

Exactive™ QuickStart Guide

About This Guide

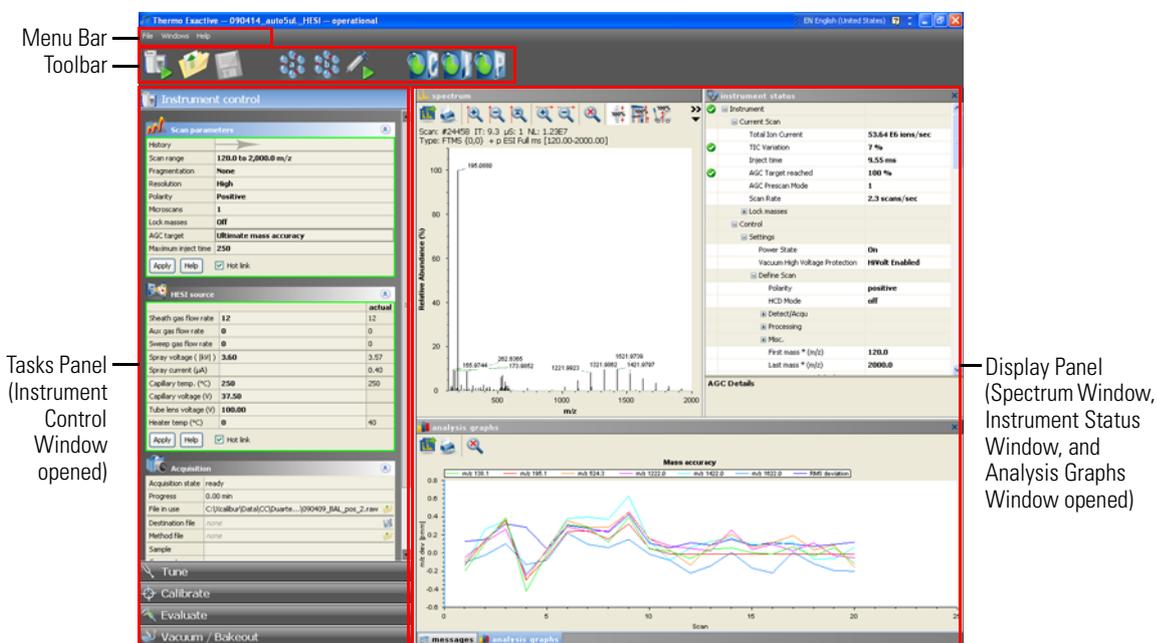
This *Exactive QuickStart Guide* provides an overview on how to set up and use the Thermo Scientific Exactive MS detector. This guide is intended for all personnel who use to operate the Exactive.

Please note that this guide does not contain any safety notices and precautionary statements. Thus it is mandatory to read also the *Exactive Operating Manual* and the *Ion Max and Ion Max-S API Source Hardware Manual*.

Exactive Tune

Exactive Tune allows operating the Exactive MS detector via the data system computer. It has the following components:

- The *menu bar* provides drop-down menus with commands for operating the program.
- The *toolbar* provides symbol shortcuts for frequently used commands.
- The *tasks panel* provides five windows: Instrument Control, Tune, Calibrate, Evaluate, and Vacuum/Bakeout. The *instrument control window* is divided into three additional windows for defining scan parameters, setting API source parameters, and acquiring and storing measurement data.
- The *display panel* provides various windows for showing real-time information about the current measurement and the instrument status.



❖ To display the Exactive Tune program

Choose **Start > Programs > Thermo Exactive > ExactiveTune**.

Preparation



Before going on, check that the following preconditions are fulfilled:

- The main power circuit breaker switch is in the On (I) position and the electronics service switch is in the Operating Mode position, the gas pressure is within the operational limit, and the vacuum levels are sufficient for operating the instrument.
- The Ion Max API source with ESI probe is installed on the MS detector. You have checked the proper position of the ESI probe in the Ion Max API source housing.
- Power supply and communication between syringe pump and MS detector are established. A clean, 500 µL Unimetrics syringe with 450 µL of calibration solution for positive ion mode is placed into the syringe pump. An infusion line is installed between the syringe pump and the grounding union that is held by the grounding bar of the Ion Max API source.



Getting Ions from Infusion Experiments

Setting up Exactive Tune



- The data system computer with the Exactive Tune software is switched on and the MS detector is placed in Standby mode.

For information about performing these tasks, see the *Exactive Operating Manual*, the *Exactive Software Manual*, or the Exactive Tune online Help, and the *Ion Max and Ion Max-S API Source Hardware Manual*.

This topic describes how to test the instrument before you can tune and calibrate your Exactive MS detector automatically.

