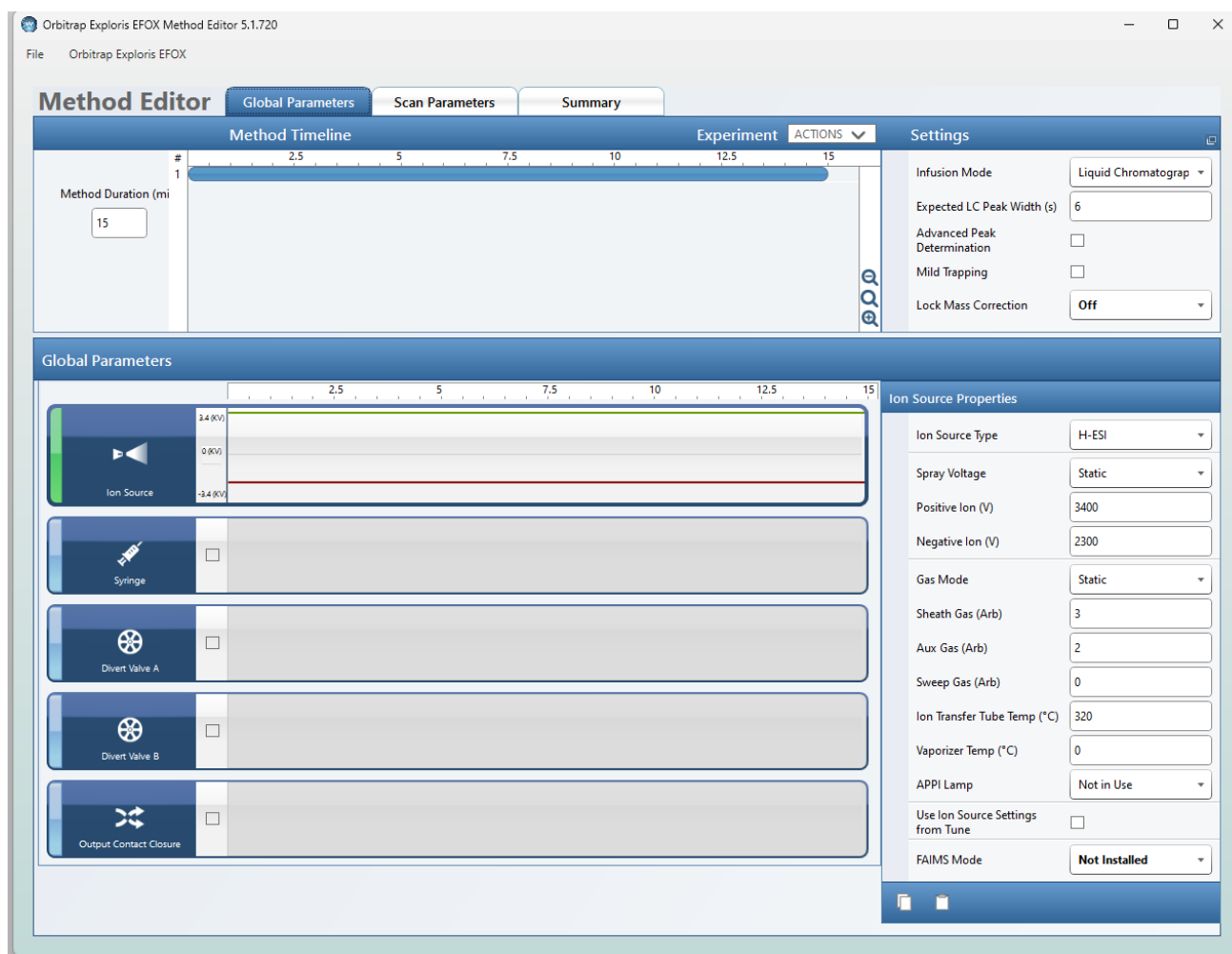
Menus

The Method Editor application window has the following menus:

- File menu

- Orbitrap Exploris menu

Figure 12 Method Editor application window



File menu

The File menu has the following commands:

Command	Description
New	Creates a new method.
Open	Displays the Open dialog box. Use the Open dialog box to find and open an instrument method file (*.meth) that already exists.
Save	Saves the (changed) settings of the active method. If a new method is created, the Save As dialog box opens.
Save As	Opens the Save As dialog box. Use the Save As dialog box to save the settings of the active method into a new file and to select the location (disk and folder) where you want to save it.
Is Method Modified	Checks if the parameters of the active method are different from the saved file. The result is displayed in a message box.

Command	Description
Validate	Checks if the active method has all parameter settings that are required for it to be executed successfully.
Create Method Summary	Creates a text file that contains all parameter settings of the active method.

Orbitrap Exploris menu

The Orbitrap Exploris menu has the following command:

Command	Description
Import Method from Raw Data File	<p>Reads the data stored in a raw data file (of an Orbitrap Exploris Series MS) and creates a method from the information therein.</p> <p>IMPORTANT: Importing the method from a raw file will replace the contents of the current method. If you do not want to replace the contents of the current method, open a new method before you import the raw file data.</p>

Global system parameters

On the Global Parameters page, you can configure the settings for the ion source, syringe pump, divert valves, and any connected devices controlled by the contact closure signal. The Method Editor application uses these settings for all scans.

NOTE

A red border around a parameter box indicates that the set value is out of range. A yellow border around a parameter box indicates that the set value is outside of the recommended range. Point to the parameter box to display the valid range. The Method Editor displays all changed set values of given parameters in bold type until you save them.

TIP

Select the **Summary** tab to view the global parameter settings.

Global MS settings

On the top left side of the Method Editor, you specify the experiment application mode and the method duration. On the top right side, you select the infusion mode and how to perform the lock mass correction.

NOTE

To use the standard internal calibration setting, you must activate the internal calibration in the Foundation Instrument Configuration window.

Parameters for the global MS settings

Parameter	Description
NOTE: These parameters appear on both the Global Parameters and Scan Parameters pages.	
Method Duration (min)	Defines the method duration in minutes. Range: 0.1–5000
Settings	
Infusion Mode	Available options are: <ul style="list-style-type: none"> Liquid Chromatography (default) Select Liquid Chromatography when the sample is introduced by LC (that is, precursors and precursor intensities change with time). Infusion Select Infusion when injecting the sample by direct infusion (that is, precursors and precursor intensities do not change with time).
Expected LC Peak Width (s)	(Infusion Mode: Liquid Chromatography) This parameter is used for a variety of features including Maximum Injection Time Mode: Dynamic, Dynamic Exclusion: Auto and scheduling AGC prescans. It is recommended to measure/determine the Expected LC Peak Width(s) from previous runs. It is taken at 10% valley (FWTM)—use Peak width as a first approach. The Expected LC Peak Width(s) has a direct influence on AGC. Please use Peak width of the narrowest peak of the chromatogram. The Expected LC Peak Width(s) measure is used to ensure proper calculations for AGC measures, that is the scheduling of periodic AGC pre-scans and receiving scan-to-scan AGC info. Range: 1–1000

