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# Direct Probe User Guide

**For VPI GC/MS Instruments**

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**ThermoFisher**  
SCIENTIFIC

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Software version: TSQ Series 4.0 and later, ISQ Series 4.0 and later, Xcalibur 4.0 and later, Exactive Series 2.9 and later.

**For Research Use Only. Not for use in diagnostic procedures.**

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## Declaration

Manufacturer: Thermo Fisher Scientific

Thermo Fisher Scientific is the manufacturer of the instrument described in this manual and, as such, is responsible for the instrument safety, reliability and performance only if:

- installation,
- recalibration, and
- changes and repairs

have been carried out by authorized personnel and if:

- the local installation complies with local law regulations,
- the instrument is used according to the instructions provided, and
- if its operation is only entrusted to qualified trained personnel.

Thermo Fisher Scientific is not liable for any damages derived from the non-compliance with the aforementioned recommendations.

## Regulatory Compliance

Thermo Fisher Scientific performs complete testing and evaluation of its products to ensure full compliance with applicable domestic and international regulations. When the system is delivered to you, it meets all pertinent electromagnetic compatibility (EMC) and safety standards as described in the next section or sections by product name.

Changes that you make to your system may void compliance with one or more of these EMC and safety standards. Changes to your system include replacing a part or adding components, options, or peripherals not specifically authorized and qualified by Thermo Fisher Scientific. To ensure continued compliance with EMC and safety standards, replacement parts and additional components, options, and peripherals must be ordered from Thermo Fisher Scientific or one of its authorized representatives.

## EMC and Safety Standards

- ITQ, and Ion Trap Series standards: EMC: EN 61326-1:2006. Safety: IEC 61010-1:2001, IEC 61010-2-081:2001
- Direct Probe Controller (DPC) standards: EMC: EN 61326-1:2013. Safety: IEC 61010-1:2001, IEC 61010-2-081:2001
- ISQ and ISQ 7000 standards: EMC EN 61326-1:2013. Safety IEC 61010-1:2010, IEC 61010-2-010:2014, IEC 61010-2-081:2015
- TSQ 9000, TSQ 8000 Evo, and TSQ Duo standards: EMC EN 61326-1:2013. Safety IEC 61010-1:2010, IEC 61010-2-010:2014, IEC 61010-2-081:2015

## Low Voltage Safety Compliance

This device complies with Low Voltage Directive 2014/35/EU and harmonized standard EN 61010-1:2001..

## FCC Compliance Statement

THIS DEVICE COMPLIES WITH PART 15 OF THE FCC RULES. OPERATION IS SUBJECT TO THE FOLLOWING TWO CONDITIONS: (1) THIS DEVICE MAY NOT CAUSE HARMFUL INTERFERENCE, AND (2) THIS DEVICE MUST ACCEPT ANY INTERFERENCE RECEIVED, INCLUDING INTERFERENCE THAT MAY CAUSE UNDESIRE OPERATION.

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**CAUTION** Read and understand the various precautionary notes, signs, and symbols contained inside this manual pertaining to the safe use and operation of this product before using the device.

## Notice on Lifting and Handling of Thermo Scientific Instruments

For your safety, and in compliance with international regulations, the physical handling of this Thermo Fisher Scientific instrument *requires a team effort* to lift and/or move the instrument. This instrument is too heavy and/or bulky for one person alone to handle safely.

## Notice on the Proper Use of Thermo Scientific Instruments

In compliance with international regulations: Use of this instrument in a manner not specified by Thermo Fisher Scientific could impair any protection provided by the instrument.

## Notice on the Susceptibility to Electromagnetic Transmissions

Your instrument is designed to work in a controlled electromagnetic environment. Do not use radio frequency transmitters, such as mobile phones, in close proximity to the instrument.

For manufacturing location, see the label on the instrument.

## WEEE Compliance

This product is required to comply with the European Union's Waste Electrical & Electronic Equipment (WEEE) Directive 2012/19/EU. It is marked with the following symbol:



Thermo Fisher Scientific has contracted with one or more recycling or disposal companies in each European Union (EU) Member State, and these companies should dispose of or recycle this product. See [www.thermoscientific.com/rohsweee](http://www.thermoscientific.com/rohsweee) for further information on Thermo Fisher Scientific's compliance with these Directives and the recyclers in your country.

## WEEE Konformität

Dieses Produkt muss die EU Waste Electrical & Electronic Equipment (WEEE) Richtlinie 2012/19/EU erfüllen. Das Produkt ist durch folgendes Symbol gekennzeichnet:



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Ce produit doit être conforme à la directive européenne (2012/19/EU) des Déchets d'Equipements Electriques et Electroniques (DEEE). Il est marqué par le symbole suivant:



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# Preface

This guide contains detailed information about using, maintaining, and troubleshooting the direct probe system.

## Contents

- [About Your System](#)
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## About Your System

Thermo Scientific systems provide the highest caliber gas chromatography/mass spectrometry (GC/MS) instrumentation available on today's market.

GC/MS represents a combination of two powerful analytical techniques: GC, which acts as a separation technique, and MS, which acts as a detection technique. Complex mixtures of individual compounds can be injected into the GC, either manually or by an autosampler, and then separated for presentation to the MS. The MS will generate a mass spectrum of the GC eluate and its components. The mass spectrum can then be used for qualitative identification as well as accurate and precise quantification of the individual compounds present in the sample.

A single-quadrupole GC/MS system provides speed and simplicity of operations and method development with powerful qualitative (full scan, library search) and quantitative (SIM) data.

A triple-quadrupole GC/MS/MS system provides the extra selectivity required for trace analysis of compounds in complex matrices.



**WARNING** Thermo Scientific systems operate safely and reliably under carefully controlled environmental conditions. If the equipment is used in a manner not specified by the manufacturer, the protections provided by the equipment might be impaired. If you maintain a system outside the specifications listed in this guide, failures of many types, including personal injury or death, might occur. The repair of instrument failures caused by operation in a manner not specified by the manufacturer is specifically excluded from the standard warranty and service contract coverage.

## Related Documentation

- *ISQ 7000 Preinstallation Requirements Guide*, PN 1R120617-0001
- *ISQ 7000 Hardware Manual*, PN 1R120617-0002
- *ISQ 7000 User Guide*, PN 1R120617-0003
- *ISQ and TSQ GC-MS Spare Parts Guide*, PN 120617-0004

The TSQ 9000 and ISQ 7000 systems include Help and these manuals as PDF files:

- *TSQ 9000 Preinstallation Guide*, PN 1R120618-0001
- *TSQ 9000 User Guide*, PN 1R120618-0002
- *TSQ 9000 Hardware Manual*, PN 1R120618-0003
- *ISQ and TSQ GC-MS Spare Parts Guide*, PN 1R120617-0004
- *Direct Probe System User Guide*, PN 1R120505-0006

### ❖ To view product manuals

If you have an ISQ 7000 or a TSQ 9000 system, open the **Manuals** folder on your desktop.

If you have a Q Exactive GC system, go to **Start > All Programs > Thermo Instruments > Manuals**.

### ❖ To open Help

- From the method editor, choose **Help**.
- If available for a specific window or dialog box, click **Help** or press the F1 key for information about setting parameters.

For more information, visit [www.thermofisher.com](http://www.thermofisher.com).

# System Requirements

Your data system must meet these minimum requirements.

System	Requirements
Hardware	<ul style="list-style-type: none"> <li>• 3.6 GHz dual-core processor</li> <li>• 16GB RAM</li> <li>• DVD drive</li> <li>• Resolution display 1280 × 1024 (SXGA)</li> <li>• 20 GB available on drive C</li> <li>• NTFS format</li> </ul>
Software	<ul style="list-style-type: none"> <li>• Microsoft™ Windows™ 10 Operating System (64-bit) English only or Microsoft™ Windows™ 7 SP1 Operating System (64-bit)</li> <li>• Microsoft .NET Framework 4.0 or later</li> <li>• Thermo Scientific™ Xcalibur™ and Foundation software<sup>a</sup></li> <li>• Thermo Scientific™ TraceFinder™ software<sup>b</sup></li> </ul>

<sup>a</sup> Check release note for compatibility with instrument control software.

<sup>b</sup> Check release note for compatibility with Thermo Foundation, Xcalibur, and instrument control software.

## Power Ratings

Mass Spectrometer (MS)

- 100-240 V ac, 50/60 Hz, 15 A max

Gas Chromatograph (GC)

- 120 V ac +6%/-10%, 50/60 Hz, 20 A max
- 230 V ac ±10%, 50/60 Hz, 10 A max

Direct Probe Controller (DPC)

- 100-230 V ac, 50/60 Hz, 2 A max

The foreline pump must be set to the line voltage that will be used at your location. Detailed instrument specifications are in the Product Specification or Product Brochure.

**Note** Always make sure that, in case of emergency, it is easy to disconnect the system's electrical cable.

## Environmental Requirements

The normal operating environment for the mass spectrometers and the direct probe controller must have the following characteristics:

- Indoor use only
- Altitude up to 2000 meters
- Maximum relative humidity between 5% and 95% up to 31 °C. The maximum relative humidity decreases linearly to 67% as the temperature climbs to 35 °C.
- Voltage variations not exceeding 10% of the nominal value
- Transient overvoltages not exceeding those specified in category II of IEC 60364-4-443.

Use the following guidelines to ensure your site has the proper environmental conditions for the system:

1. Ensure that your room temperature is 5-40 °C (41-104 °F). The analytical performance is only confirmed for temperatures between 15-35 °C (59-95 °F). For best performance, the operating temperature should be constant. Use the table below to calculate the amount of heat your system will generate and ensure your air-conditioning system can handle that amount of heat

**Table 1.** Heat output of system components

Instrument	Heat Output (BTU per Hr)	Heat Output (W)
ISQ 7000, including foreline pump	4095	1200
TSQ 9000, including foreline pump	3685	1080
TRACE 1300 and TRACE 1310	6830	2000
Q Exactive GC	5120	1500
Direct Probe Controller	819	240
Computer <sup>1</sup>	1365	400
Monitor <sup>1</sup>	85	25
AI/AS 1310	325	95
TriPlus RSH	683 <sup>2</sup> or 1366 <sup>3</sup>	200 <sup>2</sup> or 400 <sup>3</sup>

<sup>1</sup>Power requirements vary by manufacturer.

<sup>2</sup>With one power module.

<sup>3</sup>With two power modules.

2. Ensure that the relative humidity in your laboratory is between 5 and 95% with no condensation. A temperature and humidity monitor in your laboratory helps ensure that the climate is within these specifications.

3. Ensure that the air in your site is free of excess particulate matter.

For reference, the air should contain fewer than 100,000 particles (larger than 5  $\mu\text{m}$ ) per cubic meter. If the concentration is larger than this amount, dust can accumulate on electronic components. This accumulation reduces their ability to cool off properly and could cause them to overheat. If your environment is particularly dusty, we recommend that you purchase the optional dust filter for your MS.

4. Ensure that your site is free of electrostatic discharge (ESD), which may damage the electronic components of your system. Ensure your static has been discharged before touching internal components of the instrument. ESD can damage sensitive components, resulting in premature failures.

## Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

### Special Notices

Special notices include the following:







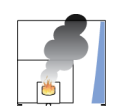
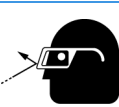

**IMPORTANT** Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.




**Note** Highlights information of general interest.

**Tip** Highlights helpful information that can make a task easier.

### Safety Symbols and Signal Words

All safety symbols are followed by **WARNING** or **CAUTION**, which indicates the degree of risk for personal injury, instrument damage, or both. Cautions and warnings are following by a descriptor. A **WARNING** is intended to prevent improper actions that *could* cause personal injury. A **CAUTION** is intended to prevent improper actions that *might* cause personal injury or instrument damage. You can find the following safety symbols on your instrument or in this guide.

Symbol	Descriptor
	<b>BIOHAZARD:</b> Indicates that a biohazard <i>will, could, or might</i> occur.
	<b>BURN HAZARD:</b> Alerts you to the presence of a hot surface that <i>could or might</i> cause burn injuries.
	<b>ELECTRICAL SHOCK HAZARD:</b> Indicates that an electrical shock <i>could or might</i> occur.
	<b>FIRE HAZARD:</b> Indicates a risk of fire or flammability <i>could or might</i> occur.
	<b>FLAMMABLE GAS HAZARD:</b> Alerts you to gases that are compressed, liquefied or dissolved under pressure and can ignite on contact with an ignition source. This symbol indicates this risk <i>could or might</i> cause physical injury.
	<b>GLOVES REQUIRED:</b> Indicates that you must wear gloves when performing a task or physical injury <i>could or might</i> occur.
	<b>HAND AND CHEMICAL HAZARD:</b> Indicates that chemical damage or physical injury <i>could or might</i> occur.
	<b>INSTRUMENT DAMAGE:</b> Indicates that damage to the instrument or component <i>might</i> occur. This damage might not be covered under the standard warranty.
	<b>LIFTING HAZARD:</b> Indicates that a physical injury <i>could or might</i> occur if two or more people do not lift an object.
	<b>MATERIAL AND EYE HAZARD:</b> Indicates that eye damage <i>could or might</i> occur.
	<b>RADIOACTIVE HAZARD:</b> Indicates that exposure to radioactive material <i>could or might</i> occur.

Symbol	Descriptor
	<b>READ MANUAL:</b> Alerts you to carefully read your instrument's documentation to ensure your safety and the instrument's operational ability. Failing to carefully read the documentation <i>could</i> or <i>might</i> put you at risk for a physical injury.
	<b>TOXIC SUBSTANCES HAZARD:</b> Indicates that exposure to a toxic substance could occur and that exposure <i>could</i> or <i>might</i> cause personal injury or death.
	For the prevention of personal injury, this general warning symbol precedes the <b>WARNING</b> safety alert word and meets the ISO 3864-2 standard. In the vocabulary of ANSI Z535 signs, this symbol indicates a possible personal injury hazard exists if the instrument is improperly used or if unsafe actions occur. This symbol and another appropriate safety symbol alerts you to an imminent or potential hazard that <i>could cause personal injury</i> .

## Hazardous Substances Precautions



**WARNING** Before using hazardous substances (toxic, harmful, and so on), please read the hazard indications and information reported in the applicable Material Safety Data Sheet (MSDS). Use personal protective equipment according to the safety requirements.

## Biological Hazard Warning Note



In laboratories where samples with potential biological hazards are handled, the user must label any equipment or parts which might become contaminated with biohazardous material.

The appropriate warning labels are included with the shipment of the instrument. It is the user's responsibility to label the relevant parts of the equipment.

When working with biohazardous materials, you are responsible for fulfilling the following mandatory requirements:

- Providing instructions on how to safely handle biohazardous material.
- Training operators to be aware of potential hazards.
- Providing personal protective equipment.

- Providing instructions for what to do if operators are exposed to aerosols or vapors during normal operation (within the intended use of the equipment) or in case of single fault situations such as a broken vial. The protective measures must consider potential contact with the skin, mouth, nose (respiratory organs), and eyes.
- Providing instructions for decontamination and safe disposal of relevant parts.



**WARNING** The user or operator is responsible for the safe handling of hazardous chemicals or biological compounds including (but not limited to) bacterial or viral samples and the associated waste, according to international and local regulations.

## Venting Toxic Gases

When analyzing toxic compounds, be aware that during the normal operation of the GC some of the sample might be vented outside the instrument through the split and purge flow vents; therefore, be sure to vent the exhaust gases to a fume hood. Consult local environmental and safety regulations for instructions in exhausting fumes from your system.

## Contacting Us

There are several ways to contact Thermo Fisher Scientific for the information you need.

❖ **To find out more about our products**

Go to [www.thermofisher.com](http://www.thermofisher.com) for information about our products.

❖ **To get local contact information for sales or service**

Go to [www.unitylabservices.com/en/home.html](http://www.unitylabservices.com/en/home.html).

❖ **To suggest changes to documentation or to Help**

- Fill out a reader survey online at [www.surveymonkey.com/s/PQM6P62](http://www.surveymonkey.com/s/PQM6P62).
- Send an e-mail message to the Technical Publications Editor at [techpubs-austin@thermofisher.com](mailto:techpubs-austin@thermofisher.com).



## Using the Direct Probes

This chapter explains how to use the direct insertion probe (DIP) and direct exposure probe (DEP) with the ISQ 7000, TSQ 9000, and Q Exactive GC mass spectrometers.

**IMPORTANT** Direct probes require an ISQ 7000 or TSQ 9000 to be equipped with a vacuum probe interlock.

The direct sample probes provide an alternate means of sample introduction that reduces or eliminates sample preparation time. The technique also requires a minimal sample amount for testing (micrograms to nanograms). This system, designed for use with a broad range of Thermo Scientific GC-MS systems, is ideal for quickly screening samples or for any application that does not require or allow a GC column separation.

The direct probes provide a quick, simple method for sample introduction directly into the mass spectrometer ion source, allowing for accurate analysis of highly polar, thermally labile solid or liquid compounds, polymers, composites, pieces of materials, or organisms. The direct probes are compatible with all modes of ionization and mass analysis: EI, CI, Full Scan, SIM, SRM, and MS/MS. Switching to a direct probe system takes less than three minutes and leaves the GC interface undisturbed.

The direct probes can determine the mass spectrum of substances that cannot usually be analyzed by gas chromatography. The samples can be thermally labile, highly active, or have physical characteristics (for example, a boiling point) that would prohibit or limit their ability to be analyzed by a GC system. The direct probes allow you to place a small amount of sample on the probe's end, which then is inserted directly into the ion source, and the sample is vaporized or pyrolyzed directly in the source, under vacuum. The direct probes not only allow rapid sample preparation and introduction but also provide an alternative to conventional methods of complex sample analysis.

The DIP can be used to analyze solid or liquid samples. The DIP is used to controllably (10 to 120 °C) heat a sample up to 450 °C in a glass or aluminum crucible in the vacuum chamber. The combination of heat and low pressure allows many compounds to enter the gas phase where they can be ionized and detected by the mass spectrometer. This approach can be useful when the traditional separation technique using a GC either takes too long or requires temperatures that degrade the sample before it can be sent into the mass spectrometer as well as to look at the volatile decomposition products of non-volatile materials, like polymers. The process of thermal decomposition in absence of oxygen is called pyrolysis.

The DEP allows you to rapidly (from 1 to 1,000 °C/s) heat the sample applied to the filament, and after flash vaporization or pyrolysis introduce solid or liquid samples directly into the ion source, which is used to rapidly analyze liquids, solids, or trace components in solid matrices.

**Note** Unless noted otherwise, all instructions for using the direct probe controllers apply to the ISQ 7000, TSQ 9000, and Q Exactive GC mass spectrometers.

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- [How the Direct Probe System Works](#)
- [Using the Probe to Analyze Cholesterol](#)
- [Loading Probe Samples](#)
- [Cleaning the Probe Shaft, Direct Probe Controller, DIP Sample Cups, and DEP Filament](#)

## How the Direct Probe System Works

The direct probe controller is an instrument used to generate analytically useful signals in collaboration with the sample probe (DIP or DEP) and mass spectrometer. The main advantage to using a probe is that it allows you to rapidly screen samples for any application that does not require a GC column separation. Another advantage is that a probe allows you to volatilize or pyrolyze a wide range of samples directly into the ion source.

To use the direct insertion probe (DIP), it must be attached to the direct probe controller. Once the probe is attached and you turn on the direct probe controller, the direct probe controller automatically identifies the type of probe attached. To program a DIP, you must set an initial temperature and hold time, a temperature ramp, and a final hold time and temperature. The DIP allows for a maximum of two temperature ramps.

The initial temperature of the DIP can be set within the range of 30 °C to 450 °C. You can program the temperature ramp in 10 °C increments from 10 °C/min to 120 °C/min. There is also a maximum heating rate that will heat the DIP tip as rapidly as possible. The final temperature must be at least 10 °C above the initial temperature if a ramp is being used. You can set the time to hold the sample at the final temperature within a range of 0 to 900 seconds. You can also use a second ramp, but it must have a final temperature of at least 10 °C above the final temperature used in the first ramp.



**WARNING BURN HAZARD:** Keeping the DIP in the instrument at 350 °C for more than 15 minutes will cause the probe tip to become very hot. Use caution when handling a probe at this temperature.

To use the direct exposure probe (DEP), it must be attached to the direct probe controller. Once the direct exposure probe is attached and you turn on the direct probe controller, the direct probe controller automatically identifies the type of probe attached. To program a DEP, you must set an initial current and hold time, a current ramp, and a final hold time and current. The software used for your particular analysis is determined when you develop your analytical method.

The initial current of the DEP can be set within a range of 0 mA to 1000 mA. You can program the current ramp within a range of 0 mA/min to 1000 mA/s. The final current must be greater than the initial current if a ramp is being used. You can set the time to hold the sample at the final current within a range of 0 to 900 seconds.



**WARNING BURN HAZARD:** Keeping the DEP above 750 mA for more than 15 minutes will cause the probe tip to become very hot. Use caution when handling a probe at this temperature.