❖ To set up the MS detector in the Exactive Tune software for tuning and calibration in the ESI/MS mode

- If you have not already done so, choose **Start > Programs > Thermo Exactive > ExactiveTune** to start the Exactive Tune program.
- In the Exactive Tune window, on the toolbar, click the **On/Off/Standby** button to take the MS detector out of the Standby mode or Off mode and turn it On. When you turn the MS detector to On, you initiate the following events:
 - The MS detector begins scanning.
 - Nitrogen flows into the ESI probe.
 - The Exactive MS detector applies high voltage to the ESI probe.
 - The spectrum window of Exactive Tune shows a real-time display of the mass spectrum.
- If necessary, open an existing tune file:
 - Choose **File > Load Tune File** to display the Open dialog box.
 - Browse for the folder C:\Xcalibur\methods\. Select a tune file, for example *ESI_installation.mstune*.
 - Click **Open** to open the file. Exactive Tune downloads the Tune Method parameters to the MS detector.
- Examine the pre-tune ESI source settings:
 - From the Instrument Control panel, open the ESI source window. See Figure right.
 - Set the *sheath gas flow rate* to 12.
 - Set the *spray voltage* to 4 kV.
 - Set the *capillary temperature* to 250 °C.
 - Set the *capillary voltage* to 60 V.
 - Set the *tube lens voltage* to 120 V.
 - Set the *skimmer voltage* to 25 V.
 - If you have made any changes in the window, click **Apply**.

		actual
Sheath gas flow rate	12	12
Aux gas flow rate	0	0
Sweep gas flow rate	0	0
Spray voltage (kV)	4.00	3.99
Spray current (µA)		0.00
Capillary temp. (°C)	250	250
Capillary voltage (V)	60.00	
Tube lens voltage (V)	120.00	
Skimmer voltage (V)	25.00	

Apply Help Hot link

Note Select the Hot link check box to apply all changes in real time. A green frame around the parameter box indicates an active hot link. ▲

- Set the scan parameters for tuning and calibration:
 - From the Instrument Control panel, open the Scan parameters window. See Figure right.
 - Enter a *scan range* between *m/z* 120 and 2000.
 - Set *Fragmentation* to *None* to specify that the ion source fragmentation option is turned off.
 - Select *High* for *Resolution*.
 - Set the *ion polarity mode* to *Positive*.
 - Set the total number of *microscans* to 1.
 - Set *Lock masses* to *Off*.
 - Select *Balanced* for *AGC target*.
 - Specify a *maximum injection time* of 250 ms.
 - If you have made any changes in the window, click **Apply** or select the Hot link check box.

History	
Scan range	120.0 to 2,000.0 m/z
Fragmentation	None
Resolution	High
Polarity	Positive
Microscans	1
Lock masses	Off
AGC target	Balanced
Maximum inject time	250 ms

Apply Help Hot link

Testing the MS Detector



The MS detector is now properly set up for tuning and calibration in the ESI/MS mode.

In this section, you can test whether the MS detector is operating properly. Infuse a low concentration calibration solution for positive ion mode – containing caffeine, MRFA, and Ultramark 1621 – into the ESI source using the syringe pump. See the *Exactive Operating Manual* for information about calibration solutions. Monitor the real-time display of the mass spectrum to ensure that a stable spray of solution enters the MS detector.

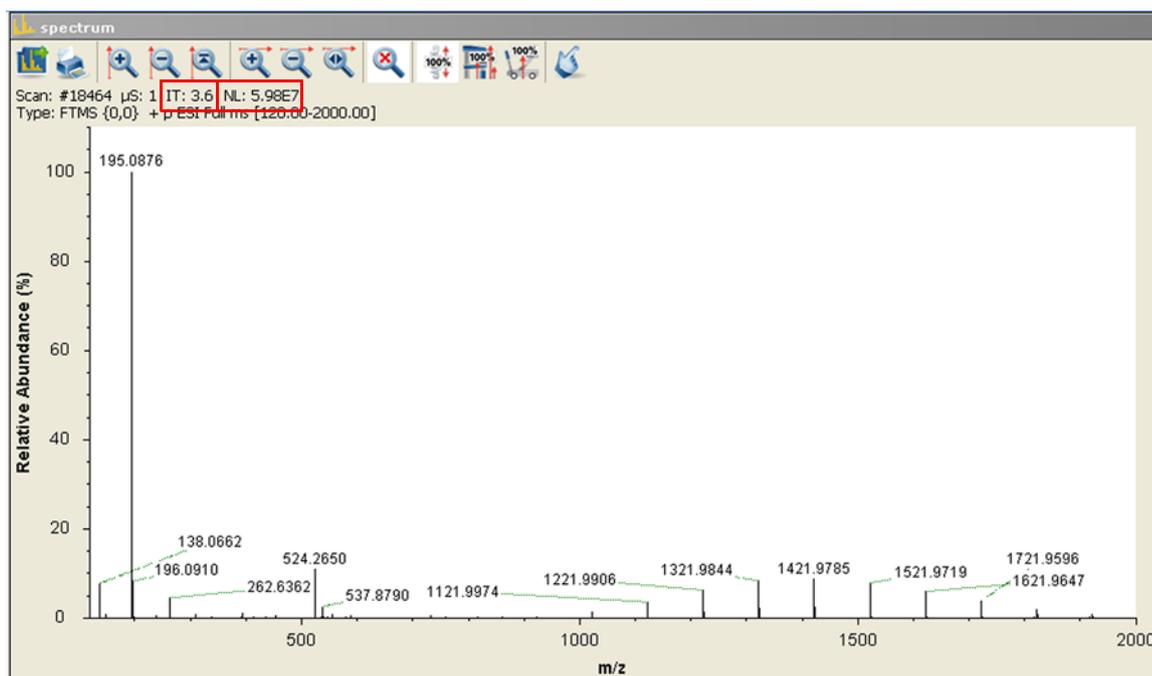
❖ To test the operation of the MS detector in ESI/MS mode