Parameter	Description
Advanced Peak Determination	<p>Select the checkbox to apply the Advanced Peak Determination (APD) algorithm. This algorithm improves the determination of the charge states and monoisotopic m/z values of isotopic envelopes.</p>
Mild Trapping	<p>Select the checkbox if you want that ions are guided through the instrument with less energy to reduce fragmentation on MS1 level. However, a lower transmission may occur for certain compounds at the cost of sensitivity. Clear the checkbox for compounds that require highest transmission and ultimate sensitivity.</p> <p>For robustness reasons, it is strongly recommend to clear the checkbox in proteomics / peptide ID or related applications.</p>
Lock Mass Correction	<p>The internal calibrant source provides an internal reference mass that is analyzed in the same analytical scan of your sample measurements. Lock mass correction provides the most accurate corrections to the data.</p> <ul style="list-style-type: none"> • Off: No lock mass correction is performed. • EASY-IC™: The internal EASY-IC source provides fluoranthene radical ions, which are used as reference masses for the positive and negative polarity lock mass correction. • User-defined Lock Mass: One or more m/z values can be defined as lock mass(es) to perform a lock mass correction during the run. Lock Mass Injection is unchecked per default. <p>When you select this option, the lock mass table appears. (Scroll down to view the table.)</p> <p>If no lock mass is found in one scan, the system will apply the last successful locking information to this scan. Time duration of last locking and lock mass correction are provided in the scan header of the individual scan.</p>
Mode	<p>(Lock Mass Correction: EASY-IC™ or User-defined Lock Mass)</p> <ul style="list-style-type: none"> • Run Start (only with Lock Mass Correction: EASY-IC™) <p>During the “prepare for run” phase, a lock mass correction is performed via a tSIM scan. Statistical manners are applied to exclude outliers and determine the best correction value. The correction is applied to all scans throughout the run. This mode enables highest acquisition speed.</p> • Scan-to-Scan

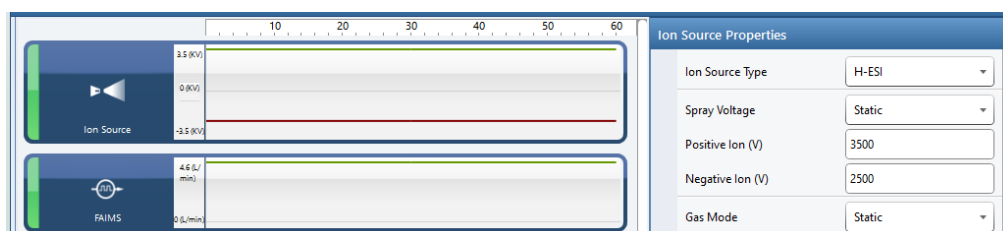
Parameter	Description
	<p>The lock mass correction is applied to every single scan. This mode delivers the highest mass accuracy. However, for the lock mass injection a certain overhead time is needed.</p> <p>The overhead time for injecting the lock mass (EASY-IC or User-defined Lock Mass) is typically 6 to 10 ms/scan.</p> <p>When EASY-IC is chosen, you can decide to apply the detected lock mass correction of the Full Scan to the subsequent ddMS2 scans by setting Use EASY-IC to Off in the scan properties of the MS2 scan.</p> <p>For User-defined Lock Mass, the Lock mass injection needs to be selected, to correct every scan (incl. ddMS2) in a Scan-to-Scan matter.</p> <ul style="list-style-type: none"> • Timed Applies the lock mass correction in the retention time window (start/stop) setting. The time window and the polarity is defined in the table that appears when you select this mode. The Timed mode provides the ability to refresh the lock mass correction multiple times during the data acquisition of a raw file (run), and therefore compromises high mass accuracy and high acquisition speed. The last successful locking information is applied to all subsequent scans until the next timed window applies and a lock mass correction is performed. If no lock mass is found, the last locking information will continue to apply.
Lock mass table	<p>(Lock Mass Correction: User-defined Lock Mass or EASY-IC: Timed)</p> <p>Use the lock mass table to:</p> <ul style="list-style-type: none"> • Specify user-defined lock masses • Specify time windows during which the lock mass correction (EASY-IC or User-defined Lock Mass) shall be applied <p>Lists can be imported (or exported to) from a CSV, a TXT, or an XML file.</p> <ul style="list-style-type: none"> • <i>m/z</i>: The lock masses' <i>m/z</i>. Range: 40–3000 • <i>t start</i> (only with Mode = Timed): The time (in minutes) to start the lock mass correction. Range: 0–Duration of active method; default: 0

Parameter	Description
	<ul style="list-style-type: none"> t stop (only with Mode = Timed): The time (in minutes) to stop the lock mass correction. Range: 0.1–Duration of active method; default: 0 Polarity: The polarity of the lock mass.
Current Lock Mass	(Lock Mass Correction: User-defined Lock Mass) Use the list to select an available lock mass list. Select Save to save the active lock mass list. Select Save As to save the active lock mass list under another name. Select Delete to delete the active lock mass list.
Mass Tolerance (ppm)	(Lock Mass Correction: User-defined Lock Mass) Defines the mass tolerance window (in \pm ppm) for detecting the lock mass defined in the table. Range: 1–20; default: 15

Ion Source Properties pane and timeline

Use the Ion Source Properties pane and the timeline to set the ion source parameters. For the time-dependent spray voltage table, you can import the settings from (or export them to) a CSV, a TXT, or an XML file.

Figure 13 Ion Source Properties (FAIMS enabled)



NOTE


If you installed the optional Thermo Scientific FAIMS Pro system, the FAIMS parameters appear in this properties pane if you select a FAIMS Mode other than Not Installed.


TIP

You can copy (📄) the ion source parameters from the Tune window and paste (📄) them to the Method Editor.



