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ISQ Series Mass Spectrometers

User Guide

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ThermoFisher SCIENTIFIC

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Software version: Thermo Foundation 3.1 SP3 and later, Thermo Xcalibur 4.0 and later, ISQ Series 3.2 SP1 and later.

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Contents

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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
- re-calibration
- 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.

CE compliance has been evaluated by Professional Testing.

- 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 61326-1:2006. Safety IEC 61010-1:2001, IEC 61010-2-081:2001
- ISQ standards: EMC EN 61326-1:2013. Safety IEC 61010-1:2010, IEC 61010-2-010:2014, IEC 61010-2-081:2015.
- TSQ 8000 standards: EMC EN 61326-1:22013. Safety IEC 61010-1:2010, IEC 61010-2-010:2014, IEC 61010-2-081:2015
- Restriction of Hazardous Substances Directive (2011/65/EU)

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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 UNDESIRED OPERATION.

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.

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WEEE Compliance

This product is required to comply with the European Union's Waste Electrical & Electronic Equipment (WEEE) Directive 2002/96/EC. 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 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 2002/96/EC erfüllen. Das Produkt ist durch folgendes Symbol gekennzeichnet:

Thermo Fisher Scientific hat Vereinbarungen mit Verwertungs-/Entsorgungsfirmen in allen EU-Mitgliedsstaaten getroffen, damit dieses Produkt durch diese Firmen wiederverwertet oder entsorgt werden kann. Mehr Information über die Einhaltung dieser Anweisungen durch Thermo Fisher Scientific, über die Verwerter, und weitere Hinweise, die nützlich sind, um die Produkte zu identifizieren, die unter diese RoHS Anweisung fallen, finden sie unter www.thermoscientific.com/rohsweee.

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Conformité DEEE

Ce produit doit être conforme à la directive européenne (2002/96/EC) des Déchets d'Equipements Electriques et Electroniques (DEEE). Il est marqué par le symbole suivant:

Thermo Fisher Scientific s'est associé avec une ou plusieurs compagnies de recyclage dans chaque état membre de l'union européenne et ce produit devrait être collecté ou recyclé par celles-ci. Davantage d'informations sur la conformité de Thermo Fisher Scientific à ces directives, les recycleurs dans votre pays et les informations sur les produits Thermo Fisher Scientific qui peuvent aider la détection des substances sujettes à la directive RoHS sont disponibles sur www.thermoscientific.com/rohsweee.

Preface

This guide contains detailed information about how to use the Thermo Scientific ISQ™ Series single quadrupole GC-MS system. The ISQ Series instruments are the ISQ LT mass spectrometer and the ISQ QD mass spectrometer. The ISQ LT system is designed to stay cleaner, longer, to maximize your instrument's uptime and improve your lab's productivity. The need to break vacuum, cool off your system, and spend hours cleaning, reassembling, and restoring the system is gone. The heated ion volume, lens stack, and ion optics path in the ISQ LT system ensure that the system stays cleaner longer, but when the system no longer meets your analytical needs, restoring performance is quick and easy. The ExtractaBrite source incorporates a cartridge that contains the repeller, source lenses, and RF lens, all of which can be removed from the instrument while still under vacuum. What once took hours, or even an entire day, is now accomplished in minutes.

Contents

About Your System

Gas chromatography/mass spectrometry (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 through the use of an optional autosampler, and then separated for presentation to the MS. The MS will then generate a mass spectrum of the GC eluent and its components, which can be used for qualitative identification, as well as accurate and precise quantification of the individual compounds present in the sample.

Related Documentation

ISQ Series includes Help and these manuals as PDF files:

- *ISQ Series Preinstallation Requirements Guide* PN 1R120555-0001
- *ISQ Series Hardware Manual* PN 1R120555-0002
- *ISQ Series User Guide* PN 1R120555-0003
- *ISQ Series Spare Parts Guide* PN 1R120555-0004
- *Q Exactive GC, ISQ Series, and TSQ 8000 Evo Direct Probe System User Guide* PN 1R120505-0006

❖ To view product manuals

Open the **Manuals** folder on your desktop.

❖ To open Help

- From the ISQ Series window in the instrument control software, choose **Help > ISQ Series 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.thermoscientific.com.

System Requirements

Your ISQ Series data system must meet these minimum requirements.

System	Requirements
Hardware	• 4.6 GHz processor with 16 GB RAM • CD/R-Rom or DVD drive • 1000 GB or hard drive • Video card and monitor capable of 1680 × 1050 resolution • Quad core processor
Software	• Microsoft™ Windows™ 7 SP1 Operating System (64-bit) • Microsoft Office™ 2013 • ISQ Series 3.2 SP1[a]

[a] Check release notes for current requirements.

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
	**BIOHAZARD:** Indicates that a biohazard *will*, *could*, or *might* occur.
	**BURN HAZARD:** Alerts you to the presence of a hot surface that *could* or *might* cause burn injuries.
	**ELECTRICAL SHOCK HAZARD:** Indicates that an electrical shock *could* or *might* occur.
	**FIRE HAZARD:** Indicates a risk of fire or flammability *could* or *might* occur.
	**FLAMMABLE GAS HAZARD:** 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 *could* or *might* cause physical injury.
	**GLOVES REQUIRED:** Indicates that you must wear gloves when performing a task or physical injury *could* or *might* occur.
	**HAND AND CHEMICAL HAZARD:** Indicates that chemical damage or physical injury *could* or *might* occur.
	**INSTRUMENT DAMAGE:** Indicates that damage to the instrument or component *might* occur. This damage might not be covered under the standard warranty.
	**LIFTING HAZARD:** Indicates that a physical injury *could* or *might* occur if two or more people do not lift an object.
	**MATERIAL AND EYE HAZARD:** Indicates that eye damage *could* or *might* occur.
	**RADIOACTIVE HAZARD:** Indicates that exposure to radioactive material *could* or *might* occur.

Symbol	Descriptor
	**READ MANUAL:** 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 *could* or *might* put you at risk for a physical injury.
	**TOXIC SUBSTANCES HAZARD:** Indicates that exposure to a toxic substance could occur and that exposure *could* or *might* cause personal injury or death.
	For the prevention of personal injury, this general warning symbol precedes the **WARNING** 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 *could cause personal injury*.

Hydrogen Safety Precautions

Hydrogen is a colorless, odorless, highly flammable gas with the molecular formula H_2. Hydrogen gas presents a hazard as it is combustible over a wide range of concentrations: at ambient temperature and pressure, this ranges from about 4% to 74.2% by volume.

Hydrogen has a flash point of - 423 °F (- 253 °C) and an auto-ignition temperature of 1,040 °F (560 °C). It has a very low ignition energy and the highest burning velocity of any gas. If hydrogen is allowed to expand rapidly from high pressure, it can self-ignite. Hydrogen burns with a flame that can be invisible in bright light.

WARNING FIRE HAZARD: The use of hydrogen as a carrier gas is dangerous. Hydrogen is potentially explosive and must be used with extreme care. Any use of hydrogen gas must be reviewed by appropriate health and safety staff and all installations of hydrogen systems must be performed to applicable codes and standards. Thermo Fisher Scientific assumes no liability for the improper use of hydrogen as a carrier gas.

Before you begin using hydrogen, you should conduct a risk assessment based on the quantity of hydrogen to be used and the conditions of your laboratory. You should ask yourself:

"What hydrogen hazards associated with this project are most likely to occur?"

"What hydrogen hazards associated with this project have the potential to result in the worst consequences?"

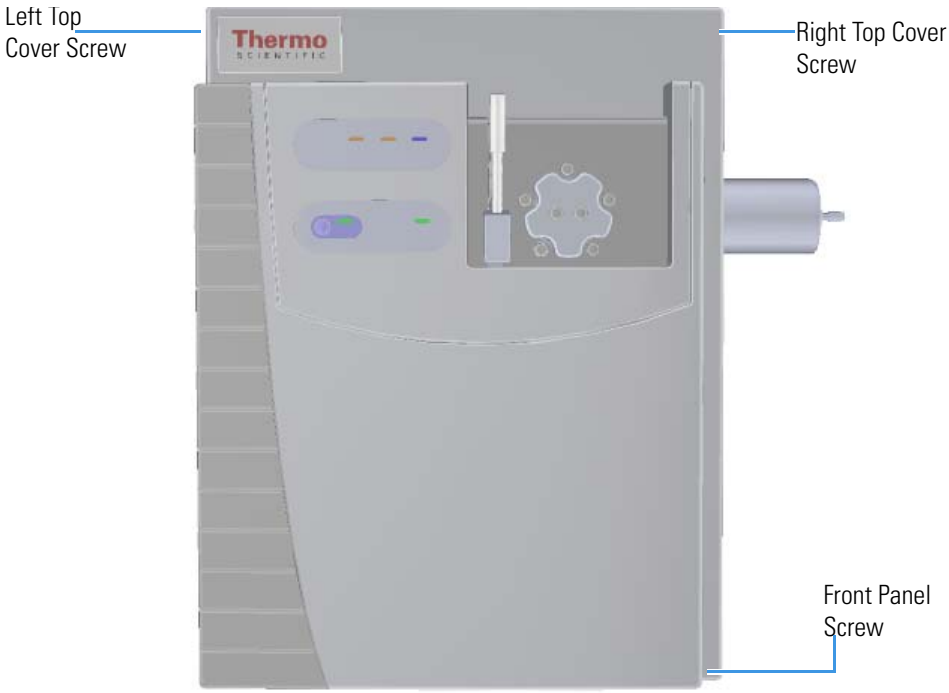
- Try to reduce or eliminate the higher risks by using the proper ventilation to remove hydrogen gas before an ignitable concentration can accumulate. You should also consider purging the hydrogen to further reduce hazards and ensure anyone who will be working with hydrogen has basic hydrogen safety training.

- As with laboratory safety in general, be sure to wear safety glasses, laboratory coats, gloves, etc. Typically there are no specific requirements for gaseous hydrogen, other than eye protection when working with a compressed gas. If working with liquid (cryogenic) hydrogen, insulated gloves and protective shoes should be worn in addition to eye protection.
- You should post "No Smoking" and "No Open Flames" signs to identify hydrogen sources and cylinders. Maintain, inspect and leak-test all hydrogen sources regularly.
- All hydrogen shutoff valves should be clearly marked and permanent hydrogen piping should be labeled as such at the supply or discharge point and at regular intervals along its length. Where hydrogen gas piping passes through a wall, the piping should be labeled on both sides of the wall.
- There should also be contingency plans in place should an incident occur.
- The site emergency response team, as well as the local fire department, should know the location of all hydrogen storage tanks.

Using Hydrogen with ISQ Series Mass Spectrometers

To use hydrogen with the ISQ Series instrument, you must always shut off the GC carrier gas before venting or turning off the ISQ Series instrument. There are three hydrogen safety screws on the ISQ Series instrument that **must** be in place. These are attached to your instrument at the factory.

Figure 1. Hydrogen Safety Screws on the ISQ Series Instrument



Make sure all the covers and panels of the ISQ Series instrument are firmly attached before powering on the ISQ Series instrument. If you vented the system, make sure the vent valve is tightly closed before powering on the system. Make sure all fittings, ferrules, and o-rings are sealed prior to powering on the system.

Hydrogen Connection Guidelines

Use the following guidelines to safely connect hydrogen to your system:

- **Piping—**Hydrogen must be delivered to equipment using appropriate piping and be done in such a way as to pose essentially no hazard to end-users. Piping systems for the delivery of hydrogen should be designed and installed by a person qualified by specific training and experience with hydrogen piping systems.

  Stainless steel is usually recommended because it is a safe, cost-effective material. Piping of *black iron* or copper must not be used, as the pipe can become brittle with age. Elastomeric/plastic tubing of various plastics and polymers should not be used, unless the tubing is approved for use with hydrogen. If elastomeric/plastic tubing is used for hydrogen gas delivery, the tubing should be tested for hydrogen permeability to minimize leakage.

  The hydrogen piping system must be flexible enough to endure routine thermal expansion and contraction. The system should also include considerations for the most severe condition of temperature and pressure expected during service. Piping and supports must be able to withstand static loading introduced by such things as ice and snow; and dynamic loading from high wind and earthquake.

  Caution should be used if burying hydrogen piping. Proper controls should be used to protect against damage and corrosion, and also to prevent Hydrogen from entering a building if there is any leakage.

- **Fittings—**All fittings must be of the proper type approved or designed for use with hydrogen gas. Use as few fittings as possible to minimize the potential for leaks. After installation, ensure that leak testing is carried out prior to system use, and on a regular basis.

  There must be no PTFE tape or other things like *plumber's putty* used to enhance a seal, as this actually is a detriment to a good seal. Ideally the best installation would use stainless steel tubing with appropriate gas-tight fittings.

  Welding is usually preferred for joints in hydrogen piping systems since welding provides a better connection and reduces the potential for leaks compared to mechanical fittings. Soft solder joints are not permitted for hydrogen systems (due to the low melting point of soft solder and its potential for brittle failure at cryogenic temperatures). Brazed joints are permitted, but such joints should be protected against the possibility of external fire.

  Tubing connections should be clamped to barbed or press-fit type connections. Hose clamps or *jubilee clamps* must not be used.

- **Valves**—All valves must be suitable for hydrogen service and for the specific operating conditions. Valves, including regulators, must not be used for hydrogen, unless they are designed and identified for such a use. Ball valves are often chosen because of their superior leak tightness through the valve seat. Pneumatic operators are usually chosen for remotely operated valves so that potential ignition sources (electricity) are remote from the valve.

  Manual shutoff valves should be provided near each point of use, within immediate reach. If a hydrogen cylinder or hydrogen generation system is located within immediate reach, a separate point-of-use shutoff valve is usually not necessary.

  Line regulators that have their source away from the point of use should have a manual shutoff valve near the point of use.

  An emergency gas shutoff device in an accessible location outside the use area should be provided in addition to the manual point-of-use valve in each educational and instructional laboratory space that has a piped gas supply system.

  If necessary, the piping system should have uninterruptible pressure relief. The pressure relief system should be designed to provide a discharge rate sufficient to avoid further pressure increase and should vent to a safe location outside or to a ventilation system exhaust.

Purchasing Hydrogen

Use the following guidelines when purchasing hydrogen:

- Hydrogen Generator—Because it minimizes the amount of hydrogen present and reduces the degree of hazard, a hydrogen generator (also called an electrolyzer) is the safest way to purchase hydrogen in the quantity used in GC/MS.

  However, to minimize the degree of hazard, the hydrogen generator must only be operated in a non-explosive environment because hydrogen buildup can be ignitable. This means that your ventilation system for the room or lab hood must maintain an air exchange rate that is at least two orders of magnitude greater than the maximum hydrogen production rate of the hydrogen generator. Be sure to follow the manufacturers' directions about proper use and maintenance of the regulator.

  To prevent the possibility of releasing hydrogen, the hydrogen generator should be set to shut down if:

  – There is a loss of flow to the ventilation system

  – A hydrogen detector alarms at 25% of the lower flammable limit of hydrogen in air.

  The oxygen exhausted by the electrolyzer should be vented to the outside as well.

- Hydrogen Cylinder—Hydrogen can be delivered in standard laboratory gas bottles or cylinders. These cylinders have a limited amount of hydrogen in them and are a safe way to transport and store hydrogen. However, compressed hydrogen gas cylinders, like all compressed gas cylinders, must be secured in an upright position, ideally with a non-combustible chain or cable. If the cylinder falls over, the valve can be knocked off and the pressurized cylinder can take off like a rocket, which leads to the release of hydrogen and possibly an explosion, severe injury, or death. Never crack a hydrogen cylinder valve to remove dust or dirt from fittings prior to attaching a regulator, as there is a risk of self-ignition.

Properly Storing Hydrogen

Storing and handling compressed hydrogen gas and cryogenic liquid hydrogen present potential health and safety hazards. Using proper storage and handling techniques is essential to maintaining a safe work environment.

Use the following guidelines when storing hydrogen:

- Store spare hydrogen gas cylinders outside and away from doors, windows, building air intake vents, structures, and vehicle routes. This precaution applies when the hydrogen is or is not in use. Indoor storage of spare hydrogen cylinders has special requirements, which is beyond the scope of this document. Documentation for each vessel should include a description of the vessel, a list of available drawings or other documents, the most recent inspection results, and the responsible person's name.
- Prevent spare cylinders from toppling by wrapping them with chains. The chains should also be protected against corrosion and excessive heat.
- Separate spare hydrogen cylinders from oxidizing gases (such as oxygen) with a 5 ft (1.5 m) tall fire barrier with a half-hour fire rating or place the cylinders at least 20 ft (6 m) apart.
- When moving hydrogen cylinders:
  - Remove the regulator and replace the cylinder valve cap before moving.
  - Move cylinders on cylinder carts or with other appropriate transport devices.
  - Never roll or drop a cylinder and never lift a cylinder by its protective cap.
- Bulk hydrogen systems include either gaseous or liquid hydrogen in fixed installations; in some gas systems a semi-permanent trailer (tube trailer) can be used. Storage vessels for compressed hydrogen gas or liquid hydrogen should be designed, constructed, tested, and maintained in accordance with applicable codes and standards. Bulk hydrogen systems represent a level of complexity again which is beyond the scope of this document; however some general guidelines are provided.

- The bulk hydrogen storage system should not be located beneath electric power lines, close to other flammable gases/liquids, or close to public areas. It should be readily accessible to authorized personnel and delivery equipment, but protected from physical damage or tampering.
- As liquid hydrogen systems also have a cryogenic hazard, additional safety considerations for the use of cryogenic liquids may be necessary.

Hydrogen Safety Codes, Standards and References

The following list of safety codes, standards and references is in no way an exhaustive list. In fact, there may be federal, state or local codes that apply to your specific location. Check with all appropriate agencies with jurisdiction before installing or using a hydrogen system.

- Air Products Safetygram #4 Gaseous Hydrogen
- ANSI/AIAA standard for hydrogen safety guidelines is AIAA G-095-2004, Guide to Safety of Hydrogen and Hydrogen Systems
- ASME B31.1, Power Piping Code
- ASME B31.3, Process Piping Code
- ASME B31.8, Gas Transmission and Distribution Systems
- BCGA Code Of Practice CP4 Industrial Gas Cylinder Manifolds and Gas Distribution Pipework
- BCGA Code Of Practice CP33 The Bulk Storage of Gaseous Hydrogen at Users' Premises
- CGA G-5, Hydrogen
- CGA G-5.4, Standard for Hydrogen Piping Systems at Consumer Locations
- CGA G-5.5, Hydrogen Vent Systems
- CGA G-5.6, Hydrogen Pipeline Systems
- CGA G-5.8, High Pressure Hydrogen Piping Systems at Consumer Locations.
- FM Global Property Loss Prevention Data Sheets 7-50: Compressed Gases in Cylinders
- FM Global Property Loss Prevention Data Sheets 7-91: Hydrogen
- IGC Doc 121/04/E, Hydrogen Transportation Pipelines System Design Features
- NASA
- NSS 1740.16 Safety Standard For Hydrogen And Hydrogen Systems Guidelines for Hydrogen System Design, Materials Selection, Operations, Storage, and Transportation
- NFPA 52, Vehicular Fuel Systems Code
- NFPA 55, Standard for the Storage, Use, and Handling of Compressed Gases and Cryogenic Fluids in Portable and Stationary Containers, Cylinders, and Tanks, 2005 Edition
- NFPA 68, Standard on Explosion Protection by Deflagration Venting
- NFPA 70, National Electrical Code

- NFPA 497, Recommended Practice for the Classification of Flammable Liquids, Gases, or Vapors and of Hazardous (Classified) Locations for Electrical Installations in Chemical Process Areas
- NFPA 13, Standard for the Installation of Sprinkler Systems
- NFPA 45, Standard on Fire Protection for Laboratories Using Chemicals
- NFPA 55, Standard for the Storage, Use, and Handling of Compressed Gases and Cryogenic Fluids in Portable and Stationary Containers, Cylinders, and Tanks
- NFPA 68, 2007 Standard on Explosion Protection by Deflagration Venting
- NFPA 69, Standard on Explosion Prevention Systems
- NFPA 91, Standard for Exhaust Systems for Air Conveying of Vapors
- NFPA 255, Standard Method of Test of Surface Burning Characteristics of Building Materials
- OSHA 29CFR1910.103 1910.103 Hydrogen

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 for information about our products.

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

Go to www.thermofisher.com (Services and Support section)

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

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

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Confirming Your GC/MS System is Working

Use the information in this chapter to confirm that your GC/MS system has power, the carrier gas rate is correct, the gas tank has enough pressure, the system has reached vacuum and temperature, and is leak-free.

IMPORTANT You need to change the GC column before setting up a method. See Chapter 2, "Changing the Column," for instruction on changing the column.

Contents

Note Many nitrile and latex gloves not certified for clean room use contain silicone mold releasing agents that will contaminate the instrument. For this reason, clean room gloves are strongly recommended when you work with the ISQ Series mass spectrometer. We recommend Cardinal Health CP100 Nitrile Cleanroom Gloves. See the *ISQ Series Spare Parts Guide* for ordering information.

Checking Power to the System

To confirm that the ISQ Series system is powered on, make sure the Power light on the front panel is solid green. If it is not lit, the ISQ Series system is not powered on. To power it on, reach around the right side of the instrument and pull up the power switch on the back. If the instrument still doesn't power on, check the electrical connections and wall outlet.

Figure 1. Checking the Power on the ISQ LT System

To confirm that a TRACE GC Ultra is powered on, make sure the front LCD panel displays information and that it is not blank. To power-on the TRACE GC Ultra, reach over the top center of the instrument and pull up on the large plastic ribbed power switch on the back. To confirm that a TRACE 1300 GC is powered on, make sure that power light on the status panel is solid green. To confirm that a TRACE 1310 GC is powered on, see if the touchscreen main menu has appeared. To power-on the TRACE 1300 or 1310 GC, reach over the top right of the instrument and pull up on the large plastic ribbed power switch on the back. If the instrument still doesn't power on, check the electrical connections and wall outlet.

Verifying the Carrier Gas Flow Rate

Once you confirm that the system is powered on, you need to verify that carrier gas rate is what you expect.

Note The TRACE GC Ultra beeps to indicate that the carrier gas is turned off or set incorrectly.

❖ To check the carrier gas flow rate

1. Access the carrier gas menu. On the TRACE GC Ultra, press the Carrier button on the front of the GC. On the TRACE 1310 GC, choose **Instrument Control** and then **Front/Back Inlet**. On a TRACE 1300 GC, open the Xcalibur software by clicking on the Xcalibur icon on the computer desktop. On the Xcalibur roadmap, select the TRACE 1300 from the instrument list in the side panel. This opens the **Status Panel**.
2. Display the column flow.

3. If the actual and set point amounts in **Col. Flow** are the same, then you have good carrier gas flow. If the amounts are different, see the troubleshooting section of your GC user documentation.