1. Click the arrow of the **Syringe Pump** button to display the Syringe Pump dialog box. See Figure right.
2. Turn on the syringe pump and set an infusion flow rate of 5 $\mu\text{L}/\text{min}$, as follows:
 - a. Select *Unimetrics* to specify the proper *syringe type*.
 - b. Select *500* in the *Volume* list box to set the proper syringe volume. Note that the Exactive MS detector automatically sets the syringe ID to its proper value of 3.257 mm.
 - c. Specify a *flow rate* of 5 $\mu\text{L}/\text{min}$.
 - d. Click **Apply** to apply the syringe parameters.
 - e. Click **Start** to start the syringe pump.

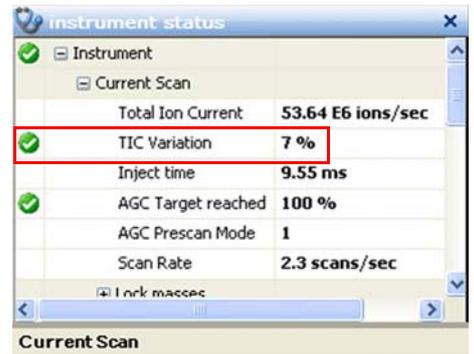
Syringe type	Unimetrics
Volume (μL)	500
Syringe inner diameter (mm)	3.257
Flow rate ($\mu\text{L} / \text{min}$)	5.000
<input type="button" value="Apply"/> <input type="button" value="Help"/>	
<input type="button" value="Manual control"/> <input type="button" value="Start"/> <input type="button" value="Stop"/>	

Note Once you have set the syringe parameters, just click the **Syringe Pump** button to switch on or off the syringe pump. ▲

3. Test the efficiency and stability of the spray of calibration solution into the MS detector. Monitor the data for the calibration solution, as follows:
 - a. In the spectrum window of Exactive Tune, observe the mass spectra of the singly-charged ions of the positive ion mode calibration solution. See Figure below. The ions are as follows:
 - Caffeine: m/z 138 (fragment), 195
 - MRFA: m/z 524
 - Ultramark 1621: m/z 1022, 1122, 1222, 1322, 1422, 1522, 1622, 1722, 1822
 - b. At the top of the spectrum window, notice the values for the inject time (IT) and normalized target level (NL). See Figure.



- c. Observe the values for NL and IT in the spectrum window. As calibration solution infuses, and the readback values fluctuate, check the status of the ion current signal:
- Is the signal present?
 - Is the signal stable? Check in the instrument status window that the TIC variation has stabilized at about 15% or less from scan to scan (green check mark). See Figure right.



If you answered “yes” to the questions in step 3c, then your MS detector is operating properly.

If you answered “no” to either of these questions, try the following troubleshooting measures:

- Ensure that the ESI probe is properly assembled and that the needle position is correct.
- Ensure that the entrance to the ion transfer capillary is clean.
- Ensure that the solution entering the probe is free of air bubbles and that the tubing and connectors are free of leaks and that they are not clogged.
- Ensure that spray voltage is appropriate.
- Ensure that discharge does not take place.

Your MS detector is operating properly in the ESI mode. You are now ready to tune and calibrate the MS detector.