The direct probe system consists of the following components:

**Figure 1.** The Direct Probe System



## Using the Probe to Analyze Cholesterol

Both the DIP Kit and the DEP Kit contain samples for analyzing cholesterol. The steps below can also be used to set up the system for the analysis of other compounds. The cholesterol sample is used for instrument qualification to ensure it is working properly. The process for using the probe to analyze cholesterol on the mass spectrometer is as follows:

- Initial Setup

- Step 1: Set Up the Direct Probe System

- Step 2: Tune and Calibrate the Mass Spectrometer

- Step 3: Replace the Repeller in the Ion Source Cartridge

- Analyzing the Sample

- Step 4: Program the Direct Probe Controller

- Step 5: Prepare the Sample
- Step 6: Create an Xcalibur Method
- Step 7: Create a Sequence File
- Step 8: Insert the Probe
- Step 9: Collect the Data
- Step 10: Remove the Probe
- Step 11: Analyze the Data

## Step 1: Set Up the Direct Probe System

To set up the direct probe system:

1. Place the direct probe controller on top of or to the side of the mass spectrometer.

**Note** If your GC/MS system has an autosampler attached to it, you may have to set the direct probe controller on the table to the left of the mass spectrometer.

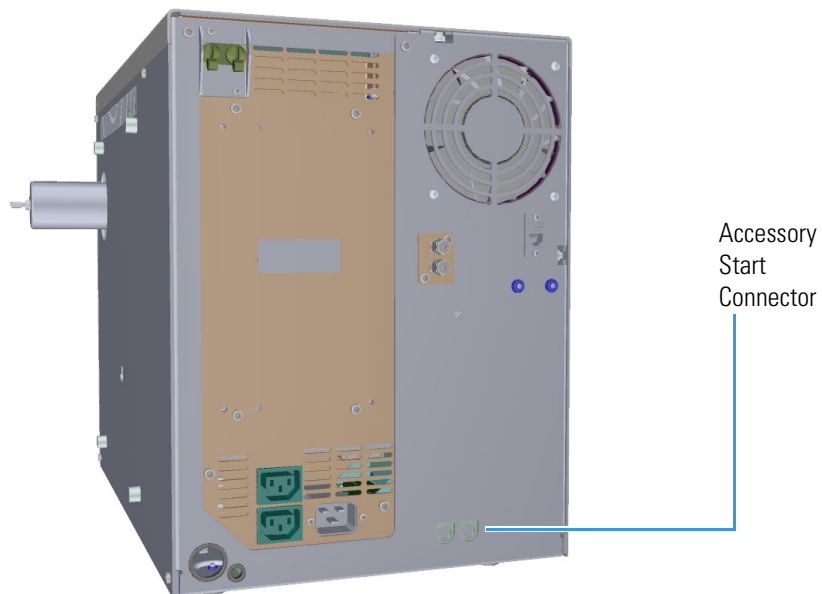
2. Use the cable in the kit to connect the direct probe controller to the mass spectrometer.
  - a. Connect the 9-pin end of the cable to the **Remote Ready** connector on the back of the direct probe controller.

**Figure 2.** Connecting the Cable to the Direct Probe Controller



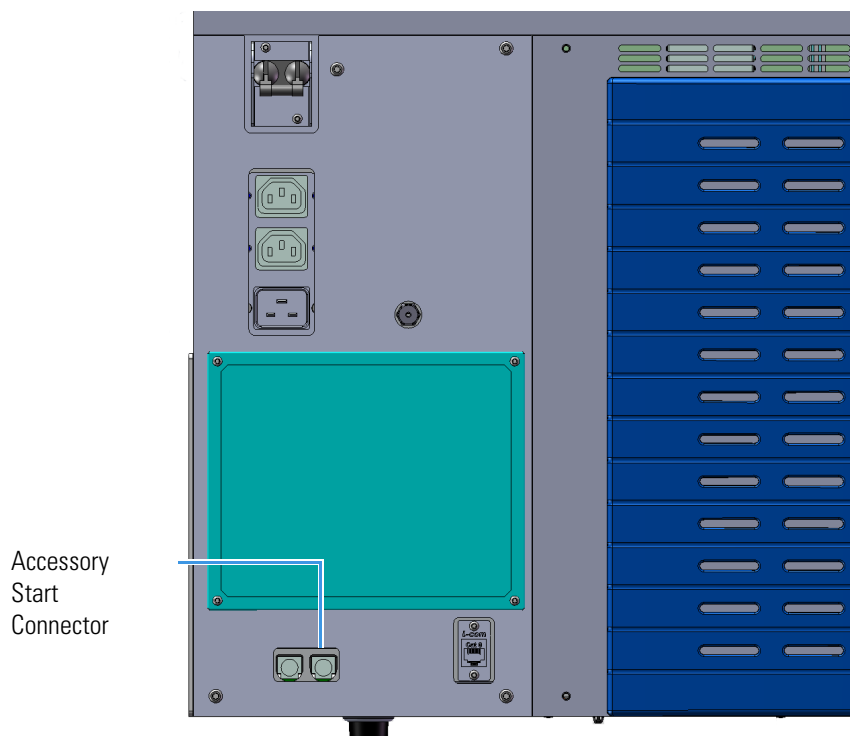
- b. If you have an ISQ 7000 mass spectrometer, connect the small round end of the cable to the Accessory Start connector on the back of the instrument.

**Figure 3.** Connecting the Cable to the ISQ 7000 Mass Spectrometer



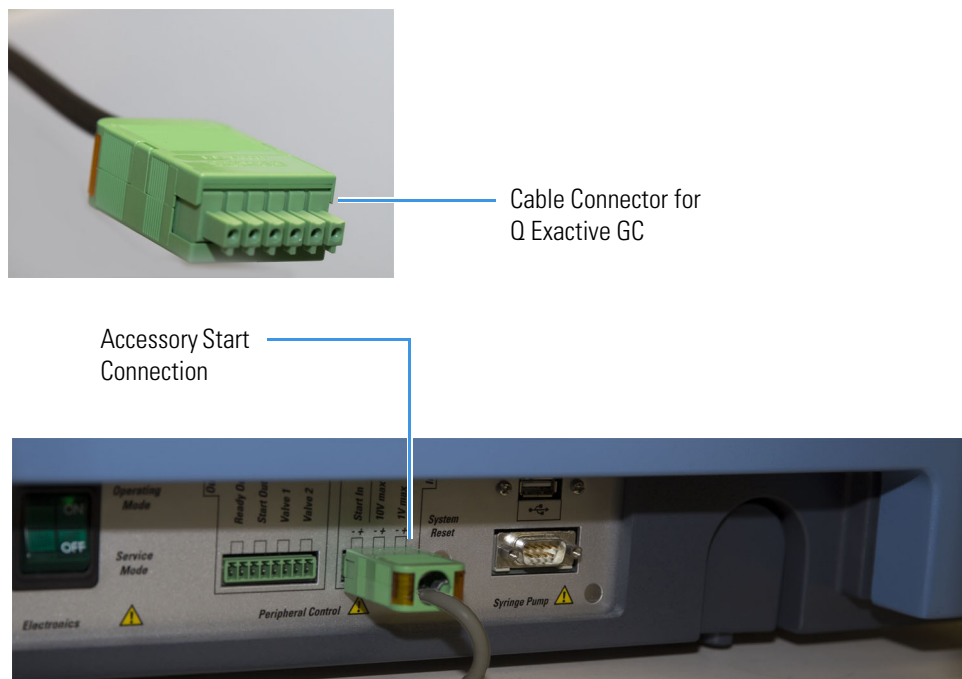
- c. If you have a TSQ 9000 mass spectrometer, connect the small round end of the cable to the Accessory Start connector on the side of the instrument.

**Figure 4.** Connecting the Cable to the TSQ 9000 Mass Spectrometer



- d. If you have a Q Exactive GC, attach the green end of the special start cable found in the Q Exactive GC Probe Kit to the Accessory Start Connector on the left side of the instrument.

**Figure 5.** Connecting the Start Cable to the Q Exactive GC Mass Spectrometer



- e. (Optional) Choose an air source to use as a probe coolant and connect it to the Air Source connector on the back of the direct probe controller.

The air source must be dry air or nitrogen at no more than 100 psi. We recommend 80 psi for normal operation. The coolant is used with the probe to rapidly cool the probe tip, which allows rapid sample throughput. However, it is not designed for subambient operation, and room temperature will affect the minimum probe tip temperature.

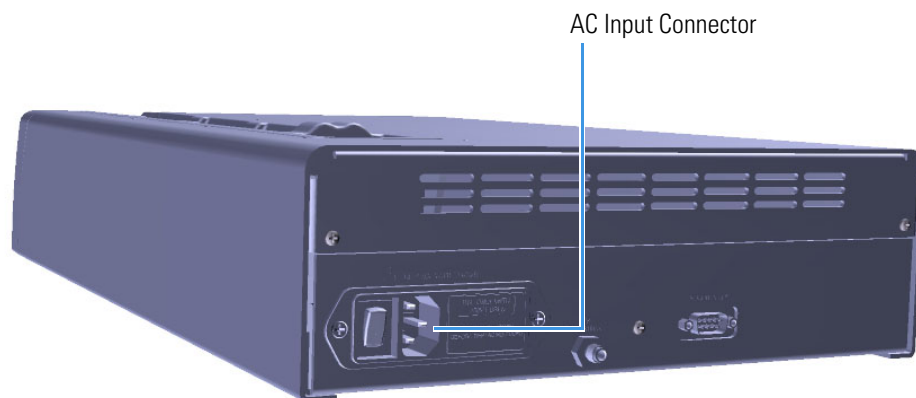
**Note** The probe tip must be able to be cooled to 35 °C for automatic cooling to complete. Press the **Start/Stop** button to cancel the automatic air cooling to less than 35 °C.

**Figure 6.** Connecting the Air Source to the Direct Probe Controller



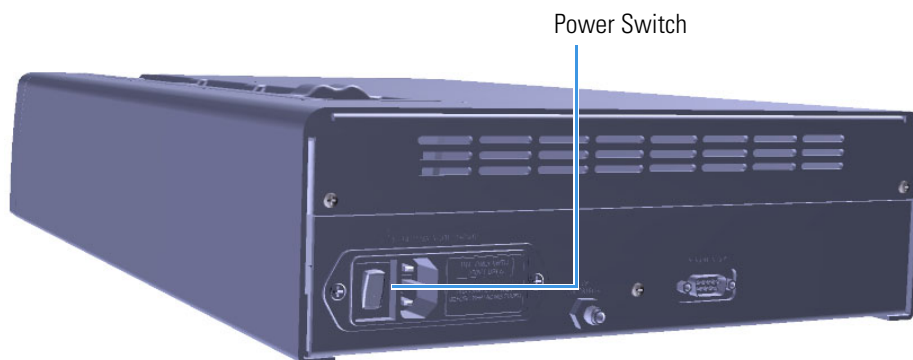
- f. Connect one end of the power cord to the direct probe controller and the other end to the electrical outlet. The power cord provides the safety grounding for the unit. Always make sure that, in case of emergency, it is easy to disconnect the electrical cable.

**Figure 7.** Connecting the Direct Probe Controller to the Electrical Outlet



- g. Press the top ( | ) of the power switch on the back of the direct probe controller to power it on.

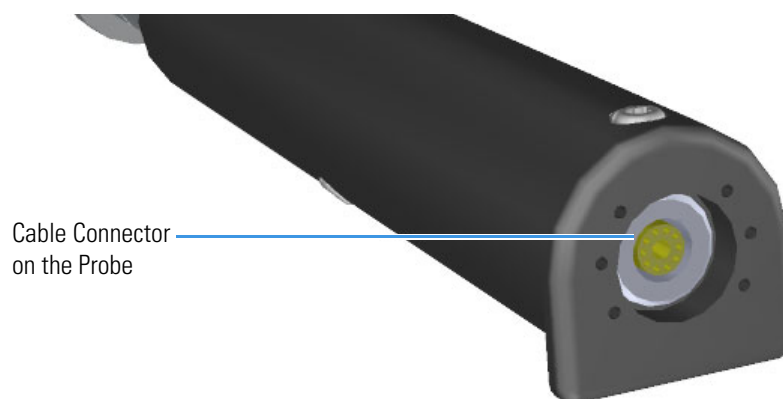
**Figure 8.** Powering On the Direct Probe Controller





3. Connect one end of the communication cable to the end of the probe.

**Figure 9.** Connecting the Communication Cable to the Probe



4. Connect the other end of the communication cable to the direct probe controller. Match the red dot located on the cable to the red dot located on the direct probe controller and firmly insert the cable until it locks in position.

**Figure 10.** Connecting the Communication Cable to the Direct Probe Controller



5. Place the probe in the cradle of the direct probe controller.

**Figure 11.** Placing the Probe in the Cradle



6. Power-on the mass spectrometer, if necessary.

## Step 2: Tune and Calibrate the Mass Spectrometer

If you are using the ISQ 7000 mass spectrometer, refer to the online help of the *Tune* software for information about determining if the instrument has been tuned and calibrated.

If you are using the TSQ 9000 mass spectrometer, click **Auto Tune** on the TSQ Series dashboard to tune and calibrate the instrument.

If you are using the Q Exactive GC mass spectrometer, tune and calibrate the instrument as described in the Q Exactive GC Software Manual.

Refer to your mass spectrometer user guide for more information about tuning the instrument.

**IMPORTANT** On ISQ 7000 and TSQ 9000 instruments, set the repeller voltage to zero and be sure that the tune file saved and used for the analysis has the repeller set to zero. If automatic tuning returns a non-zero repeller value, set the repeller to zero in the manual tune program or create a new tune type that sets the repeller to zero volts in the lens tune.

On Exactive Series instruments, the repeller must remain at the voltage set in AutoTune. The ceramic adapter must be on the probe tip to prevent shorting the repeller to the grounded probe on Exactive series instruments. If the signal appears erratic or too low with the probe in place, confirm the ceramic adapters is aligned to prevent shorting. View the report of the repeller readback voltage in the raw file status log. If it is shorted, the repeller voltage will be lower than the setpoint, but not completely at zero volts.

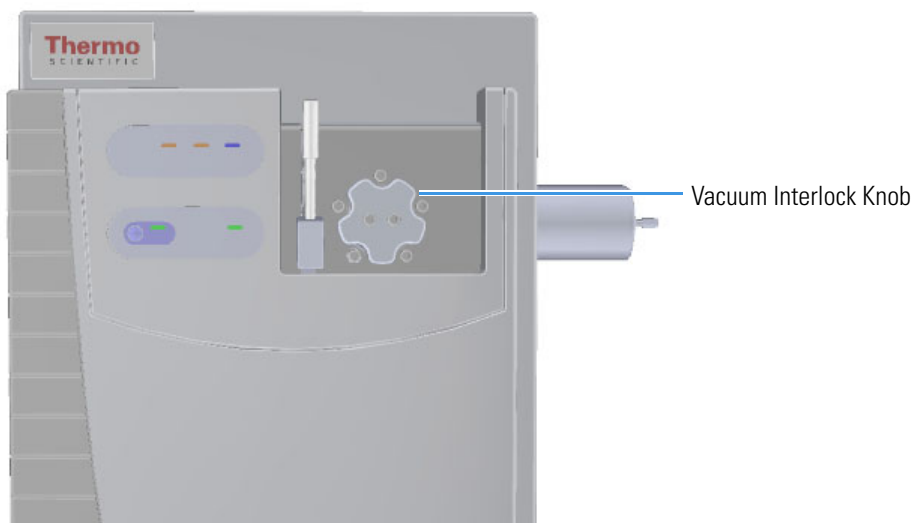
## Step 3: Replace the Repeller in the Ion Source Cartridge

To use the probe with the mass spectrometer, you must replace the repeller inside the ion source cartridge. The repeller that comes standard on the ISQ 7000, TSQ 9000, and Q Exactive GC systems does not have a hole in it. The hole in the new repeller allows the sample that is volatilized by the probe to enter the ionization region of the mass spectrometer. Your Direct Insertion Probe Kit or Direct Exposure Probe Kit contains the correct repeller to use with the probe.

❖ **To replace the standard repeller with a probe repeller**

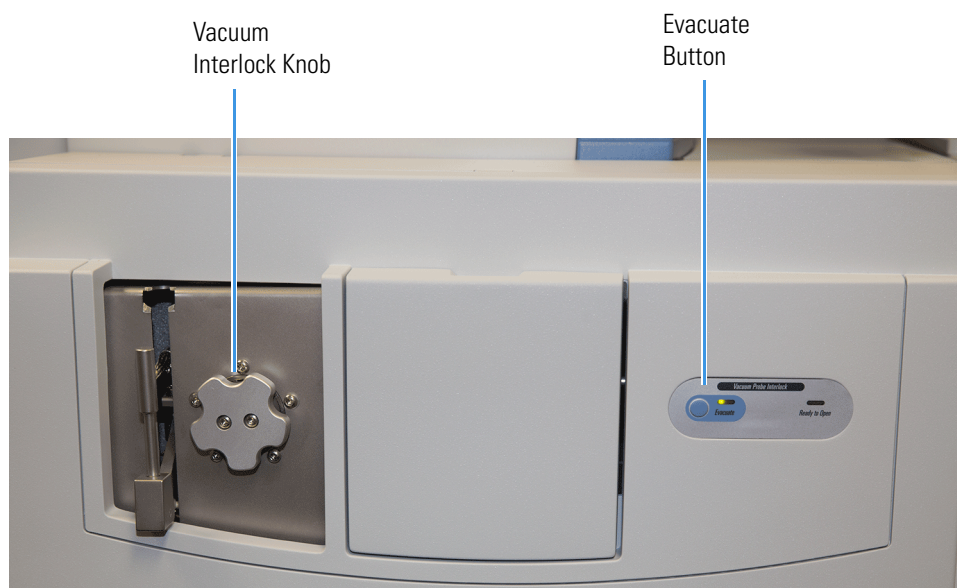
1. Twist off the round vacuum interlock knob located on the front of the instrument and set it aside. As you can see, the knob covers access to a long tunnel that goes into the instrument. This tunnel is called the vacuum interlock.

**Figure 12.** Removing the Vacuum Interlock Knob on the ISQ 7000 and TSQ 9000 Instruments



2. If you have a Q Exactive GC mass spectrometer, twist off the round vacuum interlock knob located on the interior front panel and set it aside. There is a vacuum feedthrough with a ball valve that allows access to the ion source without venting the instrument.

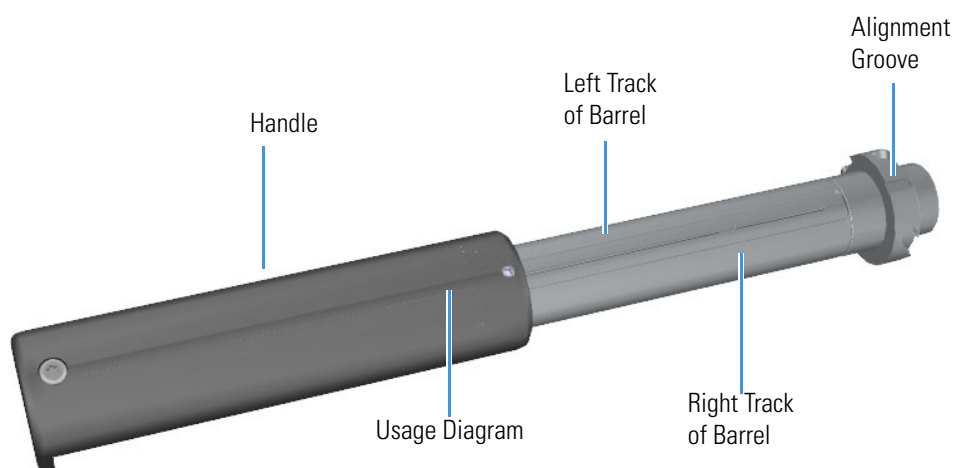
**Figure 13.** Removing the Vacuum Interlock Knob from the Q Exactive GC Instrument



**Tip** To prevent leaks through the vacuum interlock, we recommend that you leave the vacuum interlock knob attached to the instrument, except when you are removing or inserting the ion source cartridge. After you reattach the knob, press the **Evacuate** button to eliminate any air inside the vacuum interlock.

3. Get the source exchange tool out of the MS Toolkit that shipped with your instrument. It is used to remove and insert the ion source cartridge through the vacuum interlock. The tool has a large black handle on one end and a stainless steel barrel at the other. On the black handle is a diagram describing how to insert and remove the ion source cartridge from the instrument using the tool. The stainless steel barrel is cut with a track, and the black handle moves up and over the track, depending on whether you are removing or installing an ion source cartridge.