Checking Your Carrier Gas Tank Pressure

Make sure you have enough pressure in the carrier gas tank to accommodate the number of samples you plan to run. If the pressure is too low, you may run out of gas in the middle of a run, which could compromise the results of your data.

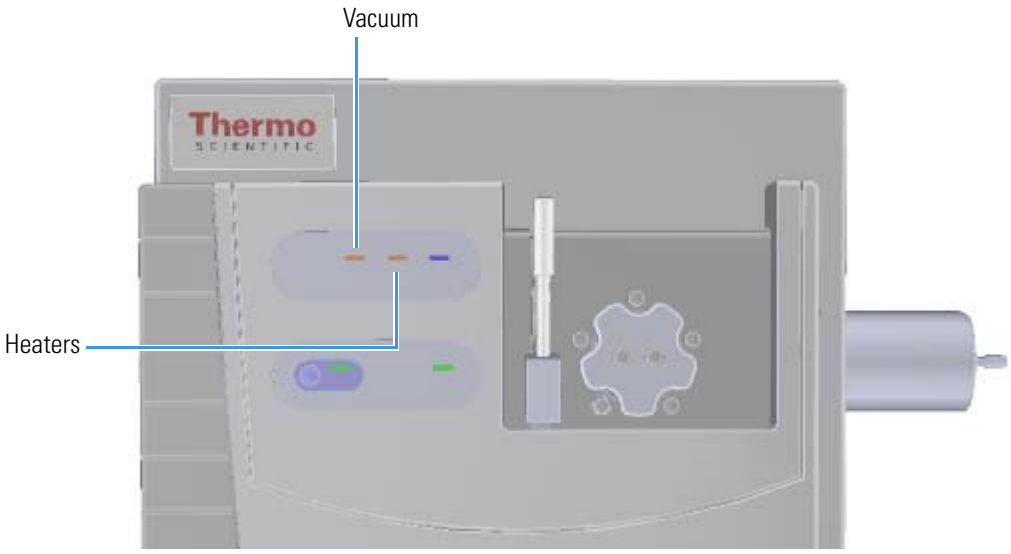
1. Locate your carrier gas tank. It might be in a different room, depending on how your lab is set up.
2. Look at the pressure gauge on the tank.
3. Ensure the pressure is more than 500 psi at the primary (or first) regulator stage. If it is not, you may want to replace the tank if you have to run a lot of samples.
4. Set the second stage regulator pressure between 80 and 100 psi (560 kPa to 700 kPa).

Checking the Vacuum and Temperature

Use the lights on the front of the ISQ Series instrument to check the vacuum and temperature of the instrument.

To check the vacuum, look at the **Vacuum** light. When the light is a solid green, the ISQ mass spectrometer is under sufficient vacuum. If it is slowly blinking orange, you have not achieved vacuum yet. If it is blinking orange quickly, you have a large leak that prevented the instrument from achieving vacuum. If this is the case, you need to find and fix the leak, then turn the power off and on. Most likely, the column nut needs to be tightened, the column was not installed correctly, or the vent valve was not completely closed.

Figure 2. Using the Lights on the ISQ Series Instrument



To check the temperature, look at the **Heaters** light. When the **Heaters** light is a solid green, the ISQ Series instrument is at temperature. If it is blinking orange, the ion source or transfer line are not at temperature. If the light is not lit, the heaters are not turned on.

Note Until the **Vacuum** light is a solid green (high vacuum is achieved), the heaters will not power on and the **Heaters** light will not be lit.

Changing the Column

The ISQ Series mass spectrometer ships with a factory-tested 15 m x 0.25mm i.d. TR-SQC column, which the Field Service Engineer uses to qualify the instrument. However, once it gets dirty, you cannot purchase a new one, so you need to install a column that accommodates the type and quantity of samples you will be running. You should choose a column that gives you the best possible resolution, analysis speed, and quantitation.

Note Many nitrile and latex gloves not certified for clean room use contain silicone mold releasing agents that will contaminate the instrument. For this reason, clean room gloves are strongly recommended when handling the column. We recommend Cardinal Health CP100 Nitrile Cleanroom Gloves. See the *ISQ Series Spare Parts Guide* for ordering information.

When determining the type of column for your particular needs, here are a few things to consider:

- **Column Material**—Columns made out of fused silica are economical and widely used. Columns made out of this material have a wide range of stationary phases and are available in many sizes that can be used with a mass spectrometer.

  Large diameter columns made of steel are widely used in process gas analysis, but they are not typically used on mass spectrometers. There are also metal-clad, fused silica columns, which have the advantages of fused silica, but the metal makes them resistant to breakage. These columns are less common and more expensive.

- **Stationary Phase**—The stationary phase is the most important consideration when selecting a column. The interaction between the stationary phase and the analyte determines how well the analytes separate from each other (resolution) and also affects how quickly the separation occurs (analysis time). Choose a stationary phase that is compatible with the nature of your analytes and the maximum GC oven temperature you are going to use.

- **Internal Diameter**—The smaller the diameter of the column, the better the separation. However, smaller diameter columns do not have as much capacity for matrix or analytes. As a result, smaller diameter columns are subject to overloading, which leads to retention time shifts and peak shape changes. Larger diameter columns can accept larger concentrations of material, but will require longer columns or slower GC oven temperature ramps (which increases the analysis time) to match the separation power of shorter columns. Typical column sizes for GC/MS have inside diameters (ID) of 0.25 mm. Smaller ID columns, such as the 0.15 and 0.10 mm, are becoming increasingly popular. Additionally, 0.32 and 0.53 mm ID columns are commonly used.
- **Film Thickness**—With larger film thicknesses, there is more capacity for the analyte. This capacity can aid in the separation of high concentration samples and in the separation of very volatile samples because thicker stationary phases allow more opportunities for the analytes to interact with the stationary phase. The optimal film thickness depends on the internal diameter of the column and desired phase ratio.

  Thick films with small internal diameters will give very strong interactions with the analytes, which can result in longer analysis time and peak tailing. Large ID columns with thin films will have very little interaction with the analytes, which will result in very fast analysis times with little separation. Typical film thicknesses are 0.25 µm for a column with an ID of 0.25 mm. Other common film thicknesses are 0.1, 0.5, and 1.0 µm.
- **Length**—The length of the column affects how much time the analyte has to interact with the stationary phase. Longer columns typically have better resolutions and higher capacities, but longer analysis times. Longer columns are also more expensive. Typical column lengths are 15 or 30 meters for GC/MS, but 100 m columns may be needed for very complex mixtures like gasoline. Very short columns (2.5, 5, and 10 m) are also available.

Note Contact your local sales representative to order a Thermo Fisher Scientific column. You can also refer to our catalog or visit our website at www.thermoscientific.com/columns.

Replacing the Factory Installed Column

Note The procedure below assumes that optional accessories designed to assist in changing column without venting the mass spectrometer are not present. For changing the column when one of these accessories is present, consult the user guide for that accessory.

❖ **To replace the factory-installed column**

Note If you are running samples, stop the acquisition before powering off the system.

1. Cool down the GC oven and injector. See the GC documentation for information. After they are cooled down, power-off the GC.

WARNING BURN HAZARD: The injector, oven, and transfer line may be hot. Allow them to cool to room temperature before touching them.

   If you are using hydrogen as a carrier gas, you must cool down and shut off the GC to prevent the buildup of hydrogen in the vacuum manifold.

2. Click **Shut Down** on the ISQ Dashboard or on the ISQ Series status page within the instrument control software.

3. Click **Yes** to continue the shutdown process. The high voltages, heaters, and turbomolecular pump power off. Once the turbomolecular pump reaches 50% speed, or five minutes elapses, the foreline pump powers off and you may vent the system.

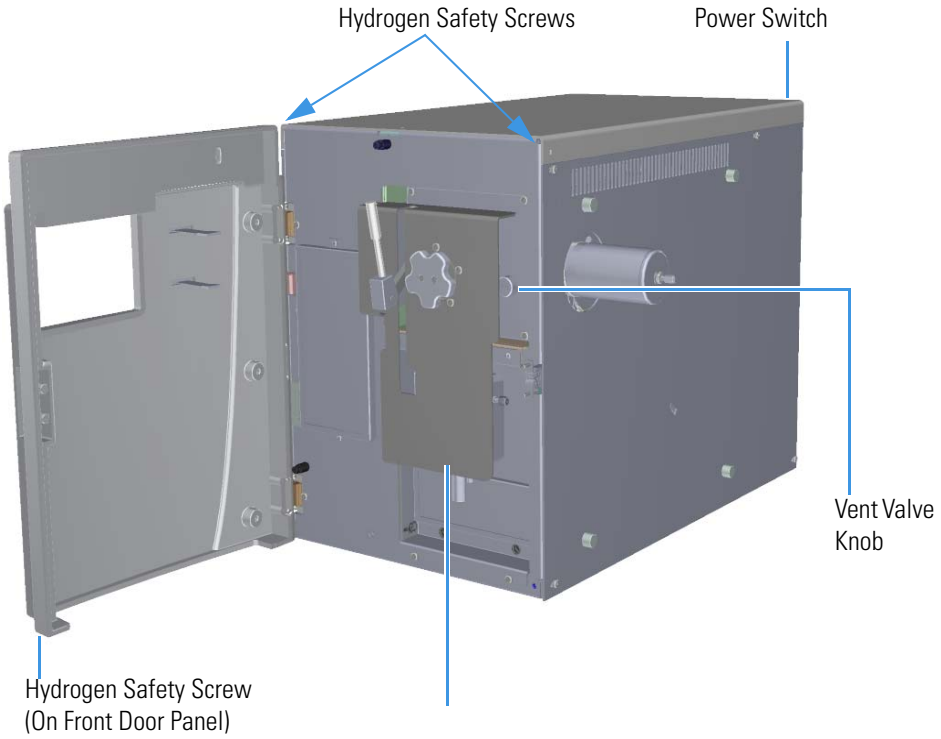
Note The amber vacuum light on the front of the instrument starts blinking rapidly, indicating that the mechanical pump has powered off after a five minute period with the turbomolecular pump off (such as when the instrument is shut down), or due to a sustained vacuum fault lasting five minutes. When the turbomolecular pump spins down below 50% speed due to the shut down process, the vacuum light turns off.

4. Reach around the right side to the back of the instrument and push down on the power switch to power-off the ISQ Series instrument.

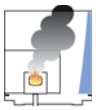
5. If you are using hydrogen as a carrier gas, unscrew the hydrogen safety screw on the front door.

WARNING FIRE HAZARD: If you are using hydrogen, do NOT reach over the top of the instrument to power it off. Instead, reach around the right side or go to the back of the instrument and flip down the power switch.

Figure 3. Powering Off the ISQ Series Instrument



6. Open the front door of the instrument.
7. Look behind the right side of the vacuum interlock shield and twist the vent valve knob one and a half times in a counter-clockwise direction to open the vent.
8. Wait five minutes for venting to complete.



CAUTION - INSTRUMENT DAMAGE Do not proceed until the instrument is vented, or pieces of the column or ferrule might blow into the instrument. To confirm that the instrument is vented, check how much the glass cover compresses the top cover o-ring in the manifold. Once the o-ring surface touching the glass is about 1 mm, it is safe to open the instrument and remove the column.

9. Turn off the carrier gas and if used, the detector gas. See the GC documentation for information about using detector gases.

WARNING BURN HAZARD: The injector, oven, and transfer line may be hot. Allow them to cool to room temperature before touching them.

10. Unscrew the transfer line nuts and remove the column.
11. Attach a blanking ferrule and nut to seal the end of the transfer line to prevent contaminants from entering the MS during column conditioning.

12. Remove the column from the column rack and from the GC.
13. Connect the new column to the injector inside the GC.

> **Note** Wear clean, lint- and powder-free gloves when you handle the column and injector ferrule.

a. Unwind the column enough to easily connect its ends to the injector and MS.
b. Wipe about 100 mm (4 in.) of the column with a tissue soaked in methanol.
c. Insert the column through the injector retaining nut and ferrule (larger end up). If the M4 retaining nut is used, slide it on the column through the side cut. Wipe the column again with a tissue soaked in methanol.

> **Tip** Slide a notched septum on the column before the injector retaining nut to make it easier to measure the proper distance between the nut and end of the column.

d. Use a scoring wafer to score and break the column about 1 cm (0.4 in.) from the end. Check for an even, flat cut using a magnifying glass. Repeat if necessary.
e. Insert a notched septum on the column to hold the retaining nut at this position. Thread the retaining nut into the injector but do not tighten.
f. Ensure that the end of the column is the proper distance into the injector. For the TRACE 1300/1310 GC the injector depths are measured from the top of the ferrule and are: splitless = 5 mm; split = 10 mm; PTV and PTVBKF = 30 mm.

> **Note** If you are using a GC other than the TRACE 1300/1310 GC, refer to the GC user documentation for the correct insertion depth.

g. Adjust the column position so that the septum contacts the bottom of the retaining nut. Use your fingers to tighten the retaining nut until it starts to grip the column.
h. Tighten the column nut finger-tight until it starts to grip the column plus a quarter turn.
i. Remove the notched septum from the column.

14. Set up the GC parameters:

a. Set the oven and injector temperature to 50 °C (122 °F).
b. Set the carrier gas flow to 1.0 mL/min.
c. Turn off vacuum compensation, which is located on the **Carrier** menu of the GC.
d. Use the column flowmeter connector to verify that there is flow through the column. If you do not have a flowmeter, dip the column outlet in a small vial of methanol. Bubbles indicate there is flow through the column. If there is no flow, check that the carrier gas is on, the GC inlet is pressurized, and the column is not plugged. If there is still no flow, consult the GC documentation or contact Technical Support.

e. Allow the column to purge for at least 10 minutes. If you used methanol to detect column flow, remove column from methanol during purge time.

f. Insert the column into the fitting of the column flowmeter connector that blocks the column flow.

15. Perform a column leak check:

a. On the TRACE 1310, select the **Leak Check** icon in the **Maintenance** menu. Otherwise, perform the leak check through the Chromatography Data System. Refer to the *TRACE 1300 and TRACE 1310 Series GC User Guide* for instructions.

Note If you are using a TRACE GC Ultra or a FOCUS GC, refer to your GC user documentation for instructions on performing a leak check.

b. Start the leak check.

The split and purge valves of the selected channel are automatically closed, and the channel is pressurized with carrier gas to the leak check setpoint.

The system monitors the pressure for one minute. If the pressure does not drop more than the maximum allowed sensitivity value, then the leak check will pass. If the leak check does not pass, use the leak detector to find and fix any leaks.

Tip Leaks can be caused by not tightening the fitting on the column flowmeter connector. Check the fitting before looking for the leak elsewhere.

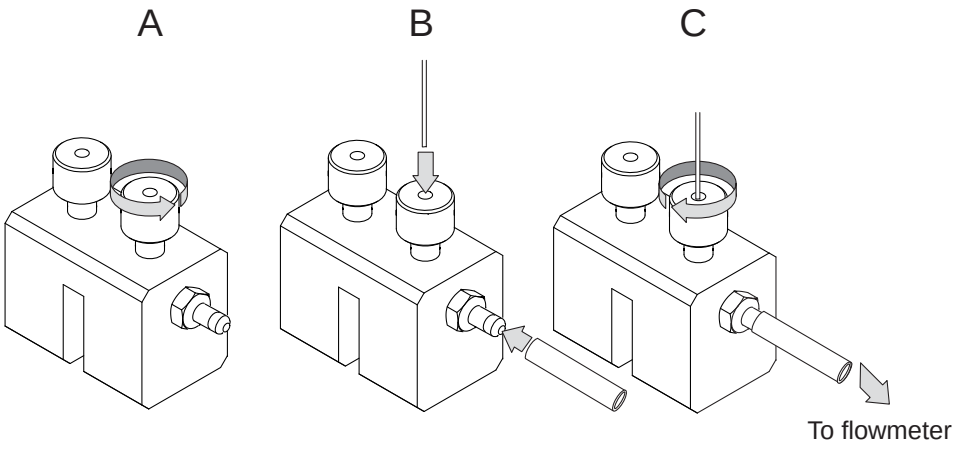
CAUTION INSTRUMENT DAMAGE: Do not allow the column flowmeter connector to exceed 80 °C (176 °F). Otherwise, it will melt and damage the instrument.

c. Repeat the leak check until no leaks are indicated.

16. Calibrate the carrier gas flow (column evaluation):

a. Carefully push the capillary column end into the flowmeter section of the column flowmeter connector. See Figure 4.

Figure 4. Column Flowmeter Connector



b. Connect the flowmeter to the dedicated fitting on the column flowmeter connector.

c. If you have a TRACE 1310, select the **Back Column** or **Front Column** button in the **Configuration** menu. Otherwise, perform the column evaluation through the Chromatography Data System. See the *TRACE 1300 and TRACE 1310 User Guide* for instructions.

d. Select **Column** and input the column's physical characteristics.

e. If a pre-/post column is present, set the length and nominal internal diameter of the pre-/post column in the same valid ranges for the column.

> **Note** For the most reproducible results, you should conduct a more detailed column evaluation. However, the following steps, while recommended, are not required.

f. Start the column evaluation:

   i. On the column page, click **Column Evaluation**.

   ii. Connect a flowmeter to the column flowmeter connector as indicated above.

   iii. Press **Start**. The inlet is pressurized to deliver a 5 mL calculated column flow.

   iv. Once the pressure has stabilized, measure the flow out of the column flowmeter connector. Enter the actual flow when prompted. The corrected column ID will be displayed.

   v. Select **Apply** and the K-factor will be adjusted to give the corrected column flow. According to the physical characteristics of the column, the system calculates and displays the relevant column K-factor. At the end of the routine, a message will indicate that the evaluation was successful.

Expect a K-factor of approximately 0.7 – 0.9 for a 15 m, 0.25 mm i.d. column (1.3 – 2.0 for a 30 m, 0.25 mm i.d. column). If the column does not report a K-factor within this range or within 0.1 units of the previous stored value, check for a leak or broken column using the leak detector. The K-factor is a measured resistance for the column. A K-factor that is too low may indicate a leak in the system, while a K-factor that is too high may indicate a blockage.

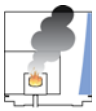
g. Fix any issues found and rerun column evaluation until an appropriate K-factor is achieved.

17. Disconnect the column flowmeter:
    a. Disconnect the column from the column flowmeter connector.
    b. Remove the column flowmeter connector, including its fittings, from the oven and set them aside.
    c. Close the GC door.
18. Condition the column before inserting it into the ISQ Series system. Column conditioning consists of passing a carrier gas through the column heated to a programmed temperature as described in the column manufacturer's instructions.
    a. If there are no conditioning instructions, perform the column conditioning by setting a final temperature 10 °C–20 °C below the column's recommended maximum temperature.

CAUTION INSTRUMENT DAMAGE: The material released from the column (column bleed) during conditioning may contaminate the ion source if the column is inserted into the transfer line during the high-temperature stage of conditioning.

WARNING FIRE HAZARD: Do not use hydrogen as the carrier gas for conditioning your column. It could vent into the oven and present an explosion hazard.

    b. Run the slow temperature program that is recommended by the manufacturer. A typical program would hold the column at 40 °C (104 °F) for 15 minutes, and then ramp at 10 °C/min (50 °F/min) up to 10–20 °C below the maximum allowed column temperature. Hold the column at this temperature for two hours.



CAUTION INSTRUMENT DAMAGE: Never exceed the column manufacturer's maximum operating temperature.

Connecting the Column to the Transfer Line

When connecting the column to the transfer line, you may use either the regular transfer line nut or the spring loaded transfer line nut with the graphite Vespel™ ferrule.

❖ To connect the column using the regular transfer line nut

1. Lower the oven temperature and allow it to cool.
2. Confirm that the MS is vented and remove the current transfer line nut and ferrule.
3. Unwind about one turn of the column from the column outlet end.

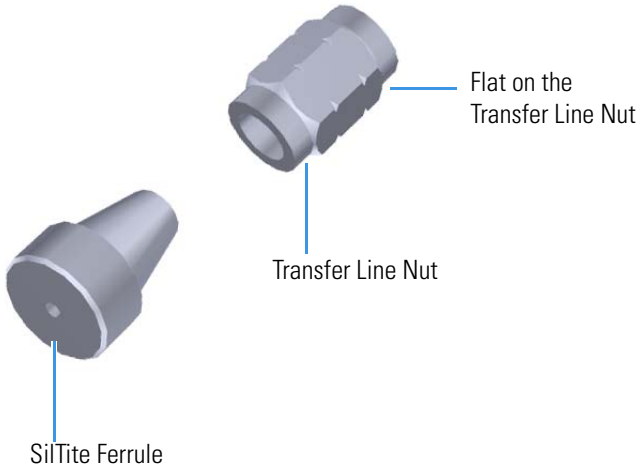
> **Note** Wear clean, lint- and powder-free gloves when you handle the column and transfer line ferrule.

4. Wipe approximately 300 mm (12 in.) of the column with a tissue soaked in methanol.
5. Choose an appropriate ferrule for the outer diameter of your column.

> **Note** If the maximum oven temperature in your method is ≥ 290 °C (554 °F), Thermo Fisher Scientific recommends using a spring loaded transfer line nut with a graphite Vespel ferrule or a SilTite™ nut and ferrule. By cycling the oven at and above this temperature, expansion and contraction of the graphite Vespel material can cause leaks in the transfer line.

6. Insert the column through the transfer line nut and ferrule, entering through the tapered end of the ferrule. Wipe the column again with a tissue soaked in methanol.

Figure 5. Transfer Line Nut and SilTite Ferrule Orientation



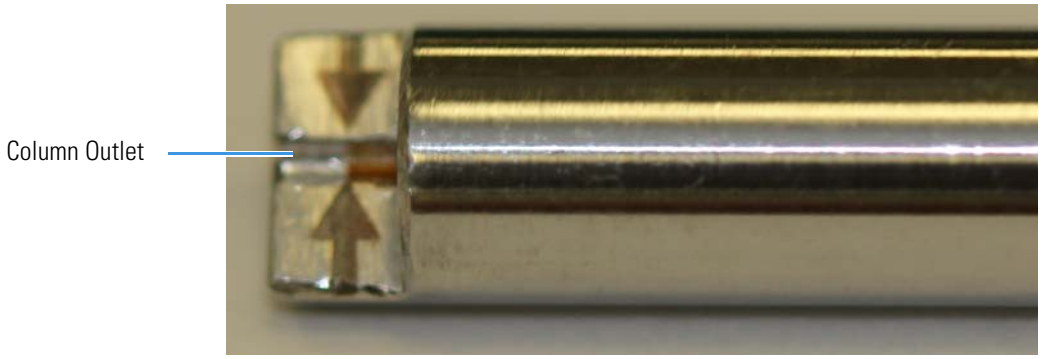
7. Insert the column into the column measuring tool (see Figure 6), which is in the ISQ Toolkit, so that it is even with the lines at the end of the column. Figure 7 indicates proper positioning of the column in the tool for accurate measuring.