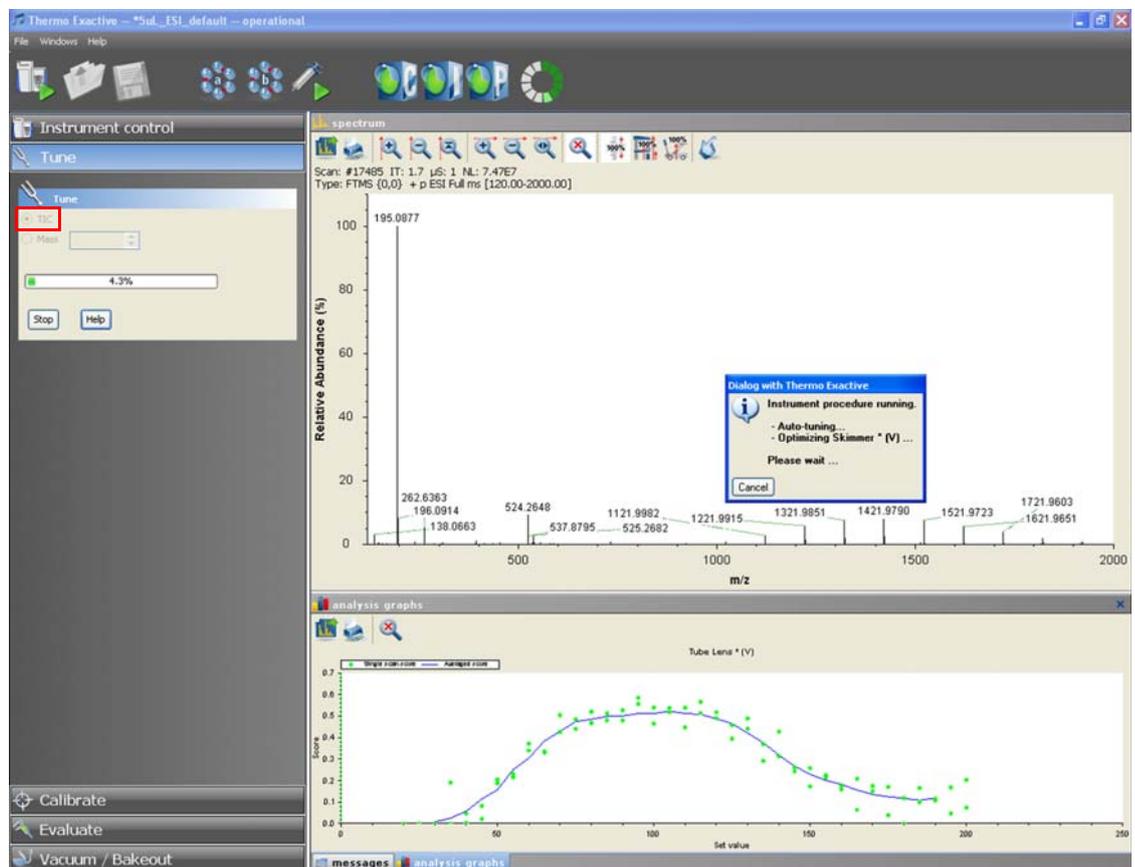
Tuning



After having established that your Exactive MS detector is working properly in the ESI mode, you can now tune the MS detector automatically. Tuning optimizes voltage settings for API source and ion transfer optics to ensure highest sensitivity. Note that tuning is compound and technique dependent.

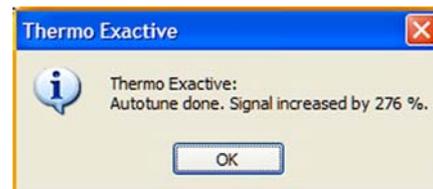
❖ To tune the Exactive MS detector

1. Click the **On/Off/Standby** button to take the MS detector out of the Standby mode or Off mode and turn it On.
2. Click the **Syringe Pump** button to start the syringe pump.





3. In the Tune window, select the TIC option button to specify that the Exactive MS detector optimizes on the total ion current (TIC) intensity. See Figure on previous page.
4. If the MS detector is equipped with a HCD collision cell, ensure that *HCD fragmentation is not activated* in the Fragmentation dialog box. (See [page 7](#).) Otherwise, the tuning procedure comprises only HCD relevant parameters.
5. Click **Tune** to start the automatic tuning procedure:
 - The Tune window indicates the completed percentage of the tuning process by a progress bar.
 - A message box informs you about the active tuning procedure and the currently tuned parameter.
 - The analysis graphs window displays a graphic representation of the tuning process for the currently tuned parameter.
 - The messages window shows a record of the tuning steps.
 - An icon appears on the toolbar to indicate an active procedure.
6. A message box informs you that the tuning procedure is completed. The message box also contains information about the factor by which the signal was increased. See Figure right. Click **OK** to close the message box.
7. Choose **File > Save Tune File as** to save the adjusted parameters in a Tune Method:
 - a. In the displayed dialog box, enter a name for the new tune file.
 - b. Click on **Save** to save the new tune parameters and to close the dialog box.



The new tune file is specific to your particular analyte and solvent flow rate. (In this example, you save settings obtained using calibration solution.) You can call the Tune Method again and use it as a starting point for optimization of the MS detector for a different analyte of interest or with a different flow rate.

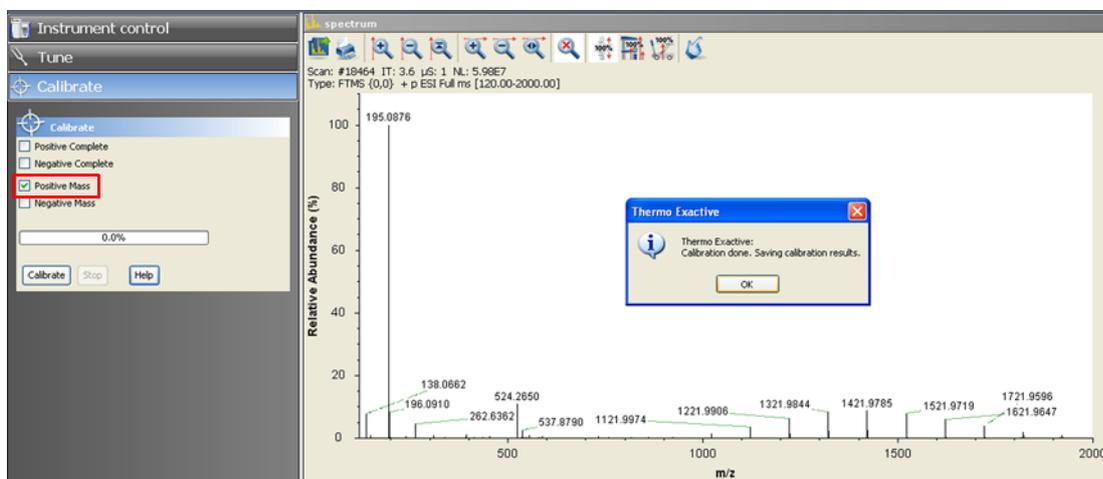
Mass Calibration

After having tuned the Exactive MS detector, it should be calibrated to ensure the mass accuracy of the results. Usually, it is sufficient to perform a mass calibration only. Before starting the calibration, check again that you have a stable spray: Focus on the injection time (IT) and on the normalized level (NL) in the header information on the spectrum window.