Parameters in the Ion Source Properties pane

Parameter	Description
Ion Source Type	<ul style="list-style-type: none"> NSI (nanoelectrospray ionization)

Parameter	Description
	<ul style="list-style-type: none"> • H-ESI (heated-electrospray ionization) • APCI (atmospheric pressure chemical ionization) • ESI (electrospray ionization) • OptiSpray • MALDI (matrix-assisted laser desorption/ionization) • DART (direct analysis in real time)
	<p>NOTE: For some ion source types (MALDI, for example), you can specify additional settings in the Define Scan subtree of the Diagnostics pane of the Tune application.</p>
Spray Voltage Spray Current	<ul style="list-style-type: none"> • Static: The spray voltage or spray current does not change with time. • Time Dependent: The spray voltage or spray current changes stepwise with time. When you select Time Dependent, a table appears where you set the time and either the spray voltage (Ion Source Type: ESI, H-ESI, NSI, OptiSpray) or spray current (Ion Source Type: APCI). <ul style="list-style-type: none"> – Time (min): The time (in minutes) when the voltage settings change to the specified value. Range: 0 to method duration – Positive Ion Range: 0-3000 Volts (NSI, OptiSpray), 0-6000 Volts (H-ESI, ESI), 0-100 μA (APCI) – Negative Ion Range: 0-2500 Volts (NSI, OptiSpray), 0-5500 Volts (H-ESI, ESI), 0-100 μA (APCI) <p>TIP: To maximize the table, click the  button in the top right corner.</p>
Positive Ion (V)	<p>The spray voltage (in volts) for the positive ion polarity mode. Range (ESI, H-ESI): 0–6000; default: 3400 Range (NSI, OptiSpray): 0–3000; default: 1800</p>
Negative Ion (V)	<p>The spray voltage (in volts) for the negative ion polarity mode. Range (ESI, H-ESI): 0–5500; default: 2300 Range (NSI, OptiSpray): 0–2500; default: 1500</p>
Pos Ion Discharge Current (μ A)	<p>(Ion Source Type: APCI) The ion discharge current (in microamperes) for the positive ion polarity mode.</p>

Parameter	Description
	Range: 0–100; default: 4
Neg Ion Discharge Current (μA)	(Ion Source Type: APCI) The ion discharge current (in microamperes) for the negative ion polarity mode. Range: 0–100; default: 10
Gas Mode	<ul style="list-style-type: none"> Static: The gas flows do not change with time. Time Dependent: The gas flows change with time. <p>When you select this option, a table appears where you can set the time (in minutes) and the flow rates for sheath gas, aux gas, and sweep gas.</p> <p>TIP: To maximize the table, select the  button in the top right corner.</p>
Sheath Gas (Arbitrary)	(Ion Source Type: ESI, H-ESI, APCI, OptiSpray) The sheath gas flow rate (arbitrary units). Range: 0–80 (H-ESI, APCI); default: 0 Range: 0–25 (OptiSpray); default: 0
Aux Gas (Arbitrary)	(Ion Source Type: ESI, H-ESI, APCI, MALDI, or DART) The auxiliary gas flow rate (in arbitrary units). Range: 0–25 NOTE: If the vaporizer temperature is set to values above 100 °C, the auxiliary gas is automatically set to 5 unless it is not set higher manually.
Sweep Gas (Arbitrary)	(FAIMS Mode: Not installed) The sweep gas flow rate (in arbitrary units). Range: 0–20
Ion Transfer Tube Temp (°C)	The temperature of the ion transfer tube (in °C). Range: 0 (ambient temperature) to 400
Vaporizer Temp (°C)	(Ion Source Type: H-ESI or APCI) The temperature of the vaporizer tube (in °C). Range: 0 (ambient temperature) to 550; default: 0
APPI Lamp	(Ion Source Type: ESI, H-ESI, or APCI) <ul style="list-style-type: none"> Not in Use: The instrument method does not use the APPI lamp. On: Switches on the installed APPI lamp. Off: Switches off the installed APPI lamp. <p>Default: Not in Use</p> <p>NOTE:</p>

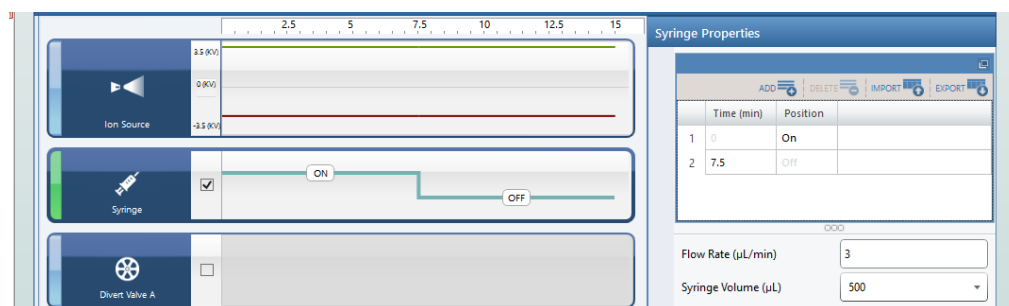
Parameter	Description
	<ul style="list-style-type: none"> • The Method Editor displays the APPI Lamp parameter under the following conditions: <ul style="list-style-type: none"> – You select a dedicated ion source type in the Instrument Configuration window. – You connect the APPI lamp's USB cable to the instrument. • The MS confirms the state of the APPI lamp before the data acquisition starts. If the ion source does not contain the APPI lamp, the data acquisition application displays an error message.
Cartridge Temp (°C)	<p>(Ion Source Type: OptiSpray)</p> <p>The infusion cartridge does not have an integrated heating element. This Cartridge Temp value will not be used.</p> <p>Range: 35–60, default: 45</p>
Use Ion Source Settings from Tune	<p>Uses the source settings that are currently set in the Tune application.</p> <p>Because the instrument method uses the source settings currently applied in the Tune application, the Method Editor application does not display the parameters.</p>
FAIMS Mode	<p>If you select a FAIMS Mode other than Not Installed, a FAIMS timeline is displayed in addition to the Ion Source timeline.</p> <p>Select one of these modes for running methods with FAIMS installed:</p> <ul style="list-style-type: none"> • Not Installed (default) • Standard Resolution: Provides the best transmission mode by setting the inner and outer electrodes to 100 °C. • High Resolution: Provides twice the resolution of the standard resolution mode by setting the inner electrode to 70 °C and the outer electrode to 100 °C. • User Defined: Displays the fields to enter the electrode temperatures.
	<p>NOTE: The electrode temperatures affect the ion separation. Although the ion transmission might be lower in the FAIMS high resolution mode, the peaks are narrower due to the electrodes' temperature differential.</p>
Total Carrier Gas Flow	<p>This parameter defines the flow of the carrier gas that propels the ions through the electrode space. The optimal gas flow for maximum transmission of ions depends on the type of ion</p>

Parameter	Description
	<p>transfer tube and is set as default for this field. In some cases, it might be beneficial to decrease or increase the carrier gas flow to optimize transmission or enhance the spray stability.</p> <p>The timing for dispersing the FAIMS and carrier nitrogen gas:</p> <ul style="list-style-type: none"> • Static: The gas flow does not change with time. • Time Dependent: The gas flow changes with time as specified in the Total Carrier Gas Flow Table. <ul style="list-style-type: none"> – Time (min): When the gas flow rate changes to the specified value. Range: 0 to the maximum method duration (5000 maximum) – Gas (L/min): The flow rate for the gas. Range: 3.5–7.7; default: 4.6 <p>This option is often used with higher gas flow rates at the beginning and the end of the chromatographic method to prevent liquid and undesired ions from entering the FAIMS electrode assembly.</p>
Total Carrier Gas Flow (L/min)	<p>(Ion Source Type: NSI, H-ESI, APCI, ESI; Total Carrier Gas Flow: Static)</p> <p>The flow rate for the FAIMS and carrier nitrogen gas. Range: 3.5–7.7; default: 4.6</p>
FAIMS Inner Electrode Temp (°C)	<p>(Ion Source Type: NSI, H-ESI, APCI, ESI; FAIMS Mode: User Defined)</p> <p>The maximum temperature of the FAIMS inner electrode (in °C). Range: 70–100; default: 100</p>
FAIMS Outer Electrode Temp (°C)	<p>(Ion Source Type: NSI, H-ESI, APCI, ESI; FAIMS Mode: User Defined)</p> <p>The maximum temperature for the FAIMS outer electrode (in °C). Range: 70–100; default: 100</p>
Ion Source Properties icons	
Copy 	Copies the ion source parameters to the clipboard.
Paste 	Pastes the ion source parameters from the clipboard.