8. Use a scoring wafer to score and break the column. Use a magnifying glass to check for an even, flat cut. Repeat if necessary.
9. Use a 5/16 in. wrench to hold the column measuring tool steady.

Figure 6. Column Measuring Tool

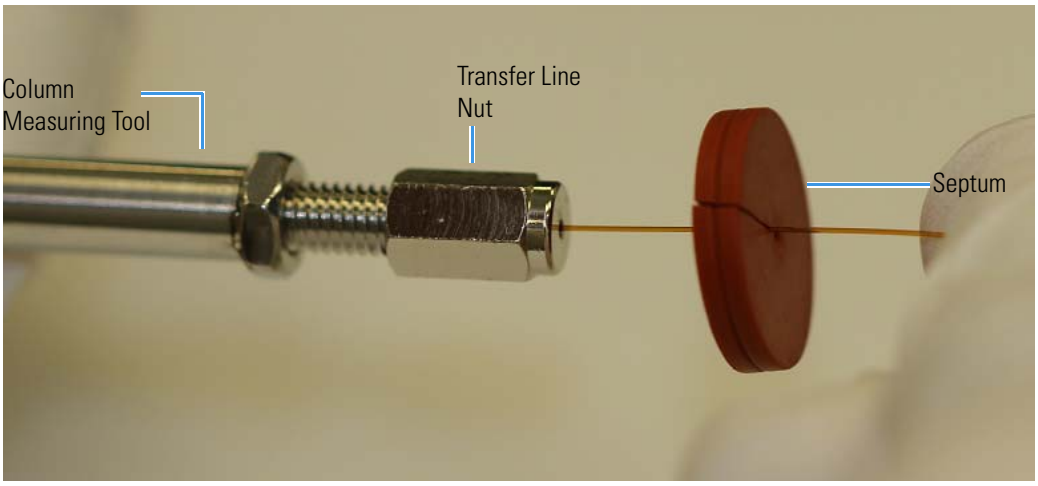
10. While holding the column measuring tool steady, tighten the transfer line nut with a 1/4 in. wrench until the column just stops moving in the ferrule.
11. Turn the transfer line nut 1 flat backward so the column is able to move in the ferrule with slight resistance.
12. Line up the outlet of the column with the arrows on the end of the column measuring tool.

Figure 7. Lining Up the Column in the Column Measuring Tool



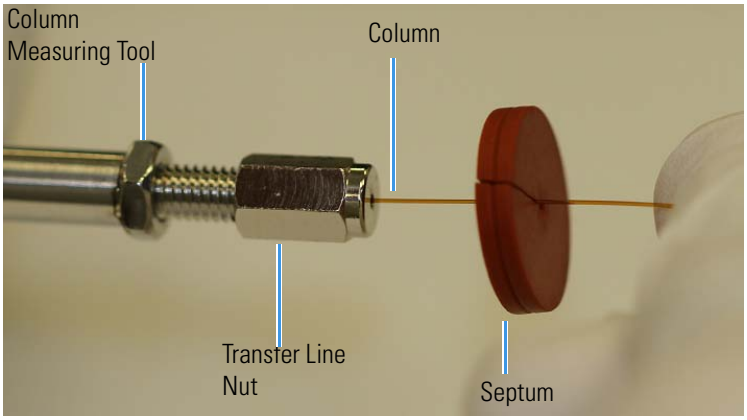
13. Place a septum with a notch cut into it behind the transfer line nut. The septum marks the place on the column where it should exit the nut.

Figure 8. Positioning the Septum



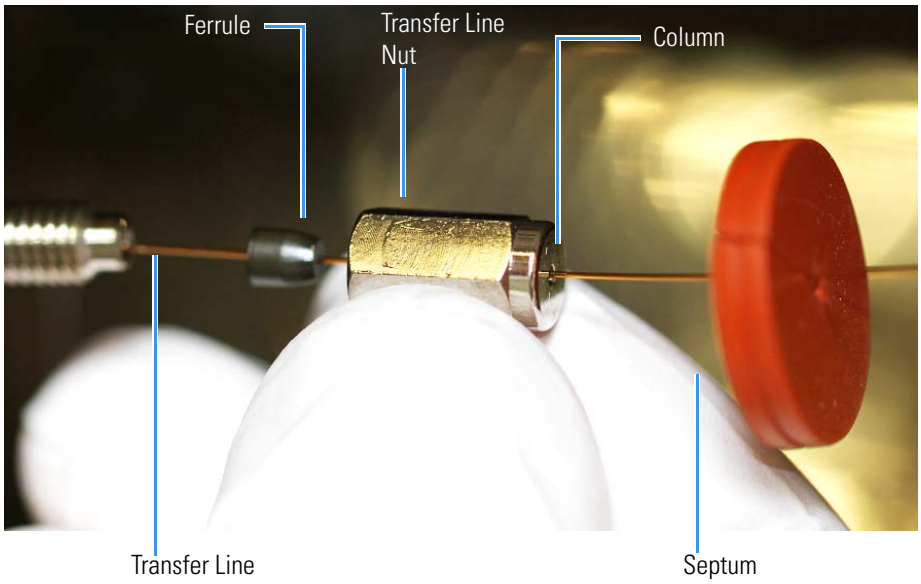
14. Pull the column back from the transfer line nut. Do not move the septum from its position on the column.

Figure 9. Pulling the Column Back from the Transfer Line Nut



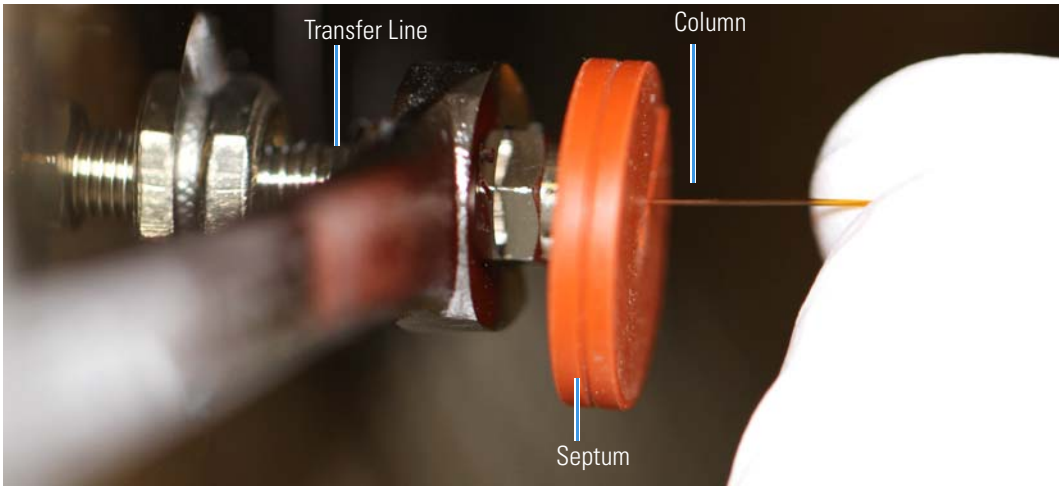
15. Loosen the transfer line nut from the column measuring tool.
16. Remove the column, transfer line nut and ferrule from the column measuring tool, making sure not to move the septum from its location on the column.
17. Insert the column into the transfer line.

Figure 10. Inserting the Column into the Transfer Line



18. Tighten the transfer line nut until it is just secure enough so that you cannot move it.
19. Loosen the nut by turning it exactly 1 flat backward.
20. Position the column in the transfer line. Use the septum as a guide to measure the correct length you should insert the column. Be careful not to change the location of the septum on the column.

Figure 11. Positioning the Column in the Transfer Line



21. Tighten the nut 1 flat forward—back to where it is secure enough in the transfer line that you cannot move it.
22. Tighten the nut 1 additional quarter turn.
23. Remove the cut septum.
24. Close the front door of the GC.

Note If you are using a SilTite ferrule, follow the instructions that come with SilTite ferrules. If you are using a graphite Vespel ferrule, they require conditioning to ensure a leak-tight seal. See the *ISQ Series Spare Parts Guide* for information about ordering these ferrules.

25. Condition the graphite Vespel ferrule:
    a. Raise the oven temperature to the maximum temperature you will operate the GC column.
    b. Wait 10 minutes.
    c. Lower the oven temperature to 40 °C (104 °F) and allow it to cool before continuing.

WARNING BURN HAZARD: The oven may be hot. Allow it to cool to room temperature before opening it. The injector will still be hot, so do not touch it.

    d. Retighten the transfer line nut.

Tip If you do not have a septum, use the following alternate procedure.

1. Tighten the nut on to the column measuring tool until the column can no longer move.
2. Continue to tighten 1/4 turn more.
3. Place the column measuring tool with the column inserted into the GC oven.
4. Confirm column flow through the column.
5. Program the column temperature to its safe conditioning temperature. Allow the temperature to remain in the final hold mode for at least 30 minutes. This will insure the ferrule is stuck to the column.
6. Cool the column and loosen the nut 1/4 turn or until the column measuring tool can be removed from the nut.
7. Unscrew the column measuring tool from the nut. The ferrule should be seated on the column.
8. Insert the column into the transfer line and tighten the nut.

9. Restore working conditions.
   a. Raise the oven temperature to the initial temperature that you will use.
   b. Turn on vacuum compensation on the GC.
   c. Twist the vent valve clockwise to close the valve. Be sure not to pinch the o-ring.
   d. If you are using hydrogen as a carrier gas, replace the front panel screw.
   e. Replace all remaining hydrogen safety screws if you are using hydrogen.
10. Power on the ISQ Series mass spectrometer.

WARNING FIRE HAZARD: If you are using hydrogen, do NOT reach over the top of the instrument to power it on. Instead, reach around the right side or go to the back of the instrument and flip up the power switch.

11. Once the ISQ Series instrument is pumped down and able to scan, click **Air & Water / Tune** on the ISQ Series Dashboard view air water spectra and look for evidence of leaks with a large *m/z* 28 signal. If you observe a leak, stop scanning and gently tighten the nut in small increments until no leaks appear when scanning.

❖ To connect the column using the spring loaded transfer line nut

Note If you use a graphite Vespel ferrule with your column, Thermo Fisher Scientific recommends using the spring loaded transfer line nut with it. See the *ISQ Series Spare Parts Guide* for ordering information.

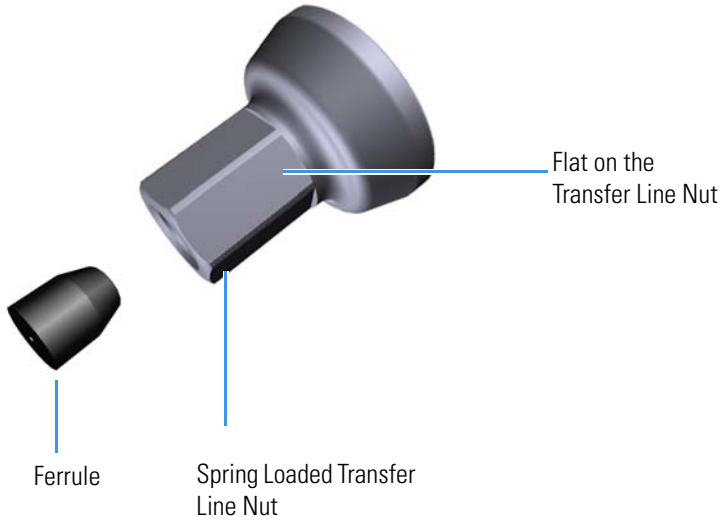
1. Lower the oven temperature and allow it to cool.

2. Confirm that the MS is vented and remove the current transfer line nut and ferrule.
3. Unwind about one turn of the column from the column outlet end.

> **Note** Wear clean, lint- and powder-free gloves when you handle the column and transfer line ferrule.

4. Wipe approximately 300 mm (12 in.) of the column with a tissue soaked in methanol.
5. Choose an appropriate ferrule for the outer diameter of your column.
6. Insert the column through the spring loaded transfer line nut and ferrule, entering through the tapered end of the ferrule.
7. Wipe the column again with a tissue soaked in methanol.

Figure 12. Transfer Line Nut and Graphite Vespel Ferrule Orientation



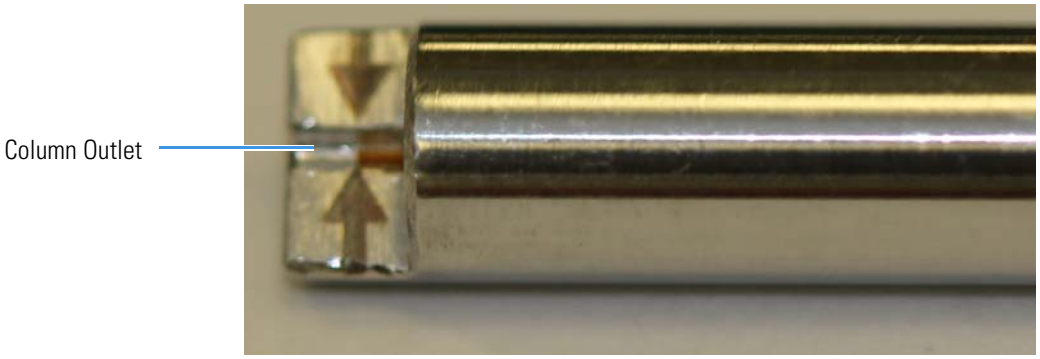
8. Insert the column into the column measuring tool (see Figure 13), which is in the ISQ Toolkit, so that it is even with the lines at the end of the column. Figure 14 indicates proper positioning of the column in the tool for accurate measuring.
9. Use a scoring wafer to score and break the column. Use a magnifying glass to check for an even, flat cut. Repeat if necessary.
10. Use a 5/16 in. wrench to hold the column measuring tool steady.

Figure 13. Column Measuring Tool

11. While holding the column measuring tool steady, tighten the spring loaded transfer line nut with a 1/4” wrench until the column just stops moving in the ferrule.

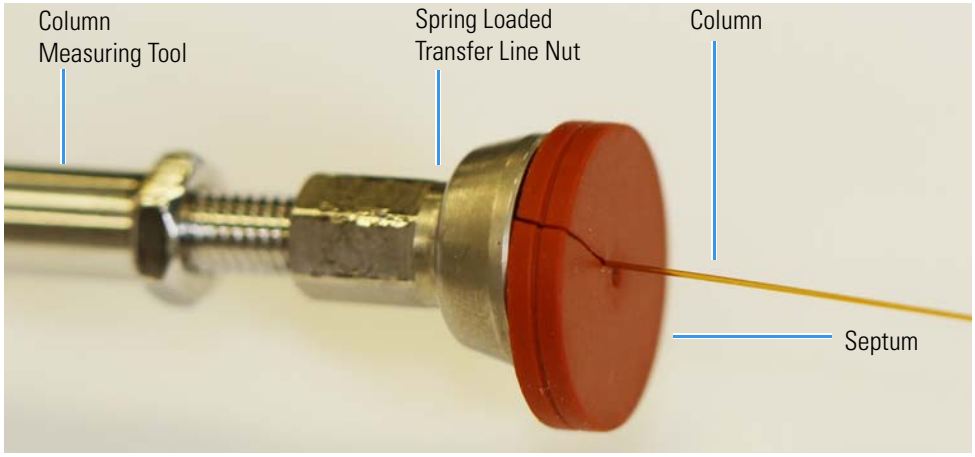
12. Turn the spring loaded transfer line nut 1 flat backward so the column is able to move in the ferrule with slight resistance.
13. Line up the outlet of the column with the arrows on the end of the column measuring tool.

Figure 14. Lining Up the Column in the Column Measuring Tool



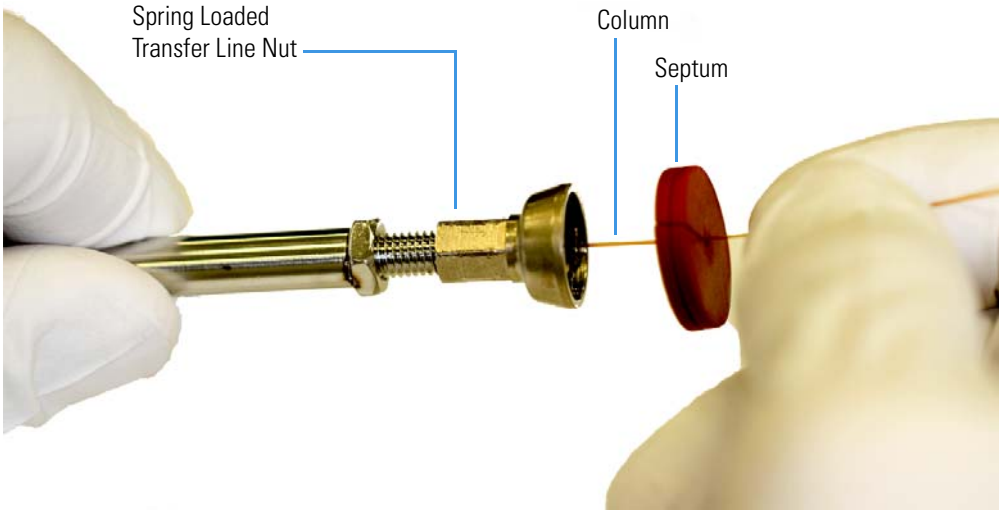
14. Place a septum with a notch cut into it behind the transfer line nut. The septum marks the place on the column where it should exit the nut.

Figure 15. Positioning the Septum



15. Pull the column back from the spring loaded transfer line nut. Do not move the septum from its position on the column.

Figure 16. Pulling the Column Back from the Spring Loaded Transfer Line Nut

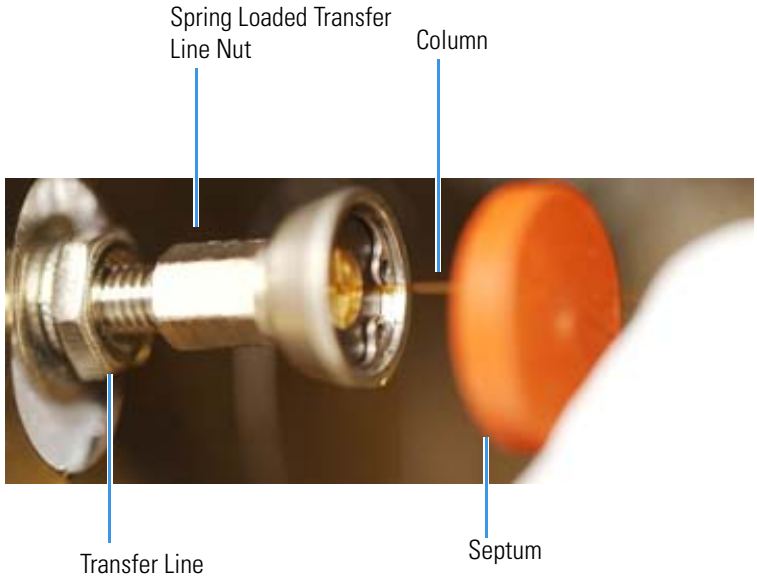


16. Loosen the transfer line nut from the column measuring tool.
17. Remove the column, transfer line nut, and ferrule from the column measuring tool, making sure not to move the septum from its location on the column.

Note The ferrule should still be able to move on the column. Use the septum to mark the correct location where the column should exit the nut.

18. Insert the column into the transfer line.

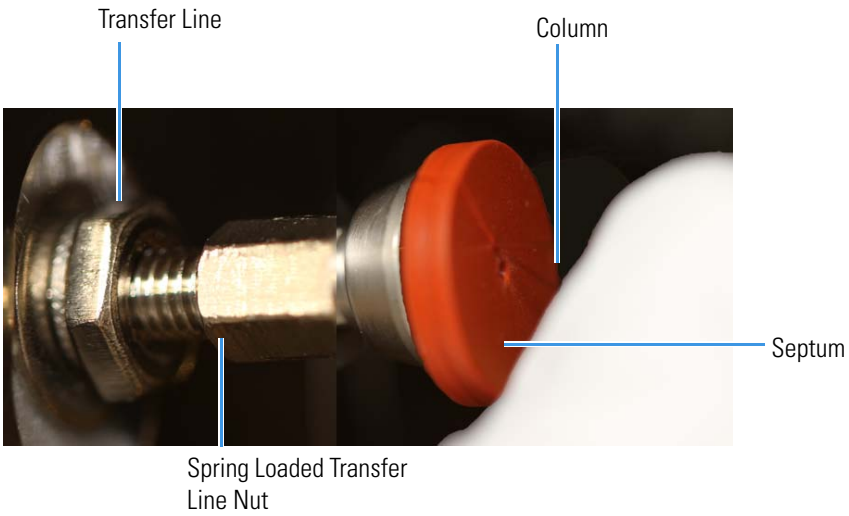
Figure 17. Inserting the Column into the Transfer Line



19. Tighten the spring loaded transfer line nut until it is just secure enough so that you cannot move it.

20. Loosen the nut by turning it exactly 1 flat backward.
21. Position the column in the transfer line using the cut septum to measure the correct length you should insert the column.

Figure 18. Positioning the Column in the Transfer Line



22. Tighten the spring loaded transfer line nut 1 flat forward—back to where it is secure enough in the transfer line that you cannot move it.
23. Tighten the spring loaded transfer line nut 1 additional quarter turn.
24. Remove the cut septum.
25. Close the front door of the GC.
26. Condition the graphite Vespel ferrule:
    a. Raise the oven temperature to the maximum temperature you will operate the GC.
    b. Wait 10 minutes.
    c. Lower the oven temperature to 40 °C (104 °F) and allow it to cool before continuing.

WARNING BURN HAZARD: The oven may be hot. Allow it to cool to room temperature before opening it. The injector will still be hot, so do not touch it.

27. Restore working conditions:
    a. Raise the oven temperature to the initial temperature that you will use.
    b. Turn on vacuum compensation on the GC.
    c. Twist the vent valve clockwise to close the valve. Be sure not to pinch the o-ring.
    d. If you are using hydrogen as a carrier gas, replace the front panel screw.

e. Replace all remaining hydrogen safety screws if you are using hydrogen.

28. Power on the ISQ Series mass spectrometer.

WARNING FIRE HAZARD: If you are using hydrogen, do NOT reach over the top of the instrument to power it on. Instead, reach around the right side or go to the back of the instrument and flip up the power switch.

29. Once the ISQ Series system is pumped own and able to scan, view air water spectra and look for evidence of leaks with a large *m/z* 28 signal. If you observe a leak, stop scanning and gently tighten the nut in small increments until no leaks appear when scanning.

3

Tuning the ISQ Series Mass Spectrometer

Tuning will improve the performance of your ISQ Series mass spectrometer. For optimum stability, you should start tuning after the lights on the front of the instrument are a solid green. These lights indicate that the instrument has reached vacuum and that it is at the last set temperature. If the system has been powered off for a period of time (a cold system), it takes longer (up to 4 hours) for the instrument components to reach stable vacuum and temperature. If you did not vent the ISQ Series instrument (system is hot), it takes approximately 30 minutes for the components to reach vacuum and temperature.