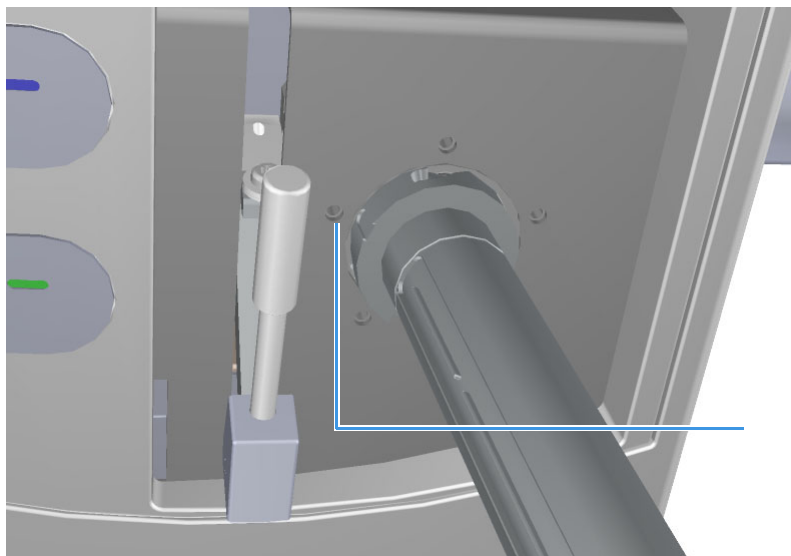
**Figure 14.** Components of the Source Exchange Tool



4. Grasp the handle of the source exchange tool in one hand and use your other hand to pull the metal barrel out and away from the handle so that the tool is fully extended.
5. Rotate the black handle to the right to lock it in place on the vacuum manifold.

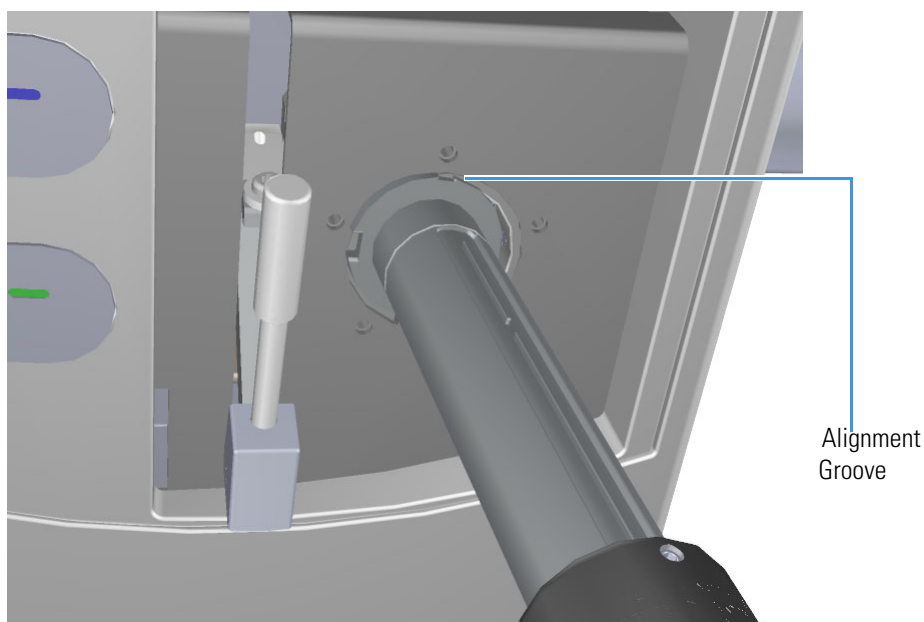
- a. With your hand around the barrel, twist the tool until the alignment groove at the end of the barrel aligns with the left screw around the slot vacated by the vacuum interlock knob.

**Figure 15.** Inserting the Source Exchange Tool



- b. Twist and push the barrel to the right until it clicks into place under the screw at the top of the slot.

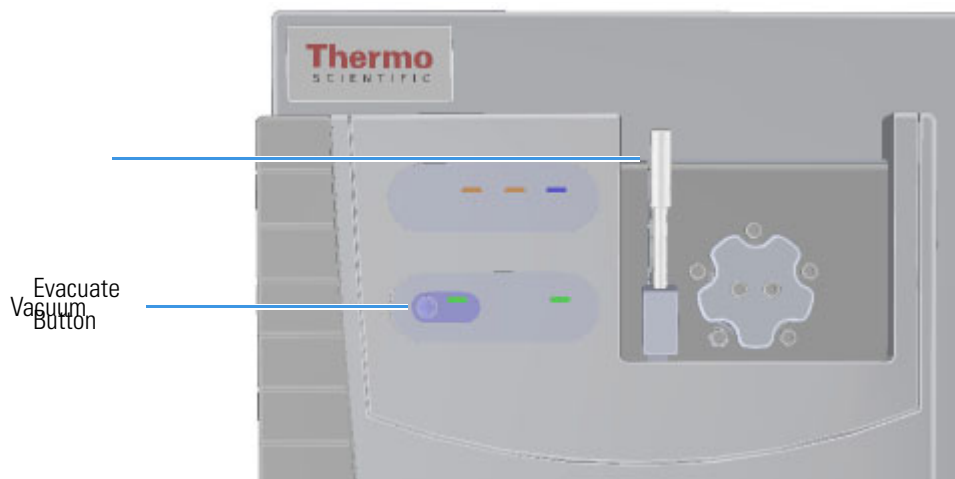
**Figure 16.** Twisting the Tool Into Place



6. Press the blue **Evacuate** button on the front of the instrument. When the Evacuate light flashes green for approximately 20 seconds and turns off, the **Ready to Open** light turns

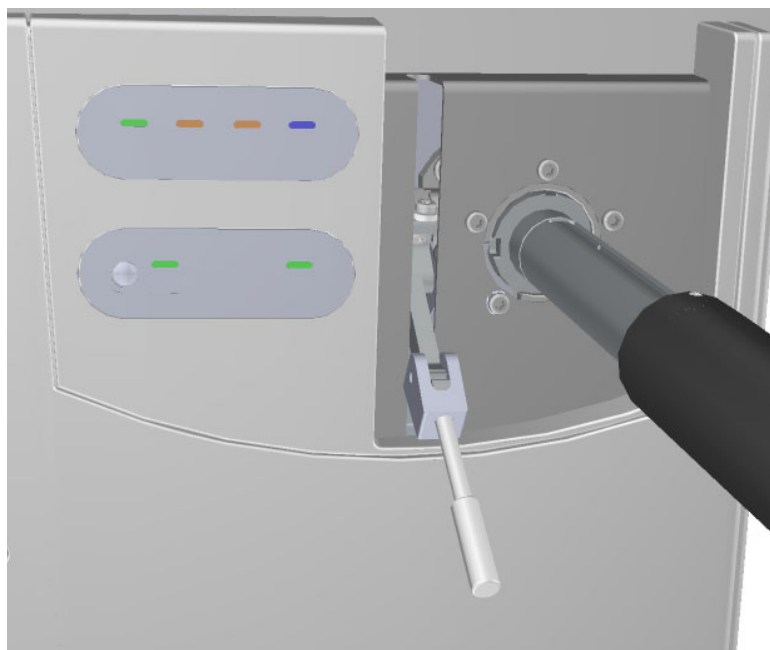
solid green. At that point, the air has been evacuated from the instrument and it is safe to open the vacuum interlock valve.

**Figure 17.** Evacuating the Instrument



7. Twist, then loosen the top part of the vacuum interlock handle to allow the entire handle to move up and down. This handle controls the interior ball valve, which seals the vacuum chamber and protects it from the outside pressure.
8. Push the handle down and then twist the top of it in the opposite direction to lock it into place.

**Figure 18.** Pulling Down the Vacuum Interlock Handle

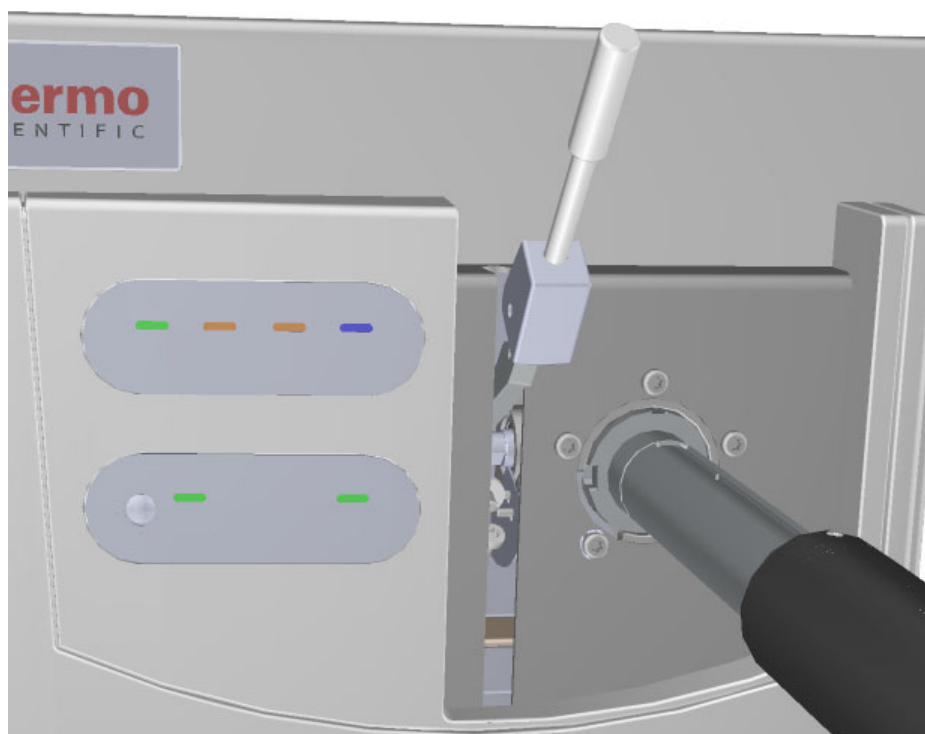


9. Pull the handle up to open the interior ball valve.

**Note** If a minute has elapsed since the **Ready to Open** light first came on, the **Ready to Open** light will turn off. You should press the **Evacuate** button and wait until the **Ready to Open** light is lit again before opening the valve. Do not open the valve if the ready to open light is not on. This may indicate a leak in the valve region. When a leak is detected during the evacuate sequence, the amber evacuate LED will flash on and off.

10. Slowly push the source exchange tool into the instrument. Because you are removing the ion volume in this step, you will go down the right side of the metal track on the barrel.

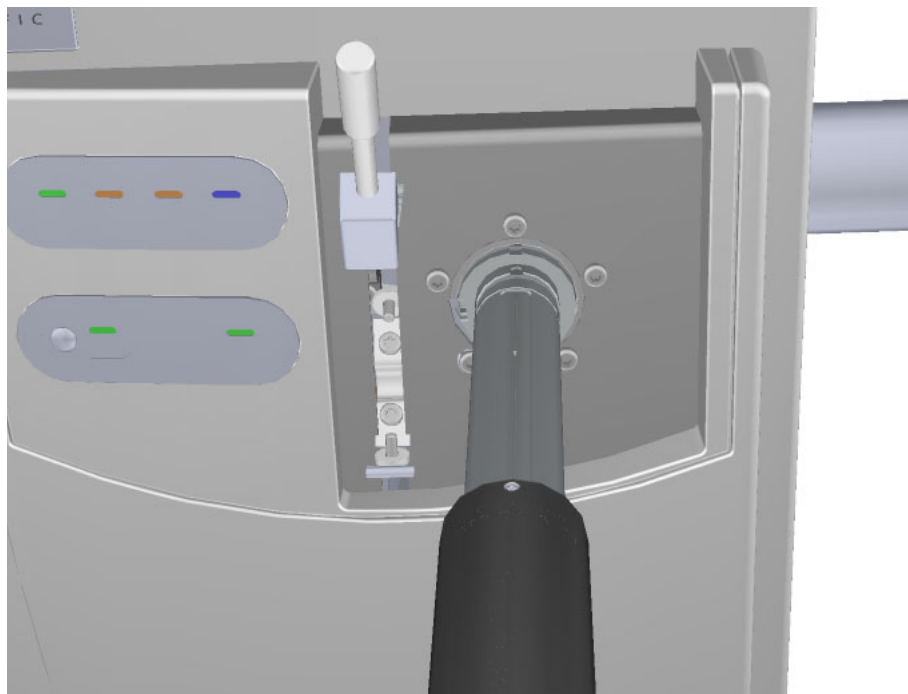
**Figure 19.** Pushing the Tool into the Instrument



11. Once the tool is all the way in (when the groove at the end of the barrel is covered by the black handle) twist the handle to the left to engage the ion source cartridge and move it onto the end of the barrel. You are essentially disconnecting the ion source cartridge from the ion source block and moving it onto the tool.

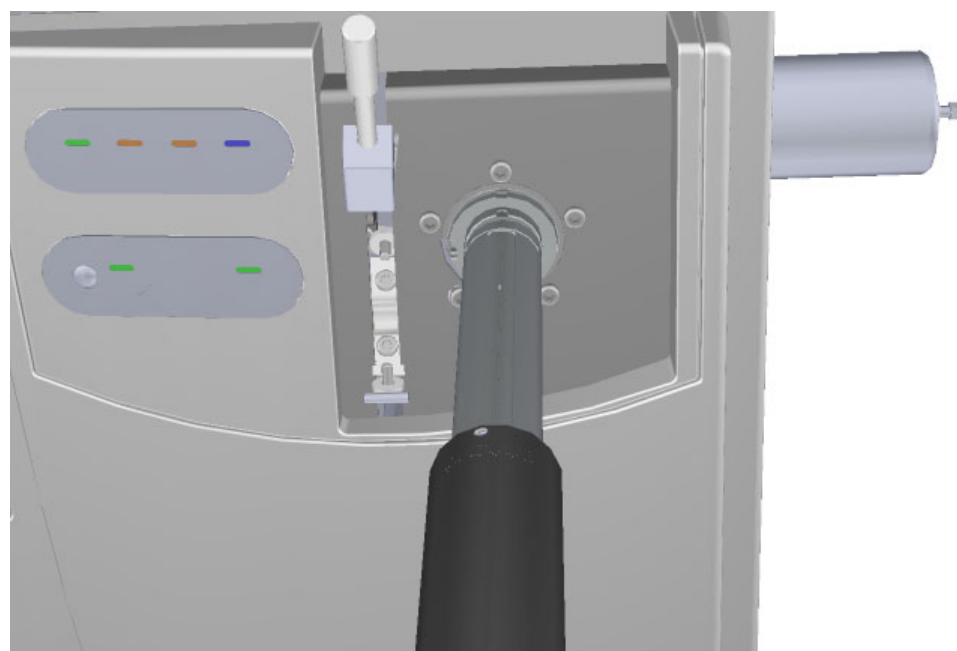
12. Pull the tool toward you and down the left side of the metal track on the barrel. As you keep pulling it toward you, more and more of the barrel will be exposed.

**Figure 20.** Pulling the Tool Down the Left Track



13. Once you reach the end of the track (when the back line at the end of the barrel is covered by the black handle), twist the handle to the left to lock it into place.

**Figure 21.** Locking the Tool in Place

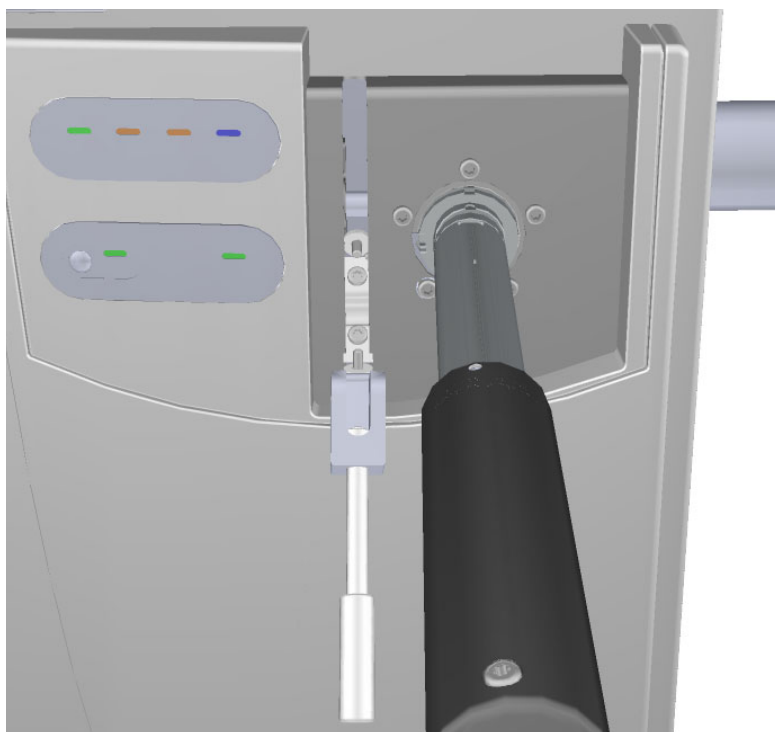




14. Pull the vacuum interlock handle down.

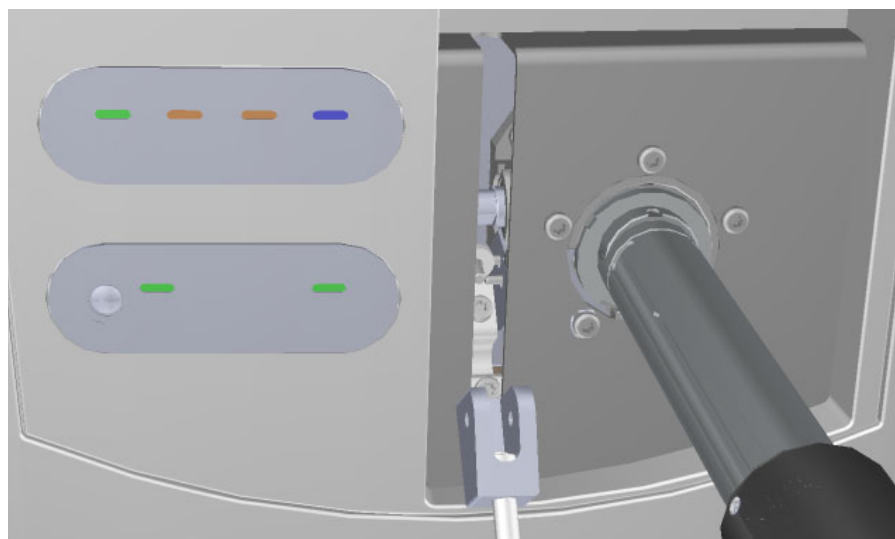
**Note** Be sure to fully extend the tool so that you do not crush the ion source cartridge with the ball valve.

**Figure 22.** Pulling the Vacuum Interlock Handle Down



15. Grasp the barrel of the tool and twist it to the left so that the alignment groove moves toward the left screw. Then pull the tool out of and away from the instrument.

**Figure 23.** Removing the Tool from the Instrument



16. Replace the plug in the VPI to prevent accidental venting and excessive leaks.
17. Let the ion source cartridge cool.
18. Hold the handle of the tool with one hand and use the other hand to pull the barrel toward you and into the handle.

**Figure 24.** Exposing the Ion Source Cartridge



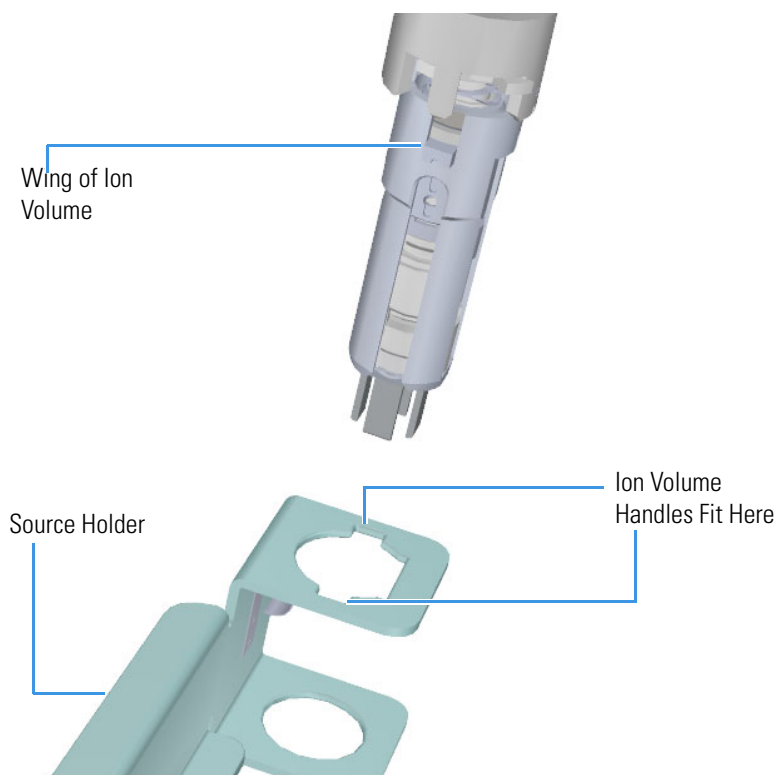
19. Invert the ion source tool so that the barrel is pointed toward the floor.

