

Q Exactive UHMR QuickStart Guide

About this Guide

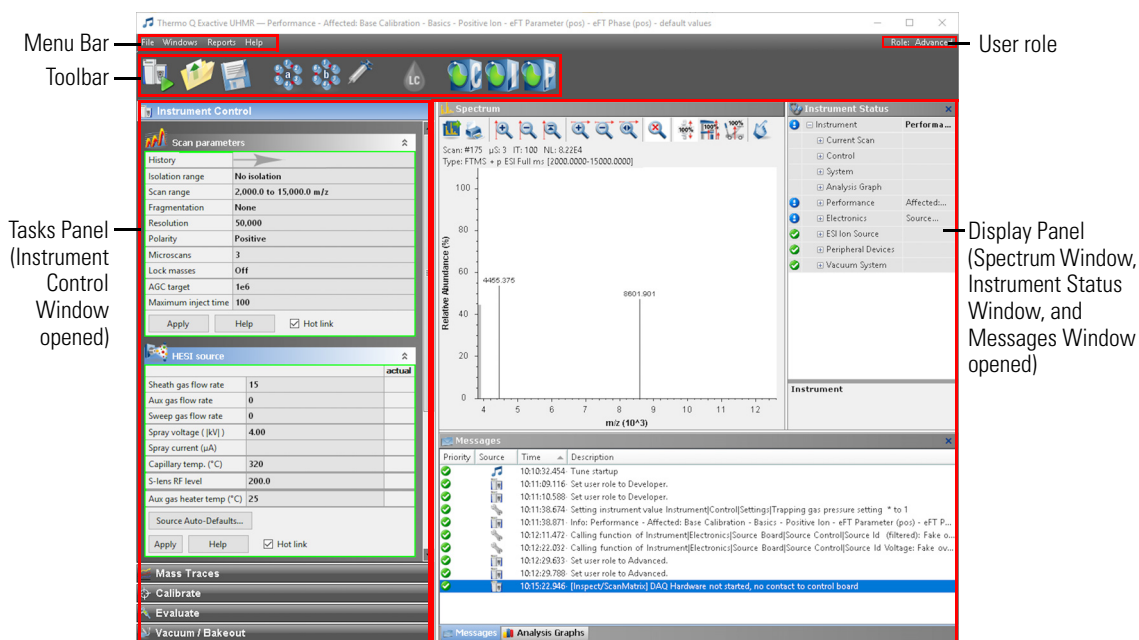
This *Q Exactive UHMR QuickStart Guide* gives an introduction on setting up and using the Thermo Scientific™ Q Exactive™ UHMR mass spectrometer. This guide is intended for all personnel who operate the Q Exactive UHMR mass spectrometer.

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

Q Exactive UHMR Tune

Use the Q Exactive UHMR Tune program to operate the Q Exactive UHMR mass spectrometer via the data system computer. It has the following components:

- The *menu bar* provides drop-down menus with commands for operating the program. On its right side it shows the active *user role*.
- The *toolbar* provides symbol shortcuts for frequently used commands.
- The *tasks panel* provides five windows: Instrument Control, Mass Traces, 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 Q Exactive UHMR Tune program

Choose **Start > Programs > Thermo Exactive Series > Tune**.

Before you go 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 HESI probe is installed on the mass spectrometer. You have checked the proper position of the HESI probe in the Ion Max API source housing.
- Power supply and communication between syringe pump and mass spectrometer are established. A clean, 500 µL Unimetrics syringe filled with CsI calibration solution 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.

Refer to the *Exactive Series Operating Manual* for instructions about preparing the CsI calibration solution.

Preparation





Getting Ions from Infusion Experiments

Setting up Q Exactive UHMR Tune



- The data system computer with the Q Exactive UHMR Tune program is switched on and the mass spectrometer is placed in Standby mode.

For information about performing these tasks, refer to the *Exactive Series Operating Manual*, the *Q Exactive UHMR Software Manual* or the Q Exactive UHMR Tune 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 Q Exactive UHMR mass spectrometer automatically.

❖ To set up the mass spectrometer in the Q Exactive UHMR Tune program for calibrating in the HESI/MS mode

- If you have not already done so, choose **Start > Programs > Thermo Exactive Series> Tune** to start the Q Exactive UHMR Tune program.
- In the Q Exactive UHMR Tune window, on the toolbar, click the **On/Off/Standby** button to take the mass spectrometer out of the Standby mode or Off mode and turn it On. When you turn the mass spectrometer to On, you initiate the following events:
 - The mass spectrometer begins scanning.
 - Nitrogen flows into the HESI probe.
 - The Q Exactive UHMR mass spectrometer applies high voltage to the HESI probe.
 - The spectrum window of Q Exactive UHMR 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 *UHMR_default.mstune*.
 - Click **Open** to open the file. Q Exactive UHMR Tune downloads the Tune Method parameters to the mass spectrometer.
- Examine the pre-tune HESI source settings:
 - From the Instrument Control panel, open the HESI source window. See figure right.
 - Set the *sheath gas flow rate* to 15.
 - Leave the auxiliary gas flow rate at 0.
 - Leave the sweep gas flow rate at 0.
 - Set the *spray voltage* to 4.3 kV.
 - Set the *capillary temperature* to 320 °C.
 - Set the *S-lens RF level* to 200.
 - Set the *auxiliary gas heater temperature* to 25 °C.
 - If you have made any changes in the window, click **Apply**.

Tip 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.

HESI source		actual
Sheath gas flow rate	15	
Aux gas flow rate	0	
Sweep gas flow rate	0	
Spray voltage ([kV])	4.30	
Spray current (µA)		
Capillary temp. (°C)	320	
S-lens RF level	200.0	
Aux gas heater temp (°C)	25	

Source Auto-Defaults... Apply Help ☒ Hot link

- Set the scan parameters for calibration:
 - From the Instrument Control panel, open the Scan parameters window. See figure right.
 - Enter a *scan range* between *m/z 2000 and 15000*.
 - Set *Fragmentation* to *None* to specify that the ion source fragmentation option is turned off.
 - Select *50000* for *Resolution*.
 - Set the *ion polarity mode* to *Positive*.
 - Set the total number of *microscans* to 3.
 - Set *Lock masses* to *Off*.
 - Select *1e6* for *AGC target*.
 - Specify a *maximum injection time* of 100 ms.
 - If you have made any changes in the window, click **Apply** or select the Hot link check box.

Scan parameters	
History	
Isolation range	No isolation
Scan range	2,000.0 to 15,000.0 m/z
Fragmentation	None
Resolution	50,000
Polarity	Positive
Microscans	3
Lock masses	Off
AGC target	1e6
Maximum inject time	100

Apply Help ☒ Hot link

The mass spectrometer is now properly set up for calibration in the HESI/MS mode.

Testing the Mass Spectrometer



In this section, you can test whether the mass spectrometer is operating properly. Infuse a 2 mg/mL CsI calibration solution into the HESI source with the syringe pump. Monitor the real-time display of the mass spectrum to ensure that a stable spray of the calibration solution enters the mass spectrometer.

Refer to the *Exactive Series Operating Manual* for instructions about preparing the CsI calibration solution.