IMPORTANT Be sure to give the ISQ Series instrument enough time to stabilize. Otherwise, you may see mass drift, mass spectral changes, or changes in the fragmentation of your data.

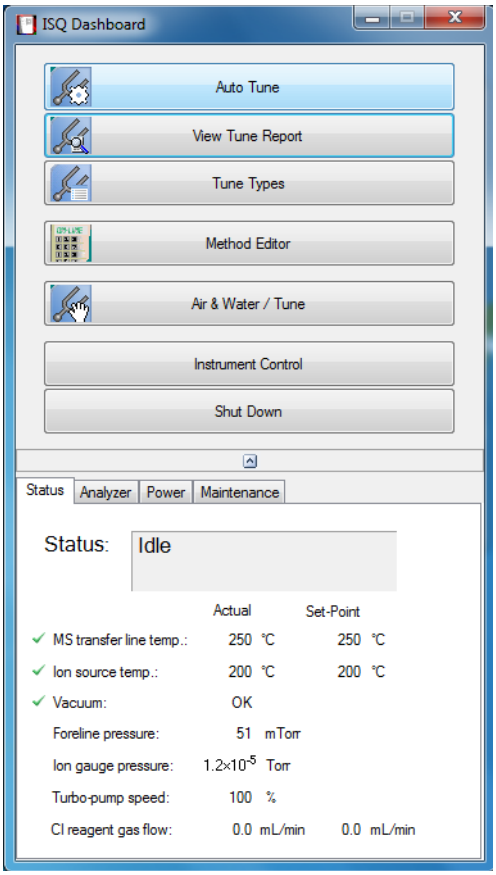
Contents

Accessing ISQ Series Autotune

❖ **To access *ISQ Series Autotune***

Open the ISQ Series Dashboard and click **Auto Tune** to open the ISQ Autotune window.

Figure 19. Accessing ISQ Series Autotune from the ISQ Series Dashboard



The list of available tune types opens.

Tune Types Included with the ISQ Series Mass Spectrometer

This section explains the different tune types in ISQ Autotune.

Daily Tune Check

Daily Tune

EI Default Tune

EI Full Tune

Fast Scan Tune

Negative CI Tune

Positive CI Tune

Daily Tune Check

Daily Tune Check—This tune check is used to check how well your last tune is performing. It is the fastest tune type. The daily tune check performs a leak check, makes sure the mass calibration is okay, and sets the detector sensitivity to generate a m/z 69 ion with an intensity of 20,000,000 counts. If your SOP allows it, you can use this tune to rapidly verify that the previous lens tune is still generating good spectra.

Daily Tune

Daily Tune—This tune is used to quickly tune the system. It performs a mass calibration and leak check, tunes the lenses and resolution, and sets the detector sensitivity to generate a m/z 69 ion with an intensity of 20,000,000 counts. You can perform a daily tune as frequently as your SOP requires. If the system is tuning to your satisfaction, then there is no need to perform the more time-consuming default or full tunes.

EI Default Tune

EI Default Tune—This tune creates a default tune file. It requires a clean instrument, starts with factory tune, and sets repeller to 0V and quadrupole voltage to a low value. This tune is used to generate a base for all the other tunes. As a result, you should only use this tune when the ion source is clean. The EI default tune will start with the tune file stored in the instrument at the factory and then perform a mass calibration and leak check, set the repeller to 0 V and tunes the lenses. The quadrupole offset voltage will be set to a low value to improve resolution, which is also tuned. The detector gain will be calibrated to generate ~ 300,000 electrons for every ion that strikes the detector. This tune is used in cases where a dirty ion source has been replaced by a clean one or where the computer has been replaced. Additionally, this tune will generate spectra that are the closest in appearance to the factory tune.

Note The EI Default Tune resets values on the instrument after you insert a clean ion source cartridge. Always follow the EI Default Tune with an EI Full Tune prior to running samples.

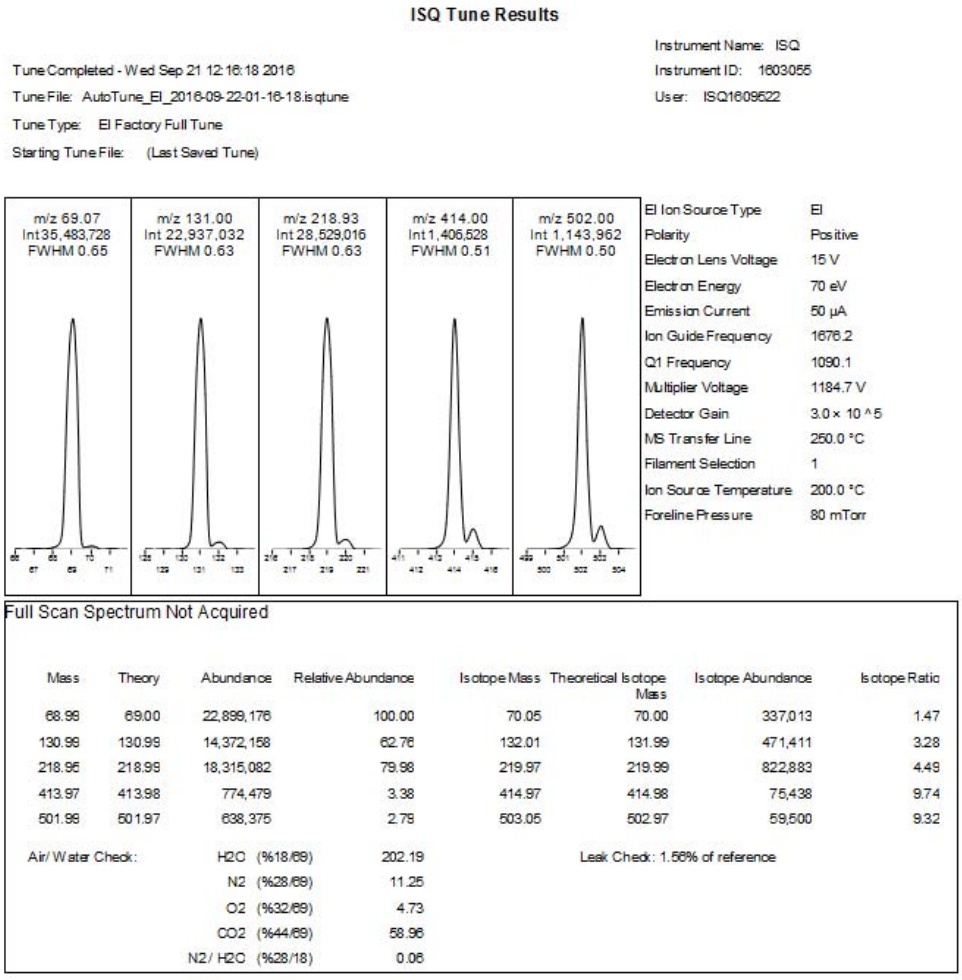
EI Full Tune

EI Full Tune—This tune is used to completely retune the system. It takes the longest amount of time to run, but the advantage of using it is that it re-optimizes nearly all the parameters affecting the signal. This type of tune will perform a mass calibration, tune the lenses and resolution, and perform a leak check. The detector gain will be calibrated to generate 300,000 electrons for every ion that strikes the detector. You should run an EI full tune when the daily

tune or the daily tune check is not adequate, when the electron multiplier is getting old (tuning to high electron multiplier voltages), or the first tune after you replace the electron multiplier. Unless your SOP requires it, this is not the best tune to use on a daily basis because of the length of time it takes to run it.

Figure 20shows a typical tune report for an EI Full Tune on a system using helium as a carrier gas.

Figure 20. Typical EI Full Tune Report



ISQ Tune Results

Instrument Name: ISQ
Instrument ID: 1603055
User: ISQ1609522

Tune Completed - Wed Sep 21 12:16:18 2016
Tune File: AutoTune_EI_2016-09-22-01-16-18.isqtune
Tune Type: EI Factory Full Tune
Starting Tune File: (Last Saved Tune)

m/z 69.07	m/z 131.00	m/z 218.93	m/z 414.00	m/z 502.00
Int 35,483,728	Int 22,937,032	Int 28,529,016	Int 1,406,528	Int 1,143,962
FWHM 0.65	FWHM 0.63	FWHM 0.63	FWHM 0.51	FWHM 0.50

EI Ion Source Type	EI
Polarity	Positive
Electron Lens Voltage	15 V
Electron Energy	70 eV
Emission Current	50 µA
Ion Guide Frequency	1676.2
Q1 Frequency	1090.1
Multiplier Voltage	1184.7 V
Detector Gain	3.0 × 10 ^ 5
MS Transfer Line	250.0 °C
Filament Selection	1
Ion Source Temperature	200.0 °C
Foreline Pressure	80 mTorr

Full Scan Spectrum Not Acquired

Mass	Theory	Abundance	Relative Abundance	Isotope Mass	Theoretical Isotope Mass	Isotope Abundance	Isotope Ratio
68.99	69.00	22,899,176	100.00	70.05	70.00	337,013	1.47
130.99	130.99	14,372,158	62.76	132.01	131.99	471,411	3.28
218.95	218.99	18,315,082	79.98	219.97	219.99	822,883	4.49
413.97	413.98	774,479	3.38	414.97	414.98	75,438	9.74
501.99	501.97	638,375	2.79	503.05	502.97	59,500	9.32

Air/ Water Check:		
	H2O (%18/69)	202.19
	N2 (%28/69)	11.25
	O2 (%32/69)	4.73
	CO2 (%44/69)	58.96
	N2/ H2O (%28/18)	0.06

Leak Check: 1.56% of reference

Typical results for an EI Full Tune are listed below.

- Peak Intensities:
  - Base Peak ≥ 10,000,000
  - *m/z* 502 ≥ 300,000
  - FWHM = 0.4–0.8
- Water Background: *m/z* 18:69 < 240%

- Repeller Voltage:
  - Helium carrier gas = 3–8 V
  - Hydrogen carrier gas = 7–15 V
- Multiplier Voltage
  - Normal Performance: < 2200 V
  - Replace Multiplier: ≥ 2200 V
- Foreline Pressure: < 100 mTorr
- Ion Gauge Pressure: $< 5 \times 10^{-5}$ Torr

Note Foreline and ion gauge are optional devices. Their pressures are dependent on column flow rate.

- Isotope Ratios:
  - *m/z* 70:69 = 0.8–3.0%
  - *m/z* 220:219 = 3.2–6.0%
  - *m/z* 503:502 = 7.5–15%
- Leak Check: < 10%

Run the EI Full Tune if you suspect a system problem. The following conditions could indicate an issue:

- Increased detector gain—Detector gain is related to multiplier voltage, so if the detector gain is increased, multiplier voltage will also increase.
- Leak check change—Leak check results change over time base on instrument conditions. Recently vented systems exposed to air should be lower than 10% after one day of pumping down. Assuming the system is leak free, the instrument leak check should constantly decrease over time until stabilizing.

Fast Scan Tune

Fast Scan Tune—This tune retunes the system with fixed Q1 voltages necessary for fast scanning. By increasing the ion energies and shortening ion flight times through the mass analyzer, this tune provides increased ion signal required to tune resolution and perform mass calibration at a high scan rate. This tune may cause high mass ions to exhibit more fronting than the other built-in tune types. This tune performs a leak check and sets the detector gain to 300,000. It does not tune the detector gain. Run a fast scan tune when scanning above 10,000 amu/s to improve the mass calibration for high mass ions.

Negative CI Tune

CI- Tune—This tune is used to analyze samples with negative CI. The standard NCI tune performs a mass calibration, then tunes the lenses and sets the resolution. This type of tune assumes you are using methane as the CI reagent gas and tunes the system with a 1.0 mL/min flow. This tune does not set the detector gain.

Note Chemical ionization tunes are very different from the electron ionization tunes. You should not use a CI tune unless your instrument has a CI ion volume and methane reagent gas installed.

Note Tunes with high repeller voltages are not recommended with CI mode. A tune file with a low repeller voltage should be loaded in manual tune and saved to the instrument before tuning in CI mode.

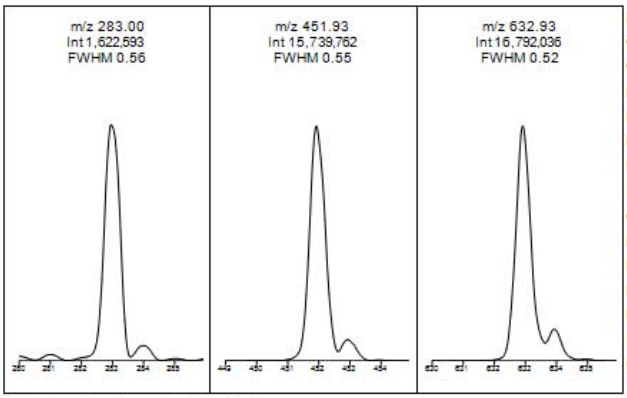
Figure 21 shows a typical CI- Tune report where methane is the CI reagent gas.

Figure 21. Typical CI- Tune Report with Methane as a CI Reagent Gas

ISQ Tune Results

Instrument Name: ISQ
Instrument ID: 1510142
User: ISQ1606626

Tune Completed - Wed Jul 20 20:51:45 2016
Tune File: AutoTune_NCI_2016-07-21-09-51-45.isqtune
Tune Type: CI- Tune (built-in)
Starting Tune File: (Last Saved Tune)



EI Ion Source Type	CI
CI Flow	1.0 mL/min
CI Gas Type	Methane
Polarity	Negative
Electron Lens Voltage	15 V
Electron Energy	70 eV
Emission Current	50 µA
Ion Guide Frequency	1663.8
Q1 Frequency	1084.7
Multiplier Voltage	1159.0 V
Detector Gain	3.0 × 10 ^ 5
MS Transfer Line	250.0 °C
Filament Selection	1
Ion Source Temperature	200.0 °C
Foreline Pressure	118 mTorr

Full Scan Spectrum Not Acquired

Mass	Theory	Abundance	Relative Abundance	Isotope Mass	Theoretical Isotope Mass	Isotope Abundance	Isotope Ratio
282.98	282.99	961,093	10.28	284.02	283.99	59,793	6.22
451.96	451.97	9,347,714	100.00	453.02	452.97	739,153	7.91
632.93	632.96	9,284,979	99.33	633.89	633.96	1,540,388	16.59

Air/Water check is not performed in

Leak check not performed

Mass Scale

Typical results for a CI- Tune using methane as the reagent gas are listed below.

- Peak Intensities:
  - Base Peak: 452 or 633
  - Base Peak ≥ 10,000,000
- CI Gas Flow: 1.0–4.0 mL/min Methane
- Emission Current:
  - 50 µA
  - Standard Set Point

  Note Emission current is an input value, and it should match the value set in the tune.
- Multiplier Voltage:
  - Normal Performance: < 2200 V
  - Replace Multiplier: ≥ 2200 V
- Foreline Pressure: < 400 mTorr
- Ion Gauge Pressure: < 1×10^{-4} Torr

  Note Foreline pressure fluctuates with CI reagent gas flow rate. As the CI reagent gas flow rate increases, the foreline pressure also increases. Ion gauge pressure also increases if an ion gauge is installed on the system.
- Isotope Ratios: *m/z* 453:452 = 5.8–11.8%

Positive CI Tune

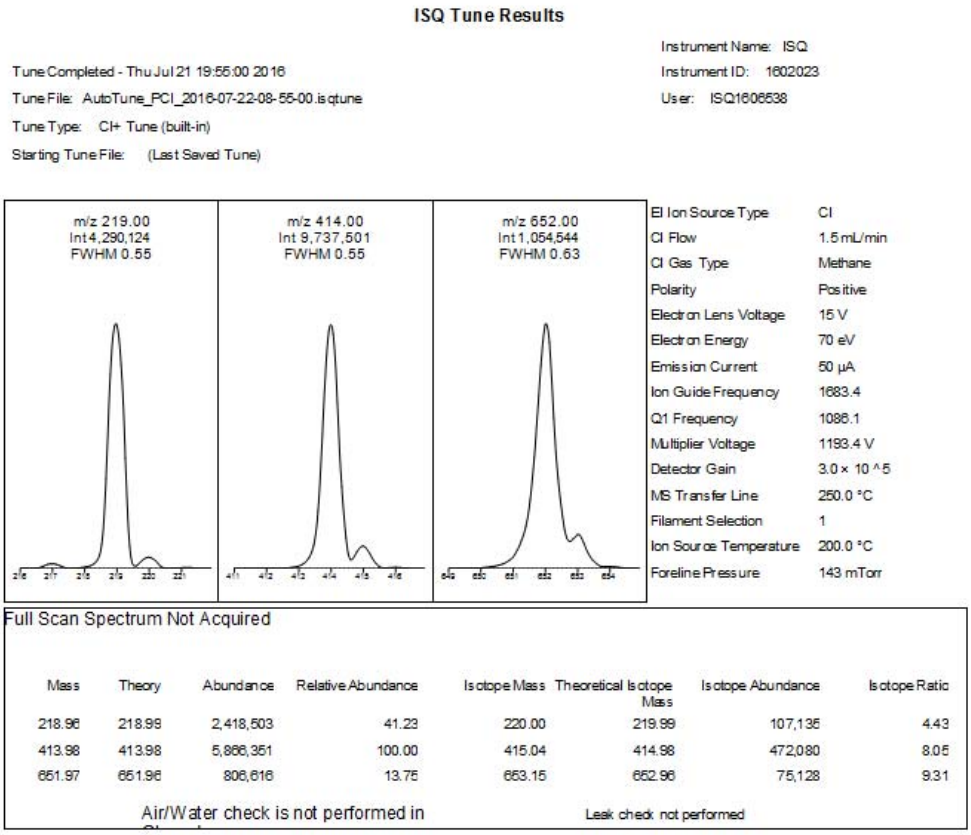
CI+ Tune—This tune is used to analyze samples with positive CI. The standard PCI tune performs a mass calibration, then tunes the lenses and sets the resolution. This type of tune assumes you are using methane as the CI reagent gas and tunes the system with a 1.5 mL/min flow. This tune does not set the detector gain.

Tip If you intend to use ammonia reagent gas, attach methane to one CI reagent gas port and ammonia to the other port. Tune the instrument using methane, then switch to the ammonia port. Allow plenty of time for the new reagent gas to purge the CI tubing before starting your analysis.

Note To add a tune type to the list, see Modifying an Automatic Tune.

Figure 22 shows a typical CI+ Tune report where methane is the CI reagent gas.

Figure 22. Typical C+- Tune Report with Methane as a CI Reagent Gas



ISQ Tune Results

Instrument Name: ISQ
Instrument ID: 1602023
User: ISQ1606538

Tune Completed - Thu Jul 21 19:55:00 2016
Tune File: AutoTune_PCI_2016-07-22-08-55-00.isqtune
Tune Type: CI+ Tune (built-in)
Starting Tune File: (Last Saved Tune)

m/z 219.00
Int 4,290,124
FWHM 0.55

m/z 414.00
Int 9,737,501
FWHM 0.55

m/z 652.00
Int 1,054,544
FWHM 0.63

EI Ion Source Type	CI
CI Flow	1.5 mL/min
CI Gas Type	Methane
Polarity	Positive
Electron Lens Voltage	15 V
Electron Energy	70 eV
Emission Current	50 µA
Ion Guide Frequency	1683.4
Q1 Frequency	1086.1
Multiplier Voltage	1193.4 V
Detector Gain	3.0 × 10 ^ 5
MS Transfer Line	250.0 °C
Filament Selection	1
Ion Source Temperature	200.0 °C
Foreline Pressure	143 mTorr

Full Scan Spectrum Not Acquired

Mass	Theory	Abundance	Relative Abundance	Isotope Mass	Theoretical Isotope Mass	Isotope Abundance	Isotope Ratio
218.96	218.99	2,418,503	41.23	220.00	219.99	107,135	4.43
413.98	413.98	5,866,351	100.00	415.04	414.98	472,080	8.05
651.97	651.96	806,616	13.75	653.15	652.96	75,128	9.31

Air/Water check is not performed in

Leak check not performed

Typical results for a CI+ Tune using methane as the reagent gas are listed below.

- Peak Intensities:
  - Base Peak: 414
  - Base Peak ≥ 1,000,000
- CI Gas Flow: 1.5–4.0 mL/min Methane
- Emission Current:
  - 25–50 µA
  - Standard Set Points

  Note Emission current is an input value, and it should match the value set in the tune.
- Multiplier Voltage
  - Normal Performance: < 2200 V
  - Replace Multiplier: ≥ 2200 V

- Foreline Pressure: < 400 mTorr

  Note Foreline pressure fluctuates with CI reagent gas flow rate. As the CI reagent gas flow rate increases, the foreline pressure also increases.

- Ion Gauge Pressure: < 1e-4 Torr
- Isotope Ratios:
  - *m/z* 415:414 = 5.8–11.8%

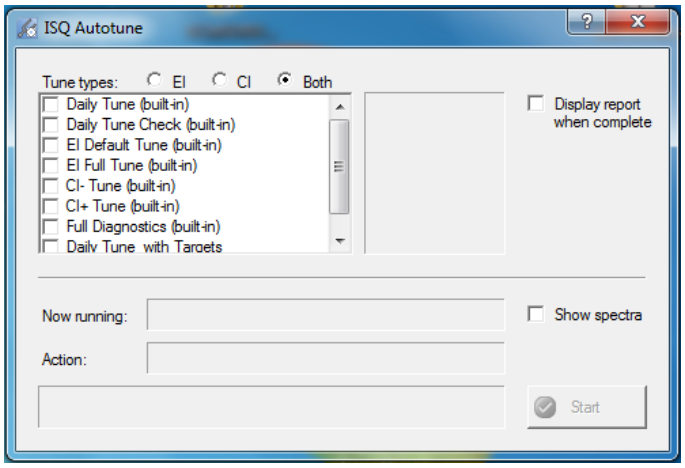
IMPORTANT Sensitivity is affected by the amount of water in the system. A recently cleaned source initially increases sensitivity in CI+, and then sensitivity drops as water is pumped out. Methane is highly reactive with water.