**Figure 25.** Inverting the Tool



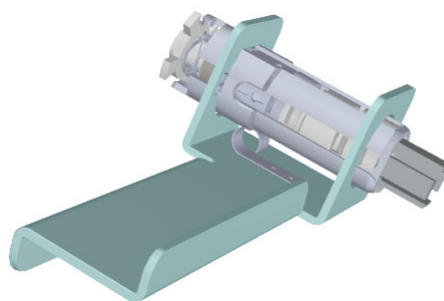
20. Slide the source holder, which is in the MS Toolkit, onto the end of the ion source cartridge. The opening of the source holder is designed to accommodate the handles of the ion volume.

**Figure 26.** Attaching the Source Holder



21. Twist the holder to disengage the ion source cartridge from the tool.  
22. Set the ion source cartridge and holder on a clean surface.

**Figure 27.** Removing the Ion Source Cartridge

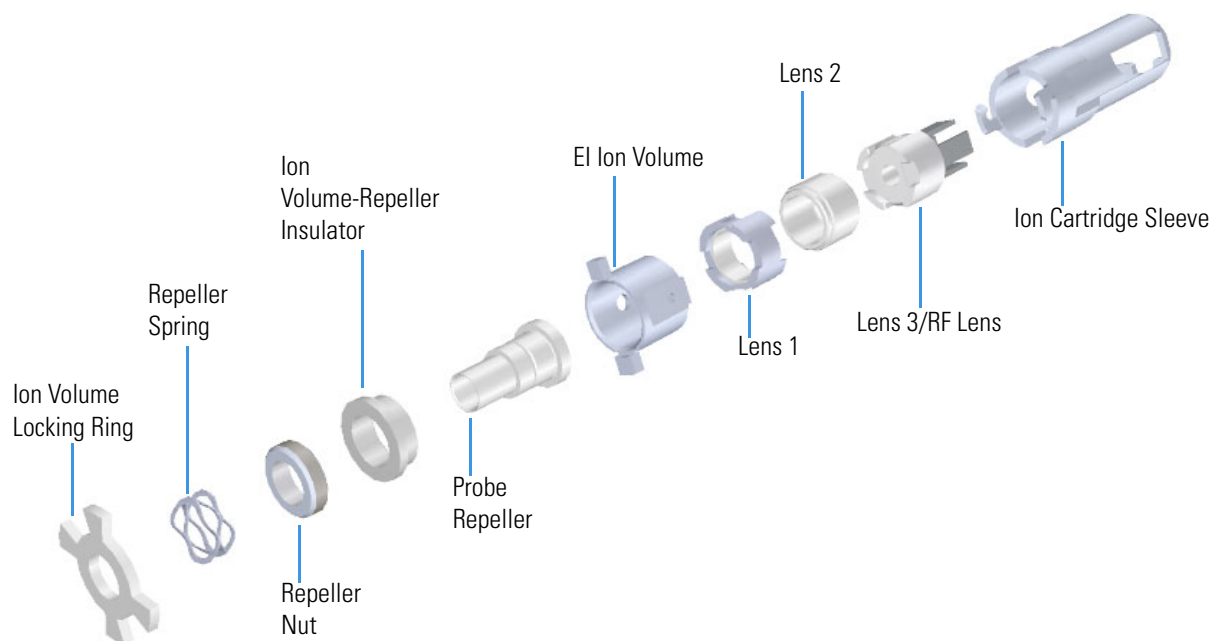


**WARNING BURN HAZARD:** The ion source cartridge may be hot.

23. Let the ion source cartridge cool down before removing it from the source holder.
24. Disassemble the ion source cartridge by removing the locking ring first, then the repeller spring, then the nut, insulator, and repeller (which comes out in one piece).

**IMPORTANT** Always wear clean, lint-free gloves when handling the ion source cartridge.

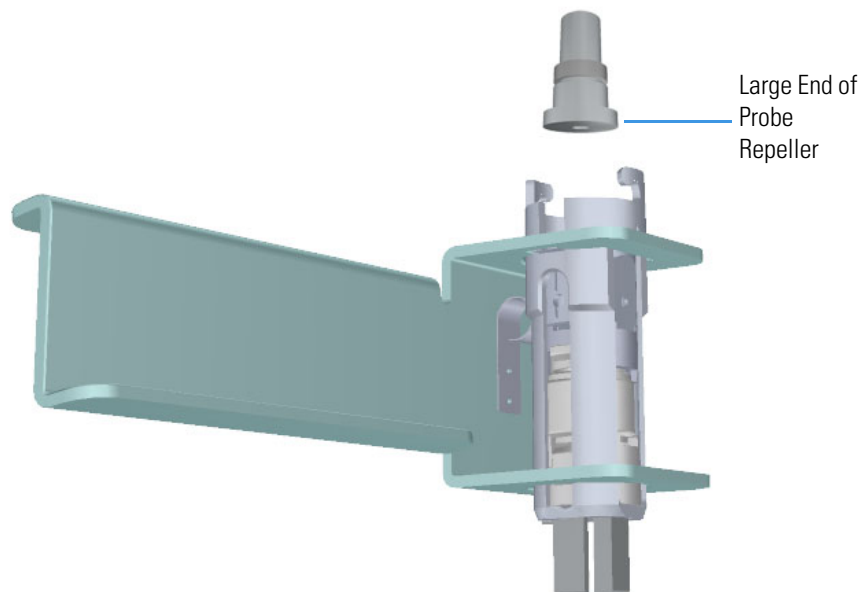
**Figure 28.** Ion Source Cartridge Components



25. Set the components on a clean work surface.
26. Replace the standard repeller (no hole) with the probe repeller.
  - a. Unscrew the repeller nut.
  - b. Slide the insulator off the repeller.
  - c. Put the insulator on the probe repeller. Make sure it goes on in the correct direction.
  - d. Screw the repeller nut down on the probe repeller until it is snug. Make sure the repeller is centered in the ceramic ring while tightening the nut.

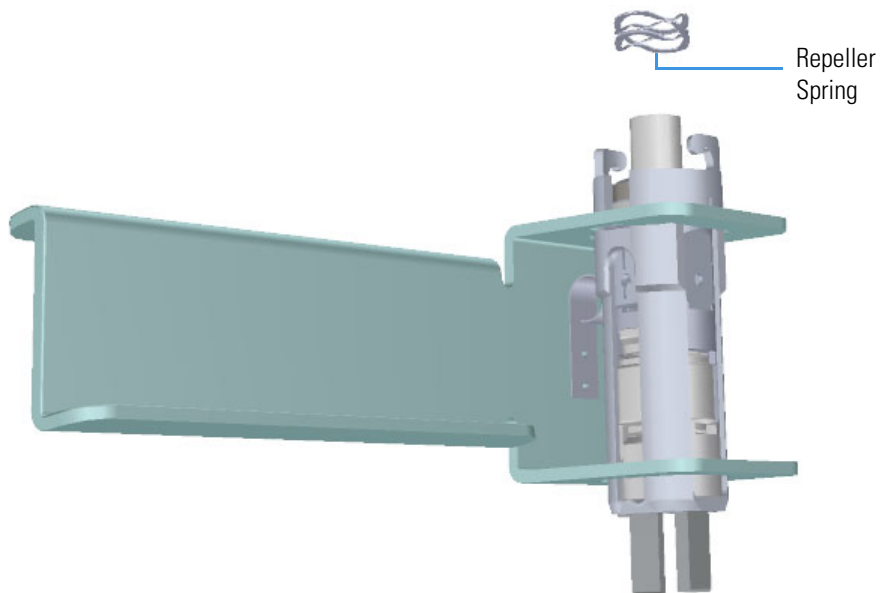
27. Insert the large flat end of the probe repeller so that it rests on top of the ion volume.

**Figure 29.** Inserting the Probe Repeller into the Source Sleeve



28. Attach the repeller spring to the probe repeller.

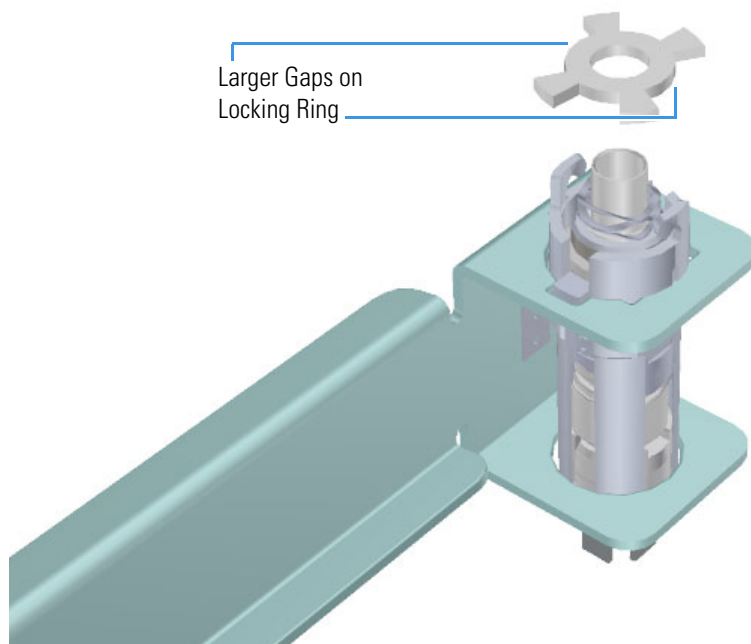
**Figure 30.** Inserting the Repeller Spring into the Sleeve



29. Place the locking ring on top of the repeller spring so that the probe repeller protrudes through the center hole on the locking ring. The hooks on the sleeve fit between the larger gaps on the locking ring.

**Note** Do not twist and lock the locking ring at this time.

**Figure 31.** Inserting the Locking Ring into the Sleeve

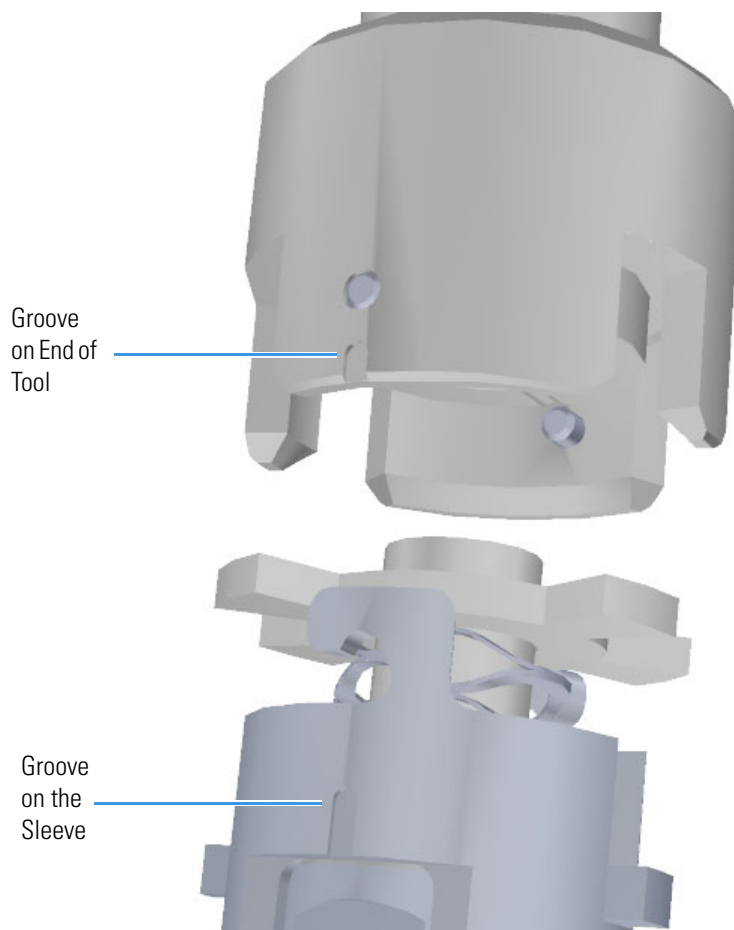


30. Hold the source holder and ion source cartridge in one hand and use the other hand to hold the source exchange tool so that the barrel is facing toward the floor. The diagram on the tool should be facing you.

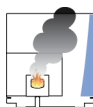
**IMPORTANT** If you try to attach the ion source cartridge to the tool without inverting it, the components may fall, so make sure you are working over a table. Otherwise, you may have to stop and clean the components again.

31. Look for a small vertical  $\frac{1}{8}$  in. line engraved on the end of the barrel. There is a similar engraved line on the sleeve of the ion source cartridge.

**Figure 32.** Aligning the Tool with the Ion Source Cartridge

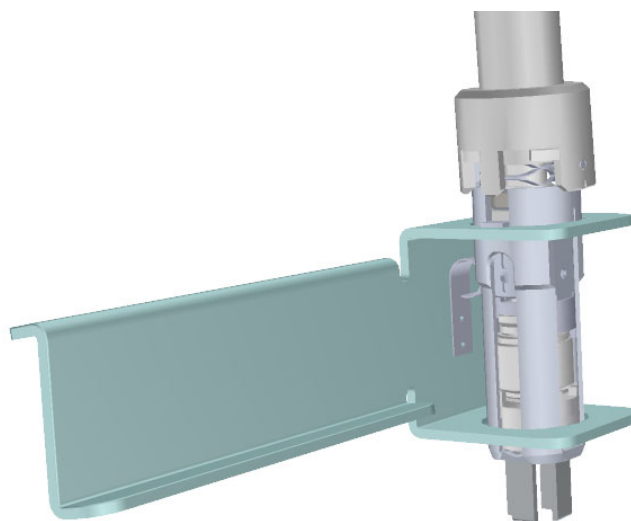


32. Position the line on the tool a half inch to the left of the line on the ion source cartridge so that the teeth of the locking ring will fit into the grooves on the end of the tool.
33. Push the ion source cartridge up into the barrel of the tool and twist it until the engraved lines are aligned. The ion source cartridge should easily slide into place with very little force.



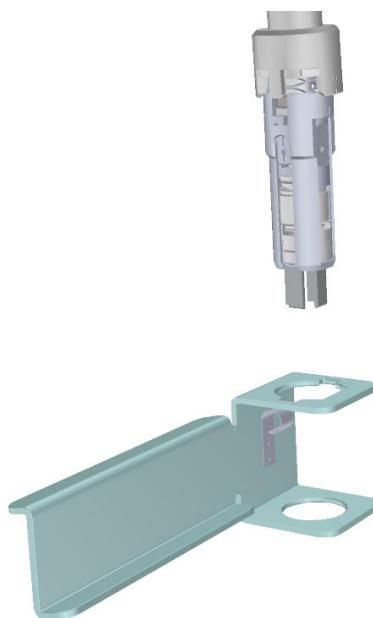
**CAUTION INSTRUMENT DAMAGE:** If you push the ion source cartridge into the barrel and it does not slide easily into position, make sure the components are assembled correctly and have not slid out of place inside the sleeve. Using too much force when inserting the ion source cartridge into the barrel can cause damage. The ion volume and lenses have keying features to assure the lens spring contacts will align with the metal parts of the lenses.

**Figure 33.** Reattaching the Ion Source Cartridge to the Tool



34. Gently pull the source holder away from the ion source cartridge.

**Figure 34.** Removing the Source Holder

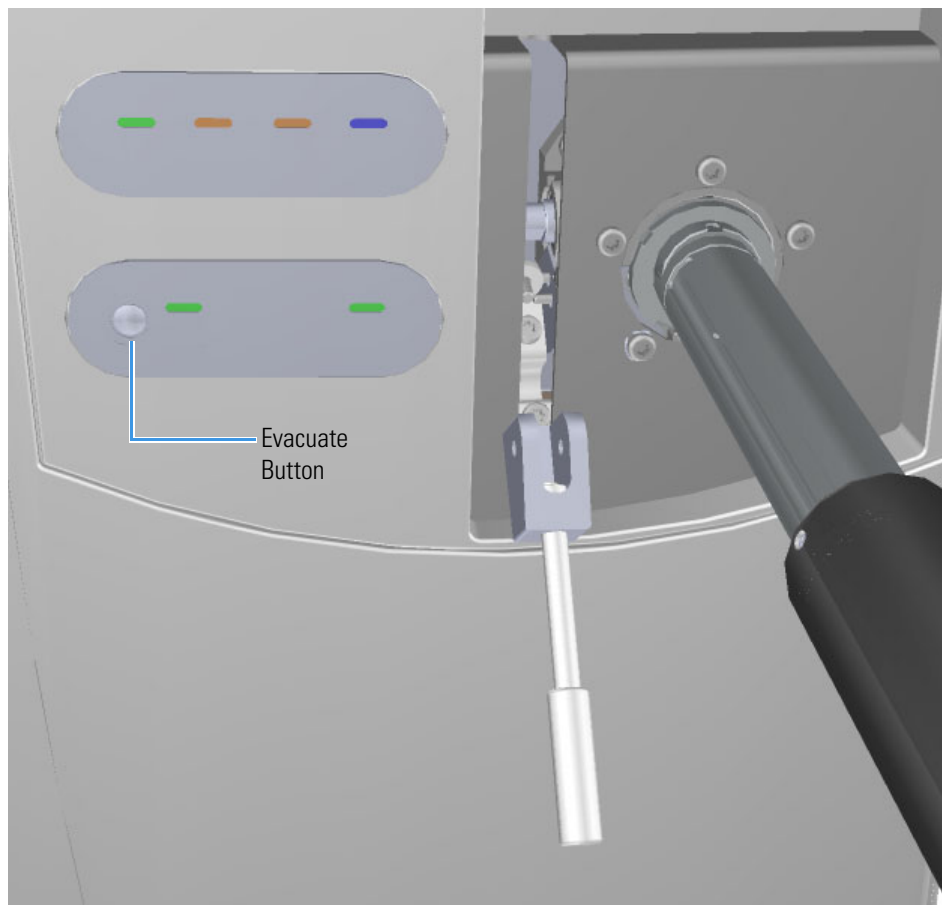


35. Turn the source exchange tool around so that the ion source cartridge is furthest away from you.
36. Firmly grasp the black handle of the source exchange tool in one hand and use your other hand to pull the metal barrel out and away from the handle so that the tool is fully extended. The ion source cartridge is now hidden inside the barrel.
37. When the barrel can go no further, twist the handle to the left to lock it into position.



38. Insert the barrel end of the source exchange tool into the vacuum interlock and twist it to the right to lock it into position.

**Figure 35.** Evacuating the Instrument



39. Press the **Evacuate** button on the front of the instrument. When the **Evacuate** light flashes green for approximately 20 seconds and turns off, the **Ready to Open** light turns solid green. At that point, the air has been evacuated from the instrument and it is safe to open it.
40. Pull the vacuum interlock handle up when the **Ready to Open** light is a solid green.
41. Twist the handle of the tool slightly to the left until it feels like it is lodged into the left-most track.

**IMPORTANT** Make sure you take the correct track on the tool or the ion volume will disassemble inside the instrument. If that happens, the tool will get stuck and you will have to shut down, vent the mass spectrometer, and manually remove the ion volume through the top of the instrument.

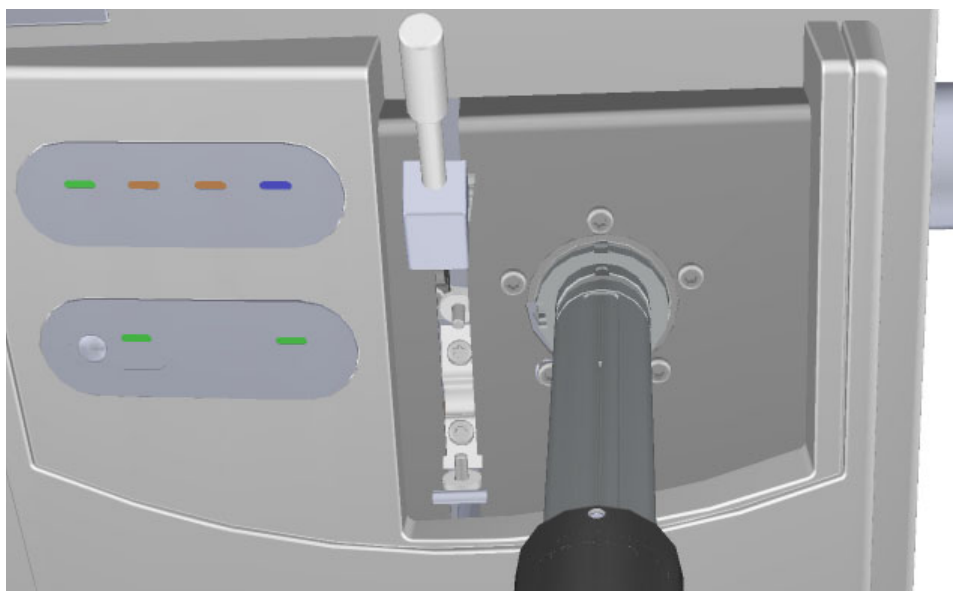
42. Push the handle toward the instrument until the end of the handle aligns with the engraved line at the end of the barrel. When you reach this line, the tool is all the way in and the ion source cartridge has been placed back onto the ion source block.