Note If the system was in Off mode before, it is necessary to put the instrument into On mode for at least 90 minutes before a mass calibration is performed. ▲

❖ To calibrate the Exactive MS detector for positive ion mode

1. Click the **On/Off/Standby** button to take the MS detector out of the Standby mode or Off mode and turn it On.
2. Click the **Syringe Pump** button to start the syringe pump.
3. In the Calibrate window, select the Positive Mass check box to specify a mass calibration for the positive ion mode. See Figure.



Positive Ion Mode Calibration





Negative Ion Mode Calibration

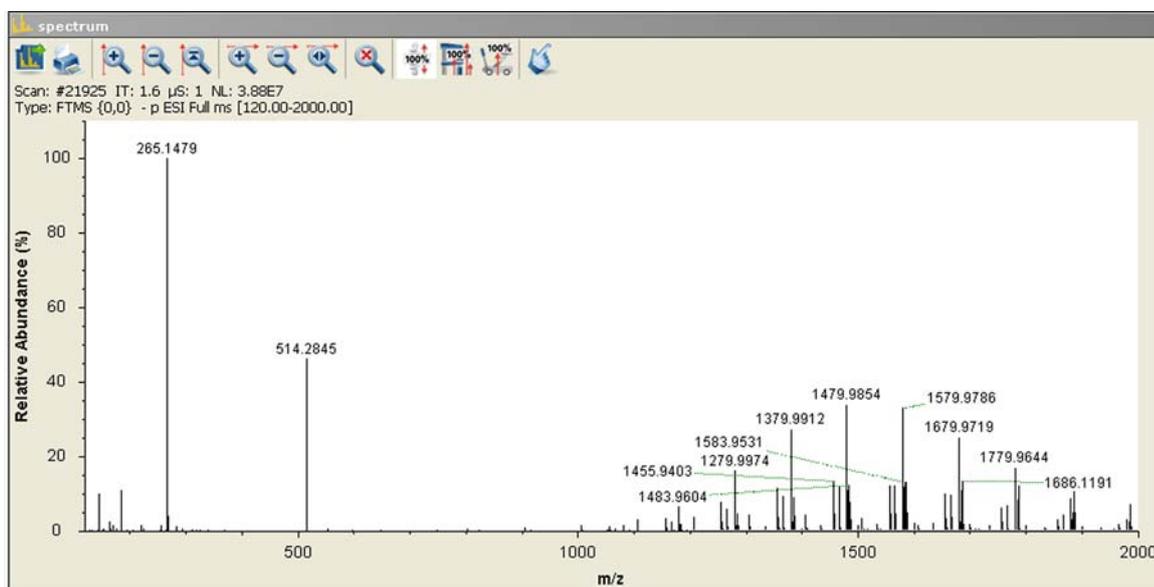


- Click **Calibrate** to start the automatic calibration procedure:
 - The Calibrate window indicates the completed percentage of the calibration process by a progress bar.
 - Exactive Tune displays a message box that informs about the active calibration procedure and the currently calibrated parameter.
 - The analysis graphs window displays a graphic representation of the calibration process for the currently tuned parameter.
 - The messages window shows a record of the calibration.
 - An icon appears on the toolbar to indicate an active procedure.
- A message box appears when the calibration procedure is completed. The message box also informs you that Exactive Tune has automatically saved the calibration results. Click **OK** to close the message box.

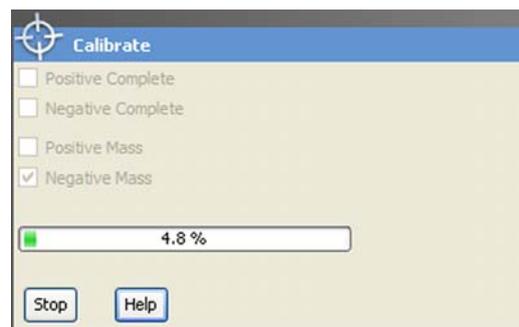
Before performing measurements in the negative ion mode, the MS detector will need to be calibrated with a calibration solution containing sodium dodecyl sulfate, sodium taurocholate, and Ultramark 1621. See the *Exactive Operating Manual* for information about calibration solutions.

❖ To calibrate the Exactive MS detector for negative ion mode

- Place a clean, 500 μL Unimetrics syringe with 450 μL of calibration solution for the negative ion mode into the syringe pump.
- Click the **On/Off/Standby** button to take the MS detector out of the Standby mode or Off mode and turn it On.
- Click the **Syringe Pump** button to start the syringe pump.
- In the spectrum window of Exactive Tune, observe the mass spectra of the singly-charged ions of the negative ion mode calibration solution. See Figure below. The ions are as follows:
 - Sodium dodecyl sulfate: m/z 265
 - Sodium taurocholate: m/z 514
 - Ultramark 1621: m/z 1280, 1380, 1480, 1580, 1680, 1780



- In the Calibrate window, select the Negative Mass check box to specify a mass calibration for the negative ion mode. Click **Calibrate** to start the automatic calibration procedure.
- The Calibrate window indicates the completed percentage of the calibration process by a progress bar. See Figure right.
- A message box informs you when the calibration procedure is completed. Click **OK** to close the message box.