Tuning the ISQ Series Mass Spectrometer

❖ **To tune the ISQ Series Mass Spectrometer using ISQ Autotune**

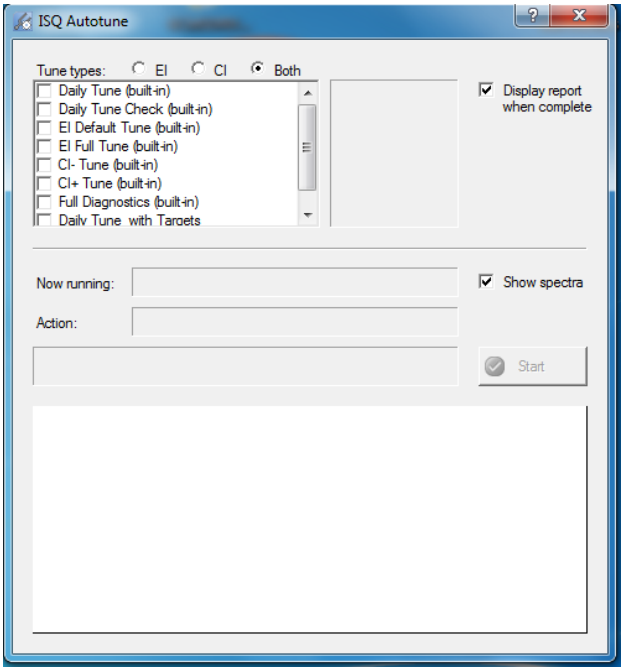
1. Select the tune type you want to use from the list of available tune types. See Figure 23.

   Figure 23. List of Available Tunes in ISQ Autotune



2. Select the **Display Report When Complete** checkbox so that you can view the tune report after running the tune.

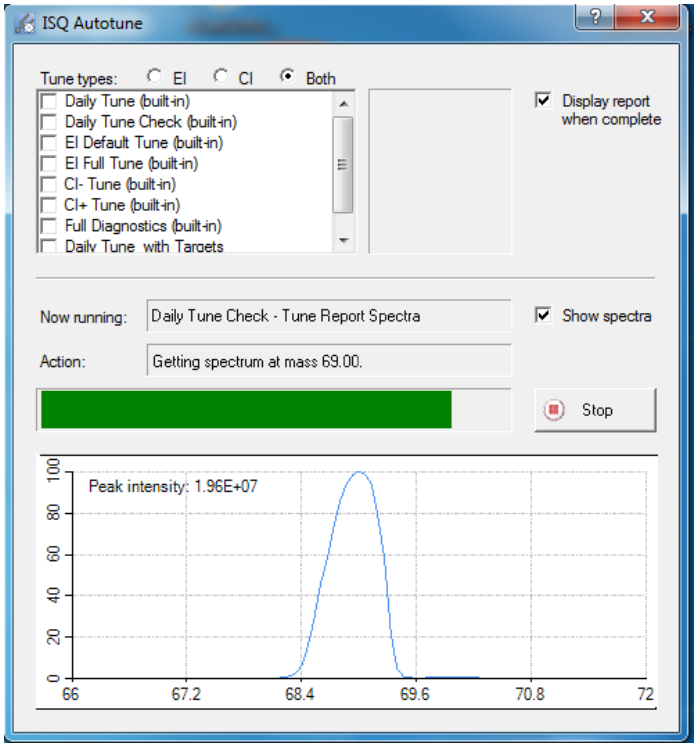
Figure 24. Displaying a Tune Report



3. Select the **Show Spectra** checkbox to show the spectra while the system is tuning.
4. Click the **Start** button to begin tuning.

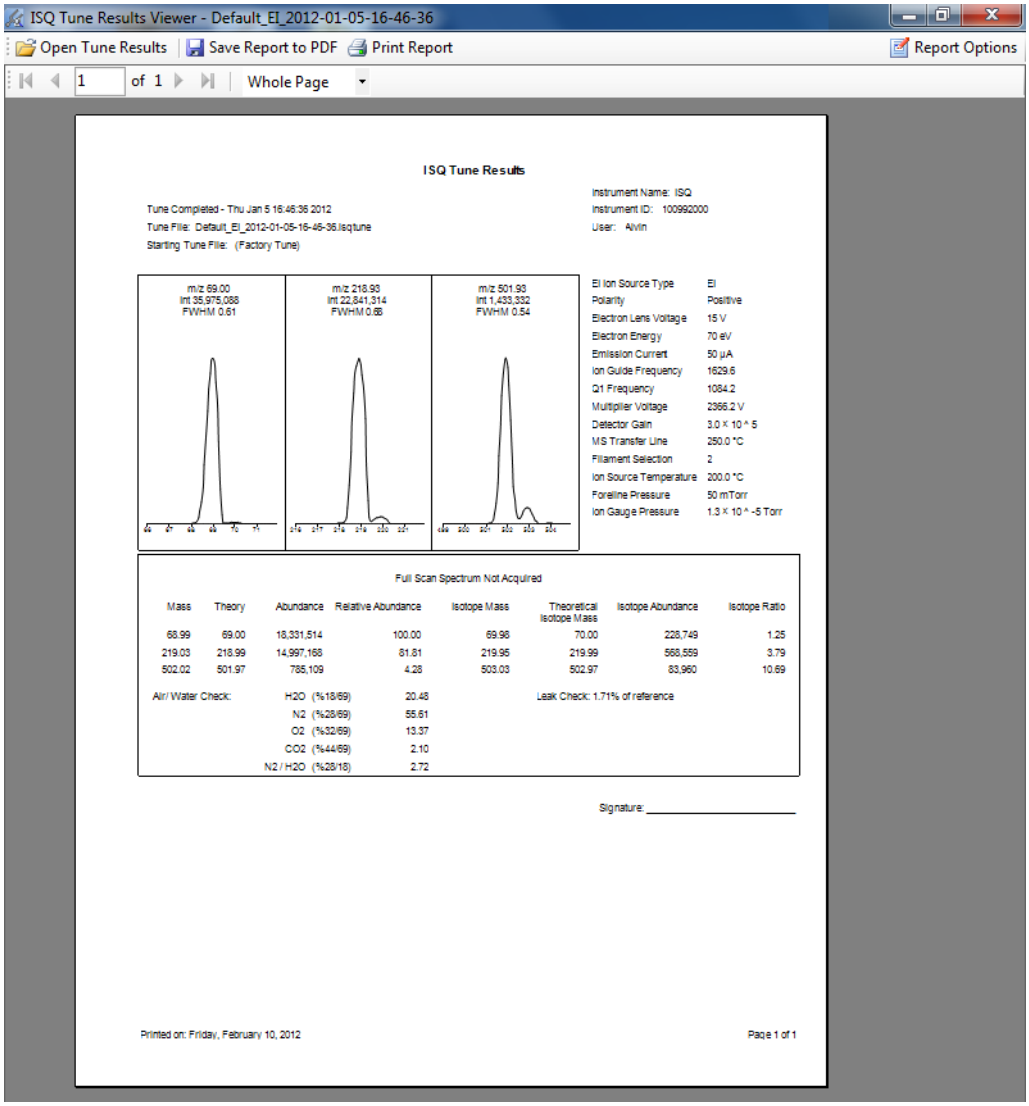
Note Make sure the power options on your computer are not set to go into Standby mode while you're acquiring data for your tune. Otherwise, it will interrupt your tune.

Figure 25. Showing the Spectra During an Automatic Tune



5. Once the tune completes, your tune report will open in the *ISQ Series Tune Results Viewer*. If you did not select the **Display Report When Complete** checkbox, you can click **View Tune Report** on the ISQ Series Dashboard and view the report.

Figure 26. Viewing a Tune Report.



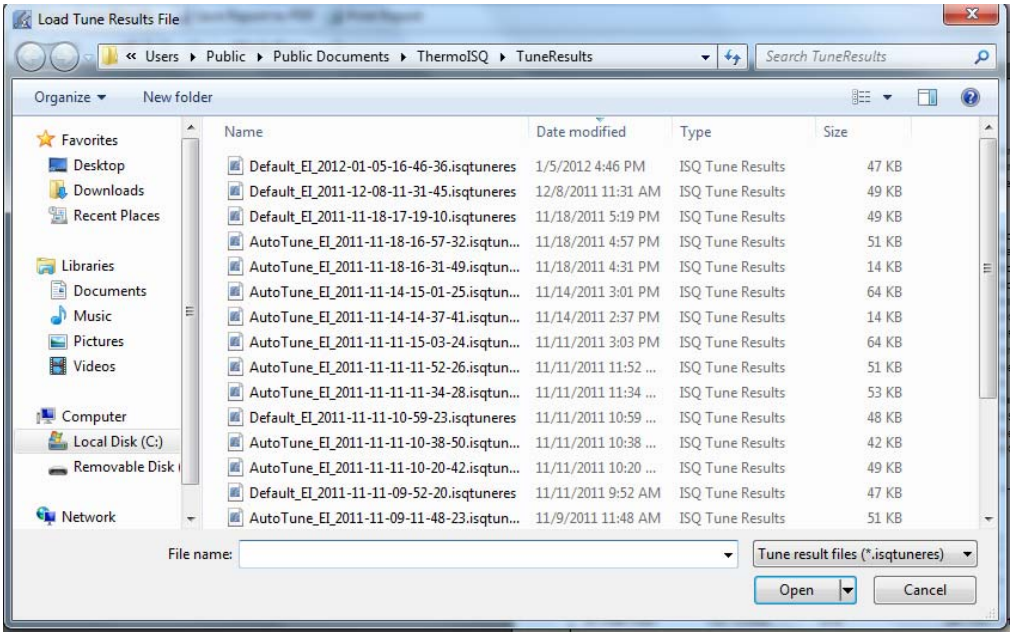
Note The **Error Action** is not diagnostic. It indicates what happens during the tune if a specific device cannot meet the tuning criteria. Any **Fail** comments on error actions do not mean the tune has failed.

6. Compare this tune with a previous tune report. Some changes in peak height are normal, but if the difference is significant, see Troubleshooting. If you have recently serviced the instrument, you most likely have a leak at the column, vent valve or near the component you just serviced.

   In the Tune Results window, you can open tune results, print report, or change the way you view the report. To save the report, click the icon and save it as a Microsoft Excel file or an Adobe Acrobat PDF file.

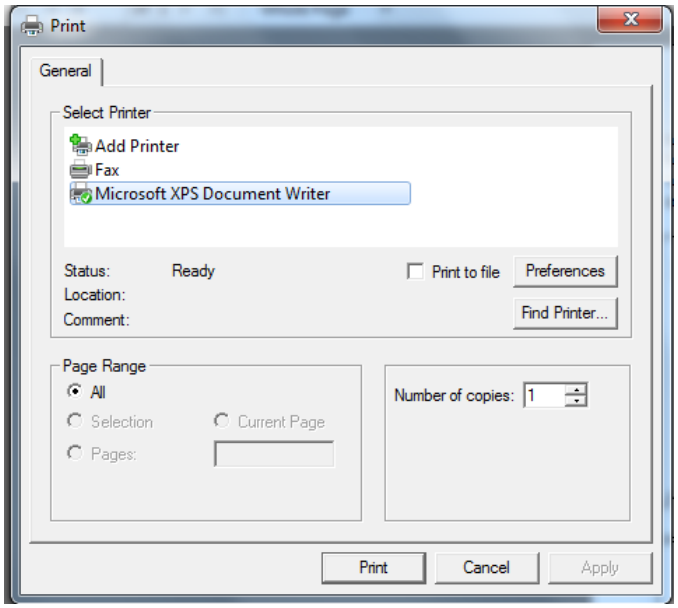
7. Click **Open Tune Results** in the top of the window and browse to another tune report on your computer. Then click **Open**.

   Figure 27. Opening a Tune Results File



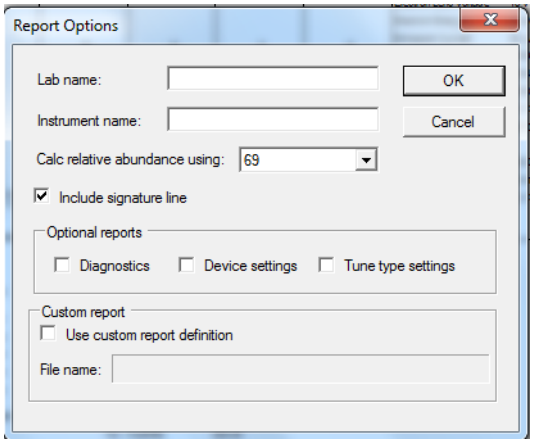
8. Click **Print Report** to open a print dialog box and print your report.

Figure 28. Printing a Tune Report



9. Click **Report Options** to select the charts and reports you want to display and change the name of your instrument. Then click **OK**.

Figure 29. Selecting the Tune Report Options



10. If the sensitivity and resolution are adequate for running the initial samples, you are ready to develop or run a method.

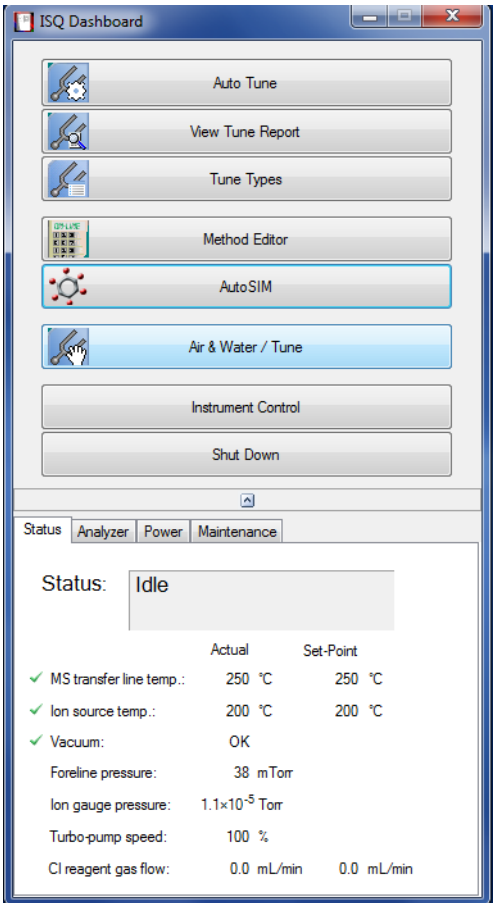
Updating Tunes for New RF Lens

If you have purchased a new lens 3/RF lens (PN 1R120574-0103), you must update the ion guide frequency in the ISQ Manual Tune utility and retune your instrument.

❖ To update the ion guide frequency using the ISQ Manual Tune utility

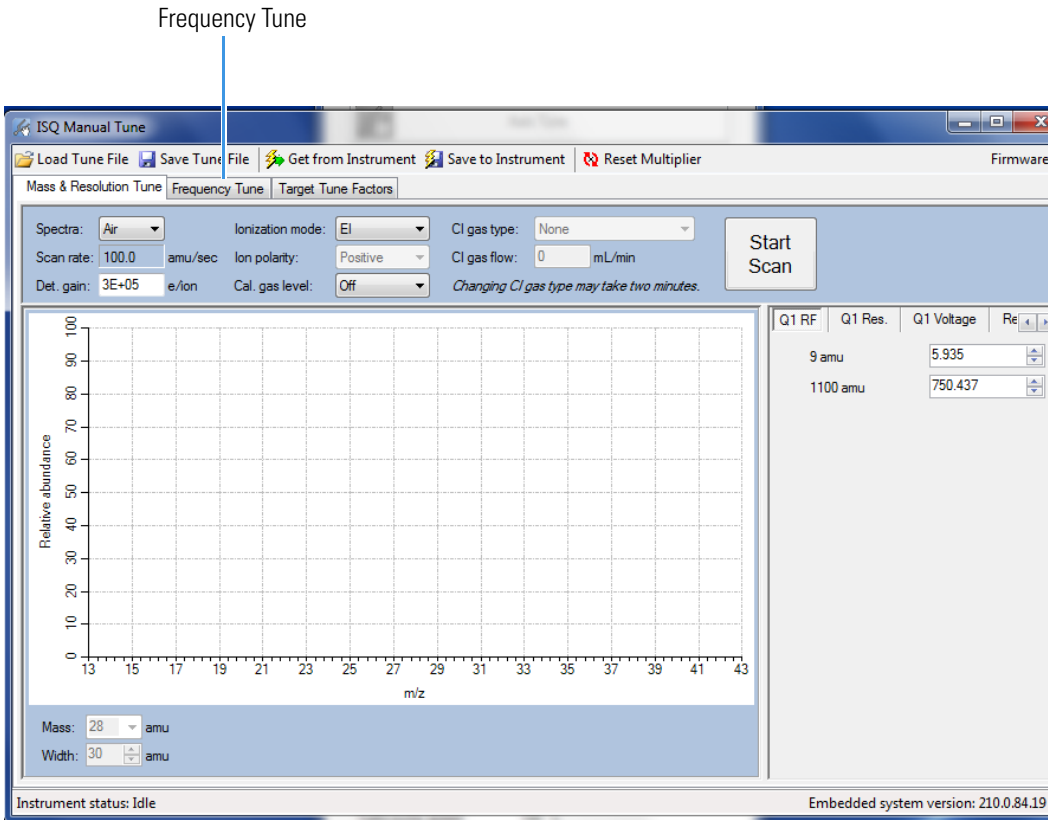
1. Open the ISQ dashboard and click **Air & Water/Tune**.

Figure 30. Accessing the ISQ Manual Tune Utility from the ISQ Dashboard



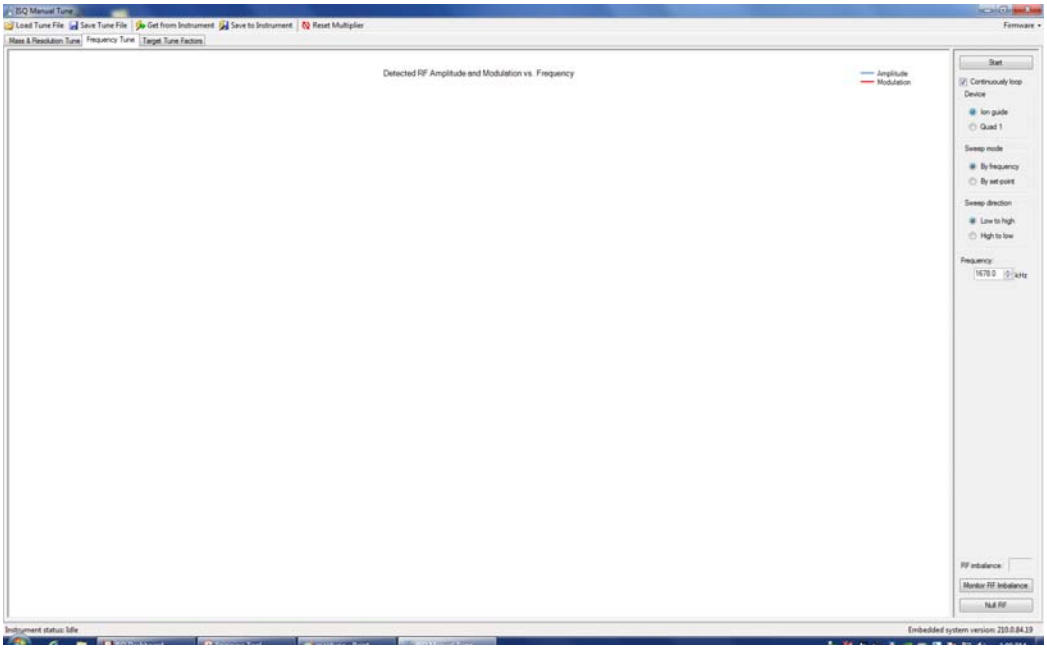
2. The ISQ Manual Tune Utility opens. Select Frequency Tune from the top menu See Figure 31.

Figure 31. Selecting Frequency Tune



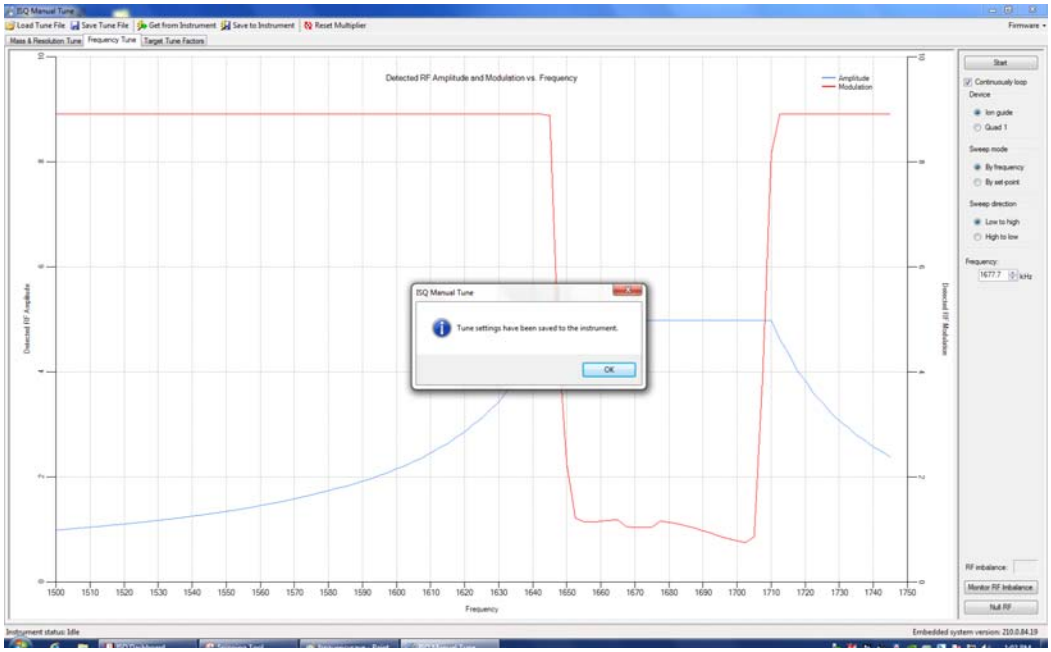
3. The Frequency Tune page opens. On the right side of the screen, under **Device**, select **Ion Guide** and then click **Start**. See Figure 32.

Figure 32. Starting the Ion Guide Frequency Tune



4. When the instrument has finished detecting the ion guide frequency, go to **Save To Instrumen**t on the top menu. A dialog box confirming that the tune settings have been saved to the instrument will open. See Figure 33.