**CAUTION INSTRUMENT DAMAGE:** Make sure the vacuum interlock handle is all the way up. Otherwise if you insert the tool with the handle down, you can damage the instrument.

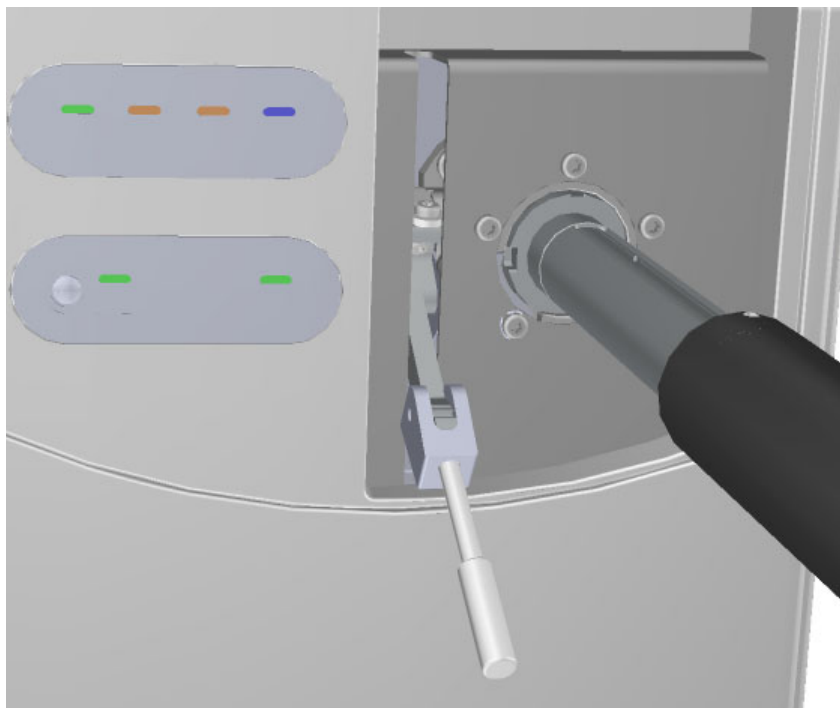
43. Twist the handle of the ion source tool to the right and pull it back toward you and down the right side of the barrel's metal track.

**Figure 36.** Pulling the Tool Down the Right Track



44. Pull the vacuum interlock handle down to close the interior ball valve.

**Figure 37.** Pulling Down the Vacuum Interlock Handle



45. Twist the end of the vacuum interlock handle and flip the handle up so that it rests next to the vacuum interlock knob location. Then twist the end again to hold it in place.

**CAUTION INSTRUMENT DAMAGE:** Do not push the handle all the way up or you will open the interior ball valve, which will cause you to lose vacuum and possibly damage the instrument.

46. Remove the tool from the instrument.
47. Reattach the vacuum interlock knob.

**Tip** To prevent leaks in the vacuum interlock, we recommend that you leave the vacuum interlock knob attached to the instrument, except when you are removing or inserting the ion source cartridge. After you reattach the knob, press the **Evacuate** button will also eliminate any air inside the vacuum interlock.

48. For optimal performance, wait at least 30 minutes for the ion source to heat up to the same temperature as the inside of the instrument. Otherwise, the masses or intensities may be different.

**Note** If you are using a Q Exactive GC mass spectrometer, tune and calibrate the instrument after replacing the repeller.

## Adding the Adapter for the Q Exactive GC to the Direct Probes

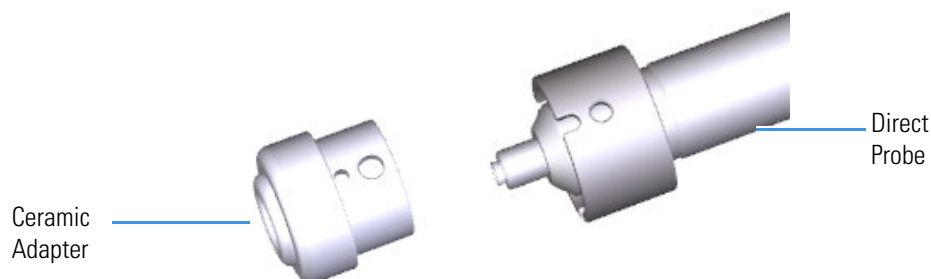
If you are using direct probes with a Q Exactive GC mass spectrometer, you must add the ceramic adapter to the end of the probe before using it. This adapter is found in the Q Exactive GC Probe Kit (PN 1R120705-0036).

### ❖ To add the ceramic adapter to a direct probe

**Note** Wear CP100 Nitrile Cleanroom Gloves when adding the ceramic adapter to the direct probe. See your mass spectrometer's spare parts guide for ordering information.

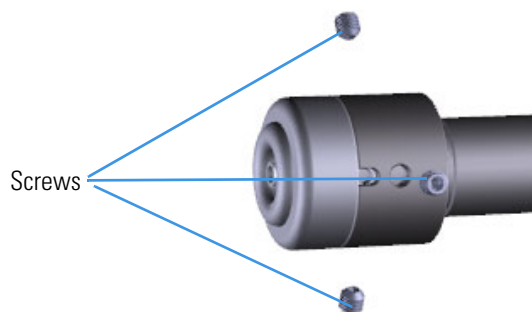
1. Place the ceramic adapter on the end of the direct probe.

**Figure 38.** Adding the Ceramic Adapter to a Direct Probe



2. Use the included wrench to tighten the three screws on the adapter.

**Figure 39.** Locating the Three Screws on the Adapter



## Step 4: Program the Direct Probe Controller

### Programming the Direct Insertion Probe

For the analysis of the cholesterol standard, which is included in your Direct Insertion Probe Kit, you must set the initial temperature to 50 °C and hold it for 60 seconds. Then you need to adjust the maximum heating rate to raise the temperature of the probe to 350 °C and hold it for 40 seconds. The second temperature ramp is not used.

#### ❖ To program the direct probe controller

1. Set the initial temperature:
  - a. Press the **Method** button on the direct probe controller.
  - b. Press the **Up** or **Down** buttons to adjust the temperature.
  - c. Press **Next** once the temperature is set and program the initial time.
2. Set the initial time by pressing the **Up** or **Down** buttons. Once the initial time is set, press **Next** and program the temperature ramp.
3. Set the temperature ramp by pressing the **Up** or **Down** buttons. Once the temperature ramp is set, press **Next** and program the final temperature.
4. Set the final temperature by pressing the **Up** or **Down** buttons. Once the final temperature is set, press **Next** and program the final hold time.
5. Set the hold time by pressing the **Up** or **Down** buttons. Once the hold time is set, press **Next** and program the second temperature ramp rate.

If the second temperature ramp rate is set to Off, press the **Next** button. The direct probe controller is now programmed and should display *DIP 1 ready*. Otherwise, repeat steps 3-5 to set the second temperature ramp, final temperature and hold time.

6. Save a method:
  - a. Press the **Prog** button.
  - b. Press the **Next** button to display *Save Method 1*.
  - c. Press the **Up** or **Down** buttons to change the number (1-9) of the method you are saving.
  - d. Once the method number is set, press **Next** to save it.

### Programming the Direct Exposure Probe

For the analysis of the cholesterol standard, which is included in your Direct Exposure Probe Kit, you must set the initial current to 0 mA and hold it for 30 seconds. Then you need to use the 20 mA/s rate to raise the current of the probe to 1000 mA and hold it for 30 seconds.

❖ **To program the direct probe controller**

1. Set the initial current:
  - a. Press the **Method** button on the direct probe controller.
  - b. Press the **Up** or **Down** buttons to adjust the current.
  - c. Press **Next** once the current is set and program the initial time.
2. Set the initial time by pressing the **Up** or **Down** buttons. Once the initial time is set, press **Next** and program the current ramp.
3. Set the current ramp by pressing the **Up** or **Down** buttons. Once the current ramp is set, press **Next** and program the final current.
4. Set the final current by pressing the **Up** or **Down** buttons. Once the final current is set, press **Next** and program the final hold time.
5. Set the hold time by pressing the **Up** or **Down** buttons. Once the hold time is set, press **Next** and program the second current ramp rate.
6. Save a method:
  - a. Press the **Prog** button.
  - b. Press the **Next** button to display *Save Method 1*.
  - c. Press the **Up** or **Down** buttons to change the number (1-9) of the method you are saving.
  - d. Once the method number is set, press **Next** to save it.

## Retrieving a Method

❖ **To retrieve a previously saved method from the direct probe controller**

1. Press the **Prog** button to display *Load Method: X* (methods numbered 1-9).
2. Press the **Up** and **Down** buttons to locate a method number.
3. Press the **Enter** button to select the method number and retrieve the method.

## Step 5: Prepare the Sample

### Preparing the sample for the Direct Insertion Probe

❖ **To prepare the cholesterol sample in the DIP Test Mix**

1. Place the sample cup holder on a level surface.
2. Insert a clean sample cup into the holder.
3. Use a syringe to inject 1 µL of the (10 ng/µL as supplied) cholesterol solution into the sample cup.

4. Wait several minutes for the solvent to evaporate.
5. Use tweezers to place the sample cup onto the tip of the probe.

### Preparing the sample for the Direct Exposure Probe

#### ❖ To prepare the cholesterol sample in the DEP Test Mix

1. Place the sample filament holder on a level surface.
2. Insert a clean filament into the holder.
3. Use a syringe to place 1  $\mu\text{L}$  of the cholesterol solution on the filament.
4. Wait several minutes for the solvent to evaporate.
5. If desired, place another drop of the (1 ng/ $\mu\text{L}$  as supplied) cholesterol solution on the filament.
6. Use tweezers to place the filament onto the tip of the probe.

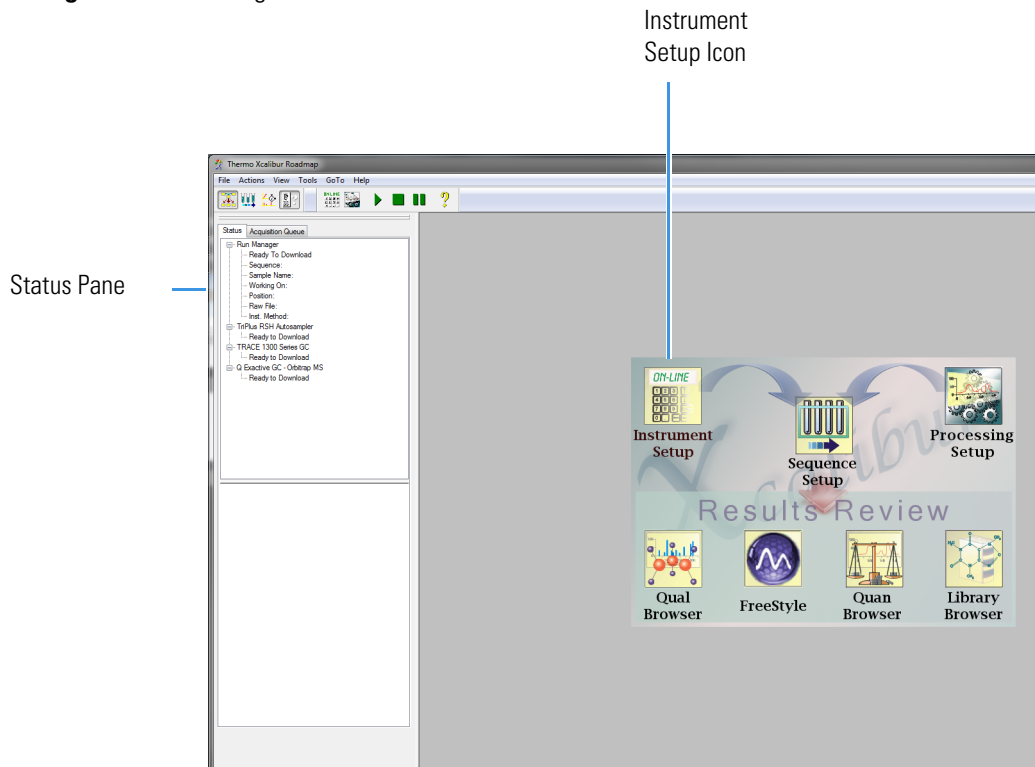
If the filament is already inserted into the tip of the probe, it is not necessary to take it out: the solution can be applied to the filament while it is inserted into the tip of the probe.

### Step 6: Create an Xcalibur Method

#### ❖ To create an *Xcalibur* method

1. Open the *Xcalibur* software and click the **Instrument Setup** icon.

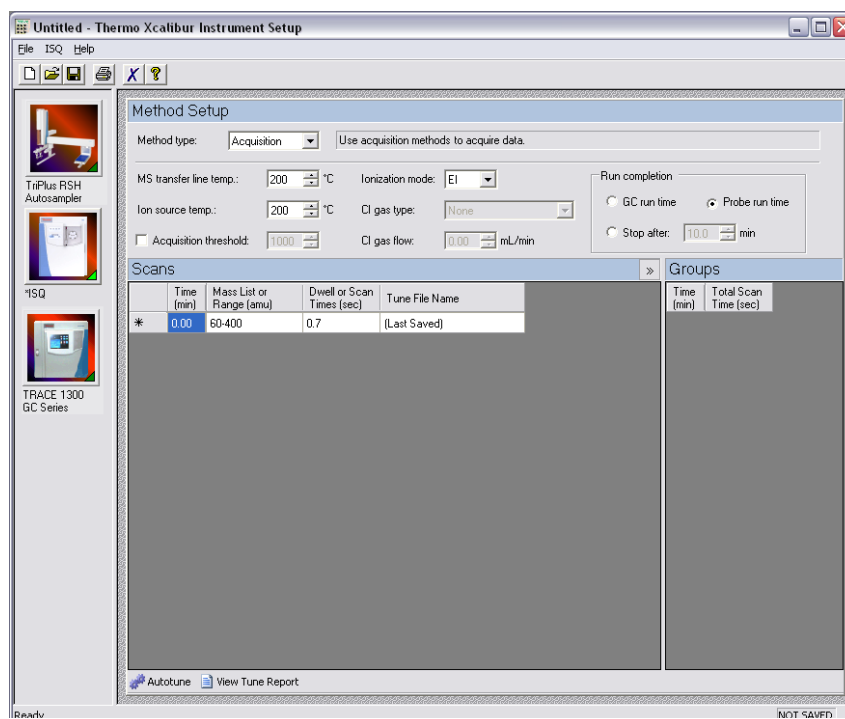
**Figure 40.** Creating an Xcalibur Method.



2. Select **File | New** from the main menu.
3. If you have an ISQ 7000 mass spectrometer and are analyzing the cholesterol standard that was included with your DIP or DEP, enter the 60-400 in the Mass List or Range (amu) column. Set the MS transfer line and the ion source temperatures to 200 °C. Select **Probe Run Time** for run completion, otherwise you must manually stop the acquisition. See [Figure 41](#).

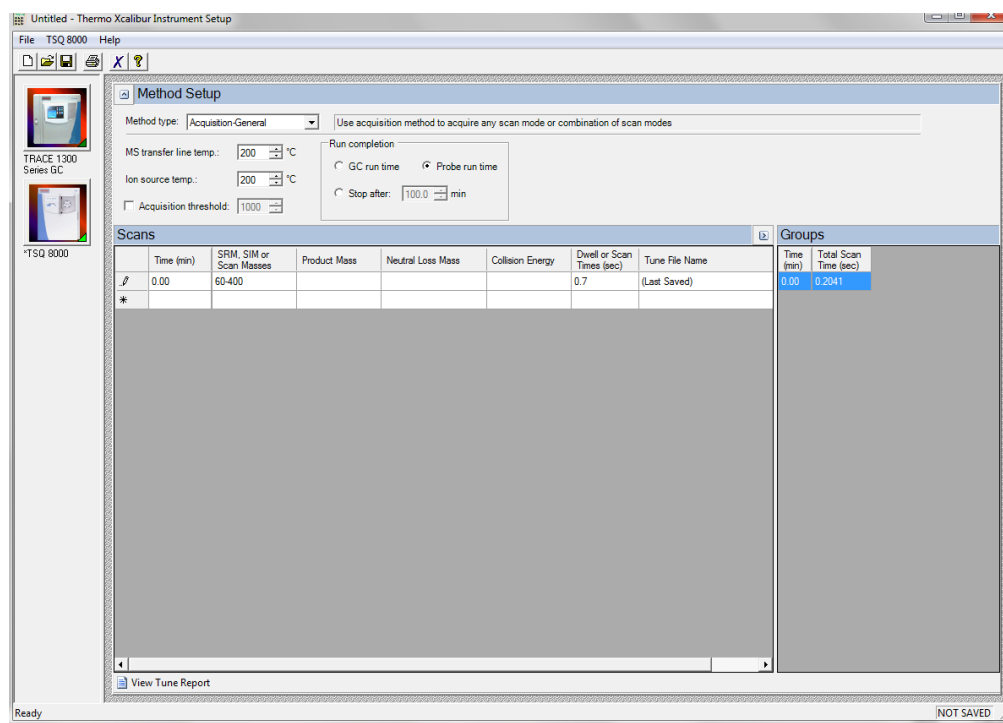


**Figure 41.** Creating a Method for the ISQ 7000 Mass Spectrometer



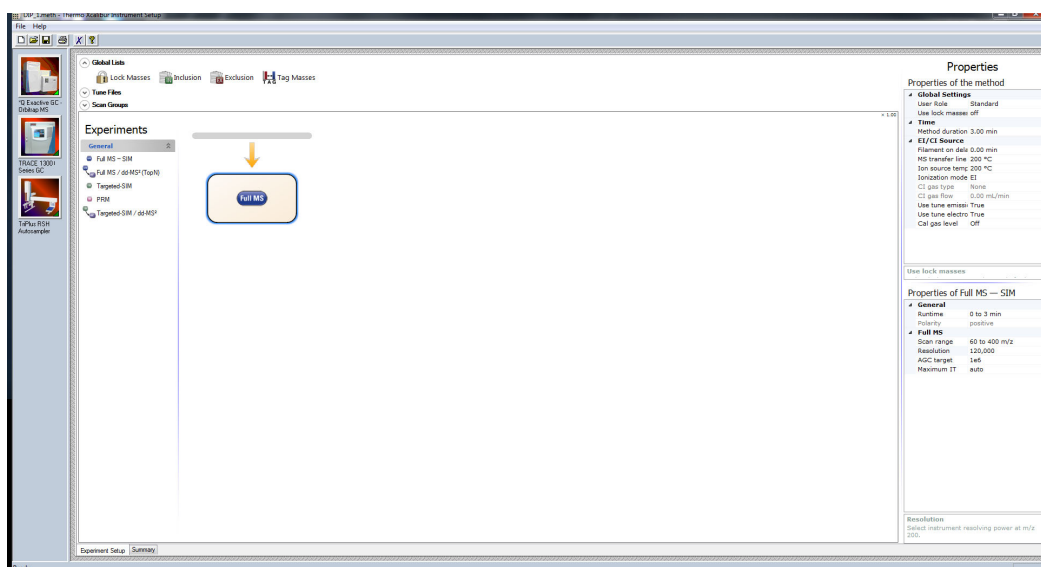
- If you have a TSQ 9000 mass spectrometer and are analyzing the cholesterol standard that was included with your DIP or DEP, enter the following parameters. Set the MS transfer line and the ion source temperatures to 200 °C. Enter 60-400 in the **SRM**, **SIM** or **Scan Masses** column. Be sure to select **Acquisition-General** from the **Method Type** pull-down menu. See [Figure 42](#).

**Figure 42.** Creating a Method for the TSQ 9000 Mass Spectrometer



5. If you are creating a new method and have the GC in the configuration, ensure that vacuum compensation is on for the GC carrier flow, otherwise, the GC carrier gas flow will be too high into the ion source. If you do not want to change the current GC settings, you can obtain the current method from the GC using a command from the instrument method editor menu.
6. Select **File | Save** from the main menu.
7. Enter the name of the cholesterol method and click **Save**. *Xcalibur* automatically adds the *.meth* extension to any method file.
8. Return to the Xcalibur Home Page window.
9. If you have a Q Exactive GC mass spectrometer and are analyzing the cholesterol standard that was included with your DIP or DEP, enter the following parameters. Set the MS transfer line and the ion source temperatures to 200 °C. Set the **Scan Range** to 60 to 400 m/z. Be sure to select **Full MS** in the **Experiments** pane. See [Figure 42](#).