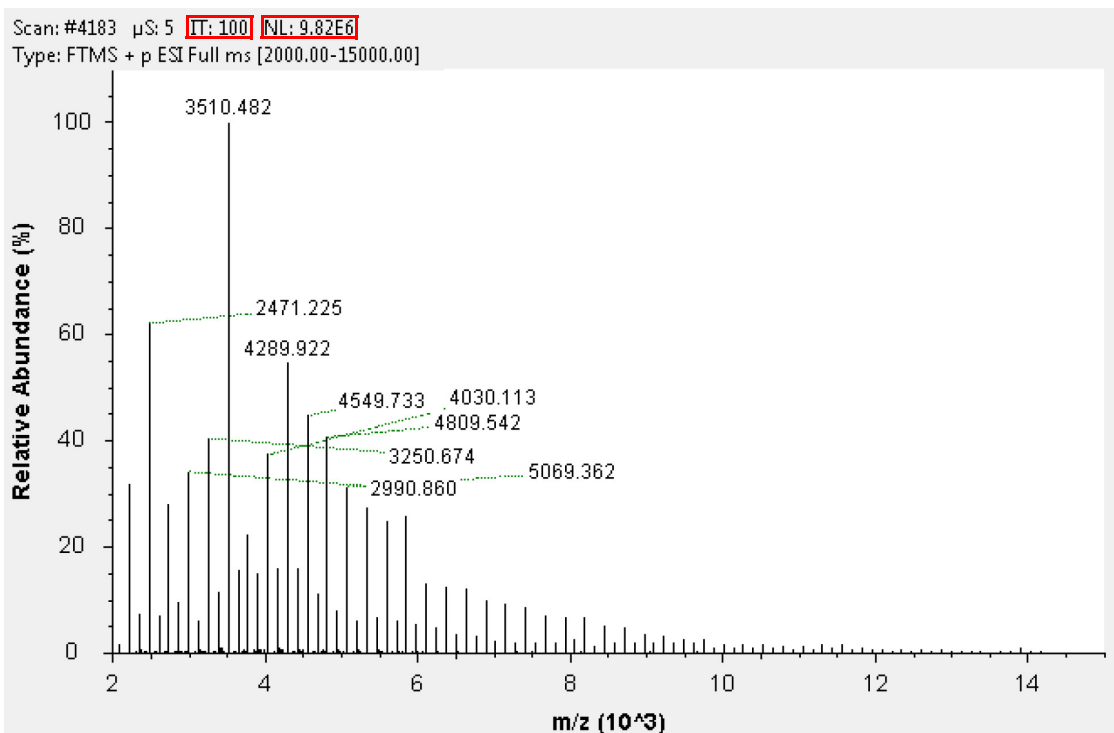
❖ To test the operation of the mass spectrometer in HESI/MS mode

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 mass spectrometer 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
Apply Help	
Manual control Start Stop	

Tip When 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 mass spectrometer. Monitor the data for the calibration solution, as follows:
 - a. In the spectrum window of Q Exactive UHMR Tune, observe the mass spectra of the CsI cluster ions. See figure below.
 - b. At the top of the spectrum window, notice the values for the inject time (IT) and normalized target level (NL).



- 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 10% or less from scan to scan (green check mark). See figure right.

If the answer to both questions in [step 3c](#) is “Yes,” then your mass spectrometer is operating properly.

If the answer to one of these questions is “No,” then try the following troubleshooting measures:

- Make sure that the HESI probe is properly assembled and that the needle position is correct.
- Make sure that the entrance to the ion transfer capillary is clean.
- Make sure 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.
- Make sure that spray voltage is appropriate.
- Make sure that discharge does not take place.

Your mass spectrometer is operating properly in the HESI mode. You are now ready to calibrate the mass spectrometer.

The Q Exactive UHMR mass spectrometer should be calibrated to ensure the mass accuracy of the results. Before you start 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.

Tip If the system was in Off mode before, put the instrument into On mode for at least 90 minutes before you perform a calibration.

The following instructions require that the User role is set to Advanced.

❖ To prepare the instrument for calibration

1. Make sure that you have a clean, unclogged HESI source.
2. Make sure that you have a clean, unclogged ion inlet.
3. If not yet done so, prepare the CsI solution as follows: 2 mg/mL CsI in 50% water : 50% isopropanol.
4. Install the HESI source on the instrument.
5. Load the tune file: *UHMR_Default.mstune*.
6. Load a 500 µL syringe with 2 mg/mL CsI solution. Connect it to the HESI inlet line and install it into the syringe pump.
7. Make sure that the instrument is in Standby mode. Confirm that the source is unclogged by pushing solution through. A droplet should format at the tip of the spray needle. If not, troubleshoot why the solution is not flowing.
8. Confirm the following settings:

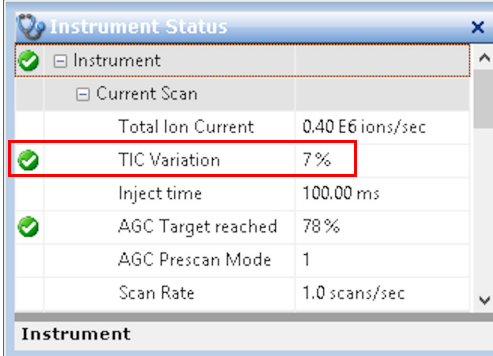
Scan parameters window

Microscans: 5
Scan Range: 2000 to 15000
Maximum Inject Time: 100
Resolution: 12500

Instrument status window

Instrument > Control > Settings > Define Scan >
Misc. > Extended trapping * (eV) UHMR: -1
Detector m/z Optimization *: High m/z
AGC Mode *: fixed

Instrument > Control > Settings > Ion transfer > In-Source Trapping Mode >
In-Source Trapping On/Off *: On
Desolvation Voltage (V) *: -150



Instrument Status	
Instrument	
Current Scan	
Total Ion Current	0.40 E6 ions/sec
TIC Variation	7 %
Inject time	100.00 ms
AGC Target reached	78 %
AGC Prescan Mode	1
Scan Rate	1.0 scans/sec
Instrument	





Instrument > Control > Settings > Ion transfer > Ion Transfer m/z Optimization >
 Ion Transfer > Target m/z: High m/z
 Inj. Fl. RF Ampl. *: 700
 Bent. Fl. RF Ampl. *: 940
 Trans. MP and HCD-cell RF Ampl. *: 900
 C-Trap RF Ampl. *: 2950

Instrument > Control > Settings >
 HCD event > C-Trap Exit Lens---Purge* (V): 2
 Trapping gas pressure setting *: 7

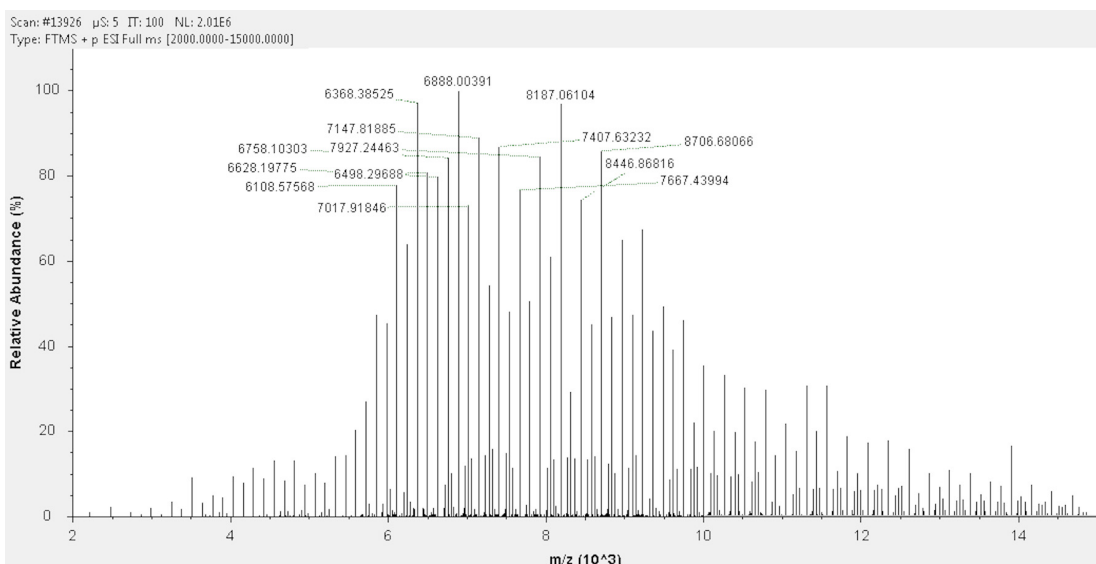
9. Click the **On/Off/Standby** button to take the mass spectrometer out of the Standby mode and turn it On.
10. Click the **Syringe Pump** button to start the syringe pump.
11. Set the Sheath gas flow rate to 15.
12. Set the spray voltage to 4.30 kV.

HESI source		actual
Sheath gas flow rate	15	15
Aux gas flow rate	0	0
Sweep gas flow rate	0	0
Spray voltage (kV)	4.30	4.34
Spray current (μA)		7.00
Capillary temp. (°C)	320	320
S-lens RF level	200.0	
Aux gas heater temp (°C)	100	108

Source Auto-Defaults...

Apply Help ☐ Hot link

13. Confirm that the syringe pump is running at 5 μL/min and that the syringe pump is pushed up against the base of the syringe.
14. Wait approximately 5 minutes for a stable spray. In positive mode, the base peak intensity should be >1E6 at 5 microscans and 100 ms IT. A typical CsI mass spectrum in positive mode is shown in the figure below.



Tip It is CRITICAL to wait for the spray to get stable. For the first few minutes of spraying, the distribution of ions and the base peak intensity will change drastically.