Syringe Properties pane and timeline

Select the Syringe checkbox to enable the table in the Syringe Properties pane. Then specify the time-dependence of the syringe pump. The Global Parameters page graphically displays the time dependence of the syringe pump on/off status as a timeline. For the syringe position table, you can import the settings from (or export them to) a CSV, a TXT, or an XML file.

Figure 14 Syringe Properties



Parameters in the Syringe Properties pane

Parameter	Description
Syringe Pump position table	
Time (min)	The time (in minutes) when the syringe pump changes between On and Off, or Off and On. Range: 0 to the maximum method duration (5000 maximum); default: 0
Position	The syringe pump is On or Off at the corresponding time. Default: On
Flow Rate (µL/min)	The volume of solution per unit of time (in micro-liters per minute) that the syringe pump injects (the syringe pump flow rate). Range: 0.5 to 500 (<i>syringe volume</i> in µL)/min; default: 3
Syringe Volume (µL)	Values: 50, 100, 250, 500, 1000, 5000, and 10 000; default 500

Divert Valve Properties pane and timeline

Select the Divert Valve checkbox to enable the table in the Divert Valve Properties pane. Then specify the time-dependence of the modular divert/inject valve. The Global Parameters page graphically displays the time dependence of the divert valve position as a timeline. For the divert valve position table, you can import the settings from (or export them to) a CSV, a TXT, or an XML file.

Figure 15 Divert Valve Properties



Parameters in the Divert Valve Properties pane

Parameter	Description
Divert valve position table	
Time (min)	The time (in minutes) when the divert valve changes position. Range: 0 to the maximum method duration (5000 maximum); default: 0
Position	The divert valve position: 1-2 (default) or 1-6.

For additional information, refer to the instrument manuals.

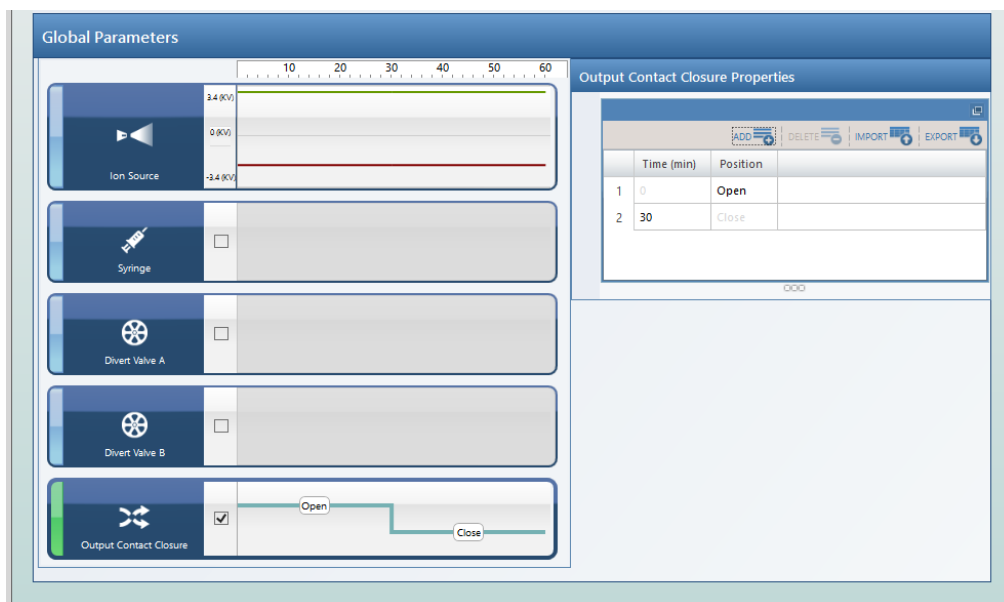
Output Contact Closure Properties pane and timeline

Select the Output Contact Closure checkbox to enable the table in the Output Contact Closure Properties pane. Then specify the time dependence of the contact closure on/off signal to an external device. The Global Parameters page graphically displays the time dependence of the contact closure on/off status as a timeline. For the contact closure position table, you can import the settings from (or export them to) a CSV, a TXT, or an XML file.

NOTE

You must configure contact closure in the Foundation Instrument Configuration window.

Figure 16 Output Contact Closure Properties



Parameters in the Output Contact Closure Properties pane

Parameter	Description
Contact closure position table	
Time (min)	The time (in minutes) when the contact closure state changes. Range: 0 to the maximum method duration (5000 maximum); default: 0
Position	The contact closure signal is Closed or Open. Default: Open

Method Editor overview

The Method Editor is below the method timeline on the Scan Parameters page. This is where you build the MS instrument method, which can have several experiments, each with its own timeline.

