

Q Exactive GC QuickStart Guide

About This Guide

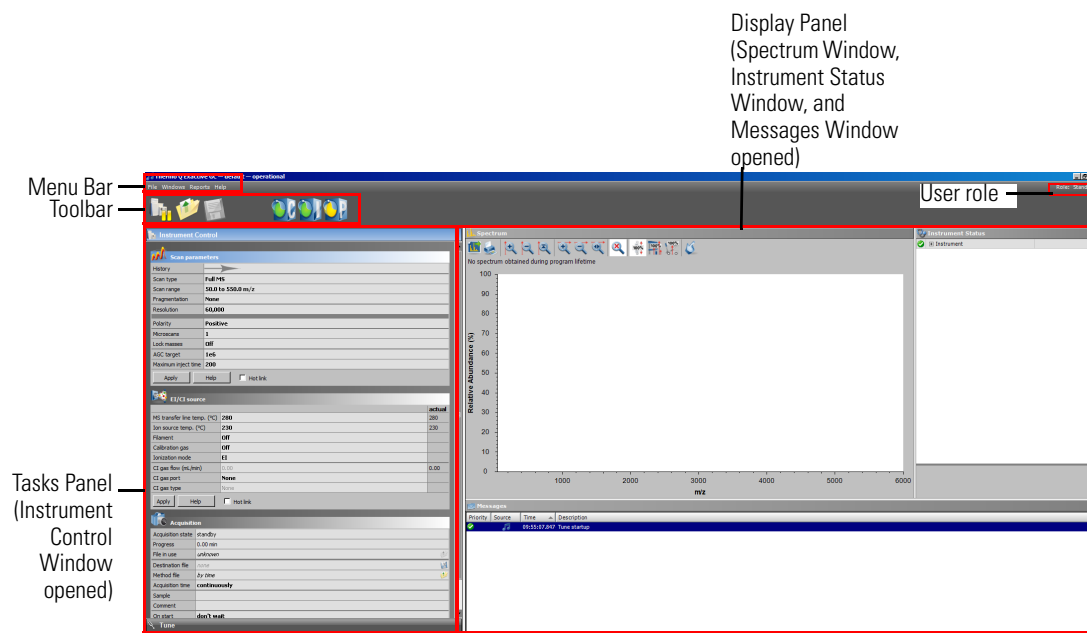
This *Q Exactive GC QuickStart Guide* gives an introduction on setting up and using the Thermo Scientific™ Q Exactive™ GC mass spectrometer. This guide is intended for all personnel who operate the Q Exactive GC mass spectrometer.

Please note that this guide does not contain any safety notices and precautionary statements. Thus, it is mandatory to read also the *Q Exactive GC Operating Manual* and the *Q Exactive GC Preinstallation Requirements Guide*.

Q Exactive GC Tune

Use Q Exactive GC Tune to operate the Q Exactive GC 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 six windows: Instrument Control, Tune, Mass Traces, Calibrate, Evaluate, and Vacuum/Bakeout. The *instrument control window* is divided into three additional windows for defining scan parameters, setting EI/CI 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 GC Tune program

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

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.

Refer to the *Q Exactive GC Operating Manual* for instructions about turning on the GC with vacuum compensation.

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

Preparation



Getting Ions from Experiments

Setting up Q Exactive GC Tune



NOTICE Unless the instrument is undergoing maintenance or service, it should be left in Standby mode when not in use. Power supplies and trap gas are stable in Standby mode. If the instrument is in the Off mode, wait 90 minutes before calibrating the system. ▲

For information about performing these tasks, refer to the *Q Exactive GC Operating Manual*, the *Q Exactive GC Software Manual* or the Q Exactive GC Tune Help.

This topic describes how to test the instrument before you can calibrate your Q Exactive GC mass spectrometer automatically.