In the CsI Calibration sub-window, select the respective check box to specify a calibration. See figure right.

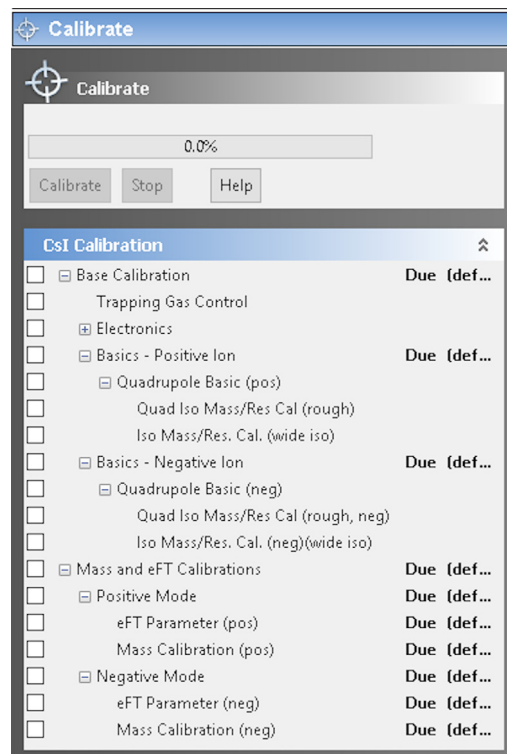
In the Calibrate window, click **Calibrate** to start the automatic calibration procedure:

- The Calibrate window indicates the completed percentage of the calibration process by a progress bar.
- Q Exactive UHMR 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 calibrated parameter.
- The messages window shows a record of the calibration.



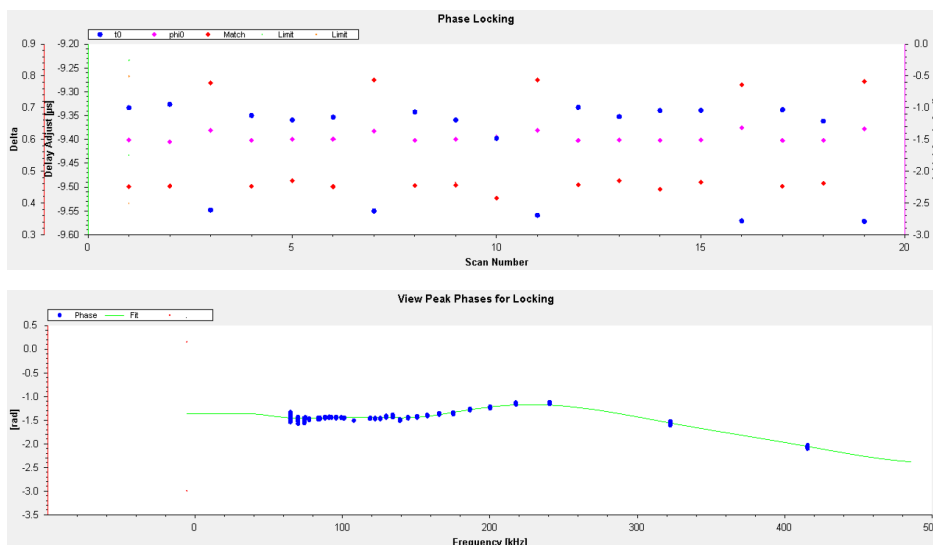
An icon appears on the toolbar to indicate an active procedure.

A message box appears when the calibration procedure is completed. The message box also informs you that Q Exactive UHMR Tune has automatically saved the calibration results. Click **OK** to close the message box.

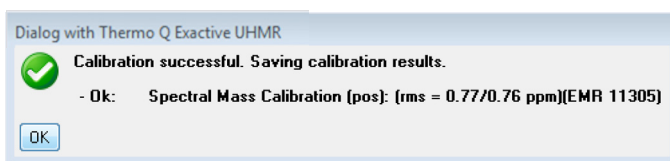
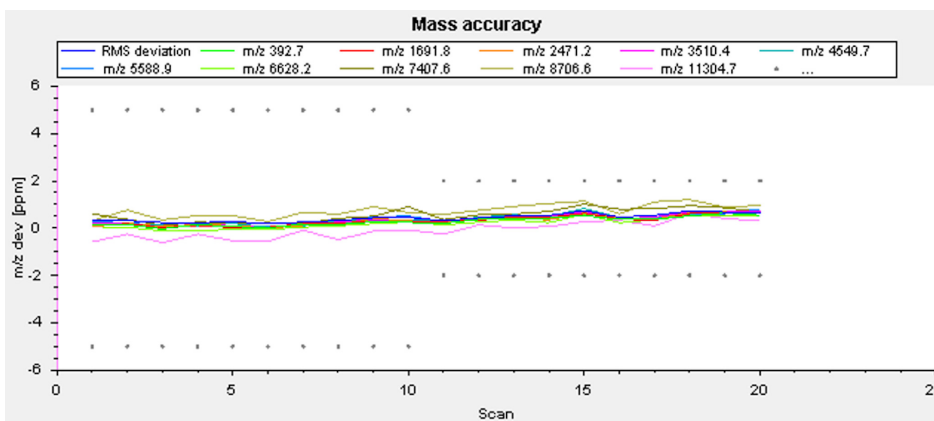


❖ To perform a mass calibration and a quadrupole calibration

1. When the spray is stable (TIC variation of <10% at 5 microscans and 100 ms IT), click the title bar of the Calibrate window to display it.
2. Run the following procedures in the CsI Calibration window:
 - a. Mass and eFT Calibrations > Positive Mode > eFT Parameter (pos).



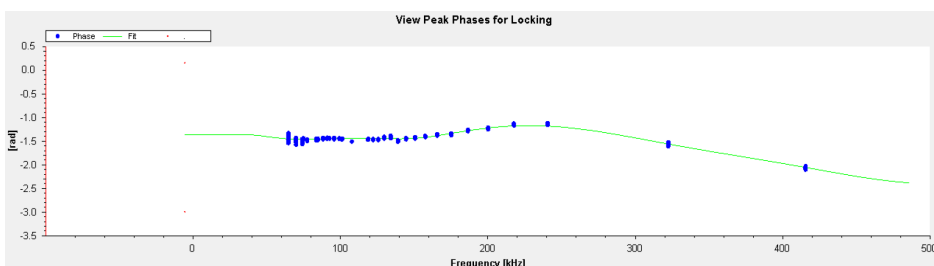
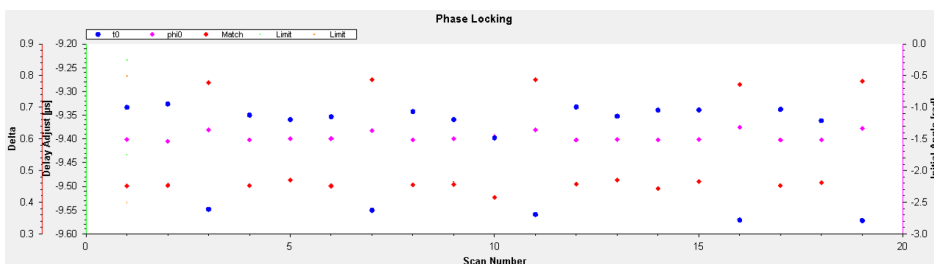
b. Mass and eFT Calibrations > Positive Mode > Mass Calibration (pos).