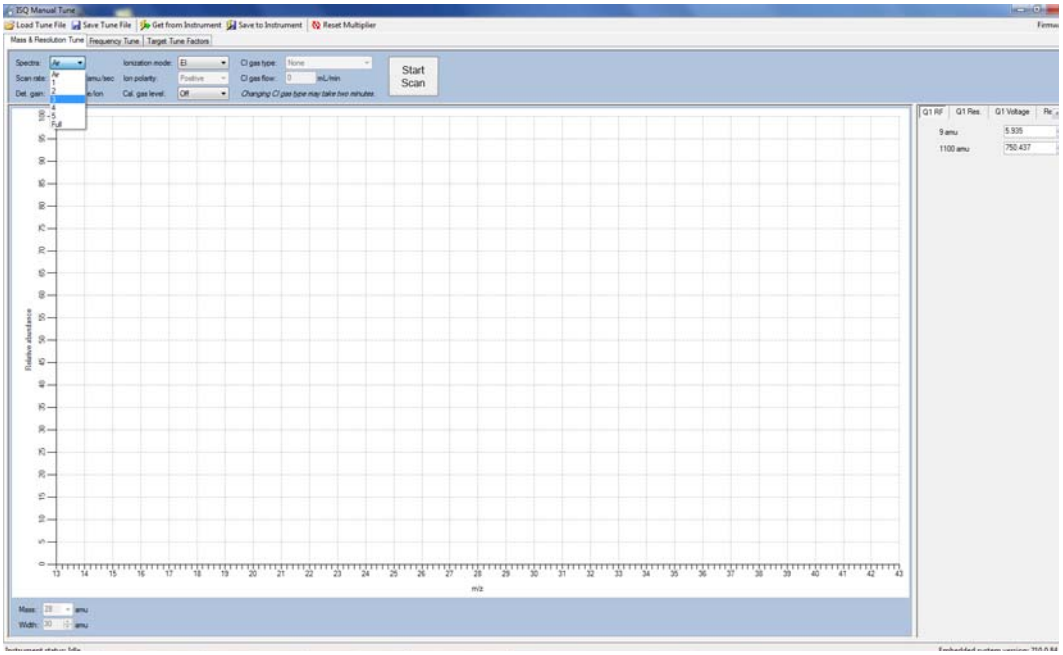
Figure 33. Saving the New Ion Guide Frequency to the Instrument



5. To confirm that the intensities are sufficient for tuning, select **Mass & Resolution Tune** on the top menu to go back to the ISQ Manual Tune home page.

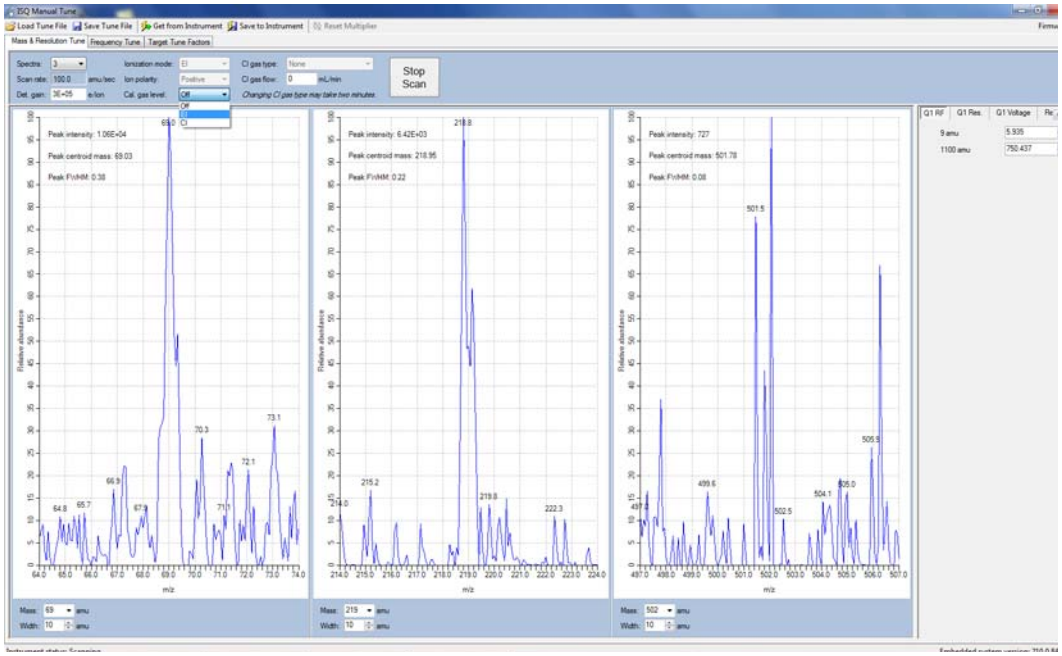
6. Choose **3** from the **Spectra** drop-down menu. See Figure 34.

Figure 34. Selecting the Correct Number of Spectra to Scan



7. Select **EI** from the **Cal. Gas Level** menu. See Figure 35.

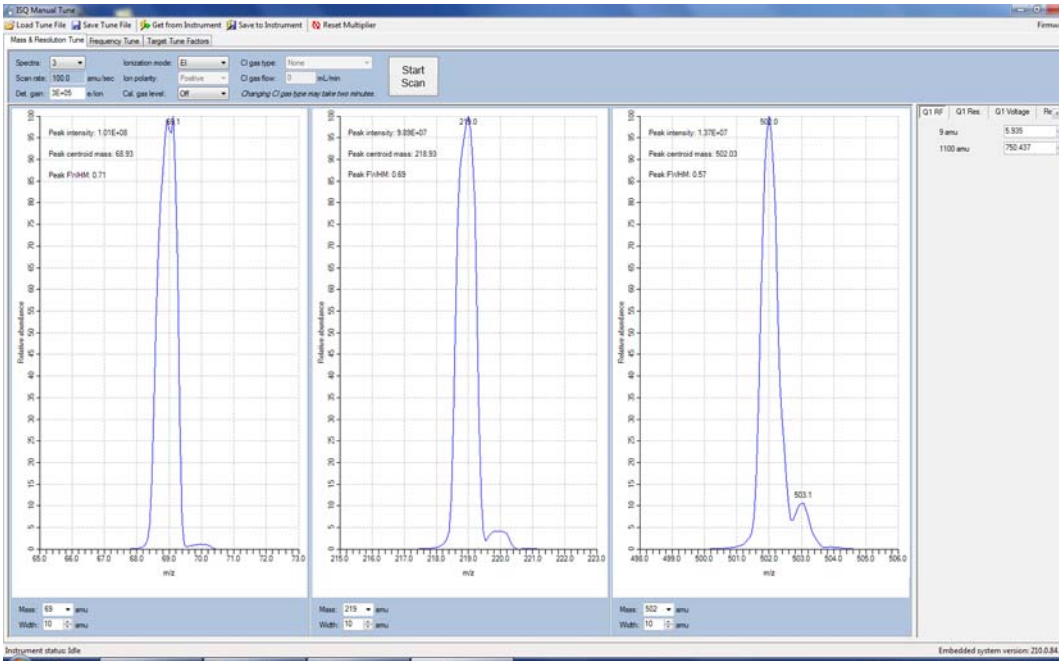
Figure 35. Setting the Calibration Gas Level to EI



8. Click **Start Scan**.

9. When the instrument has finished scanning, check the intensities for the masses 69, 219, and 502. Set the masses using the Mass drop-down menu below each spectrum. See Figure 36.

Figure 36. Checking the Mass Intensities



Note The intensities might be lower than they were at the previous frequency set for the ion guide until the lenses are tuned in Autotune.

10. Once you have confirmed the intensities are sufficient for tuning, retune the ISQ Series system.

4

Creating a Method

Once you have tuned the ISQ Series mass spectrometer, you can create a method for each component of your system. Methods are used to indicate to the GC/MS system how to collect your data.

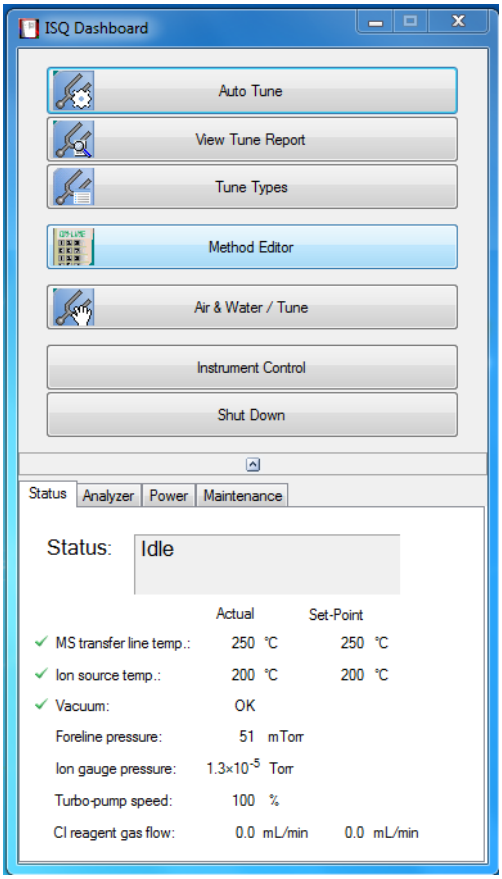
Contents

❖ To create a method for the ISQ Series mass spectrometer, GC, and autosampler

Note For information about creating a method for specific instruments and software, refer to the appropriate documentation.

1. Click **Method Editor** on the ISQ Series Dashboard to open the Method Editor. See Figure 37.

Figure 37. Accessing the Method Editor from the ISQ Series Dashboard

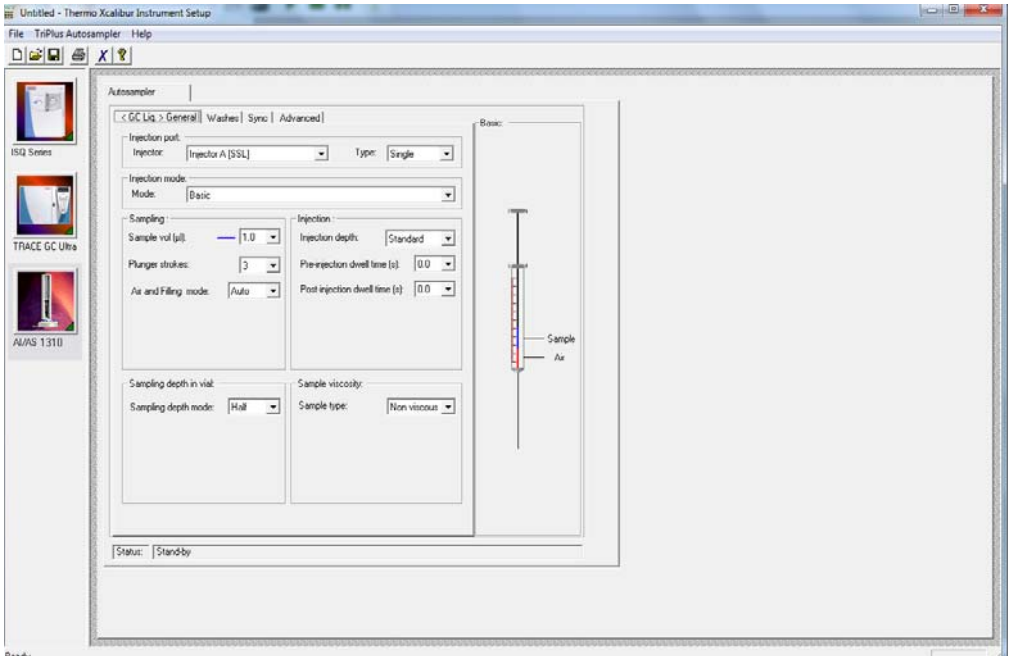


Creating a Method for the Autosampler

1. Create a method for the autosampler. Instructions for setting common parameters are below. Refer to you autosampler documentation for specific guidelines.

Note All of the configured instruments are shown in the left pane of the Xcalibur Instrument Setup window. If the instruments are not shown, you need to configure them, according to Reconfiguring Your Instrument.

Figure 38. Configuring the Autosampler



a. Configure the Sampling group:

- **Sample Volume**—Use this field to enter the sample volume to be injected into the GC. Typical values are between 0.5 and 5 µL.
- **Plunger Strokes**—Use this pull-down menu to select the number of plunger strokes to use when drawing up the sample. Air bubbles in the syringe change the amount of sample injected, which can cause signal variation in different runs. To prevent this from occurring, increase the number of plunger strokes to reduce the chance of an air pocket in your syringe. Typical values are between 3 and 10.
- **Viscous Sample**—Use this field to select Yes if your sample is viscous or No if your sample is non-viscous. With a viscous sample, the syringe is filled more slowly than if it was non-viscous. Since the amount of time saved is so small, it may be easier to just set this option to Yes.
- **Sampling Depth in Vial**—Use this pull-down menu to select the depth (Center or Bottom) at which the tip of the syringe needle will be placed in the sample vial when it is being filled. The bottom is the default and most commonly used setting.

b. Configure the Pre-Injection group:

- **Pre-Injection**—Use the **Solvent** and **Cycles** fields to set the number of solvent purges that will occur before the autosampler touches your sample. You should always have some sample rinses, either before or after injection, to make sure you do not have sample carryover from one injection to the next. You can configure the settings so that the syringe is purged with the same solvent that was used in location A, B, C or D or with solvents A and B or C and D. Typically, there is 0 or 1 cycles of pre-injection purges.
- **Sample**—Use the **Rinses** pull-down menu to select the number of times the syringe is rinsed with your sample before each injection. Rinses help ensure the sample being injected is not diluted by the residual rinse solvents. By purging the syringe with your sample before injection, the dilution is minimal. The standard setting is between 1 and 3 rinses. If you have a very limited amount of sample, however, you may want to set this field to 0 to conserve the sample.
- **Post-Injection**—Use the **Solvent** and **Cycles** fields to set the number of solvent purges that will occur after the autosampler touches your sample. You should always have some sample rinses, either before or after injection, to make sure you do not have sample carryover from one injection to the next. You can have the syringe purged with the same solvent that was used in location A, B, C or D or with solvents A and B or C and D. Typically, there are 1 to 5 cycles of post-injection purges.

c. Configure the Injection group:

- **Injection Depth**—Use the pull-down menu to select Standard or Minimum to indicate how the sample is introduced into the GC. If you select Standard, the autosampler will insert the needle all the way into the injection port. If you select Minimum, the autosampler will barely enter the injection port.
- **Pre-inj Dwell Time(s)**—Use this field to enter the time (in seconds) that the needle will be in the injection port before the plunger injects the sample.
- **Post-inj Dwell Time (s)**—Use this field to enter the time (in seconds) that the needle will be in the injection port after the plunger injects the sample.

  The two main forms of injection are a hot needle technique and a cold needle technique.

  In a hot needle injection, the needle is preheated before the sample is introduced, which causes the sample to vaporize before it leaves the needle. The vapor is then mixed with carrier gas and swept into the column.

  In a cold-needle injection, the needle is kept as cold as possible so that the sample can be introduced as a liquid stream that vaporizes in the injector. The sample vapor is produced closer to the column inlet, which can lead to better sensitivity, but worse reproducibility from one run to the next.

To use the hot needle technique, select the standard injection depth and use a pre-injection dwell time of at least 2 seconds. To use the cold needle technique, select a minimum injection depth and do not use a pre-injection dwell time. The post injection dwell time can be used to bake out the needle and ensure that all semi-volatile compounds are cleaned out of the system before the next injection is made.

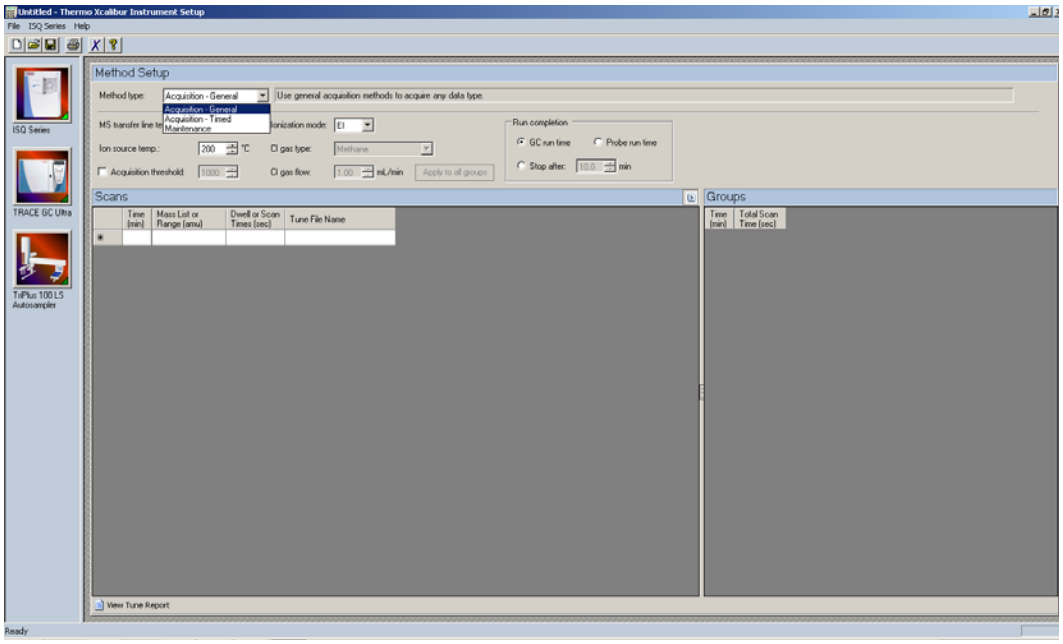
Creating a Method for the ISQ Series Mass Spectrometer

Click the ISQ Series icon in the left pane to create a method for the ISQ Series mass spectrometer.

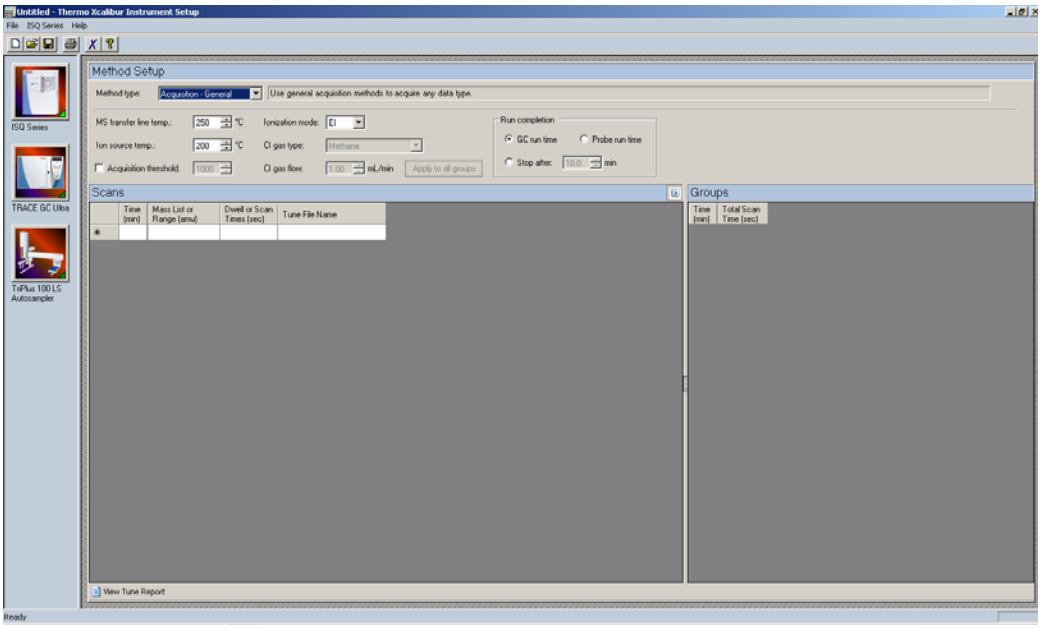
1. To create a acquisition method from the Method Type pull-down menu, select **Acquisition-General**. An Acquisition method is used to collect data.

Note The maintenance method is used to bake out or cool down the ISQ Series instrument during a sequence. This can be useful if you know you want to perform these tasks and you want to do them in an automated way as part of your data acquisition.

Figure 39. Creating a General Acquisition Method for the ISQ Series Mass Spectrometer



2. Set the **MS Transfer Line Temperature**. This field represents the temperature of the transfer line, which is the tube that contains the column as it leaves the GC oven and enters the ISQ Series mass spectrometer. The maximum allowable temperature is 400 °C. However, you will damage the column and contaminate the ISQ Series instrument if you set the transfer line temperature above the maximum allowed temperature of the column.



3. Set the **Ion Source Temperature**. You can enter a value between 0 and 350 °C. The optimal temperature depends on the analyte. Higher temperatures will keep the ion source cleaner, but will lead to increased fragmentation, which may reduce sensitivity. For most compounds, a source temperature of 275 °C (default) is adequate.

Note For best results, tune the instrument at the same temperature you will run the analyses in your method.

4. Select the **Acquisition Threshold** checkbox and enter a value for the minimum peak height for the data file, if needed. If your peak has an intensity that is below this threshold, it will not be stored. This setting may help reduce noise, but it may also alter the reported isotope ratios because the smaller isotope signals will be preferentially reduced.

5. Select **EI** from the **Ionization Mode** pull-down menu.

Note Only use CI if you have installed a CI ion volume in the ISQ Series instrument and you have connected CI reagent gas to your system. If you have CI, select a CI Gas Type from the pull-down menu and set the CI Gas Flow. (There will be a two-minute delay when you change ports for the CI Gas Type.) Typical values for Methane CI for NCI are 1.0–1.5 mL/min. For Methane CI for PCI, the gas flows are typically 1.5–2.0 mL/min.

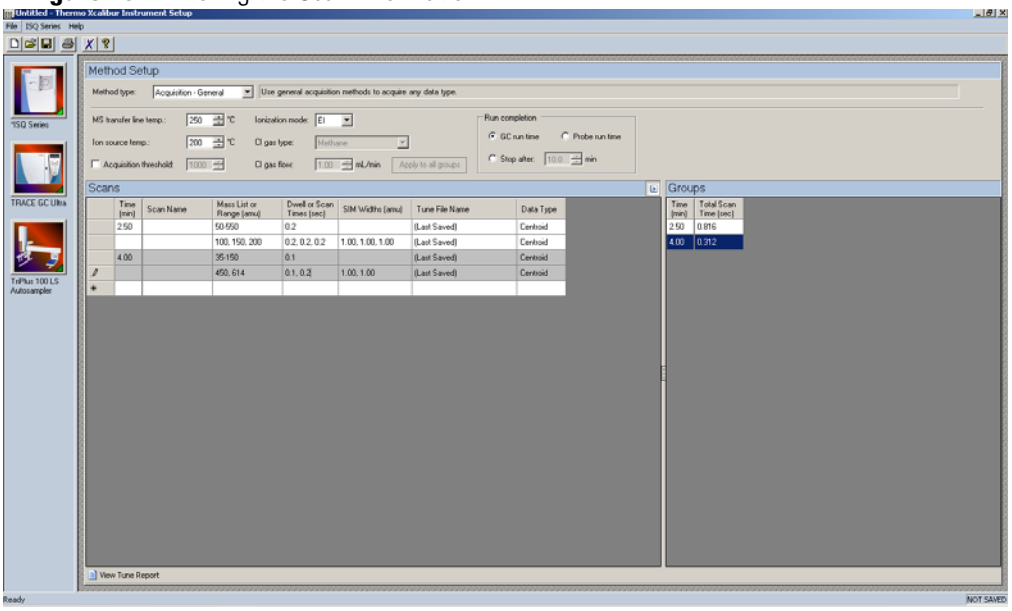
6. In the **Run Completion** group, select the action you want to occur at the end of a run:
   a. **GC Run Time**—Select this option if you want the ISQ Series system run to end when the GC run is complete. This is the most common setting.
   b. **Probe Run Time**—Select this option if you have a probe controller installed and you want the ISQ system run to end when the probe run is complete.

c. **Select Stop After**—Select this option to set the number of minutes you want the ISQ Series system to run. The end of the run can be between 0 and 1,000 minutes. This option allows you to stop the acquisition when all the compounds of interest have eluted, but the GC is still at an elevated temperature to keep the column clean. We recommend you select this option it because saves burn time on the filament.