Method Editor elements



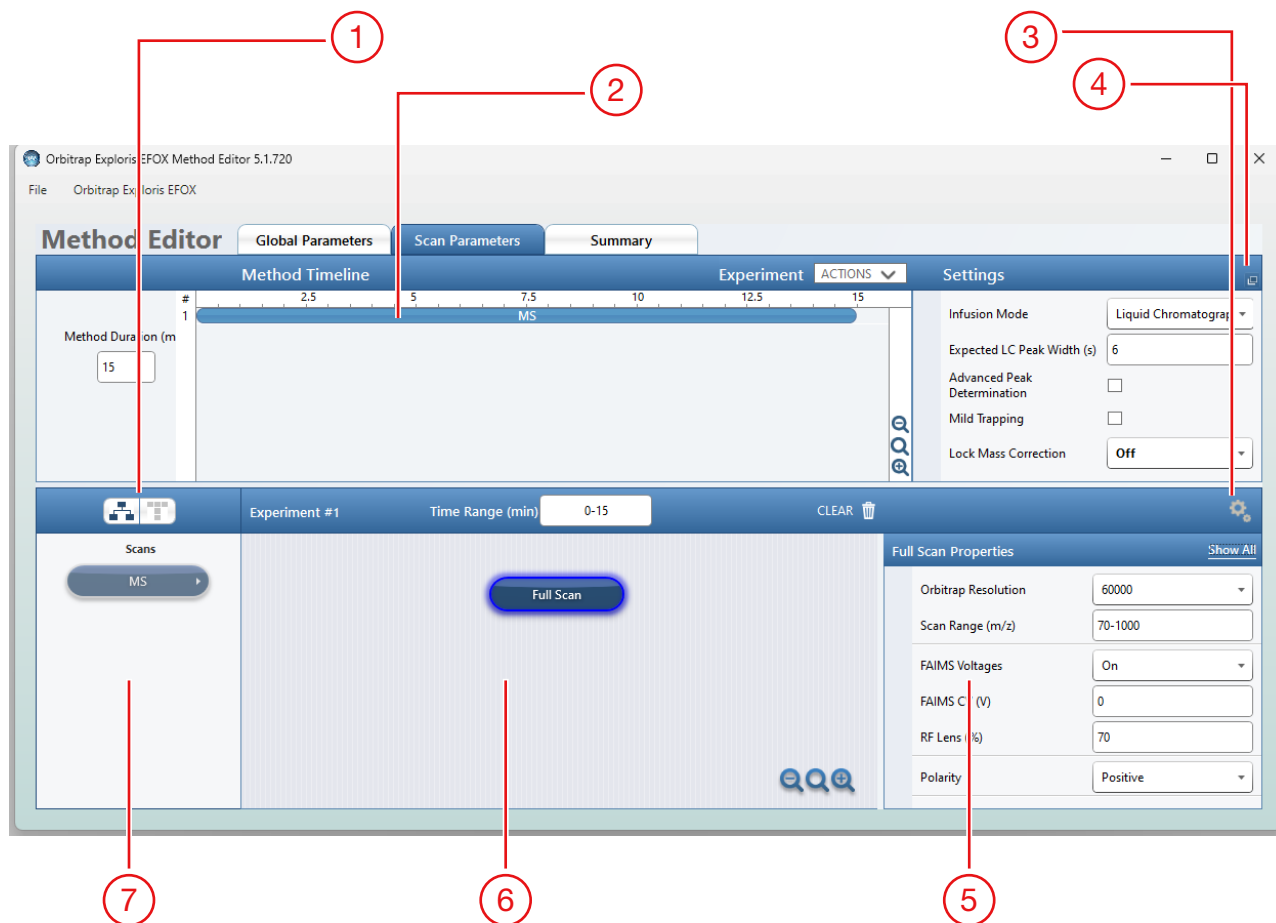
The Method Editor displays the Qualitative View (Qual View toolbar, ) in a workflow tree diagram. You can also build a workflow by selecting application-specific system templates from the Templates View toolbar (). These templates describe market-specific typical experiments.

Figure 17 Scan Parameters page



No.	Description	No.	Description
1	Select the experiment toolbar: Qual or Templates.	5	Object properties pane
2	Experiment timeline	6	Workflow area
3	Preferences icon	7	Experiment objects for the selected toolbar (Qual View shown) that you drag to the workflow area.
4	Unlock and maximize the settings.		

Set the Method Editor preferences

This topic provides information about handling parameters.

Apply parameter recommendations

When you enter a value or range that is not recommended or outside the recommended range for the selected parameter, the Method Editor application changes the border color of the affected workflow objects and parameters to yellow. Point to a yellow parameter box to display the recommended value or range.

Procedure

1. Do one of the following:
 - Right-click the parameter box and select **Apply Recommended Value**.
 - You can choose to ignore the recommendation by choosing **Ignore Recommendation**. When you use the recommended value, the box's border color returns to normal.
2. Right-click the workflow object and select **Restore Defaults**, or right-click a specific parameter box and select **Restore Default**.

Add or remove parameters from the favorites list

By default, the Method Editor shows only the preset favorite parameters in the scan properties panes. For the filter types, all parameters are shown. To add or remove parameters from the favorites list, follow this procedure.


Procedure

1. In the properties pane title bar, select **Show All if applicable**.
All parameters for the scan type appear.
2. To add or remove a favorite, select the star adjacent to the appropriate parameter (blue is selected and gray is unselected).
3. To show the modified list of favorites, select **Show Favorites**.

Experiments and instrument methods

Start by creating an instrument method (METH file) with several experiments —one scan table or workflow diagram and one timeline per experiment.

TIP

You can also start with one of the system templates, which is a complete method that you can modify. To view the system templates on the Scan Parameters page, select the Templates View icon () and select an application mode.

Add and delete experiments in a method

The instrument method can have several experiments.

Procedure

1. To add an experiment to the method, select **Add New Experiment** in the Experiment Actions list.
2. To delete an experiment from the method:
 - a. Go to the experiment by using the arrows above the workflow area.
 - b. In the Experiment Actions list, select **Delete Current Experiment**.

Save instrument methods

After creating an instrument method, save it.


Procedure

1. Select **File > Save As**.
2. In the Save As dialog box, enter an instrument method name (.meth extension), and then select **Save**.

Add custom method templates

If you want to save an instrument method as a template, save it in the Templates pane.

Procedure


1. After you build your template, select the Templates View icon ().
2. Select **Save As Template**.
3. Enter a template name and select **Save**.

The method template is added to the custom templates list.

Result

You can import the template at a later time by selecting **My Experiments** and selecting the template.

Qualitative view

On the Scan Parameters page, the Method Editor includes qualitative scan types in the left-side Qual View toolbar (.

Full Scan Properties pane



The Full Scan Properties pane sets up a full-scan type experiment—that is, signal intensity versus m/z over the specified m/z range.

NOTE

Changed set values of given parameters appear in bold type. A red table cell or red border around a parameter box indicates that the set value is out of range. Point to the parameter box to display the valid range. If you enter a value that is outside the recommended range, the box border color changes to yellow.

Parameters in the Full Scan Properties pane

Parameter	Description
Orbitrap Resolution	<p>The mass resolution of the Orbitrap analyzer, which is proportional to $1/\sqrt{m/z}$ of the mass-to-charge ratio. Mass resolution is defined as the observed m/z value divided by the smallest difference $\Delta m/z$ for two ions that can be separated: $m/z / \Delta m/z$. Spectra acquired at higher Orbitrap Resolution allow greater resolution in m/z but take longer to acquire. The selected resolution is specified for a peak at m/z 200.</p> <p>The available options are: 15 000, 22 500, 30 000, 45 000, 60 000, and 90 000.</p>
Scan Range (m/z)	<p>(Scan Range Mode: Define m/z Range)</p> <p>The range (in m/z) over which the mass analyzer detects peaks. Range: 40–1500; default: 70–1000</p> <p>TIP: Because the system optimizes the transfer of ions for the defined mass range, we recommend that you restrict the scan range to the region of interest.</p>
FAIMS Voltages	<p>(Ion Source Type: NSI, H-ESI, APCI, ESI, OptiSpray; with FAIMS Mode enabled)</p> <p>Switches On (default) or Off the FAIMS voltages.</p>
FAIMS CV (V)	<p>(FAIMS Voltages: On)</p> <p>The optimized FAIMS compensation voltage. Range: –300 to 300; default: 0</p> <p>The observed CV values on the MS are aligned with those of the other mass spectrometer platforms. This means the transmission for one m/z should be the same for the same CV setting on all platforms.</p>