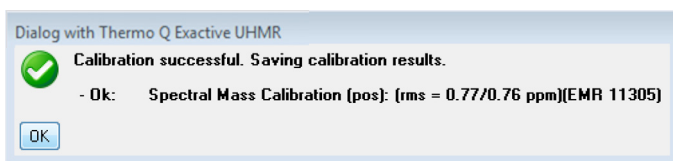
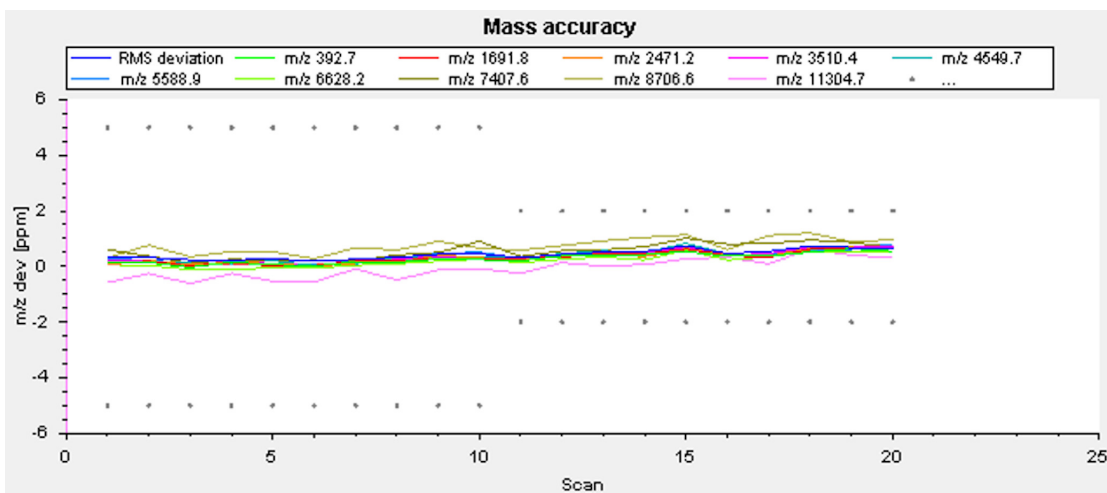
3. Switch to "Low m/z" detector mode: In the Instrument status window, select Instrument > Control > Settings > Define Scan > Detector m/z Optimization: Low m/z.

Tip You must perform the mass calibrations and the eFT calibrations in both low m/z detector mode and high m/z detector mode.

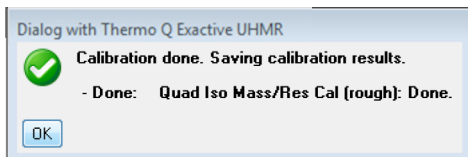
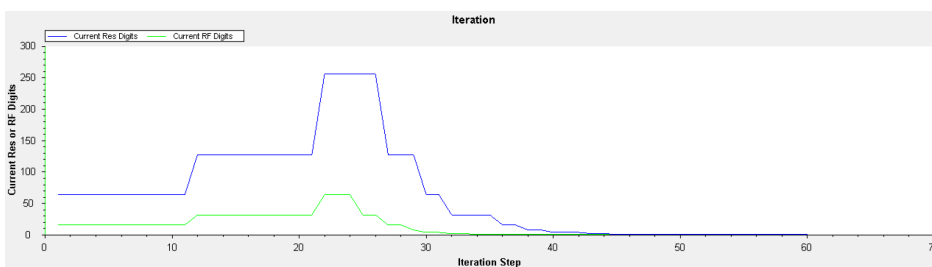
4. All eFT and Mass calibrations must be repeated. Run the following procedures in the CsI Calibration window:
 - a. Mass and eFT Calibrations > Positive Mode > eFT Parameter (pos).



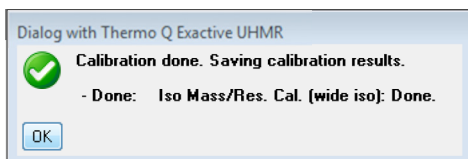
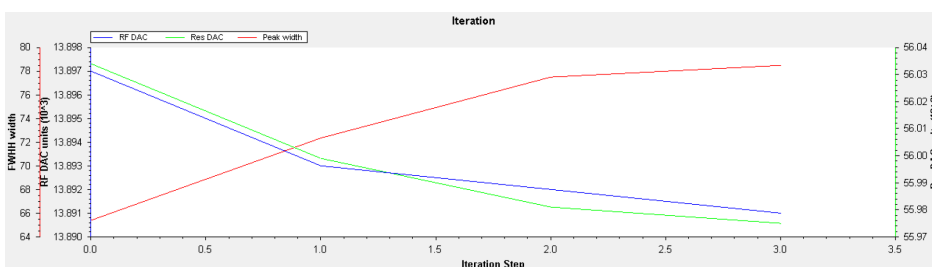
b. Mass and eFT Calibrations > Positive Mode > Mass Calibration (pos).



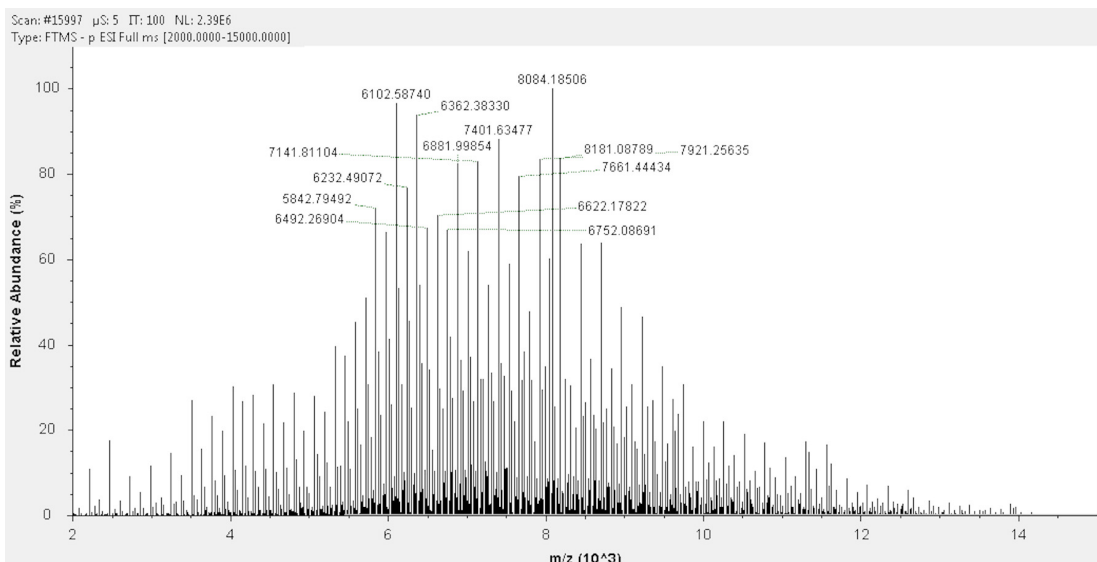
5. Run the rough quad calibration: Base Calibration > Basics–Positive Ion > Quadrupole Basic (pos) > Quad Iso Mass/Res Cal (rough).



6. Run the wide quad calibration: Base Calibration > Basics–Positive Ion > Quadrupole Basic (pos) > Iso Mass/Res. Cal. (wide iso).



7. Switch the polarity from positive to negative. Wait approximately 5 minutes for a stable spray. In negative mode, the base peak intensity should be approximately >5E5 at 5 microscans and 100 ms IT. Also confirm that the sheath gas flow rate is 15 and the spray voltage is 4.30 kV. A typical CsI mass spectrum in negative mode is shown below.



8. Confirm the following settings:

Scan parameters window

Microscans: 5

Scan Range: 2000 to 15000

Maximum Inject Time: 100

Resolution: 12500

Instrument status window

Instrument > Control > Settings > Define Scan >

Misc. > Extended trapping * (eV) UHMR: -1

Detector m/z Optimization *: High m/z

AGC Mode *: fixed

Instrument > Control > Settings > Ion transfer > In-Source Trapping Mode >

In-Source Trapping On/Off *: On

Desolvation Voltage (V) *: 150

Instrument > Control > Settings > Ion transfer > Ion Transfer m/z Optimization >

Ion Transfer > Target m/z: High m/z

Inj. Fl. RF Ampl. *: 700

Bent. Fl. RF Ampl. *: 940

Trans. MP and HCD-cell RF Ampl. *: 900

C-Trap RF Ampl. *: 2950

Instrument > Control > Settings >

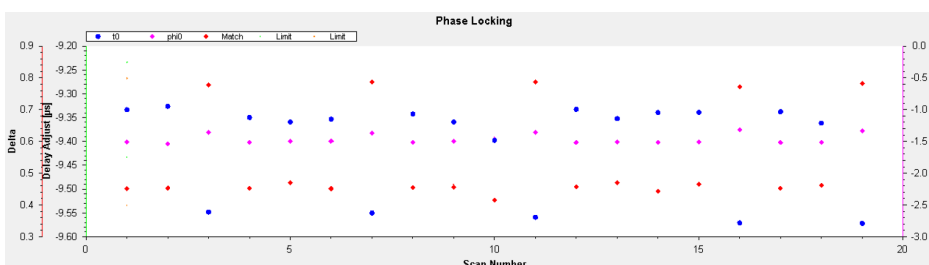
HCD event > C-Trap Exit Lens---Purge* (V): -2

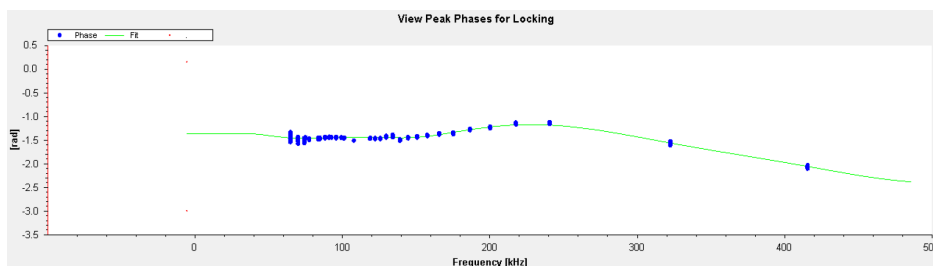
Trapping gas pressure setting *: 7

Tip It is CRITICAL to wait for the spray to get stable. For the first few minutes of spraying, the distribution of ions and the base peak intensity will change drastically.