Evaluation

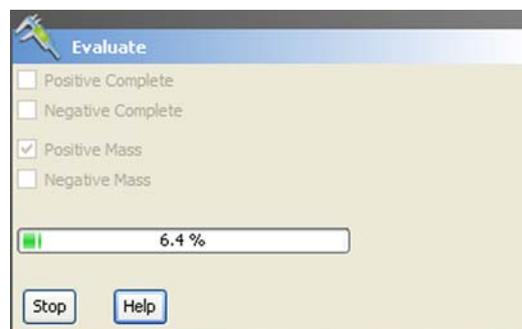


The Exactive MS detector is now ready to perform your measurements.

The Evaluate window of the Tasks panel allows performing an automatic check of the instrument calibration. The following example describes an evaluation for the positive mass calibration.

❖ To evaluate the mass calibration for positive ion mode

1. Click the **On/Off/Standby** button to take the MS detector out of the Standby mode or Off mode and turn it On.
2. Click the **Syringe Pump** button to start the syringe pump.
3. In the Evaluate window, select the Positive Mass check box to evaluate the mass calibration for the positive ion mode.
4. Click **Evaluate** to start the automatic evaluation procedure.
5. The Evaluate window indicates the completed percentage of the evaluation process by a progress bar. See Figure right.
6. A message box informs you when the evaluation procedure is completed. Click **OK** to close the message box. If the evaluation fails, calibrate the Exactive MS detector as described above.

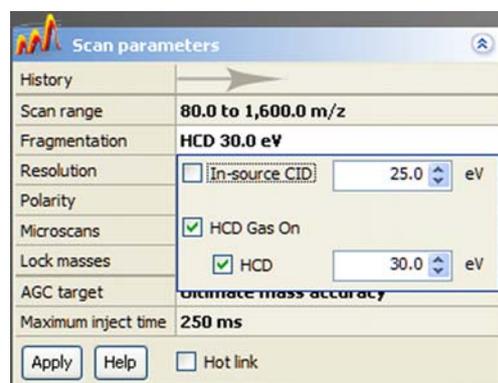


Performing AIF Experiments

Exactive allows performing All Ion Fragmentation (AIF) measurements with in-source CID and with HCD (if this option is installed). The following example describes an AIF fragmentation experiment by an instrument equipped with HCD collision cell.

❖ To set the scan parameters for an AIF fragmentation experiment with HCD

1. If necessary, tune and calibrate your Exactive MS detector as described above.
2. Set the scan parameters in the Scan parameters window:
 - a. Adjust the scan range according to your requirements (from m/z 80 to m/z 1600, for example).
 - b. Click into the Fragmentation field to display the Fragmentation dialog box:
 - i. Select the HCD Gas On check box to enable the HCD gas. See Figure right.
 - ii. Select the HCD check box and enter the desired value for the collision energy (30 eV, for example) into the spin box.
 - c. If necessary, adjust the other scan parameters according to your requirements.
 - d. If you have made any changes in the window, click **Apply** or select the Hot link check box.