Parameter	Description
RF Lens (%)	<p>For targeted CV experiments, it is recommended to use the value that was determined by a CV scan (performed in the Tune window) or optimized with single CV experiments in an HPLC run.</p> <p>Specify the value to control the RF amplitude applied to the S-lens. This value is a scaling factor applied to the nominal mass-to-voltage relationship.</p> <p>Range: 0–200; default: 70</p> <p>Decreasing the RF level decreases the transmission of high m/z ions through the S-lens, increases the transmission of the low m/z ions through the S-lens, and potentially decreases the amount of fragmentation of fragile ions in the S-lens. Increasing the RF level has the opposite effects.</p> <p>TIP: For stable ions, use the default value as a starting point. A fine tuning of the MS is recommended, however.</p>
AGC Target	<p>The Automatic Gain Control (AGC) controls the ions that are injected into the mass analyzer. The AGC target value defines the number of ions to be collected in the IRM.</p> <ul style="list-style-type: none"> • Standard (default): The system sets the recommended target (good starting point) in an automated fashion per scan type. • Custom: You can set the AGC Target in percent. <p>TIP: Please refer to the system templates for application-specific suggestions.</p>
Normalized AGC Target (%)	<p>(AGC Target: Custom)</p> <p>Specifies the Automatic Gain Control (AGC) target. This is a percentage representing the maximum number of charges to accumulate for a given analysis.</p> <p>Range: 0.001–1000; default: 100</p> <p>This is the normalized AGC Target value, it is represented as a percentage to aid in calculating the desired AGC Target. The base normalized value is different for scan types. The values are as follows:</p> <p>Scan Type = Full Scan; Normalized Base (100%), IRM = 1e6</p> <p>The IRM fills with ions until it reaches the AGC Target or the maximum injection time. The MS then transfers the ions to the Orbitrap analyzer.</p>
Absolute AGC Value	<p>(AGC Target: Custom)</p>

Parameter	Description
	<p>The absolute AGC target value is a conversion of the AGC percentage target set in the method into absolute values. This read-only value will be automatically updated according to the scan type and the target percentage.</p>
<p>Maximum Injection Time Mode</p>	<p>(Infusion Mode: Liquid Chromatography)</p> <p>Specifies the maximum injection time (max IT) that is allowed to reach the AGC Target. The IRM collects ions until it reaches the AGC Target or the max IT. The mass spectrometer then transfers the ions to the Orbitrap analyzer.</p> <ul style="list-style-type: none"> • Auto: The system calculates the maximum injection time that is available according to the chosen resolution to maximize sensitivity while maintaining the maximum scan rate (parallel acquisition). • Custom: You can specify the maximum injection time. • Dynamic: The system calculates the maximum injection time by dividing the Chromatographic peak width (in msec) by the Desired Minimum Points Across the Peak value. <ul style="list-style-type: none"> – Minimum value: If the calculated auto max IT for a single target is shorter than the detection duration of the current scan type, the instrument will acquire data in parallel acquisition mode. – Maximum value: If the calculated auto max IT for a single target is longer than the detection duration of the current scan type, the system can apply injection times up to this value, sacrificing optimally parallel acquisition.
<p>Desired Minimum Points Across the Peak</p>	<p>(Maximum Injection Time Mode: Dynamic)</p> <p>The minimum number of data points that are required across the peak. This parameter also takes the Expected Peak width into account</p> <p>When Maximum Injection Time is set to Dynamic, the instrument can dynamically increase the time beyond parallel acquisition while maintaining the number of points across the peak.</p> <p>Range: 1–1000; default: 9</p>
<p>Maximum Injection Time (ms)</p>	<p>(Maximum Injection Time Mode: Custom)</p> <p>Maximum time in milliseconds that is allowed to accumulate ions in the IRM until the AGC Target is reached.</p> <p>Range: 1–1000; default: 100</p>
<p>Microscans</p>	<p>Number of scans to average in a given spectrum.</p> <p>Range: 1–10; default: 1</p>

Parameter	Description
	<p>A microscan is one ion injection followed by ion detection. The MS sums microscans to produce one scan, which improves the signal-to-noise ratio of the mass spectral data.</p> <p>TIP: The overall scan time increases linearly with the number of microscans, significantly slowing the rate of spectral acquisition.</p>
Data Type	<p>Indicates how to collect data during the currently selected scan event:</p> <ul style="list-style-type: none"> • Profile (default): Represents mass spectral peaks as point-to-point plots, with each point having an associated intensity value. • Centroid: Represents mass spectral peaks (as a bar graph) in terms of two parameters: the centroid (the weighted center of mass) and the intensity. The normalized area of the peak provides the mass intensity data. <p>Centroiding reduces the data file size while maintaining spectral quality.</p>
Polarity	<p>The polarity mode—Positive (default), Negative, or Both—during a scan to detect ions of that polarity.</p> <p>NOTE: The polarity mode applies toward an entire experiment, and each experiment in the method can have a different polarity setting. With the Both polarity option, two scan events occur per acquisition cycle with the first scan being in positive mode followed by negative mode. If the workflow includes multiple experiments, the MS executes all experiments in positive mode for the first cycle. After that, the MS switches to negative mode.</p>
Source Fragmentation	<p>Select the checkbox to switch on ion source CID.</p> <p>An offset voltage in the ion source accelerates the ions into the background gas. Collisions with the background gas might help in the desolvation of the ions and might increase sensitivity.</p>
Energy (V)	<p>(Source Fragmentation: On)</p> <p>The collision energy (in electron volts) for ion source fragmentation.</p> <p>Range: 1–135; default: 35</p> <p>If you set the source fragmentation too high, fragmentation of ions might occur.</p>
Lock Mass Injection	<p>(Lock Mass Correction: User-defined Lock Mass)</p> <p>Lock mass injection offers the possibility to inject a defined lock mass (if provided in the solvents or similar) to the ions of the analytical scan into the C-trap and ensures the detection and locking the chosen reference mass.</p>

Parameter	Description
	<p>In general, a lock mass correction can only be performed, if the defined m/z value is detected in the performed Orbitrap scan. This means, the mass range of the scan of interest must provide the m/z value of the lock mass of interest.</p> <ul style="list-style-type: none"> • If the Lock Mass Injection checkbox is selected, the lock mass injection will be performed for all scans in the experiment. • If the Lock Mass Injection checkbox is clear, you must make sure that the lock mass is covered by at least one experiment/scan in the method. <p>The lock mass injection reduces the acquisition speed due to the associated overhead of about 6–10 ms/scan.</p>
Use EASY-IC™	<p>(Lock Mass Correction: EASY-IC™, Mode ≠ RunStart)</p> <p>If On is selected, it provides an internal reference mass that is used for mass correction during a run.</p>

Use the experiment templates

Use the left-side Templates View toolbar () to select predefined, application-specific system templates. You can then modify the experiment parameters in the Properties pane. You can also create a custom workflow template and save it as an EXP file.