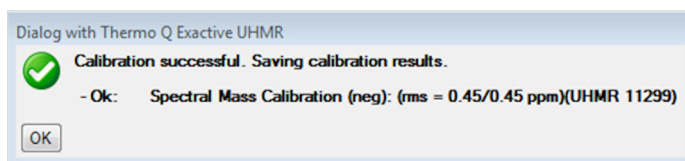
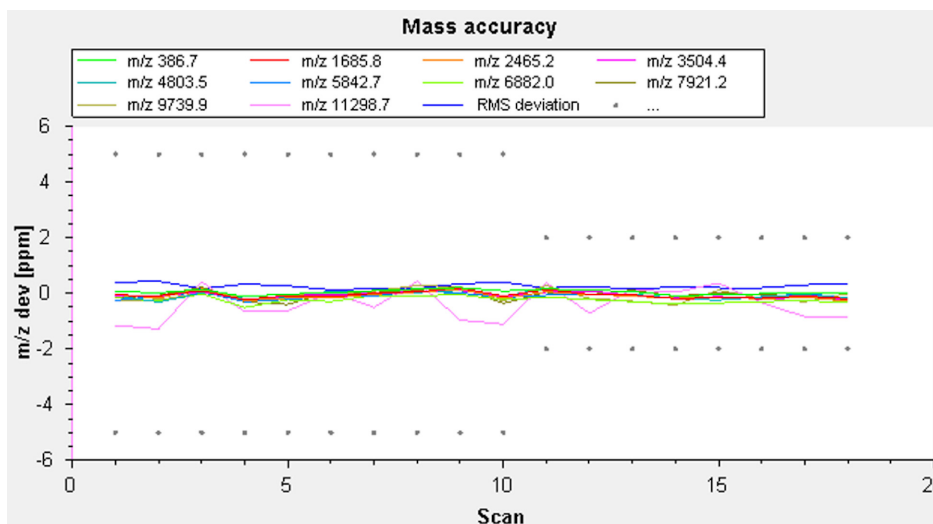
9. When the spray is stable (TIC variation of <10% at 5 microscans and 100 ms IT), run the following procedures in the CsI Calibration window:

a. Mass and eFT Calibrations > Negative Mode > eFT Parameter (neg).





b. Mass and eFT Calibrations > Negative Mode > Mass Calibration (neg)

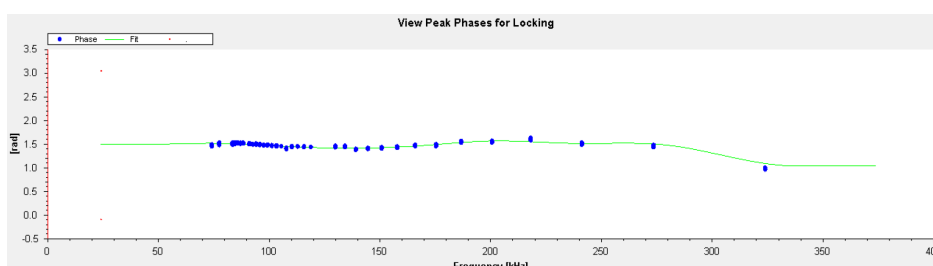
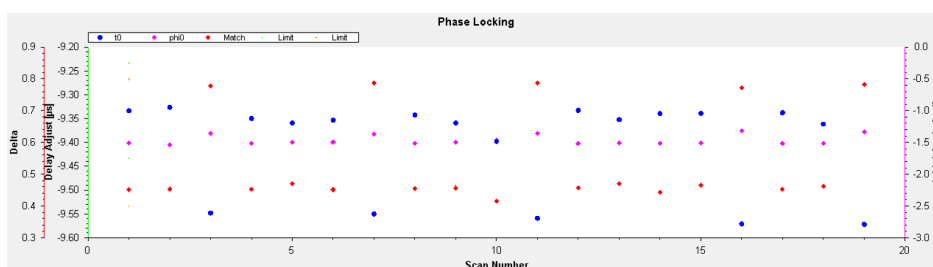


10. Switch to "Low m/z " detector mode: In the Instrument status window, select Instrument > Control > Settings > Define Scan > Detector m/z Optimization: Low m/z .

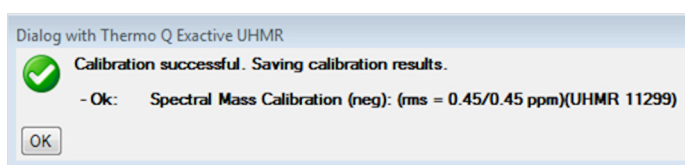
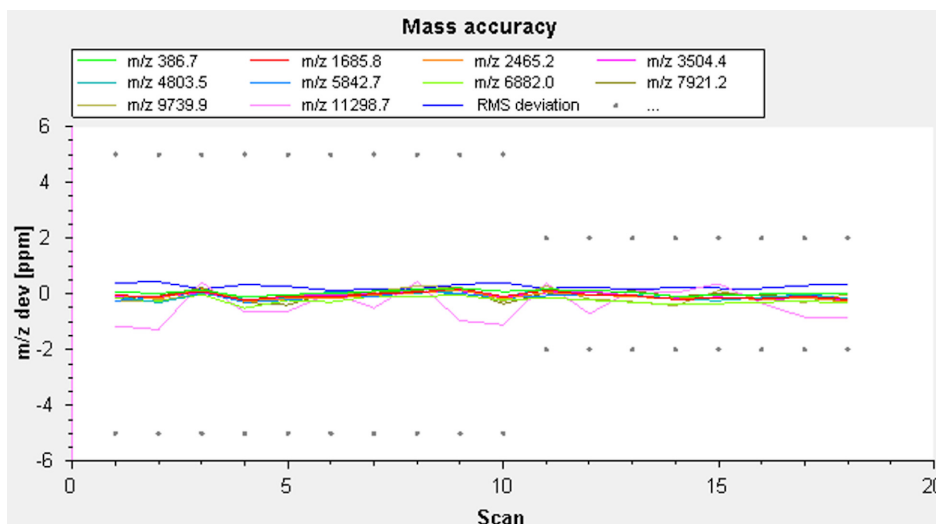
Tip You must perform the mass calibrations and the eFT calibrations in both low m/z detector mode and high m/z detector mode.

11. All eFT calibrations and mass calibrations must be repeated. Run the following procedures in the CsI Calibration window:

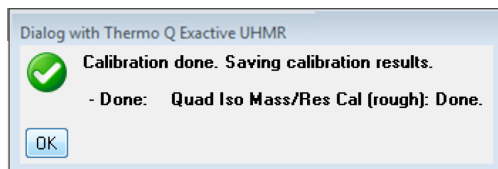
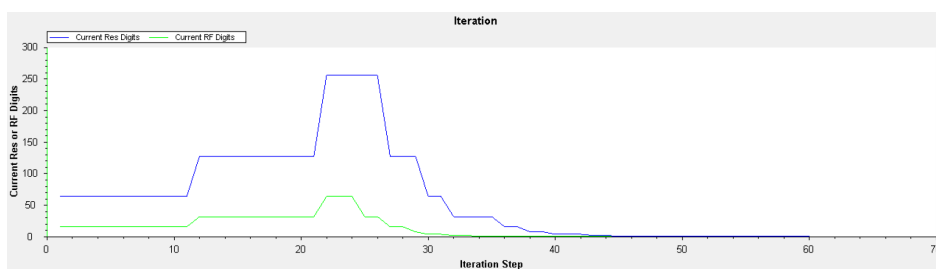
- a. Mass and eFT Calibrations > Negative Mode > eFT Parameter (neg)