Data Acquisition



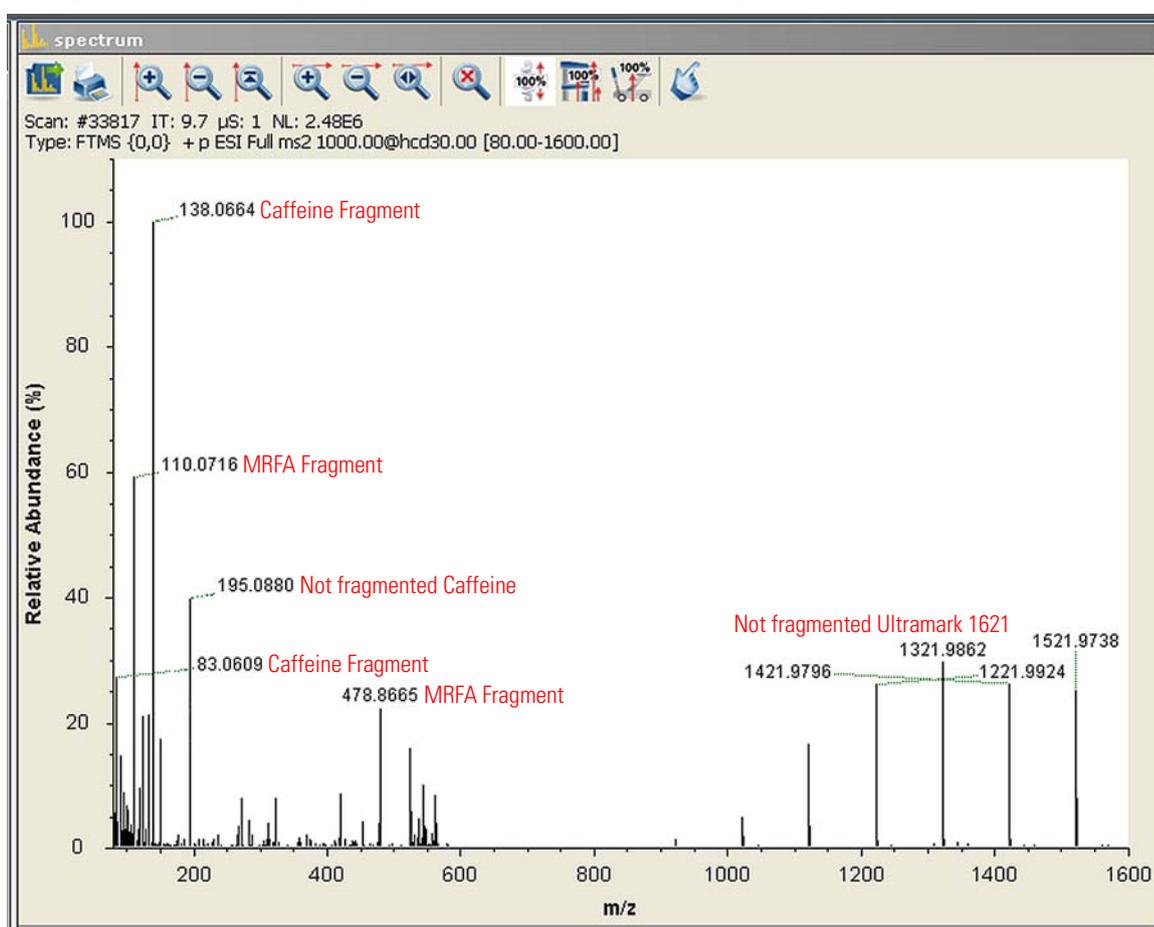
❖ To perform data acquisition

1. Specify the acquisition parameters in the Acquisition window:
 - a. In the Destination file field, type the full path (Drive:\path\file name) of the raw file that is used to store the acquired data. Alternatively, click the  button to the right of the text box to open a dialog box to browse your directories. See Figure right.
 - b. Click into the Acquisition time field to open the dialog box. Select the continuously option button to specify that the acquisition will continue until you click **Stop**. The Acquisition time field shows *continuously*.
2. Click the **On/Off/Standby** button to take the MS detector out of the Standby mode or Off mode and turn it On.
3. Click the **Syringe Pump** button to start the syringe pump.
4. In the Acquisition window, click **Start** to initiate the data acquisition. The spectrum window shows a real-time display of the mass spectrum. The data are saved in the selected raw file.
5. Click **Stop** to stop the acquisition.

Acquisition	
Acquisition state	off
Progress	0.00 min
File in use	unknown
Destination file	C:\Xcalibur\data\ESI_HCD
Method file	by time
Acquisition time	continuously
Sample	
Comment	
On start	don't wait
After acquisition	keep being on

Start Pause Help

The Figure below shows a typical spectrum of an AIF fragmentation experiment with HCD.



