

## Exactive GC Tune

Use Exactive GC Tune to operate the 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 Exactive GC Tune program

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

## 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.

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

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

**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 and Exactive GC Operating Manual*, the *Exactive GC Software Manual* or the Exactive GC Tune Help.

## Getting Ions from Experiments

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

### Setting up Exactive GC Tune

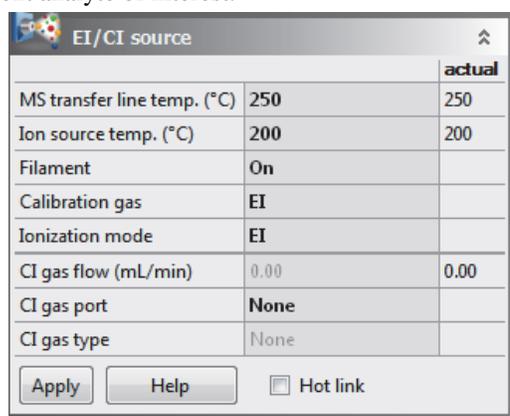
#### ❖ To set up the mass spectrometer in the Exactive GC Tune software for tuning and calibration

1. If you have not already done so, choose **Start > Programs > Thermo Exactive Series > Tune** to start the Exactive GC Tune program.

2.  In the 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 Exactive GC Tune shows a real-time display of the mass spectrum.
3. Open an existing tune file:
- a. Chose **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. 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.



		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

**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. ▲

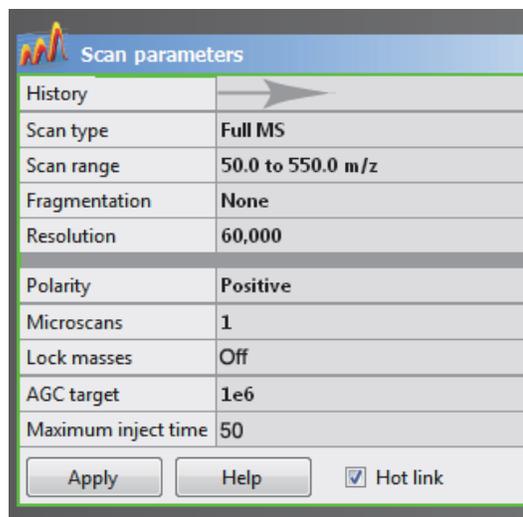
- d. Turn the *filament* to *On*.
- e. Set the *calibration gas* to *EI*.
- f. Set the *ionization mode* to *EI* or *CI* depending on the installed ion volume.
- g. If using *CI* mode, set the *CI gas flow* to *1.5 mL/min*.
- h. Set the *CI gas port* to *A* or *B* as installed.
- i. See the *Q Exactive GC and 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. ▲

5. Set the scan parameters for tuning and calibration:
- a. From the Instrument Control panel, open the Scan parameters window. See the figure on the right.
  - b. Set *Scan type* to *Full MS*.
  - c. Enter a *scan range* between *m/z 50 and 550*.
  - d. Set *Fragmentation* to *None* to specify that the HCD fragmentation option is turned off.
  - e. Select *60000* for *Resolution*.

- f. Set the *ion polarity mode* to *Positive*.
- g. Set the total number of *microscans* to *1*.
- h. Set *Lock masses* to *Off*.
- i. Select *1e6* for *AGC target*.
- j. Specify a *maximum injection time* of *50 ms*.
- k. If you have made any changes in the window, click **Apply** or select the Hot link check box.

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

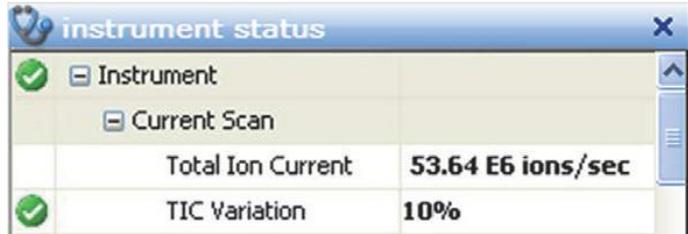


Scan parameters	
History	→
Scan type	Full MS
Scan range	50.0 to 550.0 m/z
Fragmentation	None
Resolution	60,000
Polarity	Positive
Microscans	1
Lock masses	Off
AGC target	1e6
Maximum inject time	50
Apply    Help <input checked="" type="checkbox"/> Hot link	

## Testing the Mass Spectrometer

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.