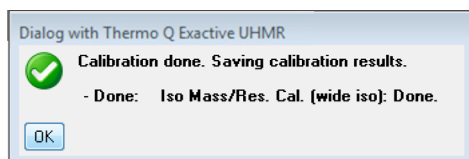
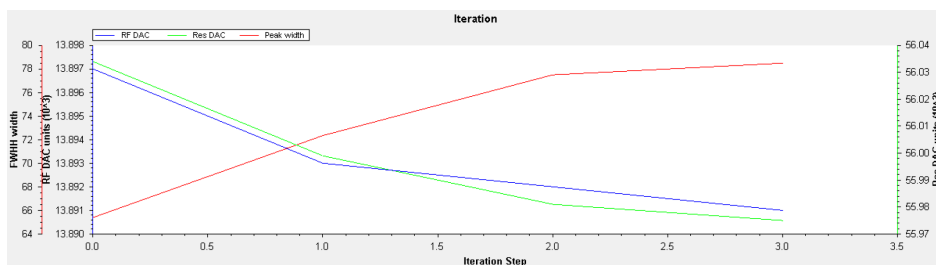
b. Mass and eFT Calibrations > Negative Mode > Mass Calibration (neg)



12. Run the rough quad calibration: Basics–Negative Ion > Quadrupole Basic (neg) > Quad IsoMass/Res Cal (rough, neg).



13. Run the wide quad calibration: Basics–Negative Ion > Quadrupole Basic (neg) > Iso Mass/Res. Cal (neg) (wide iso).



Evaluation

Evaluation of Positive Mass Calibration



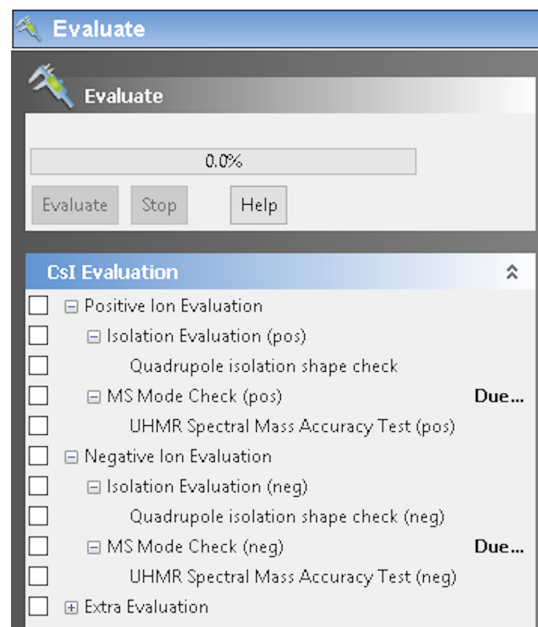
Use the Evaluate window of the Tasks panel to perform an automatic check of the instrument calibration.

The following example describes an evaluation of the positive mass calibration.

❖ To evaluate the mass calibration for positive ion mode

1. Place a clean, 500 µL Unimetrics syringe filled with CsI calibration solution into the syringe pump.
2. Click the **On/Off/Standby** button to take the mass spectrometer out of the Standby mode or Off mode and turn it On.
3. Click the **Syringe Pump** button to start the syringe pump.
4. Load the tune file: *UHMR_Default.mstune*.
5. Click the title bar of the Evaluate window to display it.
6. In the CsI Evaluation sub-window, select the respective check box to evaluate a calibration. See figure right.
7. In the Evaluate window, click **Evaluate** to start the automatic evaluation procedure.
8. The Evaluate window indicates the completed percentage of the evaluation process by a progress bar.
9. A message box informs you when the evaluation procedure is completed. Click **OK** to close the message box.

If this evaluation fails, calibrate the Q Exactive UHMR mass spectrometer as described on [page 4](#).



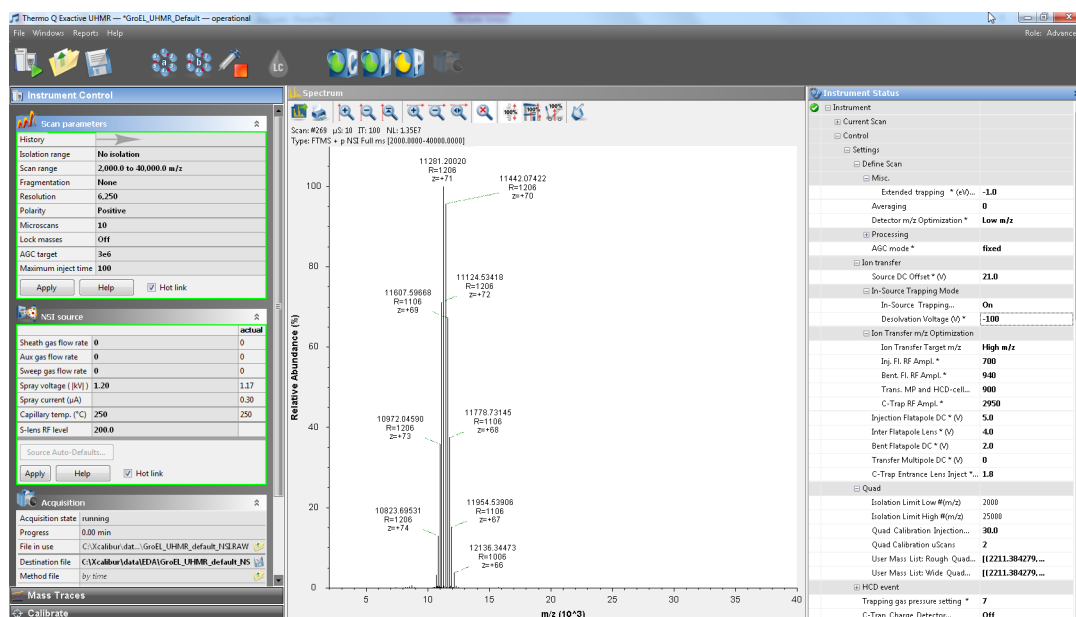
Other Experiment Types

Native Top-Down (Pseudo-MS³) Analysis of the GroEL Protein Complex

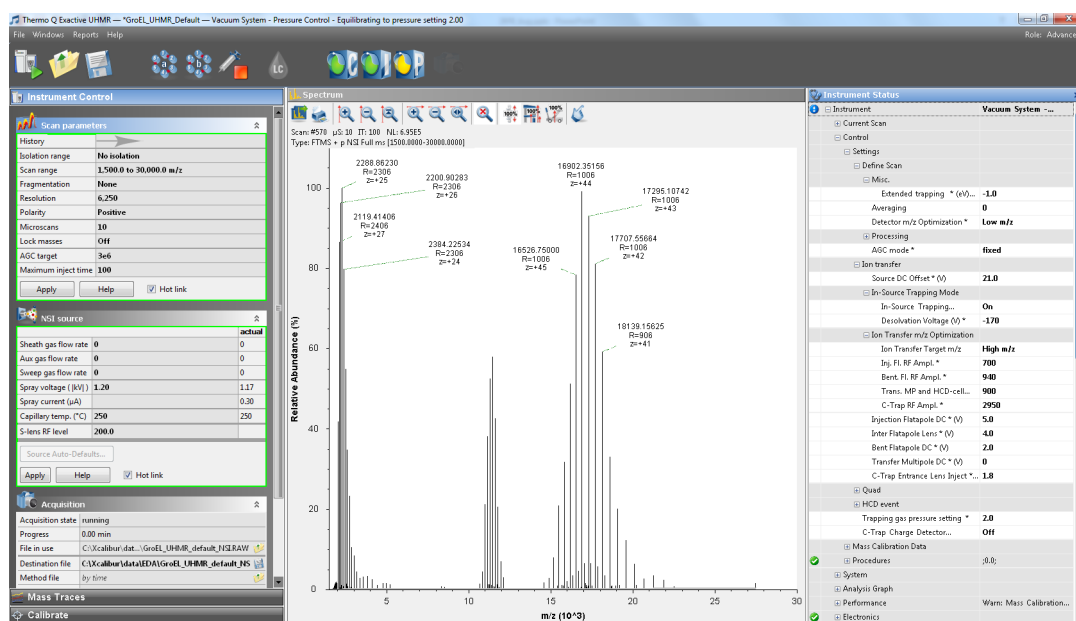
The GroEL protein complex is an 800 kDa molecular chaperone required for the proper folding of many proteins in cells. For the native top-down (pseudo-MS³) analysis of the GroEL protein complex, the static Nanospray Flex ion source is mounted on a Thermo Scientific Q Exactive UHMR mass spectrometer and the GroEL sample (typically 3–5 µL solution of ~2 µM GroEL in 50 mM ammonium acetate) is loaded into a borosilicate emitter (P/N ES387).