❖ To set up the mass spectrometer in the Q Exactive GC Tune software for tuning and calibration

1. If you have not already done so, choose **Start > Programs > Thermo Exactive Series> Tune** to start the Q Exactive GC Tune program.
2. In the Q Exactive GC 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 C-Trap and HCD cell.
 - The spectrum window of Q Exactive GC Tune shows a real-time display of the mass spectrum.
3. Open an existing tune file:
 - a. Choose **File > Load Tune File** to display the Open dialog box.
 - b. Browse for the folder C:\Xcalibur\methods\. Select a tune file.
 - c. Click **Open** to open the file. Q Exactive GC Tune downloads the Tune Method parameters to the mass spectrometer.

A tune file is specific to your particular analyte. You can load the tune file again and use it as a starting point for optimization of the mass spectrometer for a different analyte of interest.

4. Examine the pre-tune EI/CI source settings:
 - a. From the Instrument Control panel, open the EI/CI source window. See the figure on the right.
 - b. The default *MS transfer line temperature* is 250 °C.

NOTICE The MS transfer line temperature does not need to be set to the maximum oven program temperature because the outlet of the column in the MS transfer line is at reduced pressure. ▲

- c. The default *ion source temperature* is 200 °C.

NOTICE The default settings for the ions source and MS transfer line temperature are 200 °C and 250 °C. These are the minimum recommended temperatures for a typical analysis with the installed test column. For best robust operation when running samples in matrix, it is recommended to set the ion source temperature higher than the MS transfer line temperature. For example, maximum settings recommended for of high boiling analytes in matrix are: ion source temperature set to 350 °C and MS Transfer line set to 280 °C. ▲

- d. Turn the *filament* to *On*.
- e. Set the *calibration gas* to *EI*.
- f. Set the *ionization mode* to *EI* or *CI* depending on the installed ion volume.
- g. If using CI mode, set the *CI gas flow* to 1.5 mL/min.
- h. Set the *CI gas port* to A or B as installed.
- i. See the *Q Exactive GC Operating Manual* for instructions to configure the CI gas type installed at Ports A and B.
- j. If you have made any changes in the window, click **Apply**.

NOTICE 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. ▲

EI/CI source			actual
MS transfer line temp. (°C)	250		250
Ion source temp. (°C)	200		200
Filament	On		
Calibration gas	EI		
Ionization mode	EI		
CI gas flow (mL/min)	0.00		0.00
CI gas port	None		
CI gas type	None		
Apply Help ☐ Hot link			

Testing the Mass Spectrometer

5. Set the scan parameters for tuning and calibration:

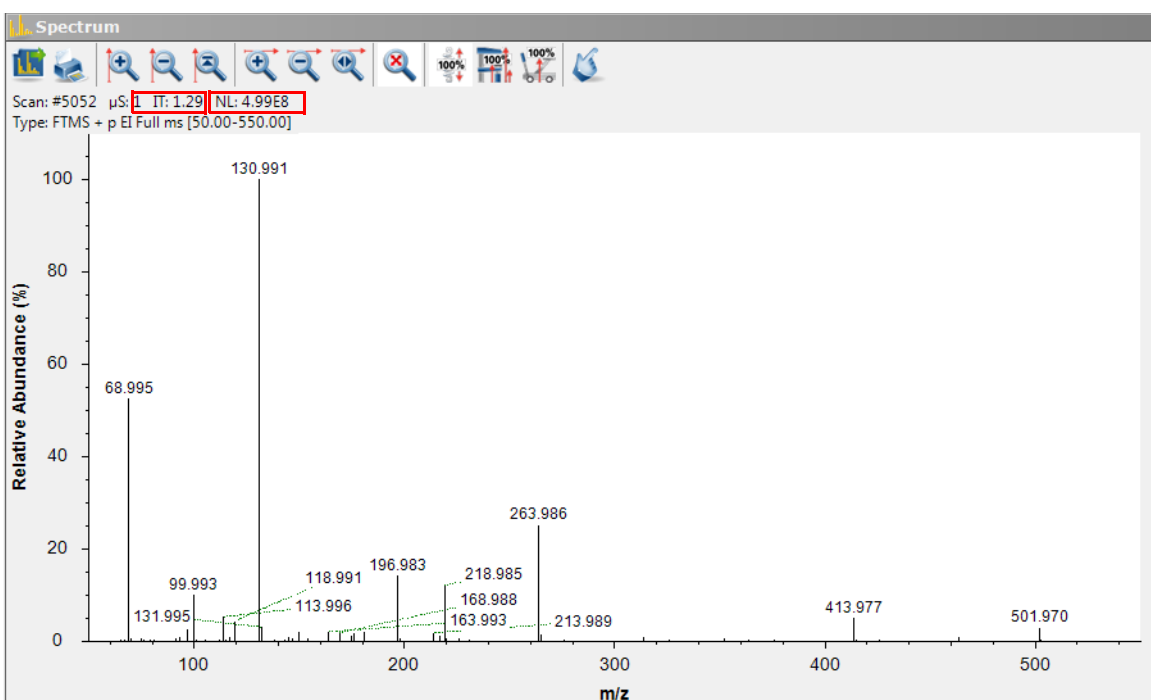
- From the Instrument Control panel, open the Scan parameters window. See the figure on the right.
- Set *Scan type* to *Full MS*.
- Enter a *scan range* between *m/z* 50 and 550.
- Set *Fragmentation* to *None* to specify that the HCD fragmentation option is turned off.
- Select 60000 for *Resolution*.
- Set the *ion polarity mode* to *Positive*.
- Set the total number of *microscans* to 1.
- Set *Lock masses* to *Off*.
- Select 1e6 for *AGC target*.
- Specify a *maximum injection time* of 50 ms.
- If you have made any changes in the window, click **Apply** or select the Hot link check box.