1. 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 modes shows the Inject Time is typically less than 5 ms and the height of the tallest peak is typically greater than  $1 \times 10^{-8}$ . See figure below.
2. 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.

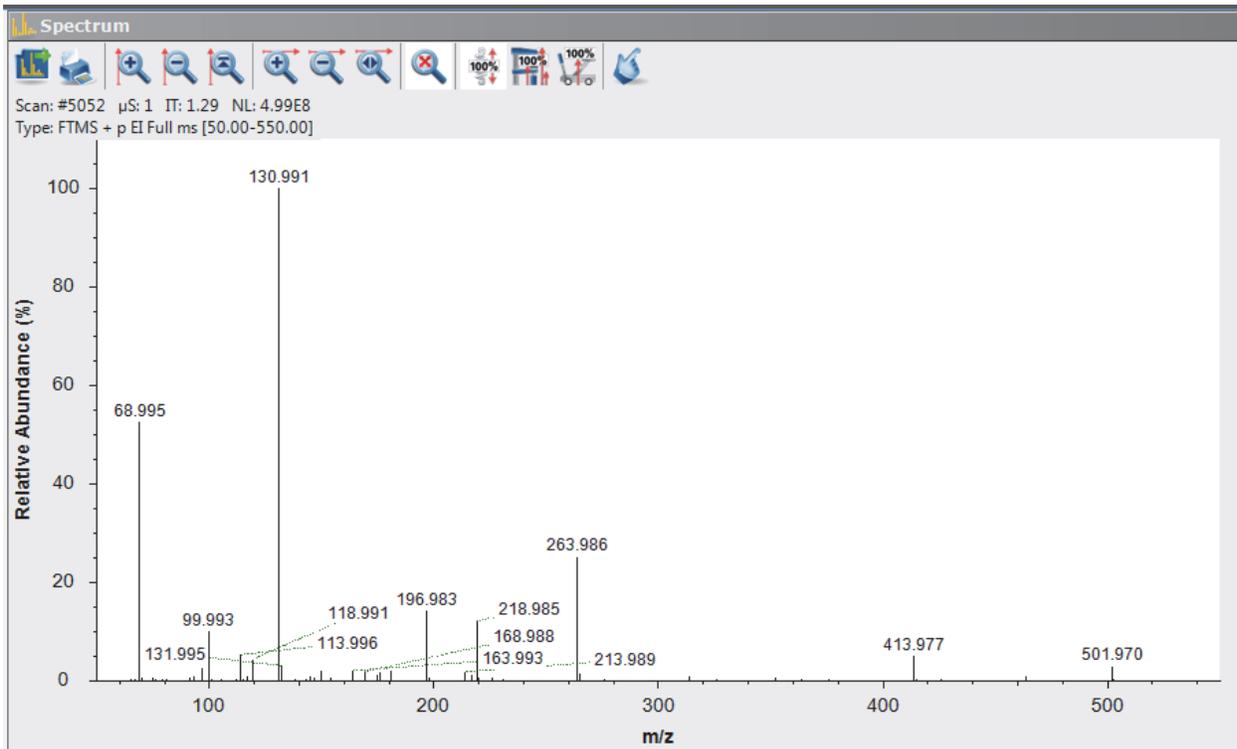


instrument status	
Instrument	
Current Scan	
Total Ion Current	53.64 E6 ions/sec
TIC Variation	10%

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

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 (nearly) ready to calibrate the mass spectrometer.

## Leak Check the MS system

The Exactive GC mass spectrometer needs to be “leak free” to ensure that background noise remain as low as possible. To conduct a leak check be sure to leave the perfluorotributylamine spectra scanning as directed above.

### ❖ To conduct a leak check on the 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 and turn it On. 
2. Turn the **Filament** on and set the Calibration Gas to EI.
3. Click the title bar of the Evaluate window to display it.
4. In the Positive Ion Evaluation sub-window, select the Leak Check check box.
5. In the Evaluate window, click **Evaluate** to start the automatic calibration procedure.
6. An icon appears in the toolbar to indicate an active procedure. 
7. A message box appears when the leak check procedure is completed.
  - The leak check will automatically pass when less than 10%, however, you should have a strong preference to it being less than 5% for the best data quality.
  - If the leak check fails, you will need to tighten the nut/ferrule adapters at either the column inlet of column exit/mass spectrometer inlet with the ¼ inch wrench (or both).