A pseudo-MS³ analysis begins by using moderate desolvation energy and transfers the intact protein complex through the mass spectrometer without fragmentation to produce its MS¹ spectrum. Next, dissociation of the protein complex into its subunits in the injection flatpole region yields the MS² spectrum. Following that, quadrupole selection and subsequent fragmentation of individual subunits in the HCD cell provide the MS³ spectrum that can be used for sequence analysis.

For the MS¹ analysis of large protein assemblies like GroEL, load the *GroEL_UHMR_Default.mstune* file from the C:\Xcalibur\methods folder. A mass spectrum similar to the one shown below should be obtained using nitrogen as trapping gas and a trapping gas pressure setting of 7.

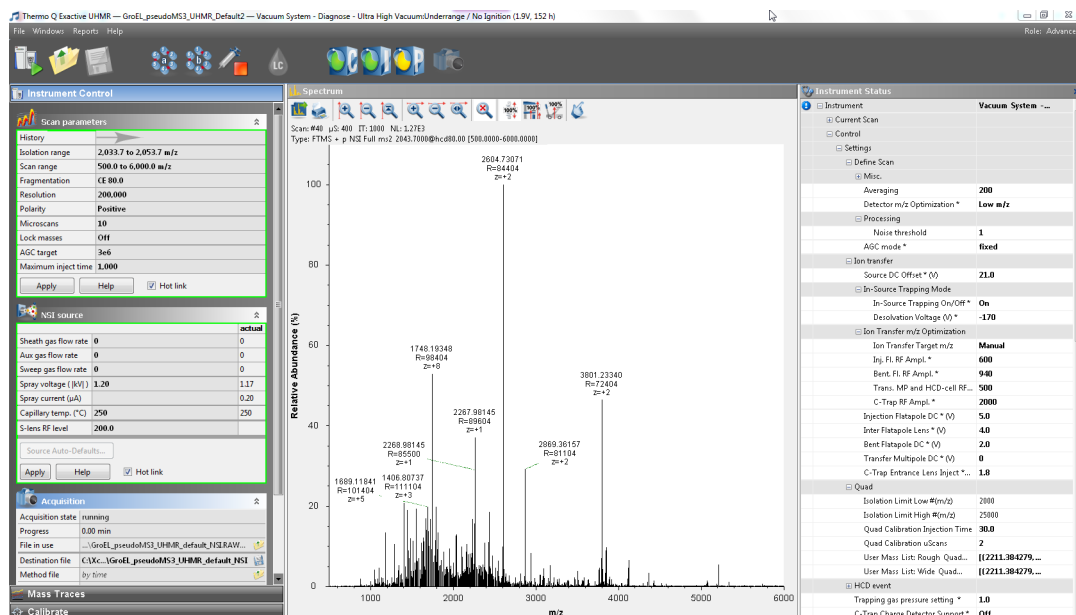


To dissociate the GroEL complex in the injection flatapole region, set the desolvation voltage to -170 V. A mass spectrum similar to the one shown below should be obtained using the Low m/z setting for the Detector m/z Optimization, nitrogen as trapping gas, and a trapping gas pressure setting of 2.



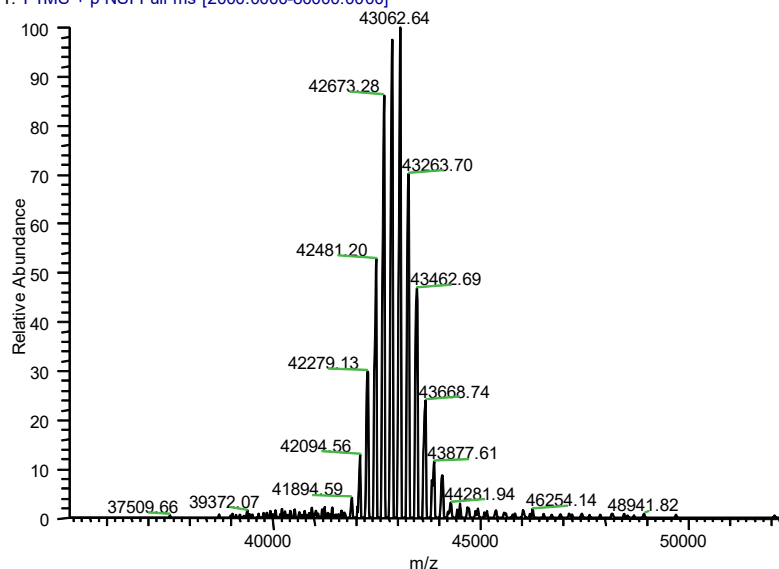
For the quadrupole selection and the HCD fragmentation of the GroEL monomer 28+, load the *GroEL_pseudoMS3_28plus_UHMR_Default.mstune* file from C:\Xcalibur\methods. A mass spectrum similar to the one shown below should be obtained using nitrogen as trapping gas and a trapping gas pressure setting of 1.

Native MS Analysis of the 9.3 MDa Flock House Virus (FHV)



For the native MS analysis of the FHV, the static Nanospray Flex ion source is mounted on a Thermo Scientific Q Exactive UHMR mass spectrometer and the FHV sample (typically 3–5 μL solution of $\sim 2\text{ }\mu\text{M}$ FHV in 50 mM ammonium acetate) is loaded into a borosilicate emitter (P/N ES387). Using the *FHV_SF6_UHMR_Default.mstune* file from the C:\Xcalibur\methods folder, a mass spectrum similar to the one shown below should be obtained when SF₆ is used as trapping gas and the trapping gas pressure is set to 6.

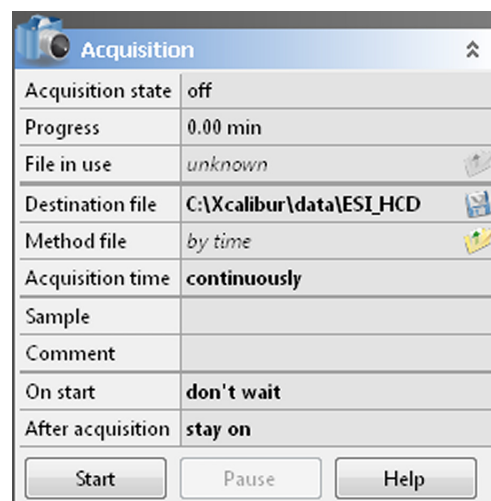
FHV_4 #133 RT: 2.56 AV: 1 NL: 2.69E4
T: FTMS + p NSI Full ms [2000.0000-80000.0000]



After you have entered the scan parameters of your experiment, you can finally start to acquire data.

❖ To perform data acquisition

- Specify the acquisition parameters in the Acquisition window:
 - 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.
 - 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*.
- Click the **On/Off/Standby** button to take the mass spectrometer out of the Standby mode or Off mode and turn it On.
- Click the **Syringe Pump** button to start the syringe pump.
- 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.
- 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	stay on
Start Pause Help	