Download new or updated system templates

Download new or updated (revised) system templates for your Orbitrap instrument from the Thermo Fisher Cloud.

Each time you open the Method Editor, the system automatically checks for new or updated system templates and notifies if there any are available. You can choose to download any new or updated template that is available. you and save them under new names.

NOTE

You must have the Thermo Scientific Almanac™ agent installed to access this feature. The agent is typically included as a component during the installation of the Xcalibur data system.

Receive new or updated system templates

Procedure

1. Make sure that you have the following:

- An active Internet connection
 - A [Thermo Fisher Cloud](#) account
2. From the Instrument Setup window, open the Method Editor.

The Method Editor window opens and the application checks for any system template updates. If new templates are available, the Download New Templates dialog box appears.

3. Click **Update**.

The system templates are downloaded to the (default) Templates folder and the Progress bar shows the download status.

4. Click **Close** when the download is complete.

TIP

You can check for updates manually at any time. From the Orbitrap Exploris menu, click **Check for New Templates**.

Save custom templates

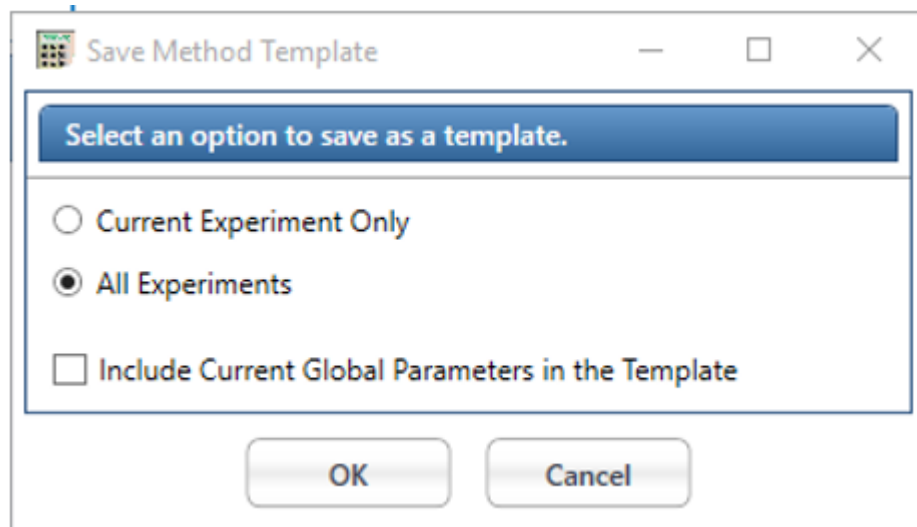
You can modify system templates and save them under new names.

Procedure

1. In the Templates View toolbar, select **Save as Template** and browse to the destination folder.
2. Enter a name for the template and select **Save**.
3. In the Save Template dialog box, enter a template name (.exp extension), and select **Save**.

A popup message “Save Method Template” appears.

Figure 18 Save Method Template dialog box



4. Select an option to save a template:
 - Current Experiment Only: If there are two or more experiments in the method timeline, only the marked experiment is saved as a Method Template.
 - All Experiments: If there are two or more experiments in the method timeline, all experiments in the method timeline are saved as a Method Template.
5. (Optional) Select the **Include Current Global Parameters in the Template** checkbox. The default state is not selected (Only the Scan Parameters of the method are saved). When selected (active), Scan Parameters and Global Parameters (such as ion source settings) are saved in the template.

Import a custom template

Procedure

1. Point to **My Experiments** to display the available templates.
2. Double-click a system template button or drag it to the **Place Scan Here** node.

System templates

The System Template folders typically contain multiple system templates, which are divided in template categories.

Use a System Template

Procedure


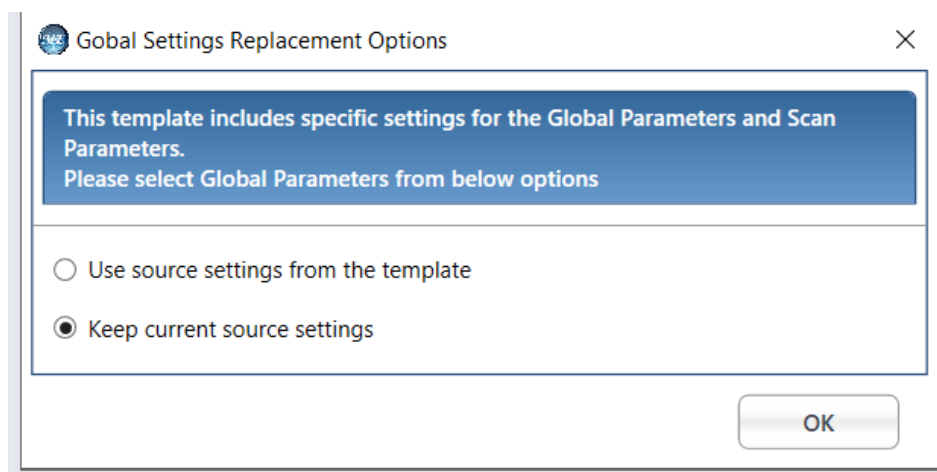
1. Select the left-side Templates View toolbar () to display the available template categories.
2. Select the triangle at the right part of the template category button to display the available system templates.
3. Double-click a system template button or drag it to the Place Scan Here node.
4. If the Global Settings Replacement Options dialog box appears, select an option:
 - Use source settings from the template
 - Keep current source settings

Figure 19 Global Settings Replacement Options dialog box





5. Select **OK** to close the dialog box.




Result

The workflow area displays the selected experiment.

Summary page

The Summary page displays the parameters for mass spectrometer setup, syringe pump, divert valves, and contact closure that you specified on the Global Parameters page and the Scan Parameters page.

Button	Description
	Print a summary report.
	Find a word on the Document View.

Button	Description
	<p>Enter the text in the field. Select the down arrow to select a find option. Select the left/right arrows to find the text on the Document View.</p>
	<p>Select the display mode of the Document View:</p> <ul style="list-style-type: none"> • Page Mode • Two Page Mode • Scroll Mode
	<p>Use the slider or select the minus/plus signs to zoom in or out the Document View.</p> <p>Or, position the mouse pointer in the pane, press and hold the <Ctrl> key, and roll the mouse wheel forward to zoom in or backward to zoom out.</p>

Display the Summary page

Procedure


1. In the Method Editor application window, select the **Summary** tab.
2. Select the tabs to display the data in a Document View or a Tree View.