**Figure 43.** Creating a Method for the Q Exactive GC Mass Spectrometer



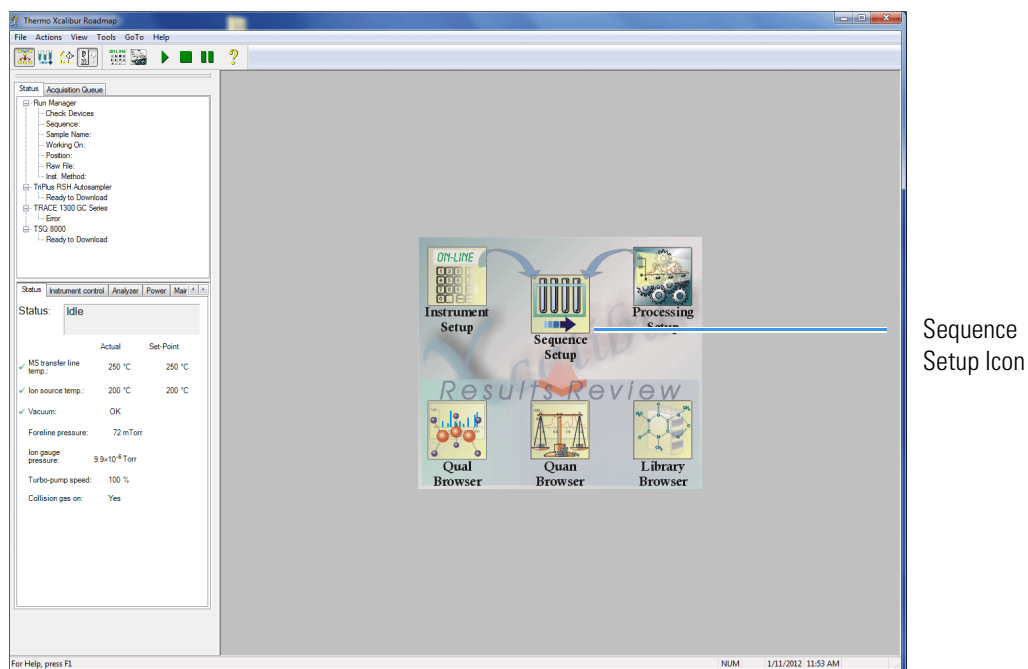
10. If you are creating a new method and have the GC in the configuration, ensure that vacuum compensation is on for the GC carrier flow, otherwise, the GC carrier gas flow will be too high into the ion source. If you do not want to change the current GC settings, you can obtain the current method from the GC using a command from the instrument method editor menu.
11. Select **File | Save** from the main menu.
12. Enter the name of the cholesterol method and click **Save**. *Xcalibur* automatically adds the *.meth* extension to any method file.

## Step 7: Create a Sequence File

### ❖ To create a sequence file

1. Open the *Xcalibur* software and click the Sequence Setup icon.

**Figure 44.** Setting up a Sequence in Xcalibur

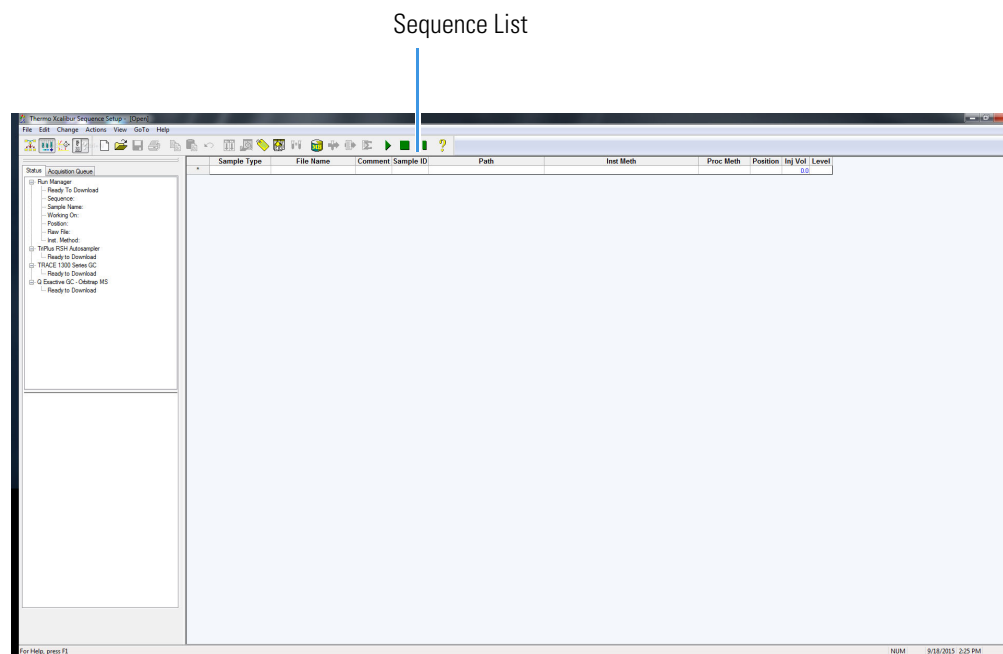


**Note** If you have devices such as an autosampler or GC configured that you are using for the probe analysis, you may want to remove them from the instrument configuration. Xcalibur will not start the run if a device is not connected or reporting an error, even if it is not needed for the analysis. See your mass spectrometer user guide for instructions on configuring your system.

2. Select **File | New** to open a blank sequence file.

3. Enter the settings shown in the figure below.

**Figure 45.** Creating a Sequence



**Note** Filenames are not case-sensitive.

4. Select **File | Save** from the main menu.
5. Enter the name of the new sequence file and click **Save** to return to the Sequence Setup window. *Xcalibur* automatically adds the *.slr* extension to the filename.

## Step 8: Insert the Probe

### ❖ To insert the probe

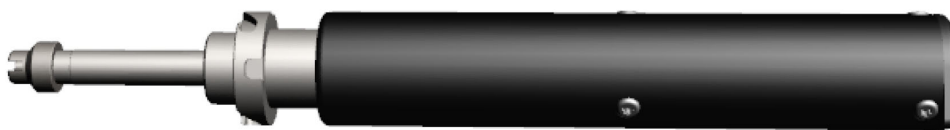
1. Twist off the round vacuum interlock knob located on the front of the instrument and set it aside. As you can see, the knob covers access to a long tunnel that goes into the instrument. This tunnel is called the vacuum interlock.

**Figure 46.** Removing the Vacuum Interlock Knob



2. Lift the probe with the sample loaded at the tip.

**Figure 47.** Direct Insertion Probe

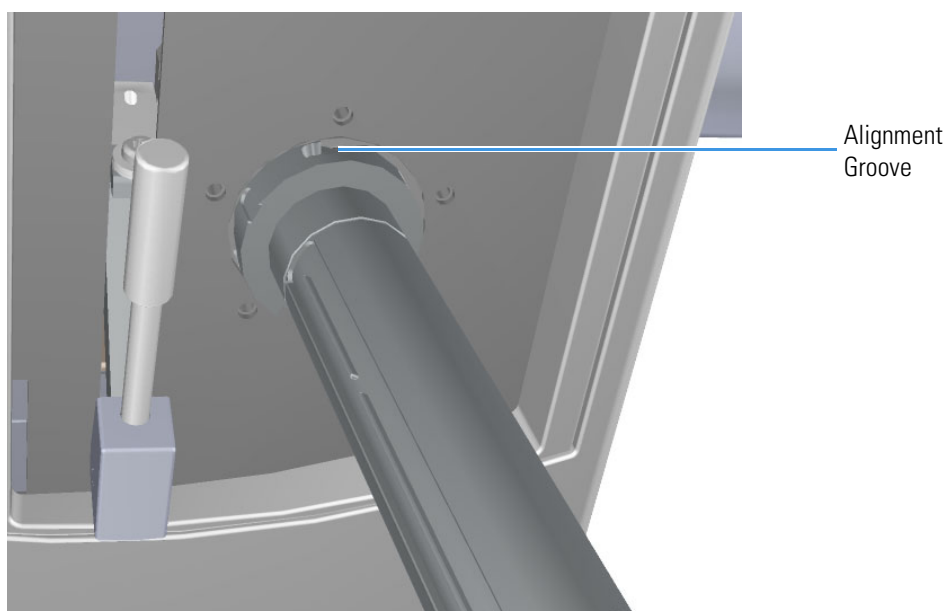


**Figure 48.** Direct Exposure Probe



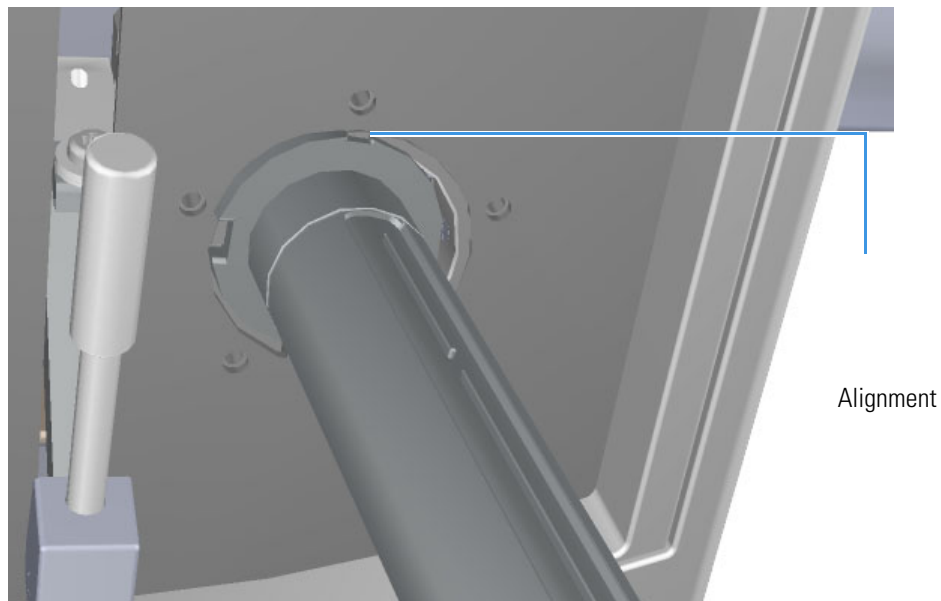
3. Grasp the handle of the probe in one hand and use your other hand to pull the metal barrel out and away from the handle so that tool is fully extended.
4. With your hand around the barrel, twist the probe until the alignment groove at the end of the barrel aligns with the left screw around the slot vacated by the vacuum interlock knob.

**Figure 49.** Inserting the Probe



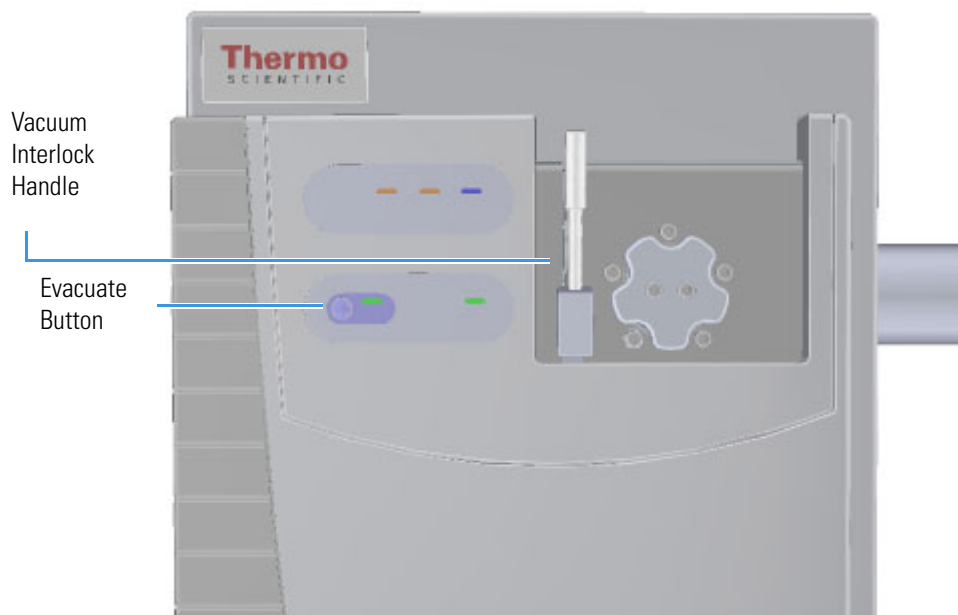
5. Twist and push the barrel to the right until it clicks into place under the screw at the top of the slot.

**Figure 50.** Twisting the Probe Into Place



6. Press the blue **Evacuate** button on the front of the instrument. When the Evacuate light flashes green for approximately 20 seconds and turns off, the **Ready to Open** light turns solid green. At that point, the air has been evacuated from the instrument and it is safe to open it.

**Figure 51.** Evacuating the Instrument

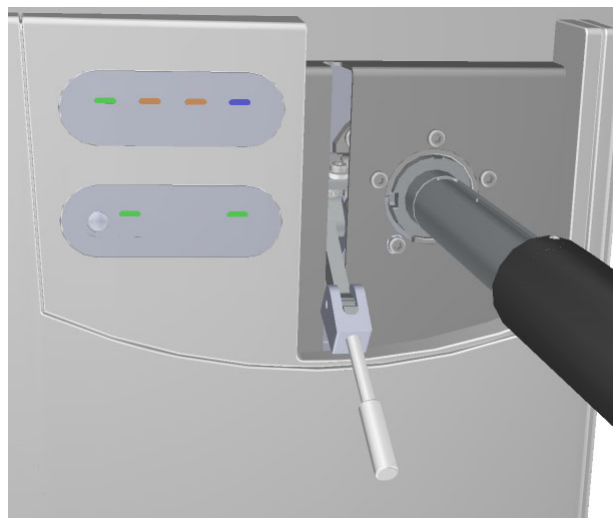




7. Twist, then loosen the top part of the vacuum interlock handle to allow the entire handle to move up and down. This handle controls the interior ball valve, which seals the vacuum chamber and protects it from the outside pressure.
8. Push the handle down and then twist the top of it in the opposite direction to lock it into place.

**Note** If a minute has elapsed since the **Ready to Open** light first came on, the **Ready to Open** light will turn off. You should press the **Evacuate** button and wait until the **Ready to Open** light is lit again before opening the valve. Do not open the valve if the ready to open light is not on. This may indicate a leak in the valve region. When a leak is detected during the evacuate sequence, the amber evacuate LED will flash on and off.

**Figure 52.** Pulling Down the Vacuum Interlock Handle



9. Pull the handle up to open the interior ball valve.
10. Slowly push the probe into the instrument.

**Note** To keep sample cool for volatile samples, do not fully insert the probe into the ion source until you are ready to press the **Start** button on either direct probe controller.

11. Once the probe is all the way in, you are ready to perform your analysis.

**Note** Because of the ceramic adapter, the probe will not insert all the way into a Q Exactive GC instrument.

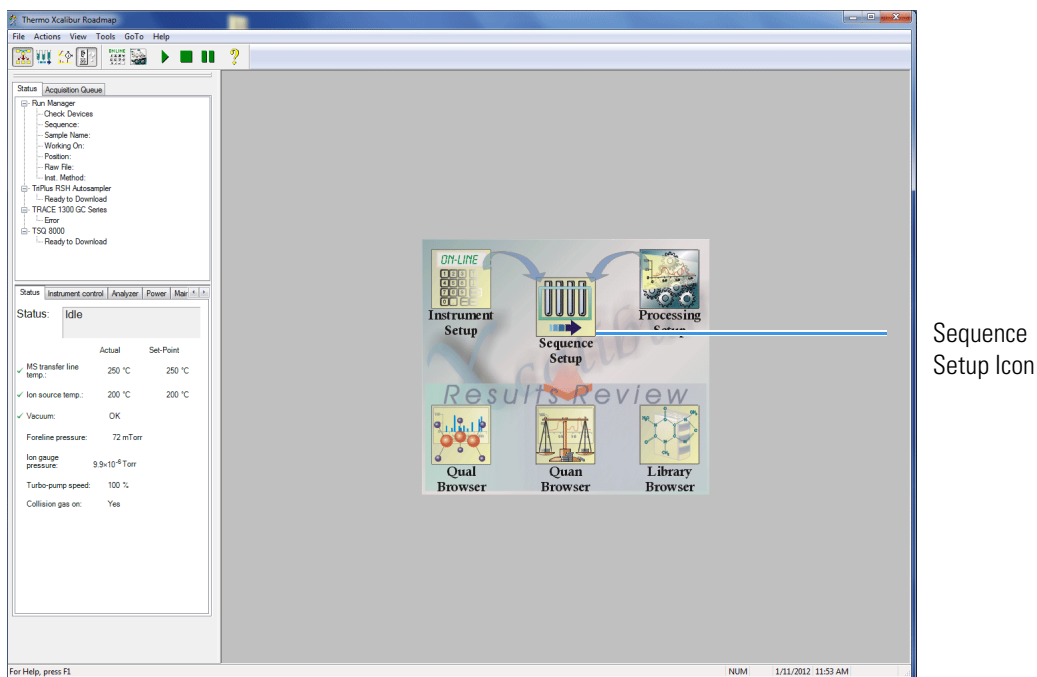
## Step 9: Collect the Data

### ❖ To collect data

1. Double-click the *Xcalibur* software icon on your computer desktop.

2. In the Xcalibur Roadmap window, click the Sequence Setup icon

**Figure 53.** Setting up Sequence in Xcalibur




**Note** If you have devices such as an autosampler or GC configured that you are using for the probe analysis, you may want to remove them from the instrument configuration. Xcalibur will not start the run if a device is not connected or reporting an error, even if it is not needed for the analysis. See your mass spectrometer user guide for instructions on configuring your system.

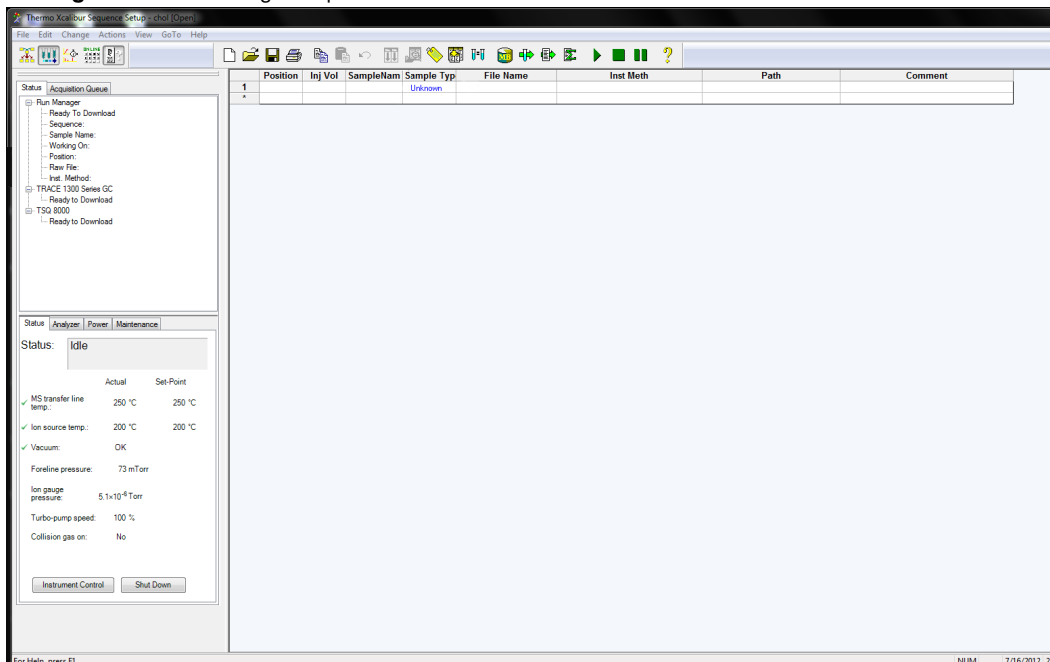
3. Load the sequence you created in [Step 7: Create a Sequence File](#) and skip to step 16 below. If you want to create a different sequence, proceed to step 4 below.

**Tip** If not all columns are visible on your Sequence Setup page, right-click on any of the columns to view and add more options.

4. In the Sequence Setup window, click in the first row of the **Sample Type** column and select a sample type from the drop-down menu. You should select Unknown when you are developing an analytical method. The other types of samples require a quantitation method.

**Note** To open an existing sequence, you can select **File | Open** from the main menu or click the  icon and browse to a sequence.