Scan parameters	
History	
Scan type	Full MS
Scan range	50.0 to 550.0 m/z
Fragmentation	None
Resolution	60,000
Polarity	Positive
Microscans	1
Lock masses	Off
AGC target	1e6
Maximum inject time	50
Apply Help ☒ Hot link	

The mass spectrometer is now properly set up for tuning and calibration in the EI mode.

In this section, you can test whether the mass spectrometer is operating properly. Admit a low flow of calibration gas for EI mode—containing perfluorotributylamine. Monitor the real-time display of the mass spectrum to ensure that the signal is stable.

- At the top of the spectrum window, notice the values for the inject time (IT) in milliseconds and the normalized target level (NL). Reasonable performance on a clean, tuned system in EI mode shows the Inject Time is typically less than 5 ms and the height of the tallest peak is typically greater than 1-e8. See figure below.



- Observe the values for NL and IT in the spectrum window. Check the ion current and readback values while scanning calibration gas:

- Is the signal present?
- Is the signal stable? Check in the instrument status window that the TIC variation has stabilized at 10% or less from scan to scan (green check mark). See the figure on the right.

instrument status	
☒ Instrument	
☒ Current Scan	
Total Ion Current	53.64 E6 ions/sec
TIC Variation	10%

If you answered “yes” to the questions in [step 2](#), then your mass spectrometer is operating properly.

Mass Calibration

Positive Ion Mode Calibration



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

- Ensure that the EI ion volume is installed in the ion source cartridge.
- Ensure that the ion source cartridge is properly inserted into the ion source block.
- Ensure that the transfer line nut is not leaking air. It will leak if cooled from high to low temperature quickly.

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

The Q Exactive GC mass spectrometer 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 ion signal: Focus on the injection time (IT) and on the normalized level (NL) in the header information on the spectrum window.

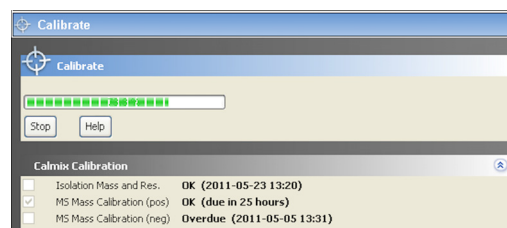
NOTICE If the system was in Off mode before, it is necessary to put the instrument into On mode for at least 90 minutes before a mass calibration is performed. The filament and calibration gas do not need to be on during this time. ▲

❖ To calibrate the Q Exactive GC mass spectrometer for positive ion mode

1. Click the **On/Off/Standby** button to take the mass spectrometer out of the Standby mode or Off mode and turn it On.

NOTICE Any time the system is placed in standby or off, the calibration gas and filament are turned off. The Calibration gas will show the last level set (EI or CI) when the system was on, when in standby or off mode and the displayed level will be set on again when the system is set On. The filament will show off when placed in Standby or Off mode and it must be set to on in order for the filament to turn on when setting the system to On. The filament and calibration gas may be turned on when in standby mode by making the appropriate selection and selecting apply or using the Hot link.