**NOTICE** You should not OVERTIGHTEN the ferrules as this will warp the fitting and create a worse leak in the system. If you do not feel comfortable performing this step, PLEASE SEEK IMMEDIATE ASSISTANCE before proceeding. ▲

8. Record the leak rate in the log book for the Exactive GC system before moving on to the next step.
9. Click **OK** to close the message box.

## Mass Calibration

The 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. ▲

### Positive Ion Mode Calibration

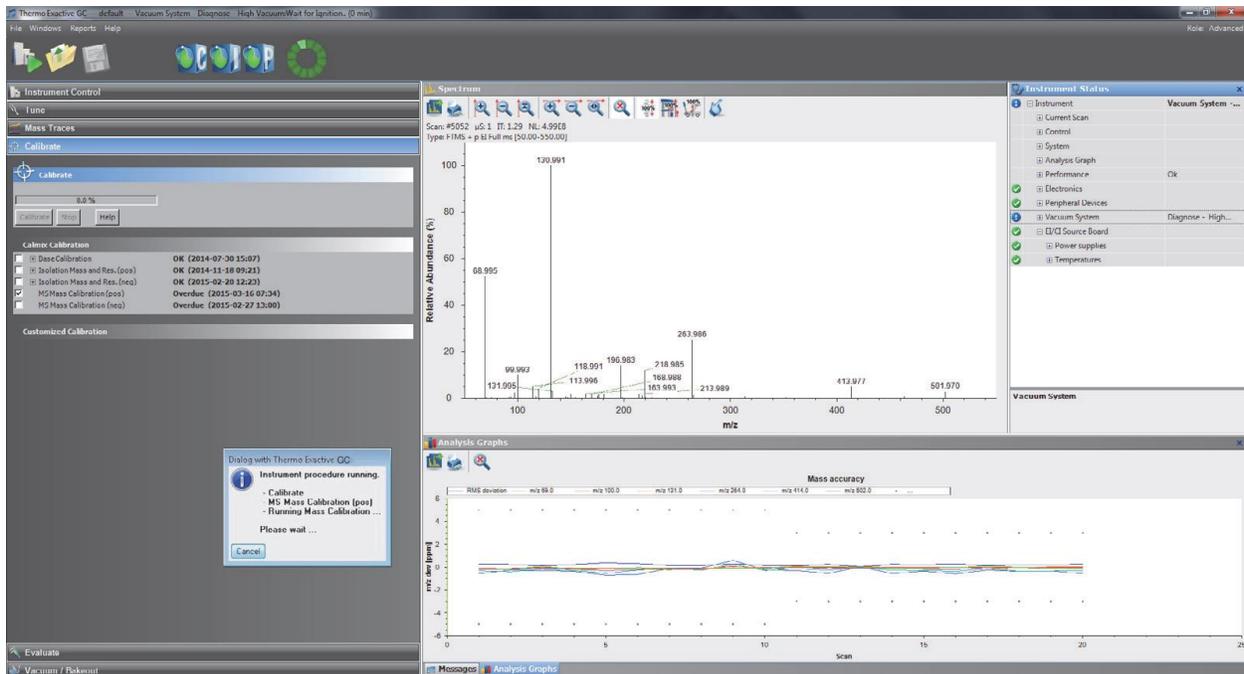
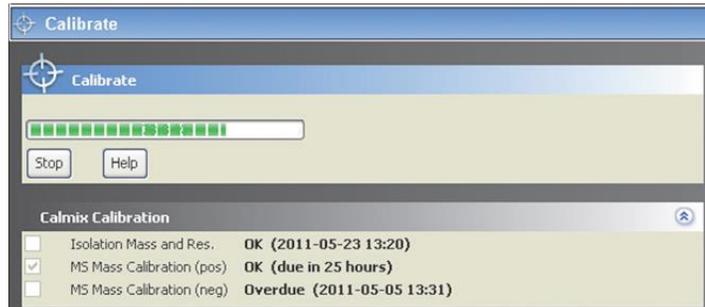
#### ❖ To calibrate the Exactive GC mass spectrometer for positive ion mode

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:
  - a. The Calibrate window indicates the completed percentage of the calibration process by a progress bar.
  - b. Exactive GC Tune displays a message box with information about the active calibration procedure and the currently calibrated parameter.
  - c. The analysis graphs window displays a graphic representation of the calibration process for the currently tuned parameter.
  - d. The messages window shows a record of the calibration.
  - e. 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.
7. A message box appears when the calibration procedure is completed. The message box also informs you that Exactive GC Tune has automatically saved the calibration results. Please record the rms error values in the Exactive GC logbook.
8. Click **OK** to close the message box.