Customer feedback

You are encouraged to report errors or omissions in the text or index. Send an email to the Technical Documentation at documentation.bremen@thermofisher.com. The PDF versions of our manuals allow adding comments with Adobe Acrobat Reader or other freely available PDF reader programs.

You can also send feedback about our product documentation through the Thermo Scientific Help Portal.

Procedure

1. Go to docs.thermofisher.com.
2. Enter the product name in the search bar and press ENTER (or select the search icon).
3. From the search results list, select a product manual or topic.
4. Select **Send Feedback** .

The Feedback window opens.

TIP

If you are not signed in to the Thermo Scientific Help Portal, your default email application will generate an email with a basic template for providing your feedback.

5. Enter your feedback in the text box.
6. (Optional) Select **Drop Files to Attach, or Browse** to upload supporting documents and images to your submission.
7. Select **Send**.

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Thermo Fisher Scientific (Bremen) GmbH, Hanna-Kunath-Str. 11,
28199 Bremen, Germany

Tel: +49(0)421 5493 0

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Revision	Release date
A	October 2025

Glossary

A

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

API source The sample interface between the liquid chromatograph (LC) and the mass spectrometer (MS).

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.

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

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.

autosampler A device that automatically loads collected samples (as for spectroscopic or chromatographic analysis) into a laboratory instrument.

C

centroid data Data used to represent mass spectral peaks in terms of two parameters: the centroid (the weighted center of mass) and the intensity. The data is displayed as a bar graph. The normalized area of the peak provides the mass intensity data.

chromatogram A single sample injection will result in a plot of detector response versus time. This plot is referred to as a chromatogram.

collision-induced dissociation (CID) A method of fragmentation where molecular ions are accelerated to high-kinetic energy and then allowed to collide with neutral gas molecules such as helium or nitrogen. The collisions break the bonds and fragment the ions into smaller pieces.

contact closure connection The cable connection is from the external peripheral device to the mass spectrometer contact closure pins (Start In and Ground). The external device sends the contact closure (start) signal to the mass spectrometer.

D

Data Dependent experiment A real-time, automated experiment that uses specified criteria to select one or more ions of interest for subsequent analysis, such as MS/MS or ZoomScan.

data-independent acquisition (DIA) A data-independent acquisition mode in which all precursor ions are fragmented.

divert/inject valve A modular valve that can be plumbed as a divert valve to control the flow direction or as a loop injector for flow injection analysis.

E

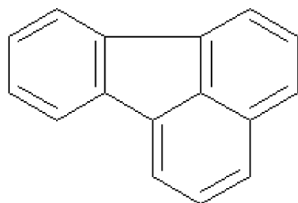
electrospray ionization (ESI) A soft ionization technique operating at atmospheric pressure. Ions are generated in solution and a high voltage is applied to generate small droplets that are then evaporated until all ions are in the gas phase.

Extensible Markup Language (XML) A general-purpose markup language that is used to facilitate the sharing of data across different information systems, particularly via the Internet.

F

FlexMix Calibration solution that is a mixture of 16 highly pure, ionizable components (mass ranges: 50 to 3,000 m/z), which is designed for both positive and negative ionization calibration.

fluoranthene A reagent anion that is used in an electron transfer dissociation (ETD) experiment. This compound is not only used as reagent ion but also as internal calibration compound.



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

full-scan type Provides a full mass spectrum 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.

H

handshake A signal that acknowledges that communication can take place.

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

higher energy collision-induced dissociation (HCD) Collision-induced dissociation that occurs in a high-pressure cell in the MS detector. In HCD, the ion optics accelerate precursor ions into a highpressure cell, where they collide with nitrogen gas. The collisions cause the ions to fragment into product ions. The ion optics then send the product ions to the mass analyzer for mass analysis. HCD produces triple quadrupole-like product ion mass spectra.

high-Field Asymmetric waveform Ion Mobility Spectroscopy (FAIMS) An optional device for separating ions at atmospheric pressure. FAIMS provides ion separation by taking advantage of compound-dependent changes in ion mobility at high electric field strengths.

I

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

ion-routing multipole (IRM) The ion-routing multipole not only functions as a collision cell for HCD fragmentation, but also as an ion storage-routing device so ions can be routed to the linear ion trap and back to the Orbitrap via the IRM cell.

isolation width The baseline width of a window for a mass peak (or peak cluster) of interest for an MS/MS or MSⁿ scan.

L

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

M

mass-to-charge ratio (m/z) Used to denote the quantity formed by dividing the mass of an ion (in u or Da) by the number of charges carried by the ion. For example, for the ion C₇H₇²⁺, m/z=45.5.

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.

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

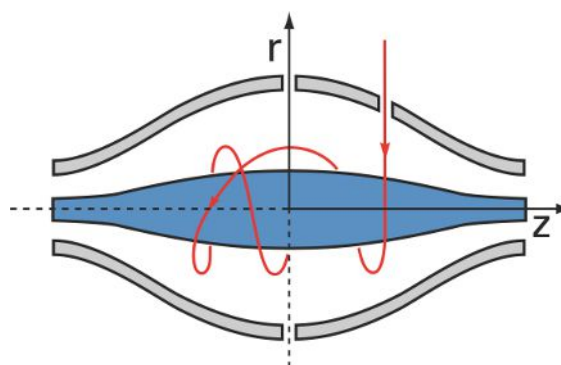
data. The number of microscans per scan is an important factor in determining the overall scan time.

N

nanoelectrospray ionization (nano ESI or NSI) A type of electrospray (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 1,000 nL/min (for dynamic nanoelectrospray, also called nanoESI nanoLC gradient separation).

O

Orbitrap mass analyzer (OT) The Orbitrap™ mass analyzer consists of a spindle-shaped 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: $m/z = k/f^2$



P

polarity The polarity of an ion can be either positive or negative: 1. positive ion: An atom, radical, molecule, or molecular moiety that has lost one or more electrons or has been protonated, thus acquiring an electrically positive charge. The charge state becomes +1, +2, +3, and so on, depending on the number of electrons lost or protons gained. 2. negative ion: An atom, radical, molecule, or molecular moiety that has gained one or more electrons, acquiring an electrically negative charge. The charge state becomes -1, -2, -3, and so on, depending on the number of electrons acquired.

product mass The mass-to-charge ratio of a product ion. The location of the center of a target production peak in mass-to-charge ratio (m/z) units.

profile data Data representing mass spectral peaks as point-to-point plots, with each point having an associated intensity value.

R

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

S

selected ion monitoring (SIM) A scan type where the mass spectrometer acquires and records ion current following the isolation of a list of singular mass-to-charge ratio values.

selected reaction monitoring (SRM) 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.

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.

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.

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

T

tandem mass spectrometry An analytical technique involving two stages of mass analysis. Initially, ions formed in the ion source are analyzed by an initial analyzer. In the subsequent stage, these mass-selected ions are fragmented, and the resulting ionic fragments are further mass analyzed. It is also called as MS/MS or MS².

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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