2. Turn the **Filament** on and set the **Calibration Gas** to EI.
3. Click the title bar of the Calibrate window to display it.
4. In the Calmix Calibration sub-window, select the MS Mass Calibration (pos) check box to specify a mass calibration for the positive ion mode. See the figure on the right.
5. In the Calibrate window, click **Calibrate** to start the automatic calibration procedure:

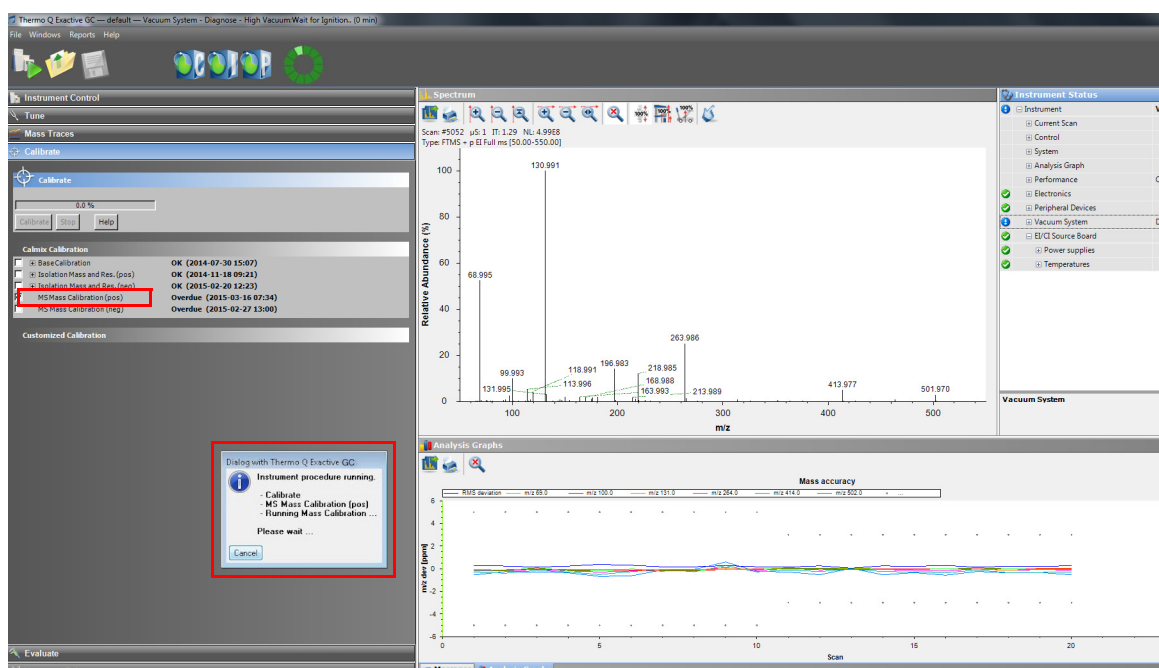


- The Calibrate window indicates the completed percentage of the calibration process by a progress bar.
 - Q Exactive GC Tune displays a message box with information about the active calibration procedure and the currently calibrated parameter.
 - The analysis graphs window displays a graphic representation of the calibration process for the currently tuned parameter.
 - The messages window shows a record of the calibration.
 - A link to a report of the calibration is available in the **Reports** menu.
6. An icon appears on the toolbar to indicate an active procedure.