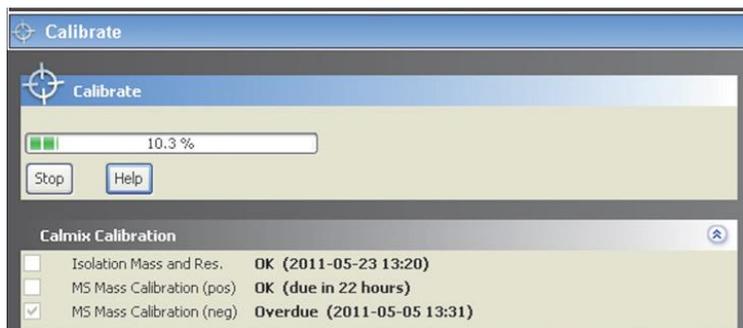


## Negative Ion Mode Calibration

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

❖ To calibrate the 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 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. Please record the rms error values in the Exactive GC logbook.
12. Click **OK** to close the message box.



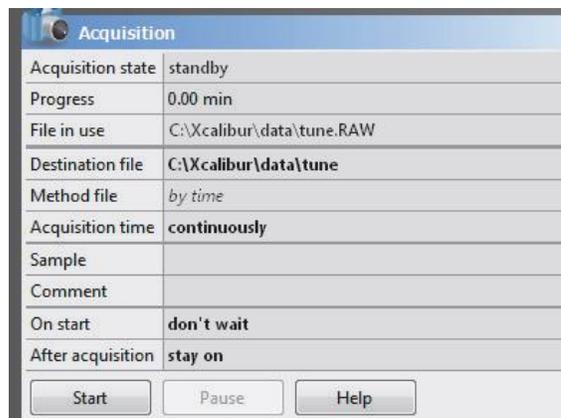
## Manual Injection/Data Acquisition

**NOTICE** This section is for those who plan to manually inject their sample into the GC system. If you intend to utilize the autosampler for your injections, please skip this section and proceed to the “After Operation” section below. ▲

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.

## **After Operation**

When the measurement is finished, Thermo Fisher Scientific recommends leaving the Exactive GC mass spectrometer in Standby mode and not in Off mode to provide the best mass accuracy for the next analysis.

### **❖ To place the Exactive GC mass spectrometer in Standby mode**

1. Turn the **Filament** off and set the Calibration Gas to Off.
2. Click the **On/Off/Standby** button to take the mass spectrometer out of the On mode and turn it Standby.

## Setting up an Automated Sequence

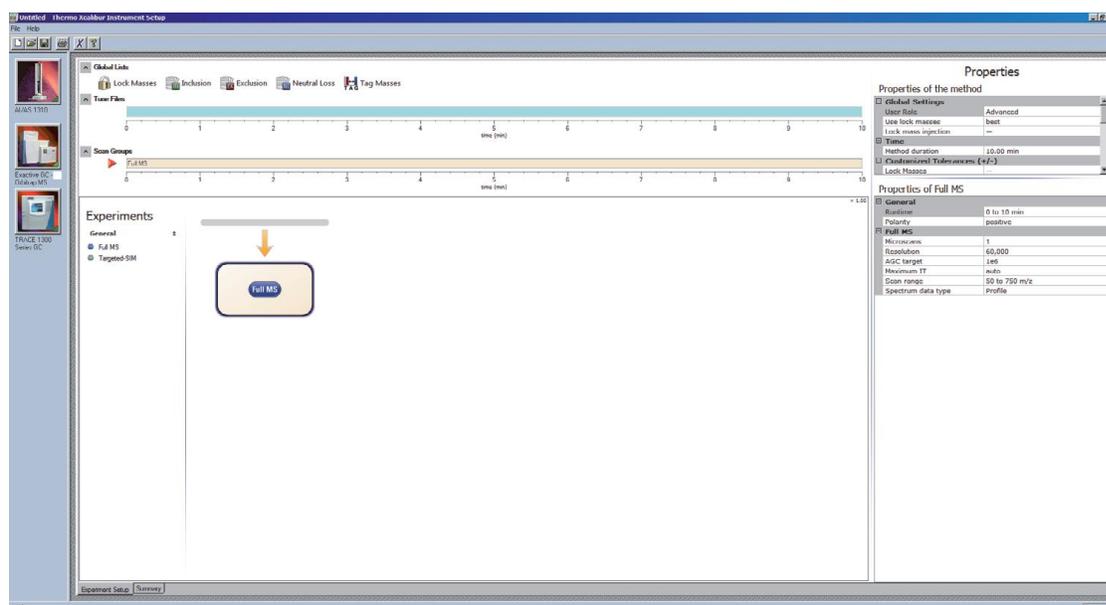
The following example describes how to create an instrument method in the Instrument Setup view of the Thermo Scientific™ Xcalibur™ software application. You can then use the instrument method to acquire data with the Exactive GC mass spectrometer. The tune file used in the instrument method is used for data acquisition. Make sure in the 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