Setting up and Running Methods

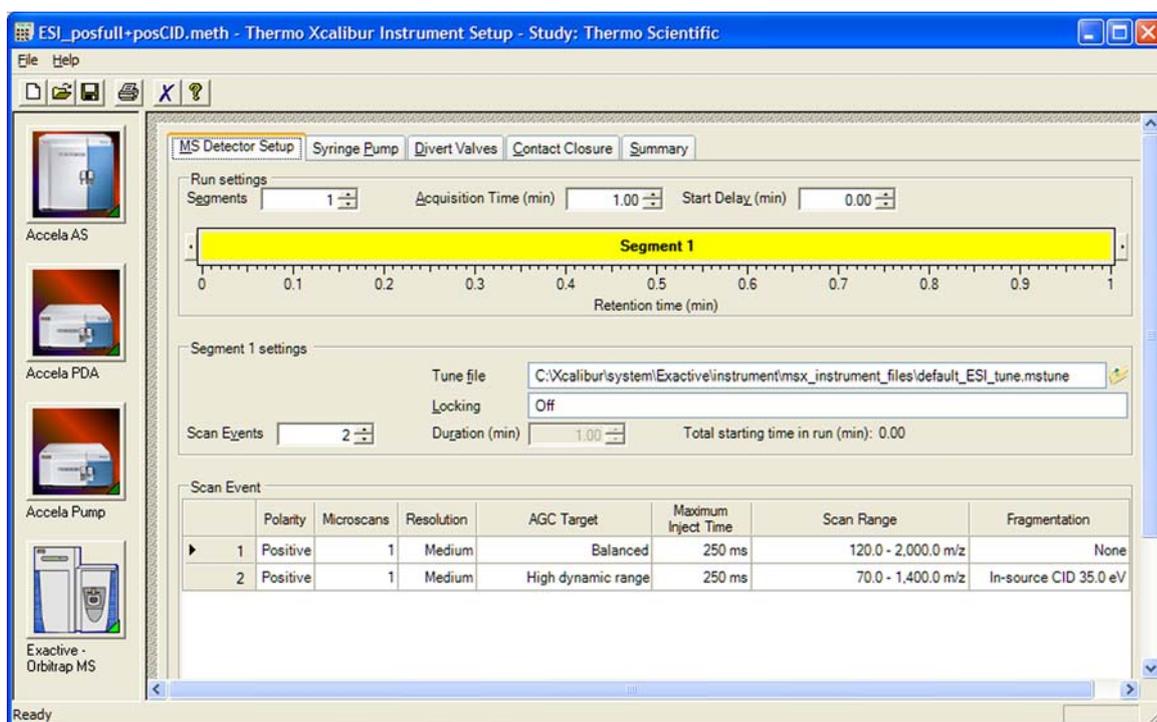
Creating the Instrument Method

In the following example, you can create an instrument method in the Instrument Setup view of Xcalibur. The method consists of one scan segment with two scan events, switching between a positive ion mode full scan and a scan with in-source CID fragmentation. Then use the instrument method to acquire data with the Exactive MS detector. The tune file used in the instrument method is used for data acquisition.

❖ To set up an instrument method

1. Display the MS Detector Setup page in Xcalibur:
 - a. Choose **Start > Programs > Thermo Xcalibur > Xcalibur** to display the Xcalibur Home Page window.
 - b. Choose **GoTo > Instrument Setup** to display the Instrument Setup window.
 - c. If necessary, click on **Exactive - Orbitrap MS** in the View bar to display the New Method page.
 - d. Click on **General MS or MSn** to display the MS Detector Setup page.
2. On the MS Detector Setup page, enter the values for the instrument method:
 - a. Enter the value for the Acquisition Time (1 minute, for example) in the spin box. See Figure below.
 - b. In the Segment settings group box, click on the  button next to the Tune file text field and browse for an existing tune file.
 - c. In the Scan Events spin box, enter 2 as the number of events.
 - d. In the Scan Event table, enter the data as shown in the following table:

	Polarity	Microscans	Resolution	AGC Target	Maximum Inject Time	Scan Range	Fragmentation
1	Positive	1	Medium	Balanced	250 ms	120–2000 m/z	None
2	Positive	1	Medium	High dynamic range	250 ms	70–1400 m/z	In-source CID 35 eV



3. Click  on the toolbar to save the new instrument method:
 - a. In the Save As dialog box, enter the name (ESI_posfull+posCID.meth, for example) and the location for your instrument method. Then click **Save**.
 - b. In the File Summary Information Dialog Box, enter a comment as header information for your instrument method. Then click **OK** to close the dialog box.

The instrument method can now be used to acquire data with your Exactive MS detector, either from the Xcalibur Sequence Setup or from the Exactive Tune window.

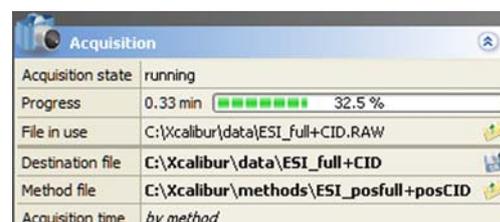
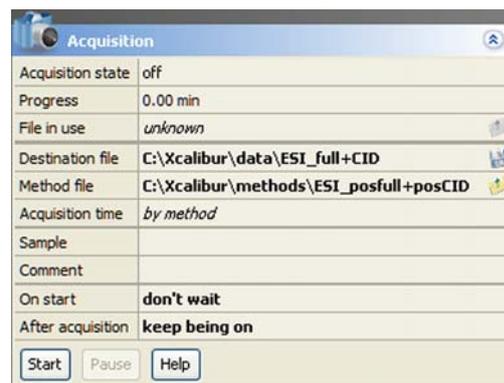
Running the Exactive



In this section, you can use the new instrument method to run the MS detector.

❖ To run the Exactive with an instrument method

1. Specify the acquisition parameters in the Acquisition window of Exactive Tune, as follows:
 - a. In the Destination file field, type the full path (Drive:\path\file name) of the raw file that is used to store the acquired data. See Figure right.
 - b. In the Method file field, click the  button to the right of the field to browse the computer for the instrument method file that was created in the previous topic. The Acquisition time field shows *by method*.
2. Click the **On/Off/Standby** button to take the MS detector out of the Standby mode or Off mode and turn it On.
3. Click the **Syringe Pump** button to start the syringe pump or start a separate pump for infusion experiments.
4. In the Acquisition window, click **Start** to initiate the data acquisition:
 - The Start button is replaced by a Stop button.
 - The Acquisition state changes to *running*.
 - A progress bar indicates the completed percentage of the acquisition. See Figure right.
 - Icons appear on the toolbar to indicate an active data acquisition.
 - If necessary, Exactive Tune appends a time stamp to the name of the destination raw file to prevent overwriting existing files.



When the Scan parameters window and the spectrum window are monitored, it is possible to view how Exactive Tune changes the values according to the settings defined in the instrument method.

The acquisition ends when the acquisition time defined in the instrument method has expired. To display the raw file with the acquired data in Qual Browser, click on the  button in the File in use field.

After Operation

After operating it is recommended to leave the Exactive in Standby mode and not in Off mode to provide the best mass accuracy for the next analysis.