Negative Ion Mode Calibration



Isolation Mass and Resolution Calibration



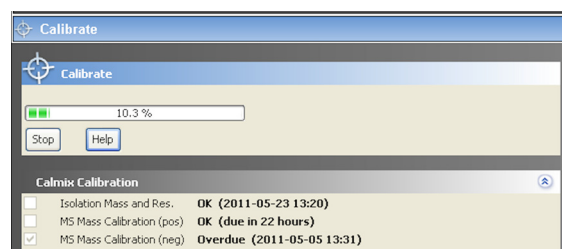
7. A message box appears when the calibration procedure is completed. The message box also informs you that Q Exactive GC Tune has automatically saved the calibration results. Click **OK** to close the message box.

Before you can start analyzing samples in MS/MS mode, you must ensure that the quadrupole isolation performance for the narrow isolation window is sufficient. Therefore, evaluate the isolation calibration for the positive ion mode as described on [page 6](#).

Before performing measurements in the negative ion mode, the mass spectrometer must be set up for and calibrated in NCI.

❖ To calibrate the Q Exactive GC mass spectrometer for negative ion mode

1. Click the **On/Off/Standby** button to take the mass spectrometer out of the Standby mode or Off mode and turn it On.
2. Insert the CI ion volume into the ion source cartridge and reinsert the ion source cartridge.
3. Change the **CI Gas Type** to methane. Use methane gas for all built-in calibrations.
4. Set the **CI Gas Flow** to 1.25 mL/min.
5. Turn on the **Filament** and CI level calibration gas.
6. In the spectrum window of Q Exactive GC Tune, observe the mass spectra of the singly-charged ions of the negative ion mode calibration gas.
7. Click the title bar of the Calibrate window to display it.
8. In the Calmix Calibration sub-window, select the MS Mass Calibration (neg) check box to specify a mass calibration for the negative ion mode.
9. In the Calibrate window, click **Calibrate** to start the automatic calibration procedure.
10. The Calibrate window indicates the completed percentage of the calibration process by a progress bar. See the figure on the right.
11. A message box informs you when the calibration procedure is completed. Click **OK** to close the message box.

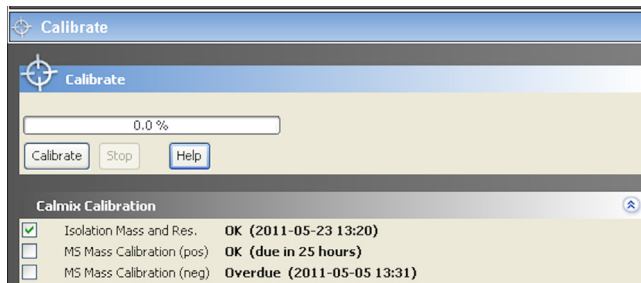


If the evaluation of the quadrupole isolation as described on [page 6](#) fails, you must calibrate isolation mass and resolution of the quadrupole. This ensures that the quadrupole isolation performance for the narrow isolation window is sufficient for analyses. The following example describes this calibration procedure for the positive ion mode.



❖ To calibrate isolation mass and resolution of the quadrupole

1. Click the **On/Off/Standby** button to take the mass spectrometer out of the Standby mode or Off mode and turn it On.
 2. Insert the CI ion volume into the ion source cartridge and reinsert the ion source cartridge.
 3. Change the **CI Gas Type** to methane. Use methane gas for all built-in calibrations.
 4. Set the **CI Gas Flow** to 1.25 mL/min.
 5. Turn on the filament and CI level calibration gas.
 6. Click the title bar of the Calibrate window to display it.
 7. In the Calmix Calibration sub-window, select the Isolation Mass and Res. check box. See the figure on the right.
 8. In the Calibrate window, click **Calibrate** to start the automatic calibration procedure.
 9. The Calibrate window indicates the completed percentage of the calibration process by a progress bar.
 10. A message box informs you when the calibration procedure is completed. Click **OK** to close the message box.
- The Q Exactive GC mass spectrometer is now ready to perform your measurements.