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. If necessary, click Exactive GC - Orbitrap MS in the View bar to display the Method Editor.
2. 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.
3. Edit the fields of the Properties pane (right) to set up the experiment parameters. Refer to the Q Exactive GC Software Manual or the Exactive GC Tune Help for a description of the available parameters.
4. Assign a tune file:
  - a. On the External Hardware pane, click the icon to expand the Tune Files pane.
  - b. In the Properties of Tunefiles pane, click the Base Tunefile field.
  - c. Click the button to open a dialog box.
  - d. Browse for the tune file (for example, ab.mstune) 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 (Full\_MS.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 GC mass spectrometer, either from the Xcalibur Sequence Setup, TraceFinder, or from the Exactive GC Tune window. The next figure shows the example of an instrument method that was set up as described above.

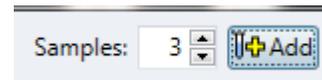


## Creating an automated sequence for data acquisition

### ◆ Configuring a sequence

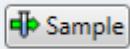
1. Open TraceFinder
  - a. Choose **Start** > Programs > Thermo **TraceFinder 4.1** > **TraceFinder 4.1 General Quan**
2. Launch the Quick Acquire Sample menu
  - a. Choose **Tools** > **Quick Acquire Sample**
3. Complete the Sequence Table with the following fields:
  - a. Filename: the name of your sample
  - b. File Path: where you want to save the data on the computer hard drive
  - c. Vial Position: the position of the vial in the tray/sample holder; the number is located in the UPPER LEFT corner of the hole
  - d. Injection volume: 1 uL for nearly all injection in both splitless and split mode
  - e. Instrument method: your preferred instrument method for analyzing the samples

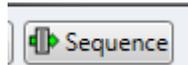
Status	Filename	File Path	Sample ID	Sample name	Commnt	Vial position	Injection volume	Instrument Method
1	Unknown1	C:\TraceFinderData\Sequences					1.000	
2	Unknown1_001	C:\TraceFinderData\Sequences					1.000	
3	Unknown1_002	C:\TraceFinderData\Sequences					1.000	
4	Unknown1_003	C:\TraceFinderData\Sequences					1.000	

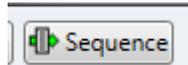


4. To add more samples to the sequence, click the “Samples: # Add button.
5. Complete all relevant fields in Step 3 above for each sample you inject. We recommend that you ALWAYS begin your analysis with a “blank” of the solvent that your samples are solvated in.
6. Once you have added the appropriate number of injections/samples to your queue, you are ready to begin the sequence.



- a. Click the Acquire: Sample  to inject a single sample from the queue after highlighting that sample of interest OR



- b. Click the Acquire: Sequence  to inject all sample in the listed spreadsheet.

7. Your start menu should look something like this:
8. Please VERIFY that the sample rows that you intend to run are referenced in the “Samples” field of this menu.
9. Also VERIFY that the “Post-run system state” is set to **Standby**.
10. You will receive a confirmation prompt that your samples have been submitted to the queue. You can “OK” this message.
11. Data will present itself in the TraceFinder window in real-time; however, only the chromatogram will be viewed.
12. You can review your data in Xcalibur > Qual Browser post acquisition.

Device Name	Use	Start Device
InPlus RSH Autosamp...	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
TRACE 1300 Series GC	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Exactive GC - Orbitrap ...	<input checked="" type="checkbox"/>	<input type="checkbox"/>