Note In Timed Acquisition mode, the run stops when the ISQ Series system has completed acquisition for the final sample in your method. These settings do not apply.

7. In the **Scans** pane, click a scan row to enter scan information.

Figure 40. Entering the Scan Information



Note If some of the columns mentioned below are not shown in the **Scans** pane, you can right-click on a heading and display them. You can also reorganize the columns by clicking on the heading of a column and dragging it to the left or right.

a. The **Time (min)** column is used to set the time that the ISQ Series system begins to acquire data after the GC starts. It is typical to have enough of a time delay to allow the solvent to get through the column before starting an acquisition.

As an example, in this method, the mass range of 50-550 amu will be scanned in 0.2 seconds. Beginning from the same start time, three different SIM masses at 100, 150, and 200 will be looked at for 0.2 seconds each. These simultaneous full scan and SIM scans will begin 2.5 minutes into the GC run. You will get a complete set of scans every 0.816 seconds.

At 4 minutes into the GC run, the scanning is completely changed. Now the full scan range is 35-150 amu, which is scanned every 0.1 seconds, and the two SIM masses are 450 and 614 amu, which are scanned for 0.1 and 0.2 seconds respectively. All these scans will repeat every 0.312 seconds until the GC run is complete.

All of these scans use the last tune file that was saved to the instrument.

b. The **Mass List or Range** column tells the ISQ Series system what masses it needs to scan. In full-scan mode, enter the start and end mass separated by a dash. In SIM mode, enter individual masses or multiple values in this field (as long as they are separated by a comma). You must put each full-scan range on a separate line.

Note Each line in a scan must only contain a Full-Scan range or individual SIM masses. They cannot be mixed in a single line.

c. In SIM mode, the **Dwell or Scan Times** column defines the amount of time (in seconds) that the ISQ Series instrument will look at your SIM ion mass. If you are in Full-Scan mode, the **Dwell or Scan Times** column determines the amount of time for each individual scan. You should set this value to have 5-20 scans across your GC peak. If you have too few scans, the GC peak area is too imprecise. If you have too many scans, the ISQ Series instrument's signal becomes less precise. The default is 0.2 s.

d. The **Tune File Name** column selects a tune file to be used for this scan. It should be the automatic tune file you created in Tuning the ISQ Series Mass Spectrometer. You can also use a specific tune file for each of the scans.

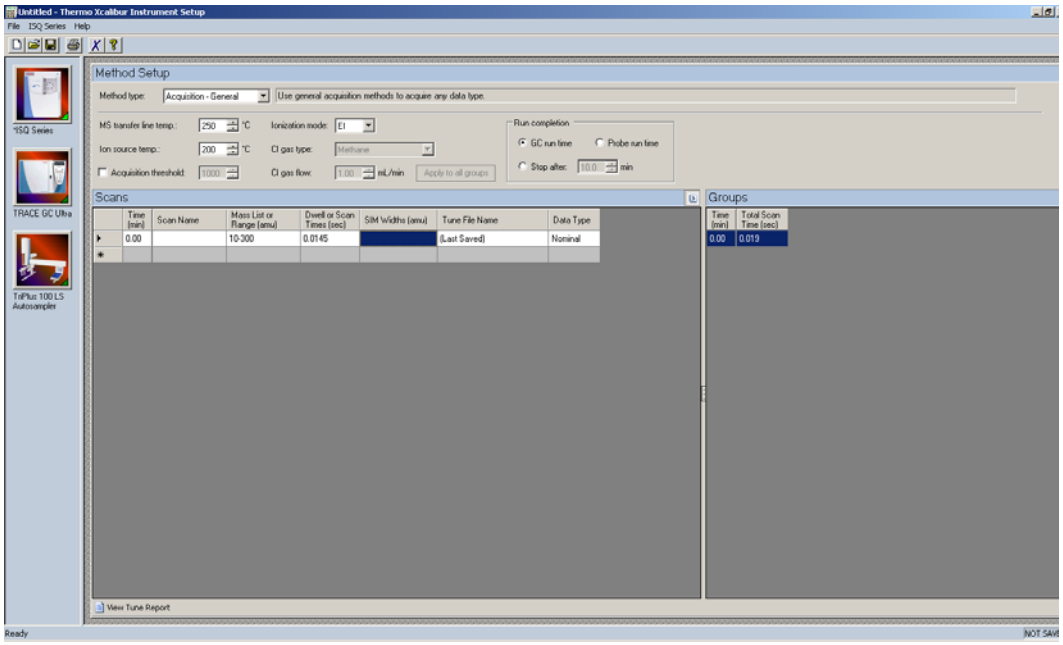
e. The **Scan Name** column contains a description of the scan. The name may be used as a label to indicate the compound used with the scan.

f. The **SIM Widths** column sets the width range of the SIM window. The range of values can be between 0.01 and 10. The default is 1 amu, which means the instrument will collect all the ions from your SIM mass +/- 0.5 amu. Narrower SIM widths lead to greater specificity.

g. The **Ion Polarity** column is only used in CI mode, which is the only mode for generating positive and negative ions. In EI mode, this column should be always be set to **Positive**.

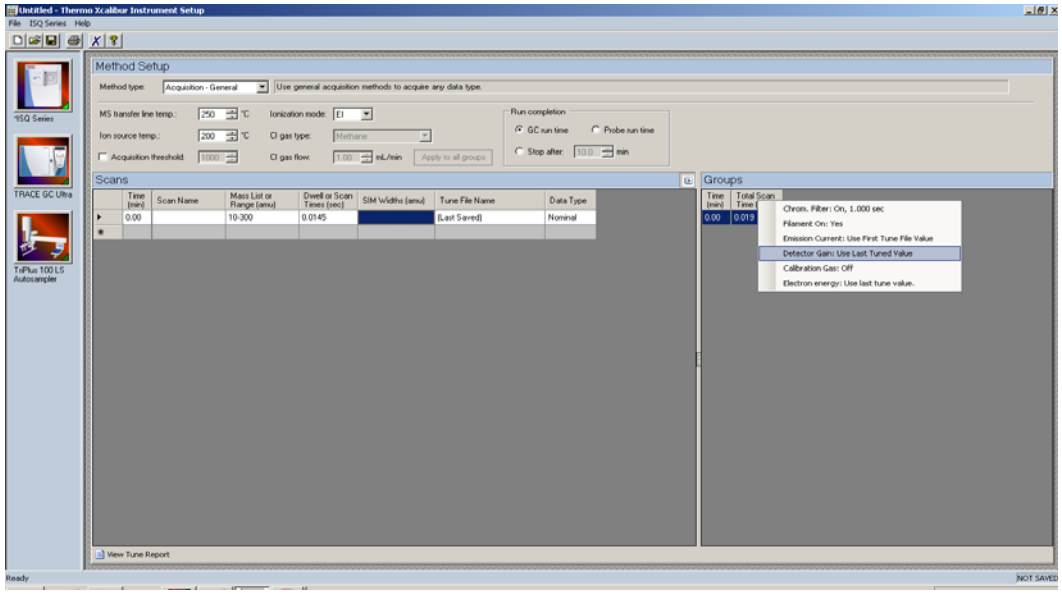
h. The **Data Type** column determines whether you want to collect **Profile**, **Centroided**, or **Nominal** mass spectra. Centroided mass spectra is most common because it is used by most of the libraries and provides the smallest data files. Profiled mass spectra provides detailed mass spectral peaks, which results in a large data file that contains details a centroided spectra does not contain. When you want to perform fast scanning (up to 20,000 amu/s), select **Nominal** from the drop-down list under **Data Type**.

Figure 41. Selecting Nominal Mass Spectra



8. In the **Groups** pane, review the information in each row. As you create scans in the **Scans** pane, information in the groups pane is automatically displayed.

Note If some of the columns mentioned below are not shown in the **Groups** pane, you can right-click on a heading and display them. You can also reorganize the columns by clicking on the heading of a column and dragging it to the left or right.



a. The **Time** column displays the time that the ISQ Series mass spectrometer begins to acquire this particular group of scans after the GC starts.

b. The **Total Scan Time** column indicates the sum of all the scans in each segment. The total scan time also contains the stabilization time that occurs between each scan. In this method, beginning 2.5 minutes into the GC run, you will get a complete set of scans every 0.816 seconds. At 4 minutes into the GC run, the scanning has completely changed.Now the scans will repeat every 0.412 seconds until the GC run is complete.

c. The **Chrom Filter On** column enables the chromatographic filter. This filter smooths spectral data as it is acquired, which may increase the signal-to-noise ratio by a factor of 2 or more. The chromatographic filter is most useful when at least four full scans are acquired across a GC peak. This setting is typically left on.

d. Use the **Chrom Filter Peak Width** column to set the peak width to match the width of the GC peak (in seconds). If the peak width is set too large, signal intensity may be reduced. The default value is 1 s.

e. The **Filament On** column turns the filament on and off in the selected segment. Turning off the filament increases the lifetime of the filament and keeps the ion source clean longer. However, no data will be collected. Use this column if you have analytes eluting before the solvent peak. You can create a segment to turn off the filament during the solvent peak to preserve the filament.

f. Use the **Emission Current** column to set the emission current used during the acquisition. For optimal analytical performance and stability, use the emission current at which the system was tuned. However, if you want to use a different emission current, deselect the **Use Tune File Emission Current** checkbox and enter a value in the **Emission Current (µA)** column. A high emission current will lead to the production of more ions, but the interaction of too many ions in the source can cause a degradation in the resolution and signal.

g. The **Use Last Tuned Detector Gain** column indicates that you want to use the detector gain set in ISQ Series Autotune or set and scanned in manual tune. If you do not need to use the gain set in ISQ Series Autotune, then you can set the gain manually. Higher gains give larger signals, but may shorten the lifetime of the detector when concentrated samples are detected.

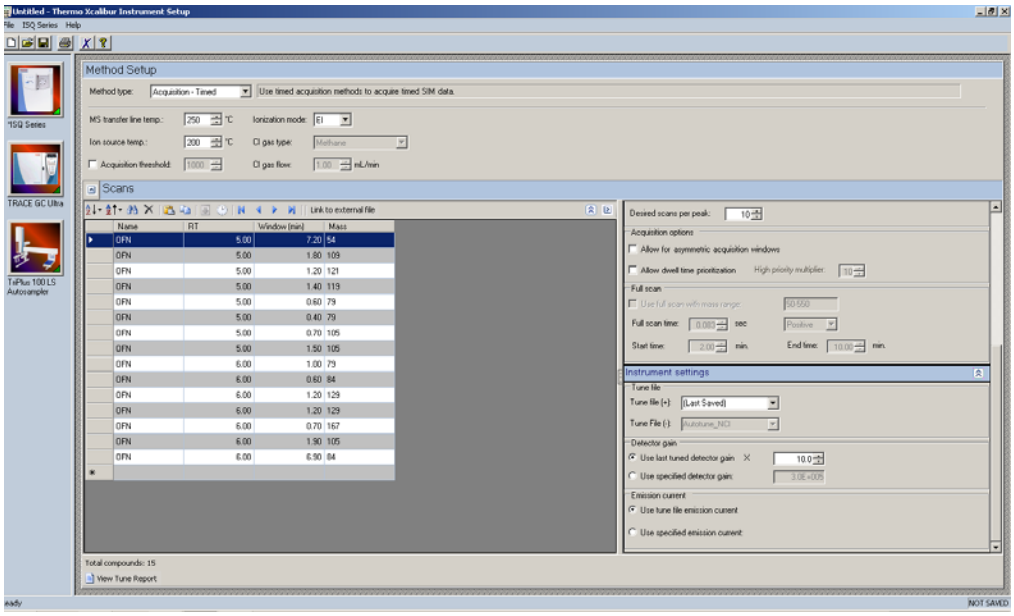
h. Use the **CI Gas Flow** column to set the flow rate of your reagent gas. Remember to set the CI gas flow in the Groups column as well as at the top of the method editor. The single value at the top of the method is sent when initializing the MS with the method.

i. Use the **CI Gas Type** column to set the type of gas attached to one or both CI gas ports (A or B). Be sure that the gas you have assigned to a port is actually attached to that port, as each CI gas has a specific calibration of flow vs. gas viscosity.

j. The **Cal Gas** column turns on the calibration gas during a run. This setting can be useful when confirming that the system is generating ions and correctly storing the data to disk. Typically, the setting is off, but you may let a low level of calibration gas into the source by selecting EI. If you have a dual-flow calibration gas module, you may select CI for a high level of gas.

9. (For **Acquisition-Timed** methods only) In the **Instrument Settings** pane, do the following:

Figure 42. Acquisition-Timed Instrument Settings



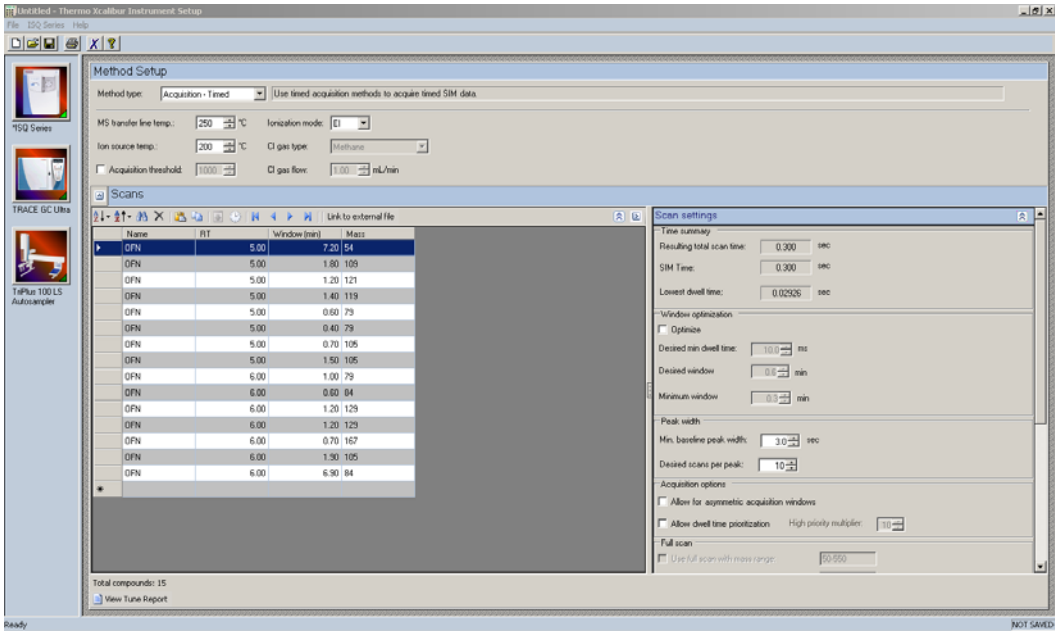
a. Use the **Tune File** to select a tune file or files to be used for this scan. If you are using EI, only the **Tune File(+)** pull-down menu is available. If you are using negative CI, select a tune file from the **Tune File(-)** pull-down menu. Choose **AutoTune_NCI** to use the most recent negative CI automatic tune file you created in Chapter 3, "Tuning the ISQ Series Mass Spectrometer," If you are using positive CI, select a tune file from the **Tune File(+)** pull-down menu. Choose **AutoTune_PCI** to use the most recent positive CI automatic tune file you created in Chapter 3, "Tuning the ISQ Series Mass Spectrometer," The software defaults to the most recent **AutoTune_NCI** or **AutoTune_PCI** tune file you created.

b. In the **Detector Gain** area, set the detector gain. Select the **Use Last Tuned Detector Gain** option to indicate that you want to use the detector gain set in ISQ Series Autotune. If you do not need to use the gain set in ISQ Series Autotune, then you can set the gain manually. Higher gains give larger signals, but may shorten the lifetime of the detector or saturate the electrometer with too much signal when concentrated samples are detected. To manually set the detector gain, select the **Use Specified Detector Gain** radio button and enter the desired value in the Detector gain box.

c. Use the **Emission Current** box to set the emission current used during the acquisition. For optimal analytical performance and stability, use the emission current at which the system was tuned. However, if you want to use a different emission current, select the **Use Specified Emission Current** radio button and enter a value in the **Emission Current (µA)** box. A high emission current will lead to the production of more ions, but the interaction of too many ions in the source can cause a degradation in the resolution and signal. The margin of error is ± 0.5 µA.

10. As appropriate, use the options in the **Scan Settings** area to further adjust your method.

Figure 43. Acquisition-Timed Scan Settings



a. The **Time Summary** section reports the resulting total scan time, the SIM time, and lowest dwell time for you method. These values are for information only and not editable.

   i. The **Resulting Total Scan Time** is the baseline peak width divided by the number of points desired across the peak. These values should be updated if your method requirements are different from the defaults.

   ii. The **SIM** time is the total length of all SIM scans for each compound in your list. This will match the total scan time unless the method also has a full scan event.

   iii. The **Lowest Dwell Time** is the actual lowest dwell time achieved by the method settings. When the **Optimize** check box is selected, if the actual lowest dwell time is considerably lower than the requested dwell time, then the minimum window has been reached, and if the actual lowest dwell time is considerably higher than the requested dwell time, then the requested window has been reached.

b. The **Window Optimization** pane allows access to the window optimizer settings. When the optimize button is checked, acquisition windows will be set automatically based on the acquisition window and dwell time targets set in this pane. For complex SIM methods, this option will help ensure a method is created that can achieve the requested scans per peak.

   Algorithm Details: If the **Optimize** checkbox is checked, the SIM acquisition windows in the method are set to the **Desired Window** unless the **Desired Min Dwell Time** cannot be met with the number of **Requested Scans Per Peak**. If this occurs, then the acquisition windows are reduced until either the **Desired Min Dwell**

Time is met, or the **Minimum Window** is reached. If the **Minimum Window** is reached first, the **Minimum Dwell Time** is reduced until the **Requested Scans Per Peak** is achievable. If the absolute minimum dwell time on the instrument, which is 0.5 ms, is reached before the requested points across the peak are achieved, the **Minimum Window** is lowered until the **Desired Scans Per Peak** criteria are met or until the absolute allowed minimum window on the instrument is reached, which is 0.24 min. In this very rare case you must reduce the number of **Desired Scans Per Peak**, increase the **Min Baseline Peak Width**, or reduce the number of transitions contained in your method before you will be allowed to save your method.

Settings: **Optimize** is not checked by default. When Optimize is checked, you can adjust the settings in the pane to optimize your method. When the box is not checked, you must manually input your acquisition windows. The default values for the window optimizer should give reasonable results for normal methods. The default values are:

Desired Min Dwell Time—10 ms

Desired Window—0.6 min

Minimum Window—0.3 min

- Change the minimum dwell time using the **Desired Min Dwell Time** combo box. If your method has many transitions, you may want to reduce the desired minimum dwell time. Note that the wider the acquisition windows in your method, the shorter the average dwell time will be.
- Change the desired acquisition window in the **Desired Window** combo box. The desired window is the amount of time to scan for a transition around a given retention time to ensure that compound will be observed. The desired window can be set from 0.24–5 min. Set the window wide enough so that a retention time shift will not cause you to miss any compounds. Include extra time in this window if there is any uncertainty in compound retention times in the method. Note that the longer the dwell time for your compounds, the narrower your acquisition windows will be.
- Change the minimum acquisition window in the **Minimum Window** combo box. The minimum window can be set from 0.24–5 min. This is the smallest amount of time that should be scanned for a transition around a given retention time so that you are confident the compound will be observed. Set the minimum window to the lowest safe value to prevent compound retention times from drifting outside the acquisition window.

Note If the dwell time limit is reached and the minimum acceptable window is forced below the 0.24 min limit, the method will fail, and a smaller list must be used.

c. Under **Peak Width**, you can change the minimum baseline peak width and desired scans per peak. These values are used to calculate the total scan time, which includes

the SIM time and the full scan time. The minimum baseline peak width should be set roughly to the shortest chromatographic peak time in your analysis.

d. Under **Full Scan**, you indicate if a Full Scan is to be run along with SIM. The Mass Range, Scan Time, Start and End Time can be set after the Use Full Scan button is selected. The Full scan time will reduce the SIM time without increasing the total scan time. If you only want to use full scan for part of your method, you can enter full-scan start and end times.

e. Under **Acquisition Options**, select the **Allow for Asymmetric Acquisition**s check box to add extra time to the beginning or end of an acquisition window without affecting other timing in your scan. When you select this option, Pre-width and Post-width columns are added to your method. Enter the extra times in these columns.

> **Note** This option is only available when the method optimizer is not active.

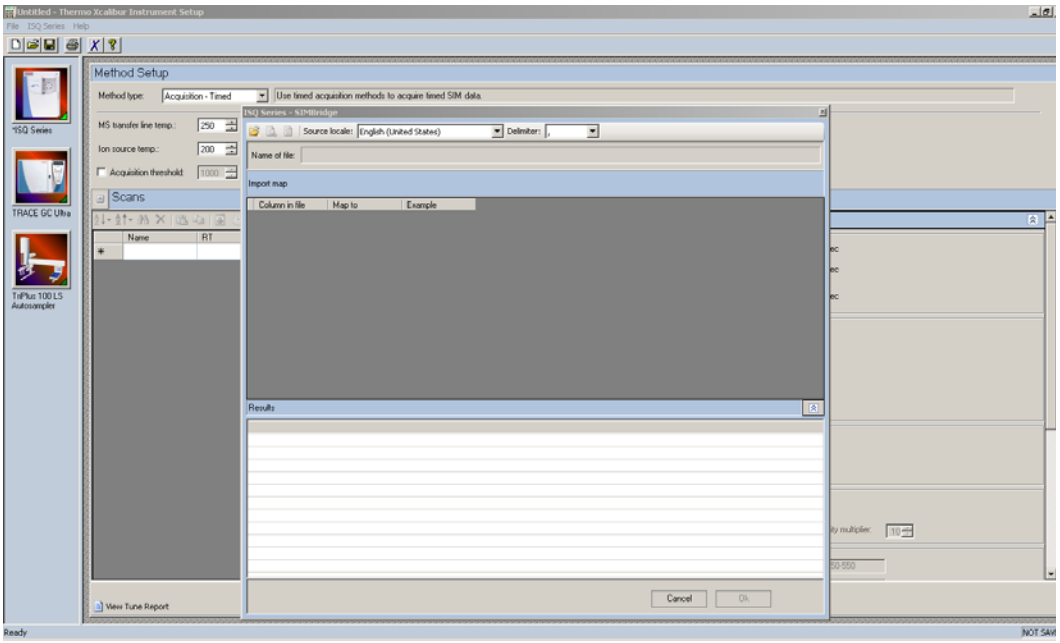
Select the **Allow Dwell Time Prioritization** check box to increase the dwell times for selected scans. The choices for each scan are Normal or High. Giving a scan high priority increases its dwell time by the value you set in the High Priority Multiplier box.

f. If you want to link to an external method, select the **Link to External File** check box. You may link to a .csv or .xml method file.