Evaluation

Evaluation of Positive Mass Calibration

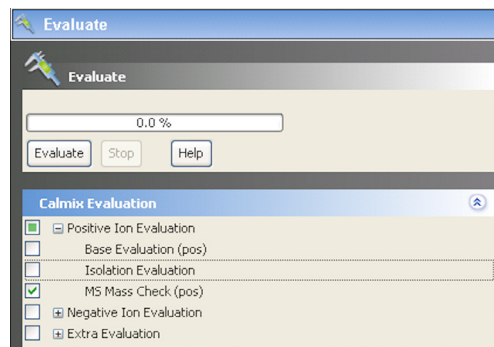


Use the Evaluate window of the Tasks panel to perform an automatic check of the instrument calibration.

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

❖ To evaluate mass calibration for positive ion mode

1. Click the **On/Off/Standby** button to take the mass spectrometer out of the Standby mode or Off mode and turn it On.
2. Turn on the filament and calibration gas.
3. Click the title bar of the Evaluate window to display it.
4. In the Calmix Evaluation sub-window, select the MS Mass Check (pos) check box to evaluate the mass calibration for the positive ion mode. See the figure on the right.
5. In the Evaluate window, click **Evaluate** to start the automatic evaluation procedure.
6. The Evaluate window indicates the completed percentage of the evaluation process by a progress bar.
7. 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 GC mass spectrometer as described on [page 4](#).

This example describes the evaluation of the isolation calibration for the positive ion mode.

❖ To evaluate the isolation calibration for positive ion mode

1. Click the **On/Off/Standby** button to take the mass spectrometer out of the Standby mode or Off mode and turn it On.
2. Turn on the filament and calibration gas.

Evaluation of Isolation Calibration

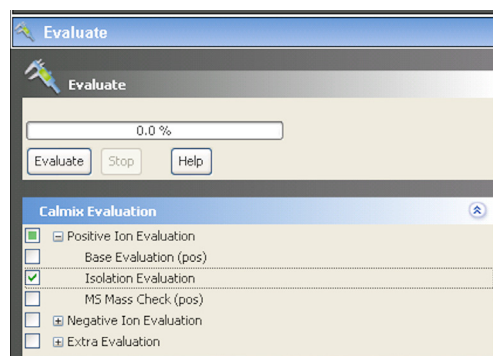


Other Experiment Types

Performing MS/MS Scans

- Click the title bar of the Evaluate window to display it.
- In the Calmix Evaluation sub-window, select the Isolation Evaluation check box. See the figure on right.
- In the Evaluate window, click **Evaluate** to start the automatic evaluation procedure.
- The Evaluate window indicates the completed percentage of the evaluation process by a progress bar.
- A message box informs you when the evaluation procedure is completed. Click **OK** to close the message box.

If this evaluation fails, please perform an “Isolation Mass and Resolution” calibration as described on [page 5](#).

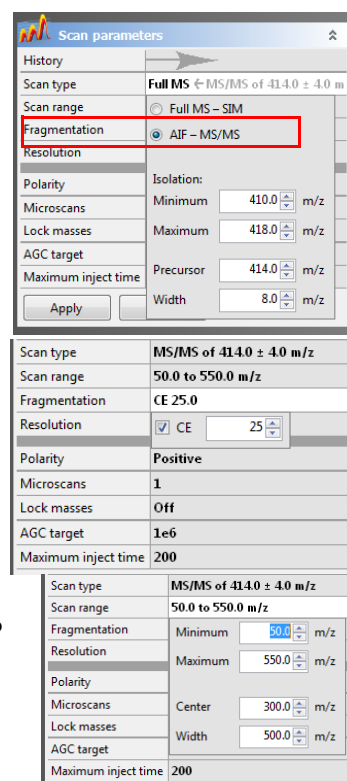


In the previous examples, the Q Exactive GC mass spectrometer operates in the full scan mode. The following examples show the mass spectrometer operating in other scan modes.