Setting Up and Running Methods

Creating the Instrument Method

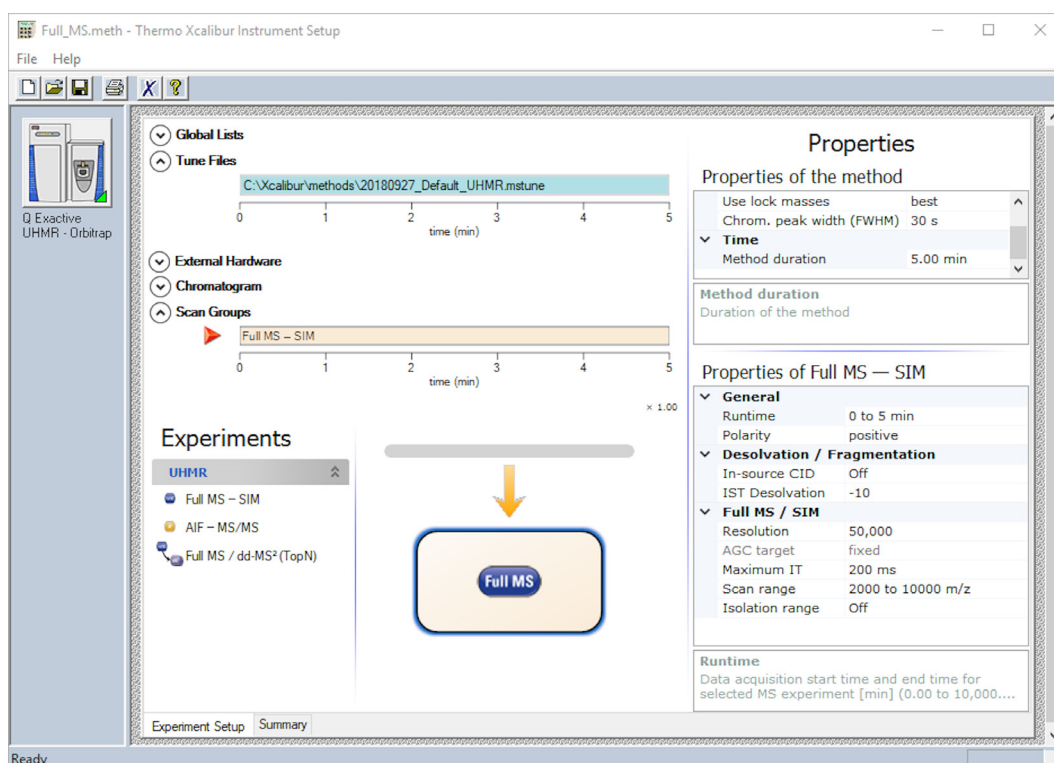
The following example describes how to create an instrument method in the Instrument Setup view of Xcalibur. You can then use the instrument method to acquire data with the Q Exactive UHMR mass spectrometer. The tune file used in the instrument method is used for data acquisition. In the Q Exactive UHMR Tune software, make sure that your tune file has appropriate settings and save it accordingly.

❖ To set up an instrument method

- Display the Instrument Setup page in Xcalibur:
 - Choose **Start > Programs > Thermo Xcalibur > Xcalibur** to display the Xcalibur Home Page window.
 - Choose **GoTo > Instrument Setup** to display the Instrument Setup window.
 - If necessary, click **Q Exactive UHMR - Orbitrap MS** in the View bar to display the Method Editor.
- In the Method Editor, drag an experiment symbol (for example, Full MS - SIM) from the Experiment pane (bottom left) to the gray bar in the Graph pane. A corresponding time bar is displayed in the Scan Groups pane.
- Edit the fields of the Properties pane (right) to set up the experiment parameters. Refer to the *Q Exactive UHMR Software Manual* or the Q Exactive UHMR Tune Help for a description of the available parameters.
- Assign a tune file:
 - On the External Hardware pane, click the  **Tune Files** icon to expand the Tune Files pane.
 - In the Properties of Tunefiles pane, click the Base Tunefile field.
 - Click the  button to open a dialog box.
 - Browse for the tune file and assign it to the experiment.
- Click  on the toolbar to save the new instrument method:
 - In the Save As dialog box, enter the name (Full_MS.meth, for example) and the location for your instrument method. Then click **Save**.
 - 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 Q Exactive UHMR mass spectrometer, either from the Xcalibur Sequence Setup or from the Q Exactive UHMR Tune window. The figure below shows the example of an instrument method that was set up as described above.

Performing Data Dependent Full MS/dd-MS² (TopN) Experiments

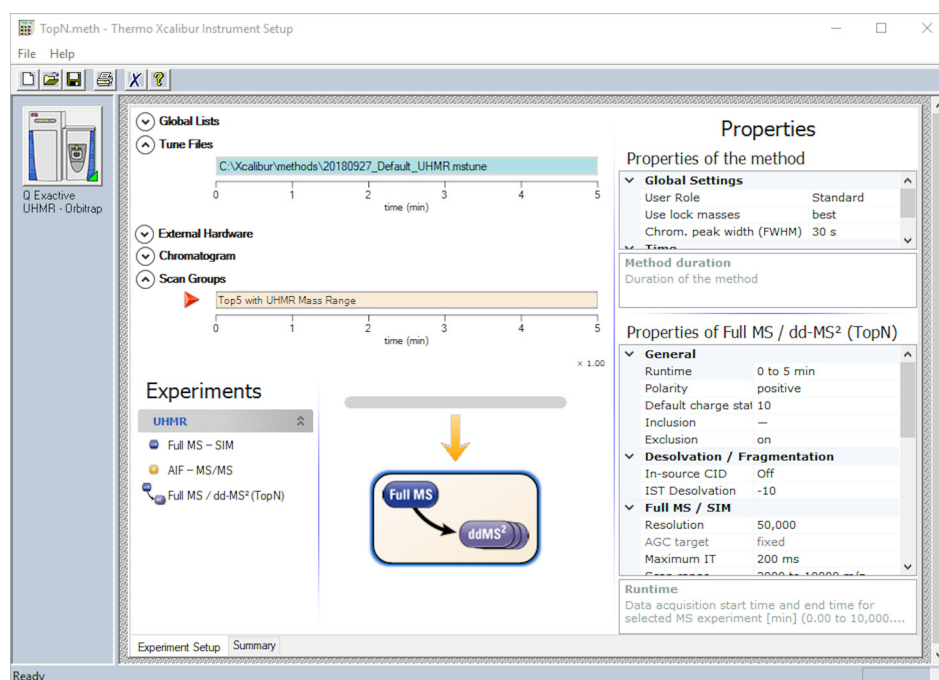


❖ To set the scan parameters for a Full MS/dd-MS² (TopN) Experiment

1. Display the Instrument 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. Click **Q Exactive UHMR - Orbitrap MS** in the View bar to display the Method Editor.
2. In the Method Editor, drag a Full MS/dd-MS² (TopN) symbol from the Experiment panel (bottom left) to the gray bar in the Graph pane. A corresponding time bar is displayed in the Scan Group pane.
3. Edit the fields of the Properties panel (right) to set up the experiment parameters. Refer to the *Q Exactive UHMR Software Manual* or the Q Exactive UHMR Tune Help for a description of the available parameters.
4. Assign a tune file:
 - a. On the Global Settings pane, click the **Tune Files** icon to expand the Tune Files pane.
 - b. In the Properties of Tune files pane, click the Base Tune file field.
 - c. Click the button to open a dialog box.
 - d. Browse for the tune file and assign it to the experiment.
5. Click on the toolbar to save the new instrument method:
 - a. In the Save As dialog box, enter the name (TopN.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 Q Exactive UHMR mass spectrometer, either from the Xcalibur Sequence Setup or from the Q Exactive UHMR Tune window. The figure below shows the example of an instrument method that was set up as described above.

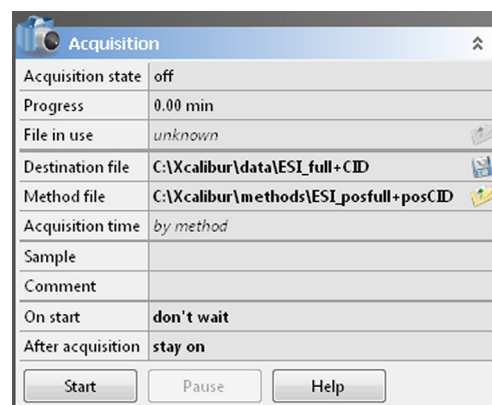
Running the Mass Spectrometer



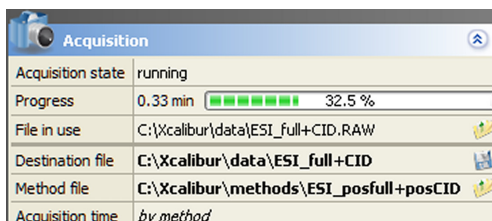
This section shows an example of how to use an instrument method to run the Q Exactive UHMR mass spectrometer.

❖ To run the Q Exactive UHMR mass spectrometer with an instrument method

- Specify the acquisition parameters in the Acquisition window of Q Exactive UHMR Tune, as follows:
 - 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.
 - 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*.
- Click the **On/Off/Standby** button to take the mass spectrometer out of the Standby mode or Off mode and turn it On.
- Click the **Syringe Pump** button to start the syringe pump or start a separate pump for infusion experiments.



- 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, Q Exactive UHMR 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 Q Exactive UHMR 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 the  button in the File in use field.

After Operation

When the measurement is finished, Thermo Fisher Scientific recommends that you leave the Q Exactive UHMR mass spectrometer in Standby mode and not in Off mode to provide the best mass accuracy for the next analysis.