**Figure 54.** Entering Samples in Xcalibur



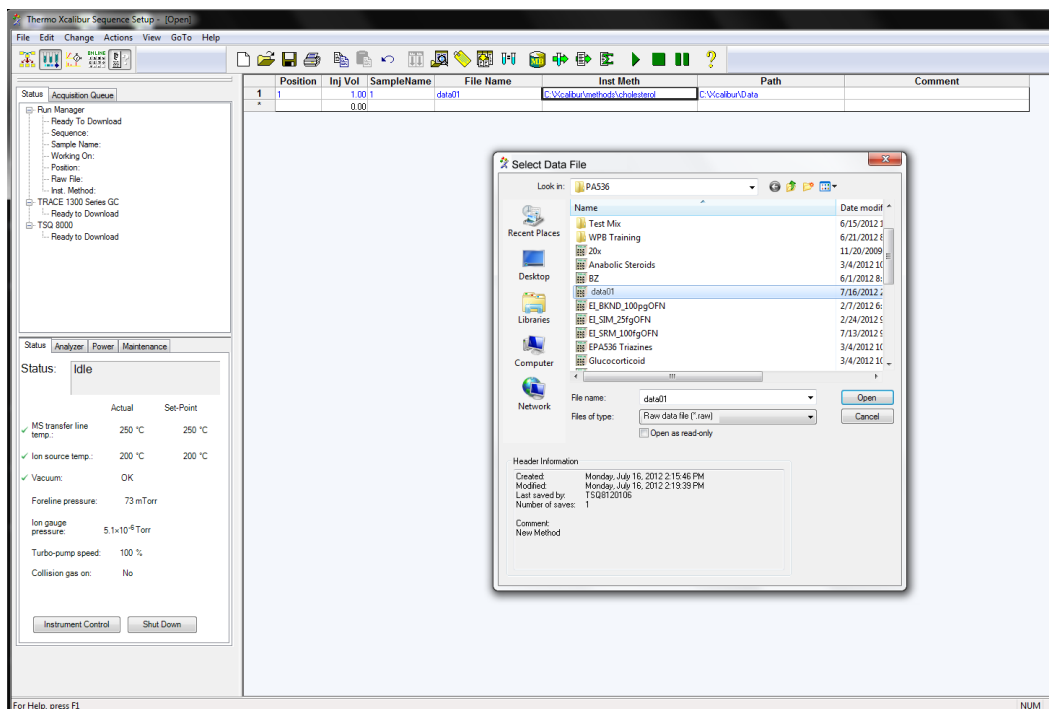
Once you select a sample type, default information automatically appears in some of the other columns. You can also right-click on the field to clear, copy, or paste into the field, as well as add and delete rows.

**Note** If you do not have some of the columns discussed here, you can add them by selecting **Change | Column Arrangement** in the main menu.

5. Click the first row in the **File Name** column and enter a file name for your first data file.

**Note** Your file name cannot contain spaces.

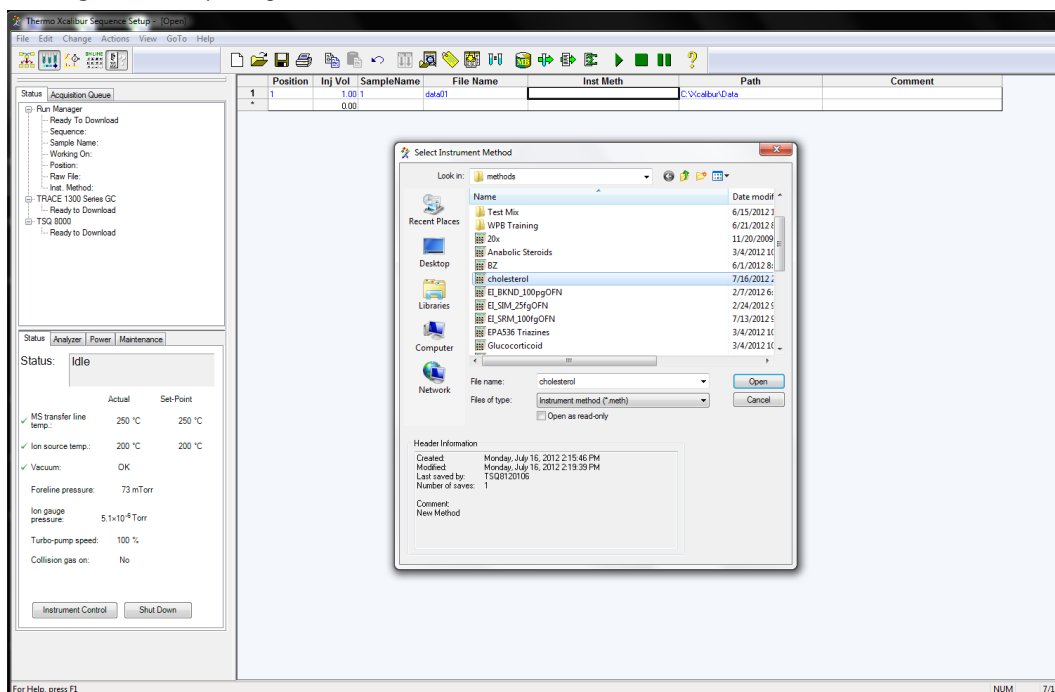
**Figure 55.** Selecting a Data File in Xcalibur





**Tip** You can double-click in the **File Name** field and browse to a raw file on your computer.

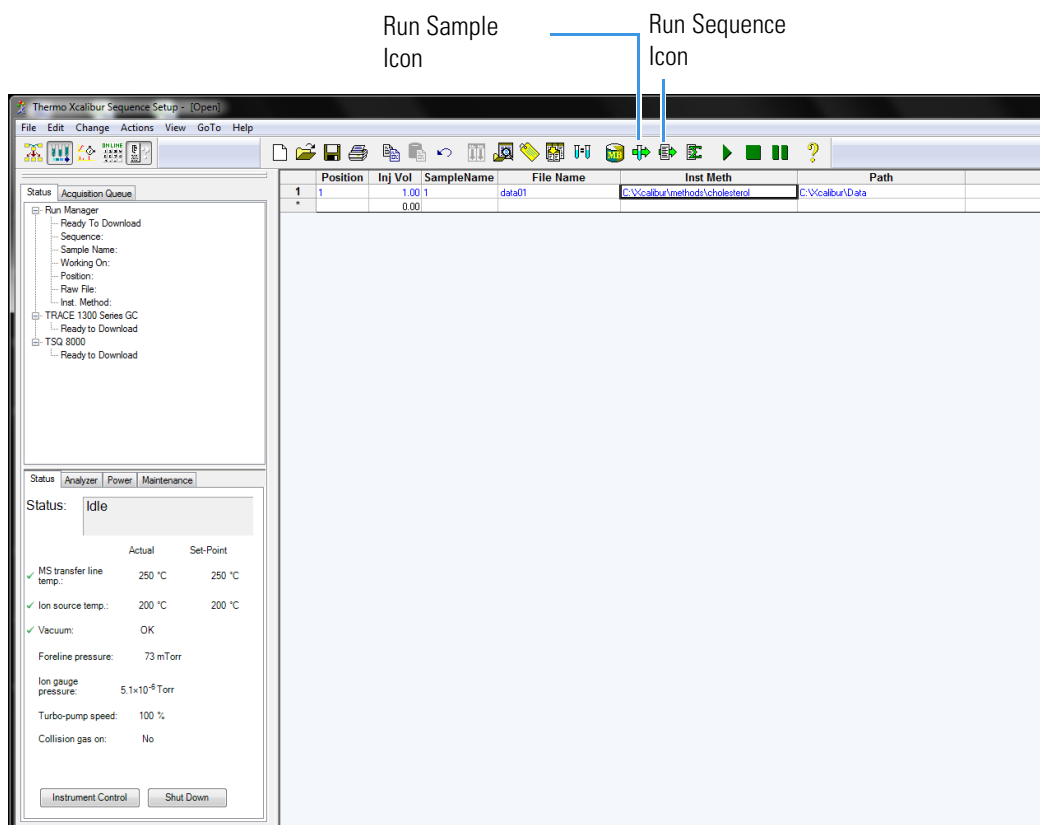
6. Click past the **Sample ID** column, which typically contains the number of the sample. Since you are in the process of developing your method, you do not have that many samples so you do not have to enter anything in this field. You can right-click on the field to clear, copy, or paste a number, as well as add and delete rows.
7. Double-click the first row in the **Path** column and select the folder in which to store your data files. You can also right-click on the field to clear, copy, or paste into the field, browse to a folder on your computer, or add and delete rows.
8. Double-click the first row in the **Instrument Method** column and navigate to the folder containing your instrument method. Select the instrument method and click the **Open** button. You can also right-click on the field to clear, copy, or paste into the field, browse to a folder on your computer, or add and delete rows.

**Figure 56.** Opening an Instrument Method



9. Click past the **Processing Method** column, which typically contains the path to your processing method. You are in the process of developing your method and the processing method is created after your method is finalized.
10. Click past the **Level** column, which is only used when you have a processing method. Since you are in the process of developing your method, you do not have the processing method defined yet.
11. Now that you have set up one row of your sequence, you can stop or you can continue adding tasks. Click in the next row and repeat steps 2-10 for each additional task in your sequence. Remember, you can right-click in most fields to add more rows as you go.
12. Once you have created a sequence of all your samples, select **File | Save** or click the  icon to save the sequence.
13. Enter a comment and click **OK**.
14. Click the row you want to run and select **Actions | Run This Sample** from the main menu. You can also just click the  icon on the main toolbar.

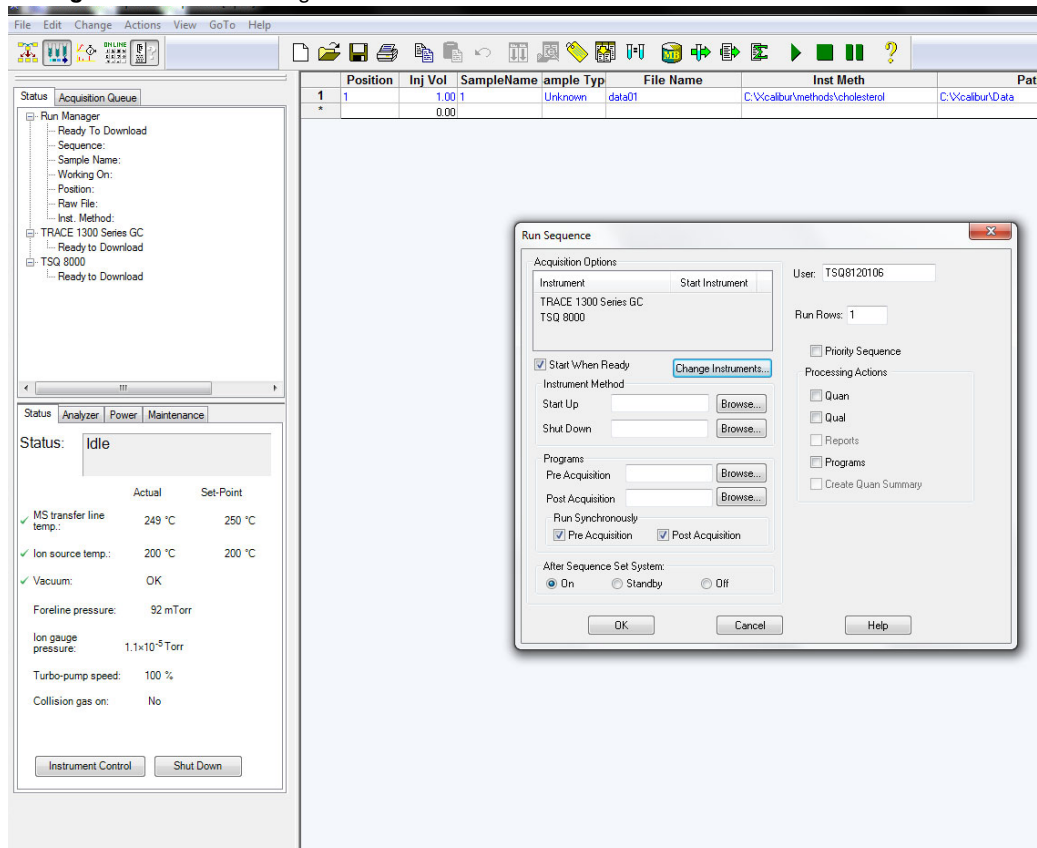
**Figure 57.** Starting a Sample or Sequence Run in Xcalibur



To run the whole sequence, select **Actions | Run Sequence** from the main menu. You can also just click the  icon on the main toolbar.

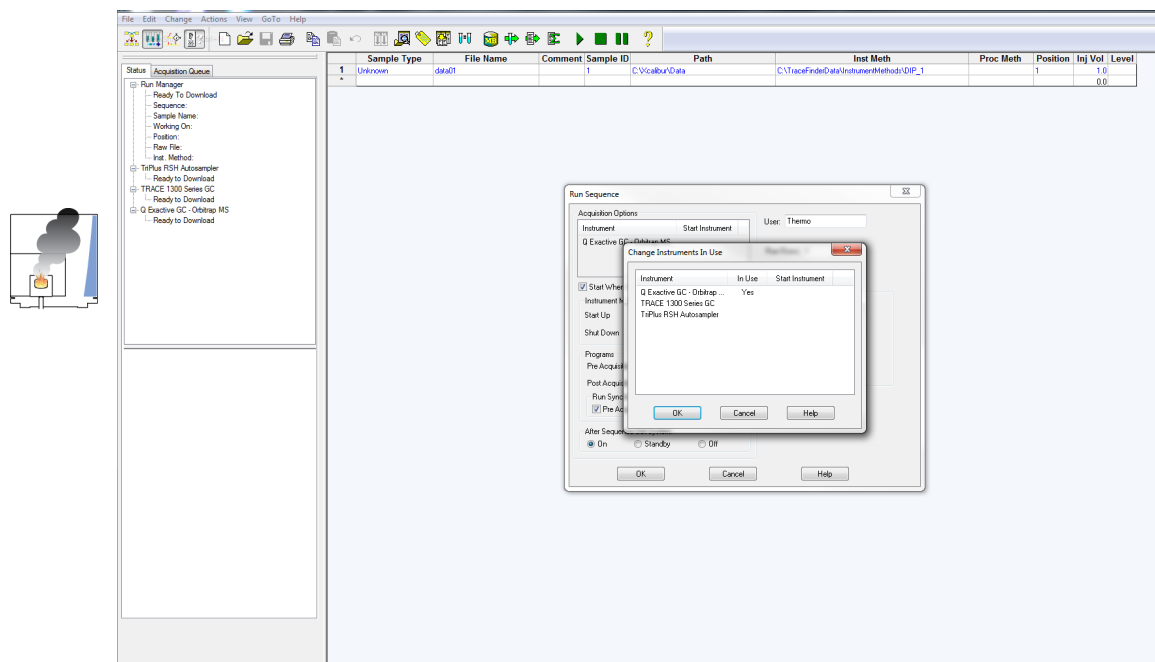
15. In the Run Sequence window, customize the way you want your sequence to run.

**Figure 58.** Customizing a Run



- Select the **Start When Ready** checkbox to run the sequence when the instruments are ready.
- Click **Change Instruments**. Ensure that the **In Use** column reads **Yes** next to the name of the mass spectrometer. If it does not, click the mass spectrometer name in the **Instrument** column. See [Figure 59](#). Then click **OK**.

**Figure 59.** Setting the Instrument in Use to the Mass Spectrometer



- c. In the **Instrument Method** group, you can browse to the sequence to be used when the instrument starts up or shuts down.
- d. Configure the **After Sequence Set System** group:
  - **On**—Select this option to leave the instrument fully ready after an acquisition.
  - **Standby**—Select this option to leave the instrument fully ready after an acquisition.
  - **Off**—Select this option to shut down the MS using the automatic shutdown sequence built into the instrument firmware. This will turn off high voltages, heaters, and vacuum pumps.
- e. In the **User** field, you can change your user name. The user name defaults to the name that was used to log into the computer.
- f. In the **Run Rows** field, enter the rows to be run in the sequence. Each value must be separated by a hyphen.
- g. Select the **Priority Sequence** checkbox if this sequence has priority over any other sequence. If someone else's sequence is running in the background, this setting puts your sequence ahead of theirs.
- h. In the Processing Actions group, select **Quan**, **Qual**, **Reports**, **Programs** or **Create Quan Summary**. Since you are developing your method, you can skip this setting.

**Note** The **Reports** checkbox is only enabled when you select the **Qual** checkbox. The **Reports** and **Create Quan Summary** checkboxes are only enabled when you select the **Quan** checkbox.

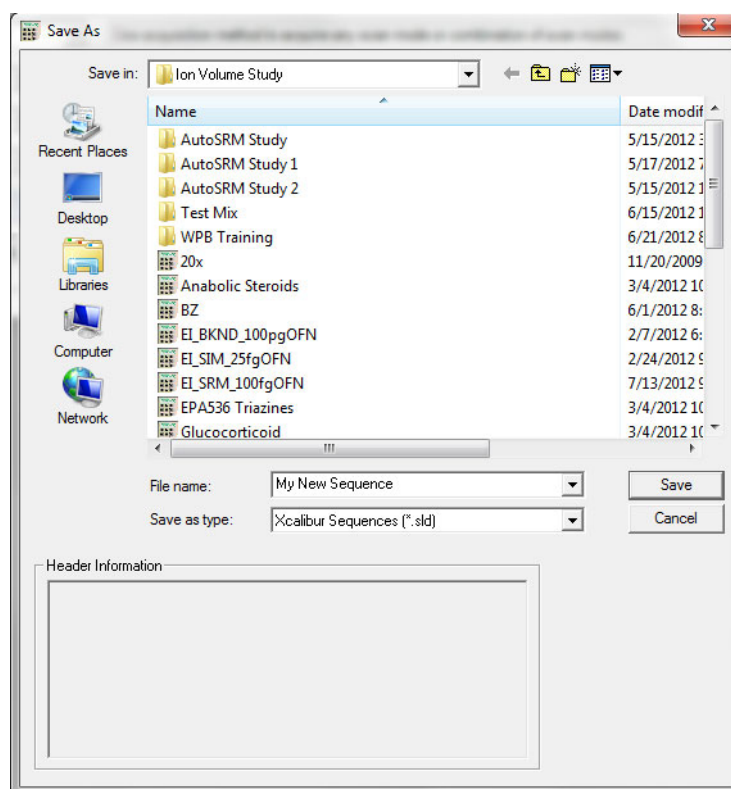


16. Click the **OK** button to run the sample or sequence, and store your data on the computer.
17. Push the **Start/Stop** button on the direct probe controller.

**Tip** Manual control is available for controlling the temperature of the Direct Insertion Probe during a temperature program. To pause the current program's ramp, press **Enter** on the probe controller. This holds the probe at its current temperature. Now you can manually adjust the temperature up and down between the low and high points of the temperature ramp by pressing the **Up** and **Down** buttons on the probe controller. Press the **Start** button during the paused temperature state to resume the program at the current ramp. Note that these manual control options are not available when the ramp rate is set to **MAX**.

18. In the Save As window, select a location on your computer that you want to store your sequence. Then click the **Save** button.

**Figure 60.** Saving a Sequence on Your System



19. Once your sequence completes, you are ready to explore your data.

## Step 10: Remove the Probe

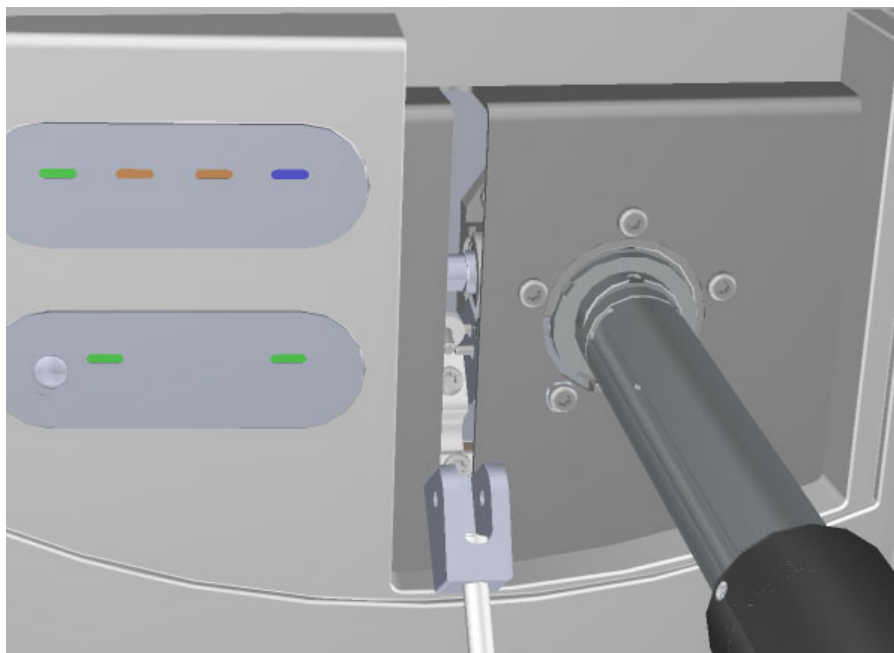
❖ **To remove the probe**

1. Pull the probe slowly away from the instrument. Before proceeding to the next step, make sure the probe is fully extended.

**CAUTION INSTRUMENT DAMAGE:** Do not remove the probe when it is hot (above 100 °C) or you may damage the inlet valve vacuum seals. Also, make sure the probe is fully extended before closing the ball valve to prevent damage to the ball valve and the probe.