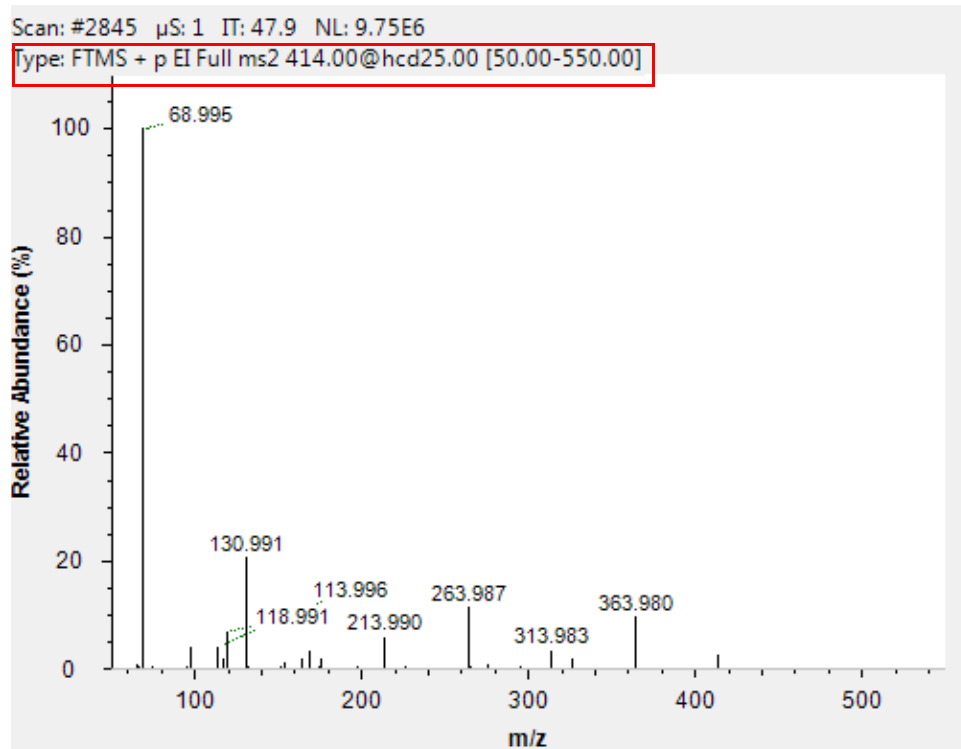
The following example shows an MS/MS experiment. Precursor ions that were selected by using the quadrupole are sent to the HCD collision cell of the Q Exactive GC mass spectrometer. Here, they are fragmented with Collision Energy (CE)

❖ To perform an MS/MS experiment

- If necessary, calibrate your Q Exactive GC mass spectrometer as described above.
 - Set the scan parameters in the Scan parameters window:
 - In the Scan type field, select **AIF-MS/MS**. See the figure on the right.
 - In the Isolation area, enter a value for the precursor mass (m/z 414, for example).
 - Enter a width not larger than 10 amu (m/z 8, for example). Note that the scan type changes to MS/MS.
 - Click into the Fragmentation field to display the Fragmentation dialog box.
 - Select the HCD check box and enter the desired value for the collision energy (45, for example) into the spin box. See the figure on the right.
 - Click into the Scan Range field to display the Scan Range dialog box.
 - Adjust the scan range according to your requirements (from m/z 50 to m/z 550, for example). See the figure on the right.
 - If necessary, adjust the other scan parameters according to your requirements.
 - Click **Apply** or select the Hot link check box.
- Perform the data acquisition as described on [page 8](#).



The figure below shows an example of a typical MS/MS spectrum.



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

❖ To perform data acquisition from Tune

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 the figure on the 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 mass spectrometer out of the Standby mode or Off mode and turn it On.
3. Turn on the filament.
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	standby
Progress	0.00 min
File in use	C:\Xcalibur\data\tune.RAW
Destination file	C:\Xcalibur\data\tune
Method file	by time
Acquisition time	continuously
Sample	
Comment	
On start	don't wait
After acquisition	stay on
Start Pause Help	