2. Close the ball valve by pulling down the vacuum interlock handle.

**Figure 61.** Pulling Down the Handle



3. Remove the probe from the vacuum interlock.



**WARNING BURN HAZARD:** Keeping the probe in the instrument at 350 °C for more than 15 minutes will cause the probe tip to become very hot. Use caution when handling a probe at this temperature.

4. Reattach the vacuum interlock knob.

**Tip** To prevent leaks through the vacuum interlock, we recommend that you leave the vacuum interlock knob attached to the instrument, except when you are removing or inserting the ion source cartridge. After you reattach the knob, press the **Evacuate** button to eliminate any air inside the vacuum interlock.

**Figure 62.** Reattaching the Vacuum Interlock Knob



5. Close the front door of the instrument.

## Step 11: Analyze the Data

### Analyzing Data Acquired Using a Direct Insertion Probe

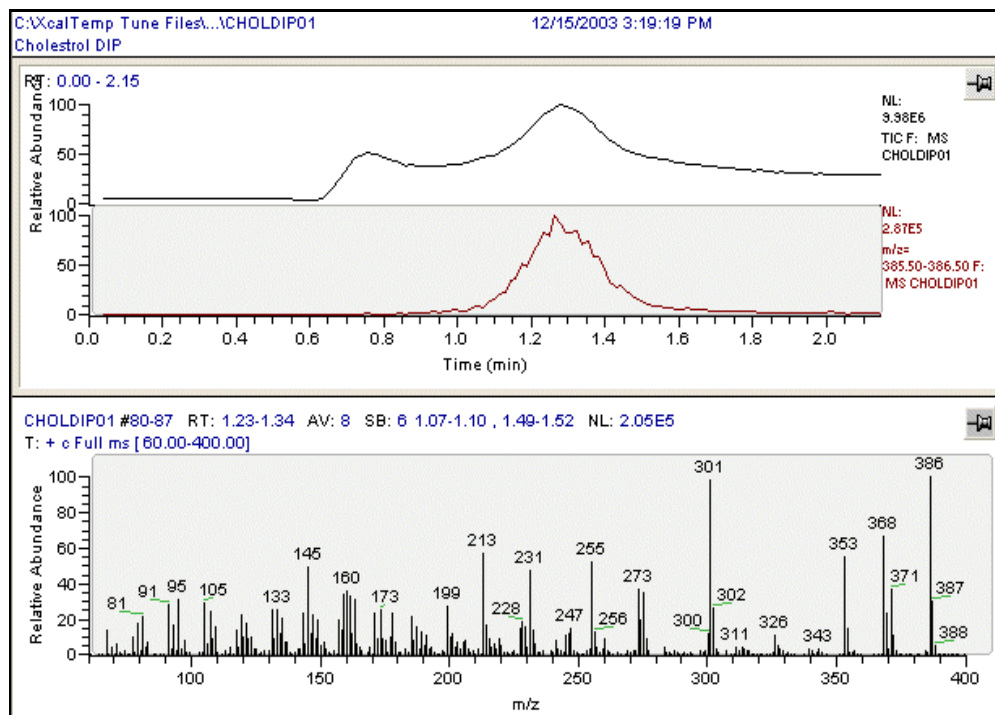
❖ **To analyze the data file acquired with a DIP**

**Note** To analyze another cholesterol sample, repeat the steps in [Step 5: Prepare the Sample](#) and make the appropriate addition to the sequence table.

1. In the Sequence Setup window, click the **Roadmap** icon to return to the Xcalibur Home Page.
2. Select **Qual Browser** to open the Qual Browser window. Refer to the Qual Browser documentation to learn how to use Qual Browser techniques.

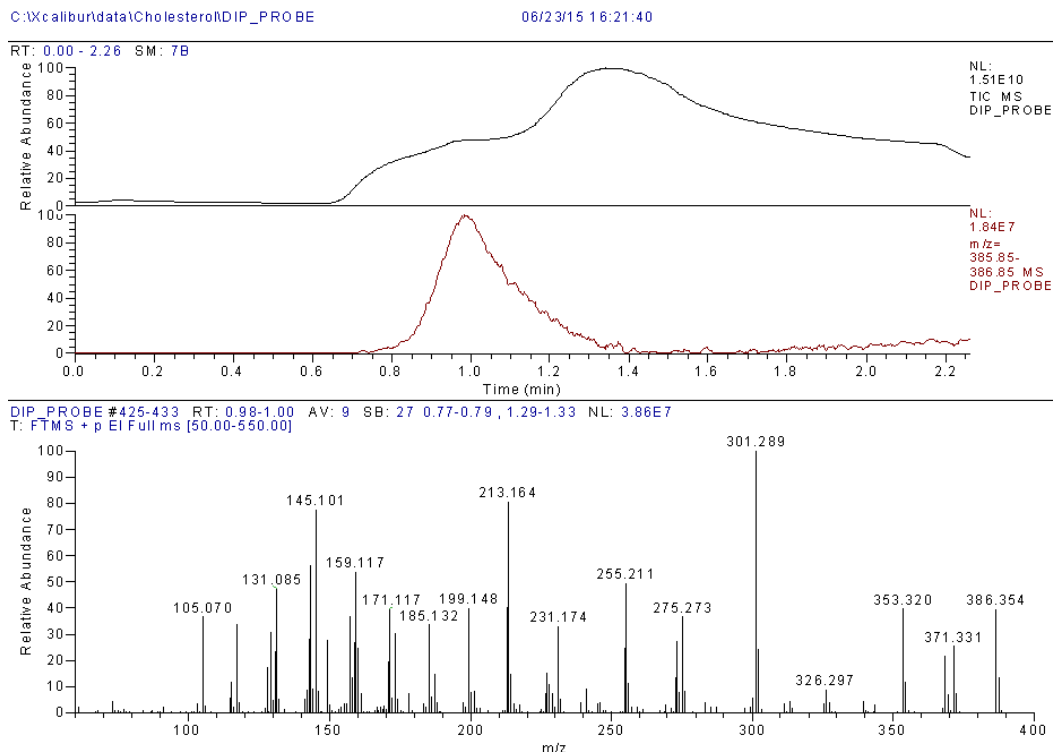
3. Select **File | Open** and select the raw data file you just ran. Your cholesterol sample should look similar to the data shown in the figure below.

**Figure 63.** Analyzing Your DIP Data in Qual Browser



4. The figure below shows accurate mass spectra acquired using a DIP on the Q Exactive GC instrument.

**Figure 64.** Analyzing DIP Accurate Mass Data Acquired on the Q Exactive GC System



5. You can use the mass calculator in the **Tune** program to calculate the accurate mass of your compound of interest from its formula.

**Figure 65.** Using the Mass Calculator to Determine Accurate Mass of a Compound

**Mass Calculator**

Compound

Formula: C27H46O

Type: Chemical formula

Species: 1+

Polarity: ☒ positive ☐ negative

m/z: 386.35432 Composition: C27 H46 O

## Analyzing the Data Acquired Using a Direct Exposure Probe

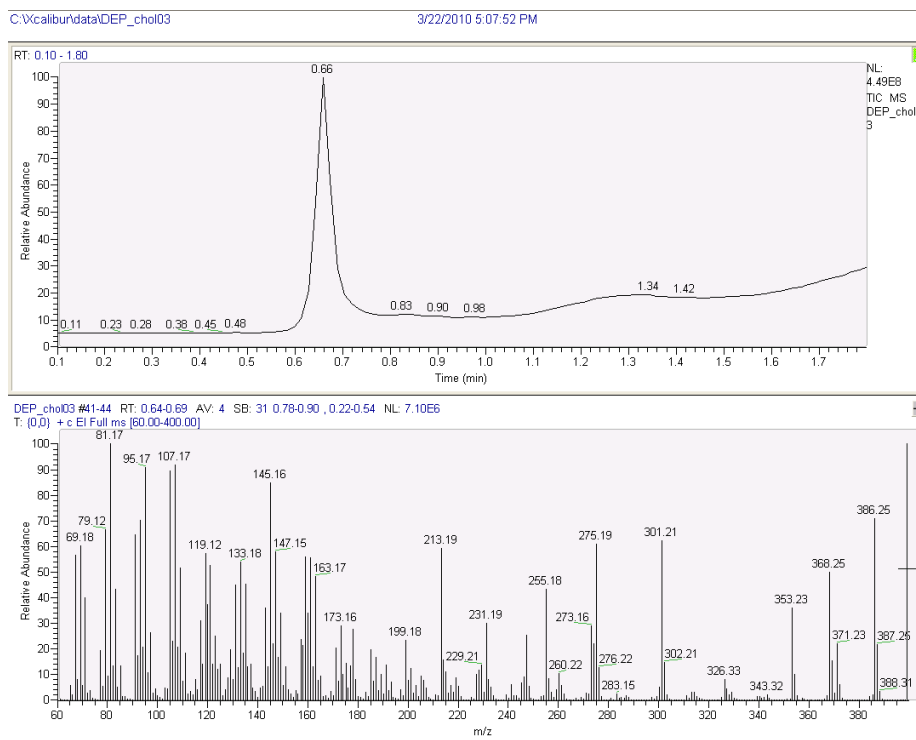
### ❖ To analyze the data file acquired using a DEP

**Note** To analyze another cholesterol sample, repeat the steps in [Step 5: Prepare the Sample](#) and make the appropriate addition to the sequence table.

1. At the Sequence Setup window, click the **Roadmap** icon to return to the Xcalibur Home Page.

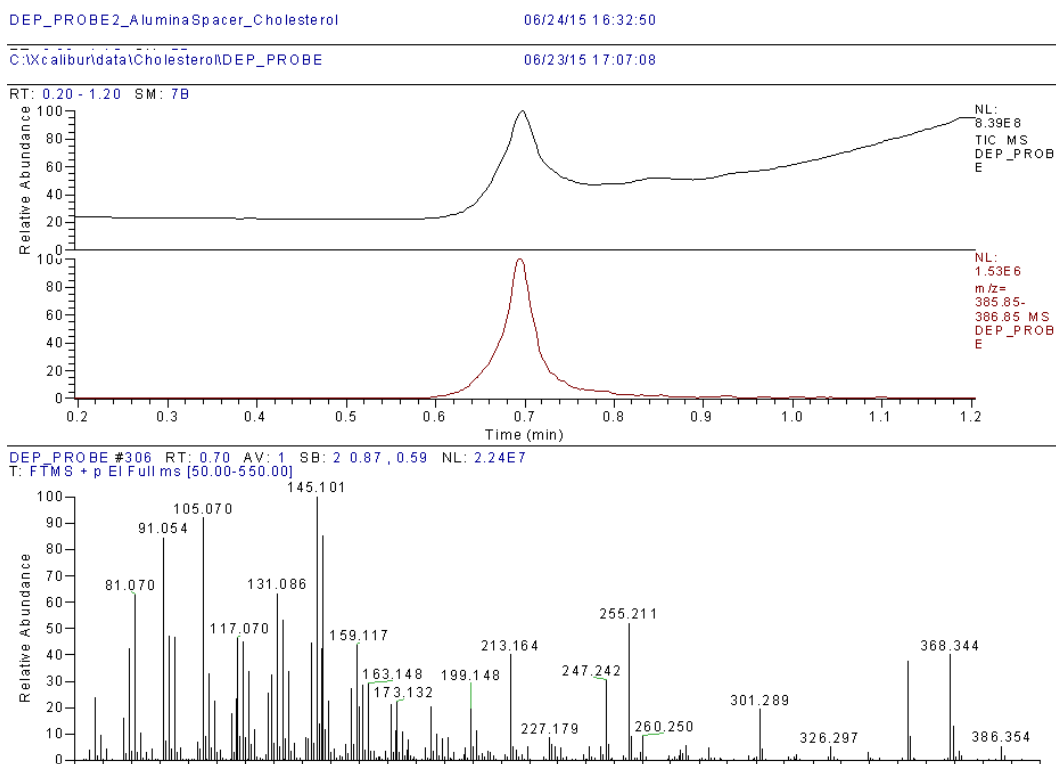
2. Select **Qual Browser** to open the Qual Browser window. Refer to the Qual Browser manual to learn how to use Qual Browser techniques.
3. Select **File | Open** and select the raw data file you just ran. Your cholesterol sample should look similar to the data shown in the figure below.

**Figure 66.** Analyzing Your DEP Data in Qual Browser



4. The figure below shows accurate mass spectra acquired using a DEP on the Q Exactive GC instrument.

**Figure 67.** Analyzing DEP Accurate Mass Data Acquired on the Q Exactive GC System



5. You can use the mass calculator in the **Tune** program to calculate the accurate mass of your compound of interest from its formula.

**Figure 68.** Using the Mass Calculator to Determine Accurate Mass of a Compound

Mass Calculator

Compound

Formula: C27H46O

Type: Chemical formula

Species: 1+

Polarity: ☒ positive ☐ negative

m/z: 386.35432 Composition: C27 H46 O

## Loading Probe Samples

### Loading Samples into a Direct Insertion Probe

You can load liquid or solid samples into your DIP. However, before loading samples, use the following precautions to avoid contaminating the ion source.

1. Inspect the DIP tip and the sample cup holder.
2. Clean the DIP tip prior to loading the first sample. A DIP tip can get contaminated by handling or from previous sample runs.

**Figure 69.** Cleaning the Probe Tip



3. Handle sample cups with clean tweezers or wear gloves. Use one sample cup for each sample to avoid cross-contamination. Make sure the sample cup is clean.
4. Place the sample cup holder on a level surface.

**Note** The sample cup holder can be used to hold several samples at one time and can be carefully warmed on a laboratory hot plate to evaporate the solvent from liquid samples.

5. Use clean tweezers to place a sample cup into the sample cup holder.
6. Put a liquid or solid sample into the sample cup.
  - a. Liquid samples may be injected directly into the sample cup. However, you may choose to dilute your liquid sample in a solvent, in which case please wait till the solvent evaporates before inserting the probe into the MS.
  - b. Put solid samples directly into the sample cup or you can dissolve solid samples in a solvent.
  - c. Do not overload sample cups. If your samples are in powder form, get easily electrostatically charged, or will be subjected to pyrolysis, it may be advantageous to use aluminum crucibles (PN 0568761) and crimp the top of the crucible with tweezers or pliers. This will prevent sputtering of the sample while letting the vapors and gases to still exit the crucible. The aluminum crucibles, unlike the glass ones, do not have a flared-up rim that prevents them from sinking into the tip. Do not push the aluminum crucibles all the way down the sample cup holder to be able to easily retrieve them after the analysis is finished. Excess powder type samples can electrostatically move through the analyzer and create performance issues.
  - d. Use small samples to help keep the system clean, thereby reducing the amount of maintenance required. Small samples also keep the background low. In general, the sample size should be 1  $\mu$ L or less to minimize contamination of the ion source.
  - e. Sample along the walls may evaporate at a different temperature than sample at the bottom, and could therefore distort the evaporation profile.



- f. Avoid putting sample on the outer wall of the sample cup. This contaminates the probe tip.

## Loading Liquid Samples on the Direct Insertion Probe

### ❖ To load liquid samples on the DIP

1. Use a syringe to inject the liquid sample into a sample cup.
2. Wait for the solvent to evaporate.
3. Use a pair of clean tweezers to insert the sample cup into the probe tip. You can use an infrared or incandescent bulb as a heat source to increase the evaporation rate of your solvent. However, be careful not to warm the sample droplet too quickly, or the solvent could sputter and cause sample loss.

## Loading Solid Samples on the Direct Insertion Probe

### ❖ To load solid samples on the DIP

1. Put the solid sample into the sample cup and compress it into the bottom.
2. Use a pair of clean tweezers to insert the sample cup into the DIP tip.

## Loading Liquid Samples on the Direct Exposure Probe

Apply liquid samples directly to the sample loop or the end of the filament on the DEP.

### ❖ To load liquid samples on the DEP

1. Use a 10  $\mu$ L syringe to draw 1 or 2  $\mu$ L of sample.
2. Apply the liquid onto the filament loop. The loop will support the resulting bead of liquid.



**CAUTION INSTRUMENT DAMAGE:** Allow sample solvent to evaporate completely before inserting the DEP into the mass spectrometer.

3. Evaporate the solvent carefully, so that the sample remains deposited uniformly on the tip of the filament wire.

You can use an infrared or incandescent bulb as a heat source to increase the evaporation rate of your solvent. However, be careful not to warm the sample droplet too quickly, or the solvent could sputter and cause sample loss.

### Loading Solid Samples on the Direct Exposure Probe

❖ **To load solid samples directly onto the filament of the DEP**

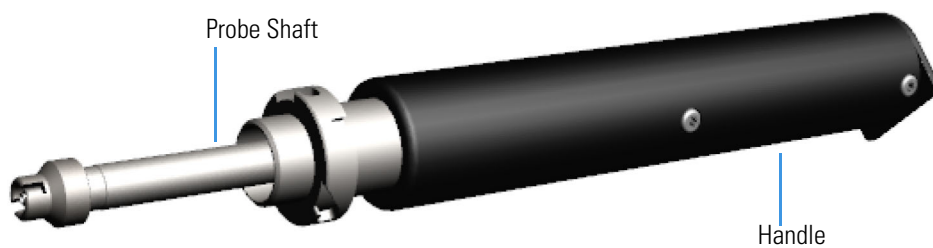
1. Load solid samples with a dry syringe needle, or use a piece of clean wire or capillary column to collect some of the sample.
2. Lightly touch the sample to the filament being careful not to bend or damage the filament.

### Cleaning the Probe Shaft, Direct Probe Controller, DIP Sample Cups, and DEP Filament

To clean the probe shaft, direct probe controller, and sample cups:

1. After your probe has thoroughly cooled, check it for obvious residue or abrasions. You should examine and clean your probe and sample cups often to ensure the integrity of your experiments.
2. Clean the probe shaft.
  - a. Use a soft cloth to wipe the probe handle and shaft.

**Figure 70.** Cleaning the Probe Shaft



- b. Use a soft cloth dampened in a mild detergent and water solution to remove dirt. Never use abrasive cleaners.
  - c. Wipe the probe with water to remove detergent residue.
  - d. Allow the probe to dry completely before reusing.
3. Clean the sample cups on the DIP.

**Note** If you want to reuse your sample cups, clean them before reusing them. Dirty sample cups can lead to false-positive data in subsequent runs.

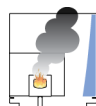
- a. Clean the sample cups in an ultrasonic solvent bath.
- b. Dry the sample cups in a drying oven at 250 °C.
- c. Store clean sample cups in a clean glass container.
- d. Use clean tweezers to handle the clean sample cups and avoid contamination.

## 4. Clean the filament on the DEP.



**CAUTION INSTRUMENT DAMAGE:** Always start and clean the probe when it is in a vacuum, and the vacuum manifold is sufficiently pumped down.

- a. Examine and clean your filament wire often to ensure the integrity of your experiments.
- b. Insert the probe with the filament to be cleaned into the mass spectrometer. Press the Clean button on the direct probe controller, which runs 1200 mA through the filament to burn off any residues. The residues are typically removed after one or two cleaning cycles.



**CAUTION INSTRUMENT DAMAGE:** Supplying current greater than 100 mA to the filament in the presence of air can burn out the filament.

**Figure 71.** Pressing the Clean Button



## 5. Clean the direct probe controller.

- a. Power-off and unplug the direct probe controller.
- b. Use a clean, damp cloth to wipe the direct probe controller.
- c. Dry off the direct probe controller and plug it back in.
- d. Power-on the direct probe controller.



## Ordering Spare Parts

To order spare parts for your direct probe system, contact Customer Service or your local sales representative.

Description	Thermo Scientific Part Number	Qty
Direct Sample Probe Controller, with both Direct Insertion and Direct Exposure Sample Probes	1R119300-0800	1
Direct Probe Controller and Direct Exposure Probe	1R119300-0600	1
Direct Probe Controller and Direct Insertion Probe	1R119300-0500	1
Direct Insertion Probe (requires controller)	1R120439-0050	1
Direct Exposure Probe (requires controller)	1R120439-0060	1
Direct Probe Controller	1R119300-5000	1
Probe Repeller	1R120406-4400	1
Test Sample, DEP/DIP	1R120339-0001	1
DEP Filaments Pkg/5	119322-0001	1
DIP Sample Cups (glass crucibles with flared-up rim) Pkg/25	119329-0001	1
DEP Sample Cups (aluminum crucible, crimpable, straight) Pkg/200	0568761	1
Fuses, T2A, low-insertion force, 250 V	76706-0200	2
Cable from Direct Probe Controller to Probe	119303-0001	1
Holder for DIP Sample Cups (fits 6) and DEP Filaments (fits 6)	119342-0001	1
9-pin Cable	119378-0032	1
Q Exactive GC Series Probe Kit	1R120705-0036	1
– Start Cable: Q Exactive GC to Probe <sup>a</sup>	1R119378-0032	1
– Ceramic Adapter <sup>a</sup>	1R120601-1000	1



<sup>a</sup> Available only as part of Q Exactive GC Series Probe Kit (1R120705-0036)