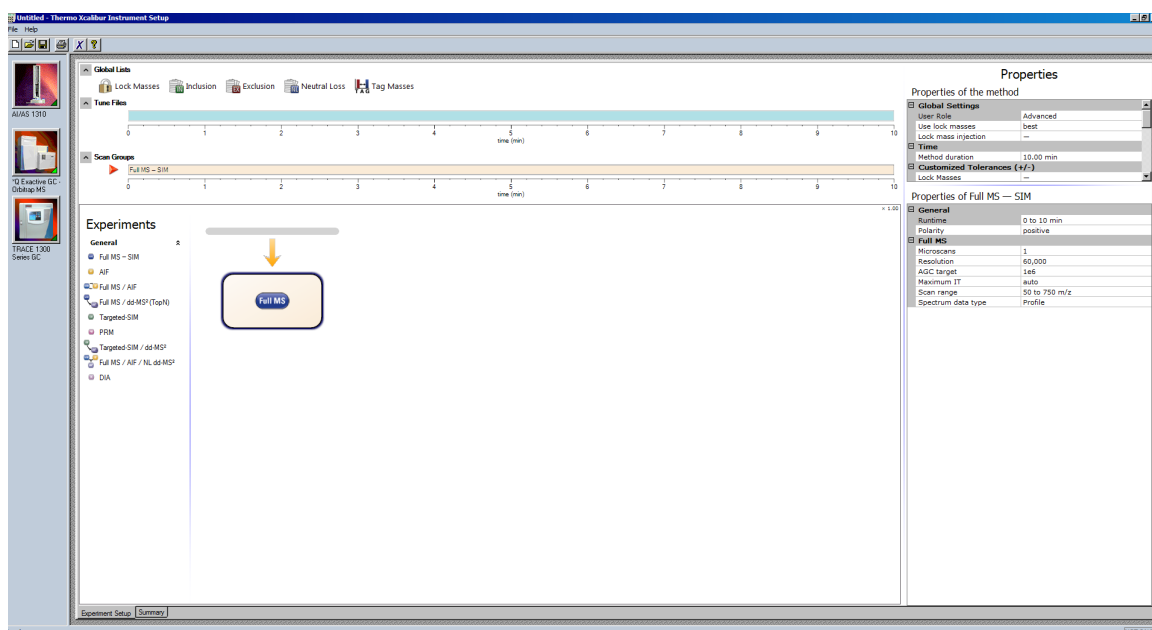
The following example describes how to create an instrument method in the Instrument Setup view of the Thermo Scientific™ Xcalibur™ software application. You can then use the instrument method to acquire data with the Q Exactive GC mass spectrometer. The tune file used in the instrument method is used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

Creating the Instrument Method

❖ 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 GC - 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 Experiments pane (lower 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 GC Software Manual* or the Q Exactive GC 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 (for example, ab.mstune) 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 GC mass spectrometer, either from the Xcalibur Sequence Setup or from the Q Exactive GC Tune window. The next figure shows the example of an instrument method that was set up as described above.



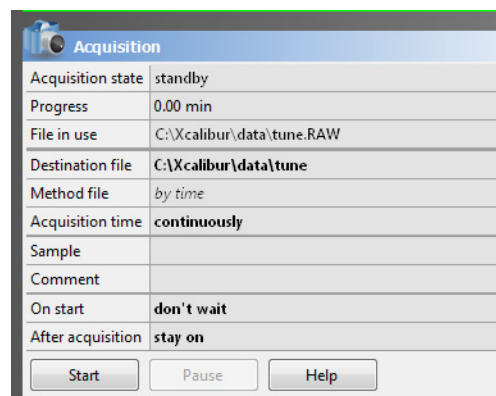
Running the Q Exactive GC Mass Spectrometer



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

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

1. Specify the acquisition parameters in the Acquisition window of Q Exactive GC 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 the figure on the 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 mass spectrometer out of the Standby mode or Off mode and turn it On.



Acquisition	
Acquisition state	standby
Progress	0.00 min
File in use	C:\Xcalibur\data\tune.RAW
Destination file	C:\Xcalibur\data\tune
Method file	by time
Acquisition time	continuously
Sample	
Comment	
On start	don't wait
After acquisition	stay on
Start Pause Help	

If necessary, Q Exactive GC 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 GC 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 leaving the Q Exactive GC mass spectrometer in Standby mode and not in Off mode to provide the best mass accuracy for the next analysis.