> **Note** In order to edit the scans within the ISQ Series Method Editor, clear the **Link to External File** check box.

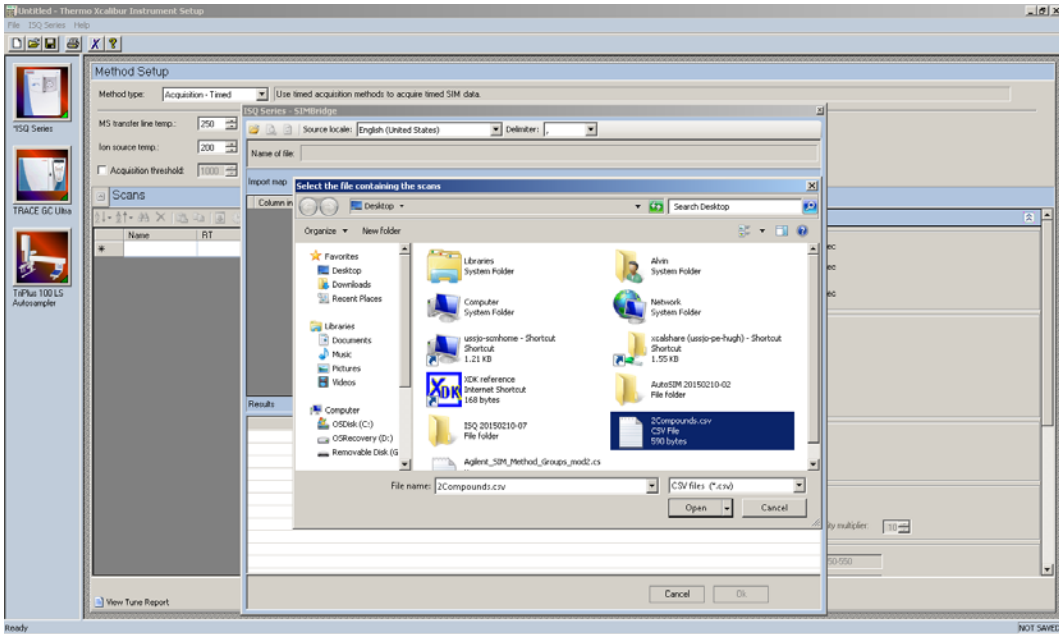
g. After clicking **Link to External File**, the **SIMBridge** dialog box opens. Choose the language of your method file from the **Source Locale** drop-down menu.

Figure 44. Setting the Source Language of Method Files using SIMBridge



h. Browse to your file.

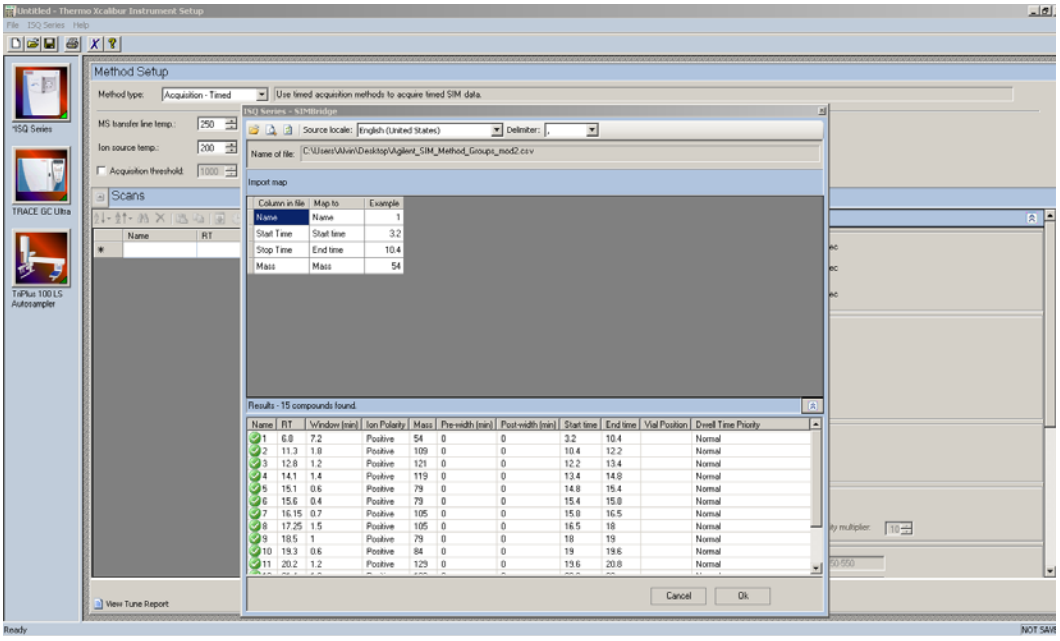
Figure 45. Linking to an External File using SIM Bridge



i. Click **Open** to open the method in SIMBridge.

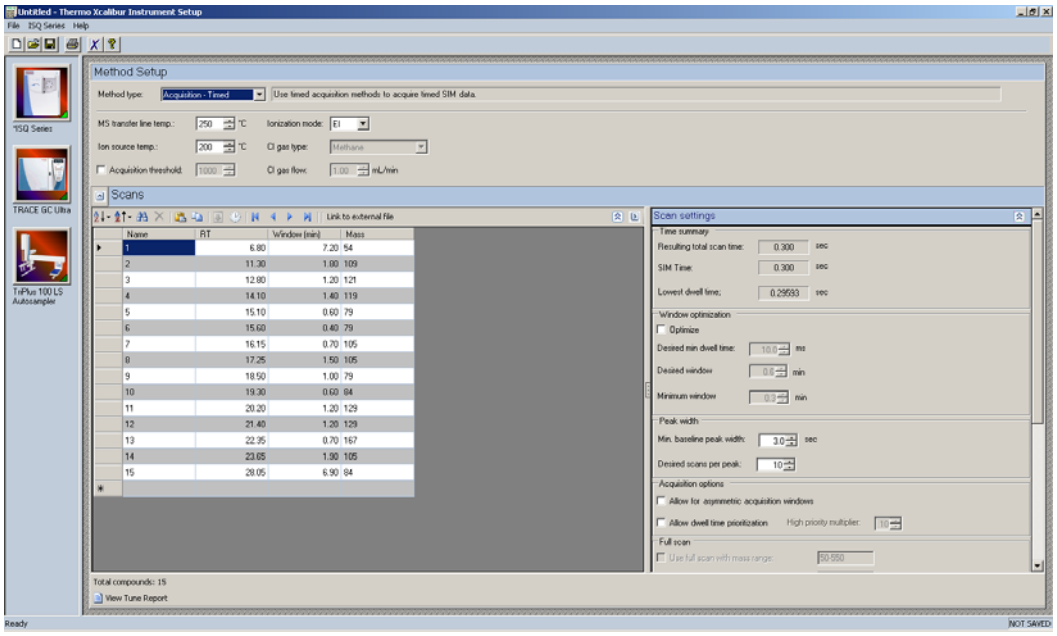
j. If necessary, change the method headings in your original file to match those in the method editor. A green check mark appears when your method is validated.

Figure 46. Changing Method Headings in SIMBridge



k. Click **Open** and the external method will be opened in the method editor.

Figure 47. Viewing a Linked File in the Method Editor



l. Either enter the analyte name in the **Name** column (referring to the analyte name) or, in your external file, enter the analyte name in the first column. You may also right-click this window to search for an analyte within your method. This function is useful if you need to edit an analyte in a complex method.

m. In the **RT** column, enter retention times for SIM methods. The retention time is the time it takes an analyte to pass from the column inlet to the detector.

n. In the **Window (min)** column, set the acquisition times. Smaller acquisition windows increase sensitivity but can cause you to miss your peak if set too small. Changing the window size only affects sensitivity if it reduces the number of compounds analyzed in a segment. If the windows do not overlap, you will not notice an improvement by reducing the acquisition window.

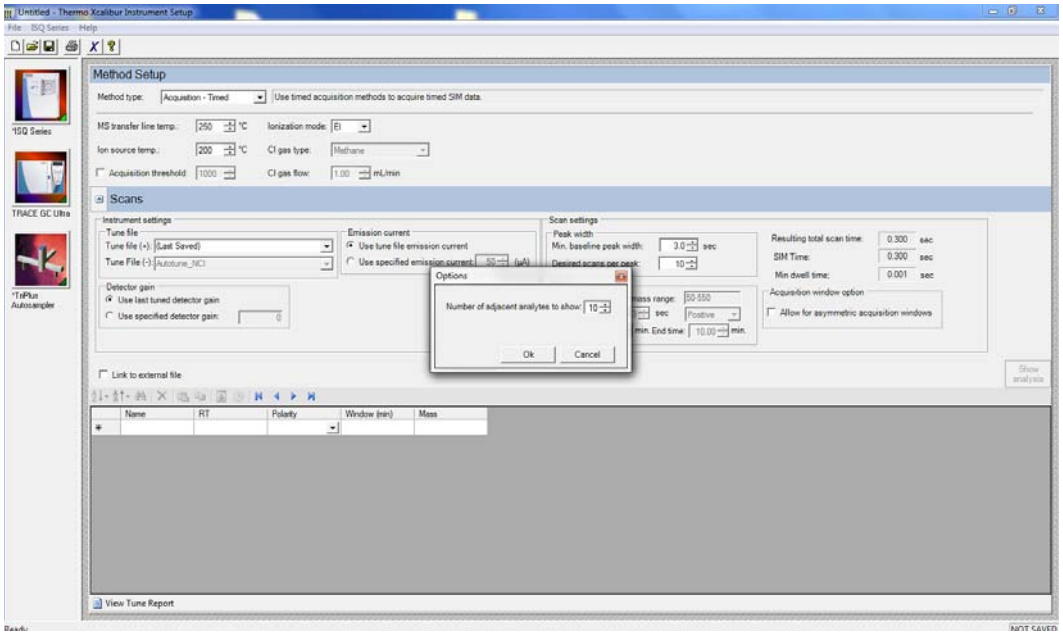
o. In the **Mass** column, enter the mass of the ion you wish to monitor.

p. Use the **Ion Polarity** column if you are using CI mode to tell the instrument to generate positive or negative ions. Only use this column if you are using CI mode. In EI mode, this column should always be set to **Positive**.

q. You may set the number of adjacent analytes to show in your method by using the **Number of Adjacent Analytes to Show** selection box found in the **Options** dialog box accessed by the **ISQ Series** main menu. See Figure 48. View the number of analytes you set in the **Show Analysis** view.

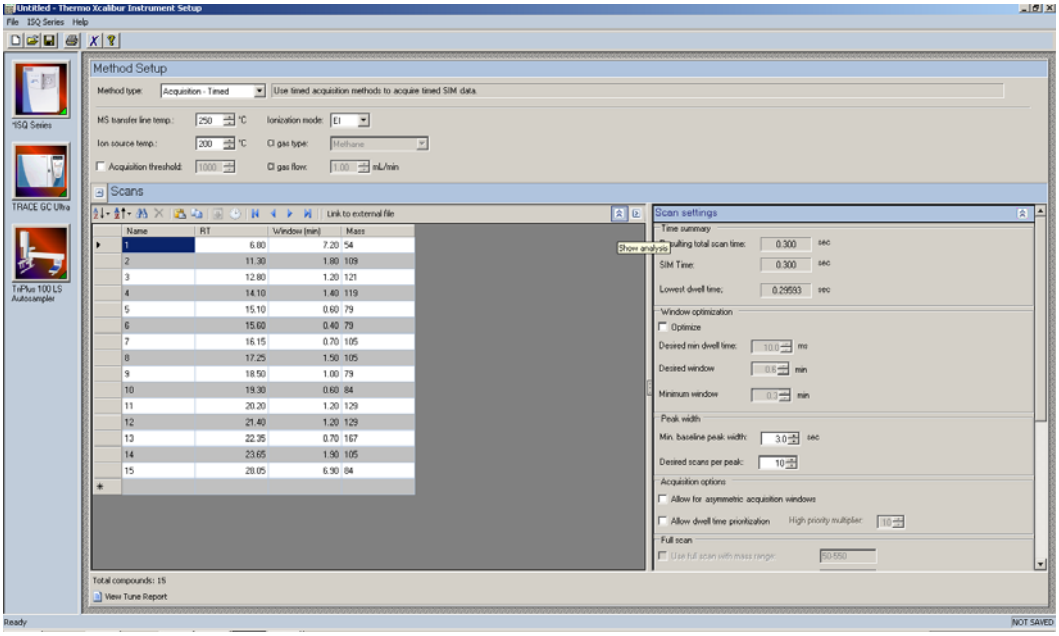
Adjustments in this column are for display purposes only and will not affect your acquisition.

Figure 48. Options Dialog Box



r. Click **Show Analysis** (see Figure 49) to validate your method. A chart appears with all your analytes by name and in order of their start times.

Figure 49. Accessing the Show Analysis Feature



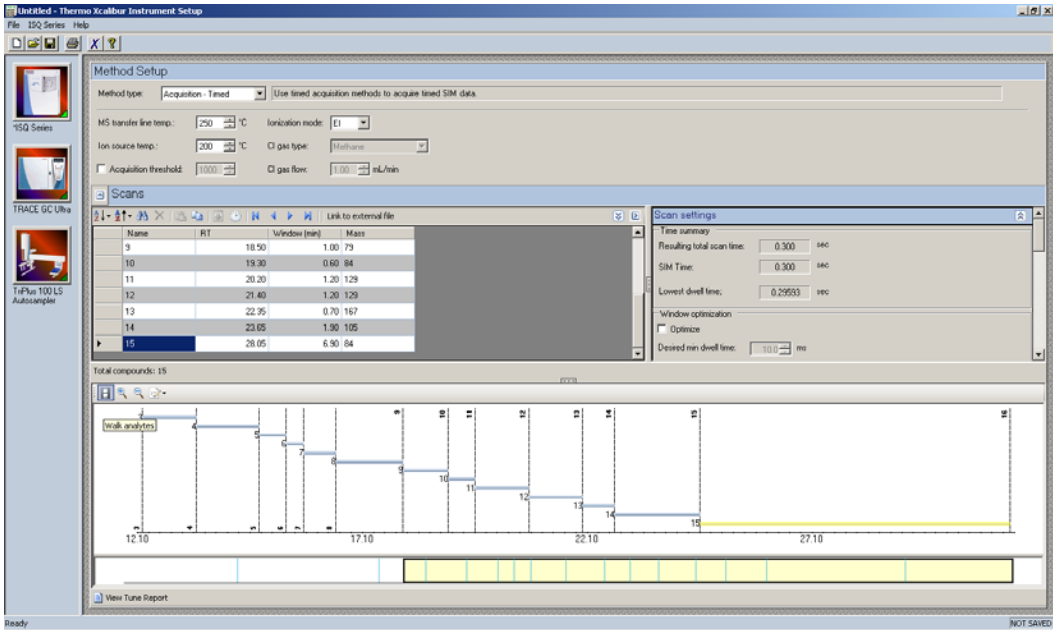
s. Use the scrolling window at the bottom of the screen to view all your analytes' expected retention times. Resize the window to view more analytes by dragging one side of the window out. As the scrolling window is decreased in size, fewer analytes are shown in the analytes chart. Increasing the size of the scrolling window allows you to view more analytes in your method. If the number of analytes retention times being viewed exceeds 50, an evenly spaced sample of the analytes shows through the window.

You may also click the ladder icon walk your analytes: have the software automatically run through your list of analytes. Click the ladder icon again to stop the process at any time.

Tip If your SIM windows are too congested to achieve the total scan time at the minimum dwell time, the segments are highlighted in red. This warning shows that there is not sufficient time in the segment to scan all events. In this case, the method fails to validate or save and a caution icon appears near the scans title. To correct this, reduce the number of overlapping compounds or change window times. When the peak bars are highlighted orange, this is a caution that there will be fewer scans across the peak than desired.

Also, if your list contains duplicate compounds, the middle of one of the duplicate peak bars will show an orange crossed pattern, instead of the usual white. Delete one of the duplicate compounds to avoid problems with data analysis.

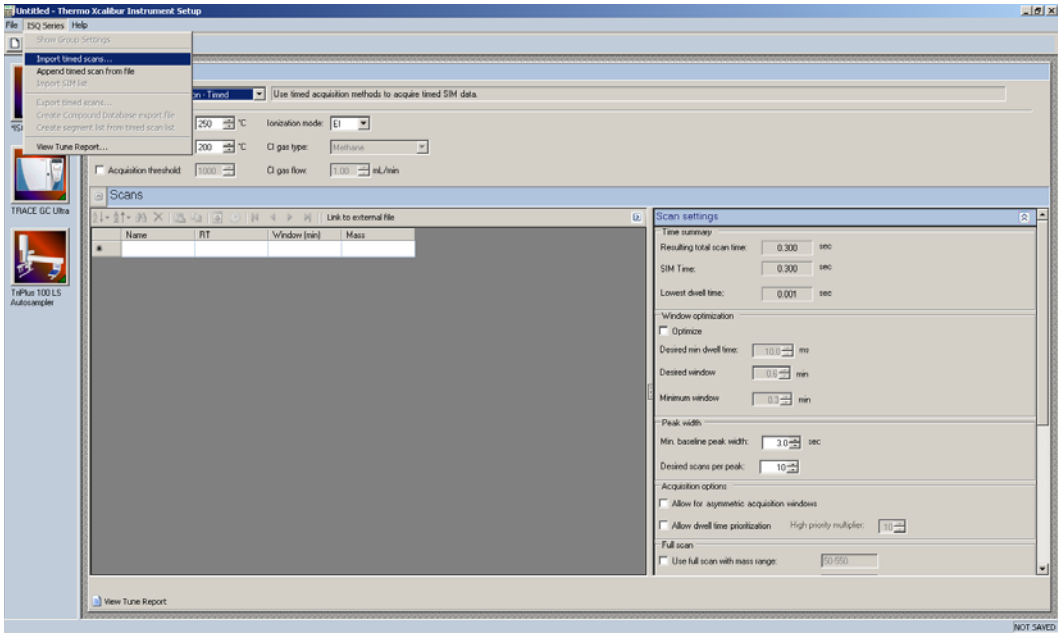
Figure 50. Validating a Method



11. To import an TSQ 8000™ or TSQ Quantum™ Series MS method, choose **ISQ Series | Import MS Method**. See Figure 51.

> **Note** This will only import the MS part of the method. You must set the GC and autosampler parameters.

Figure 51. Importing Methods



12. Choose **ISQ Series | Import Timed Scans** to import .csv or .xml files of previous methods. The software will only load files in valid formats. If your file is not valid you will receive an error message and will not be able to import the file into the Method Editor.

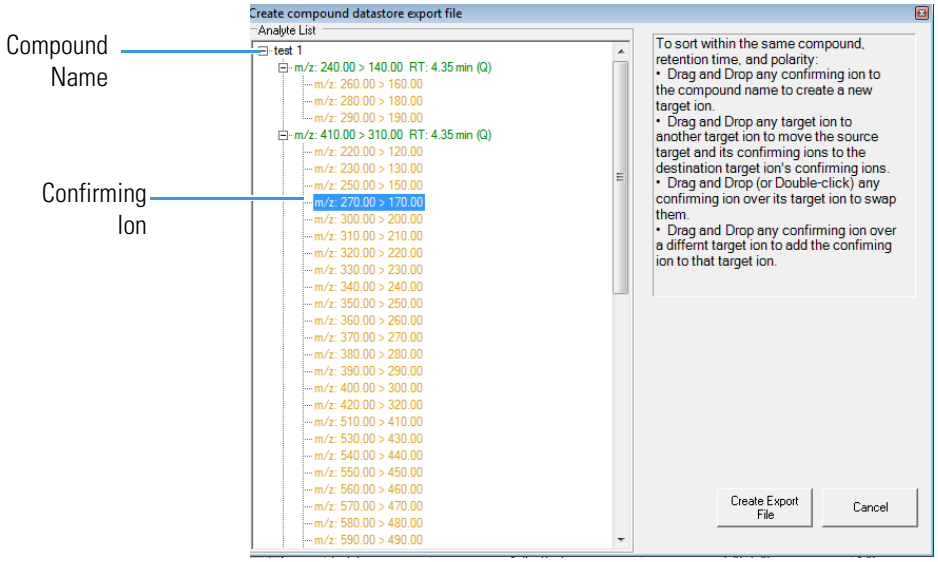
> **Tip** Export a timed scan list to see an example of a valid format.

13. Choose **ISQ Series | Append Timed Scan from File** to add scans from previous methods to the end of your open scan list. As above, you may import .csv or .xml files. The software will only load files in valid formats. If your file is not valid you will receive an error message and will not be able to import the file into the Method Editor.

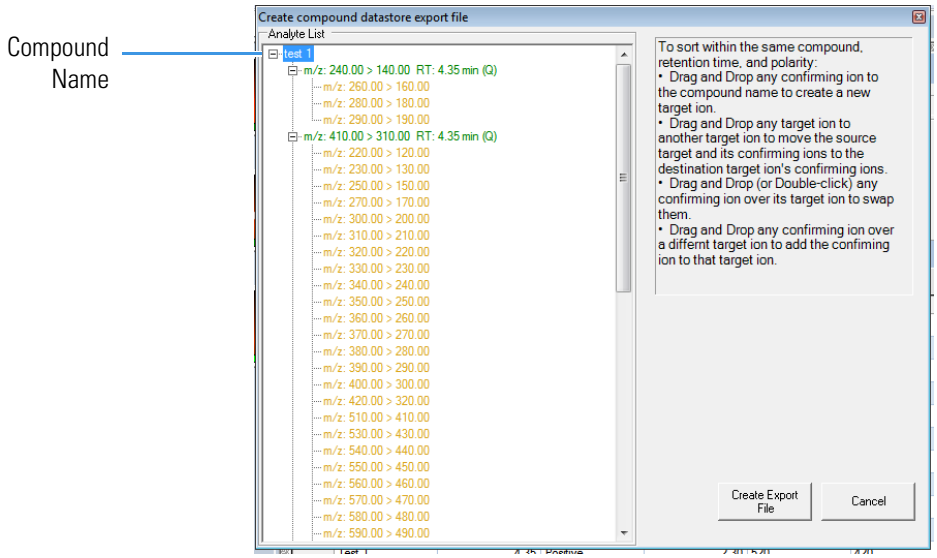
14. Choose **ISQ Series | Export Timed Scans** to export your method as a .csv file. If you prefer editing your methods in spreadsheet applications, you may want to use this option.

15. Choose **ISQ Series | Create Compound Data Store Export File** to prepare your file for the compound data store in the TraceFinder application.

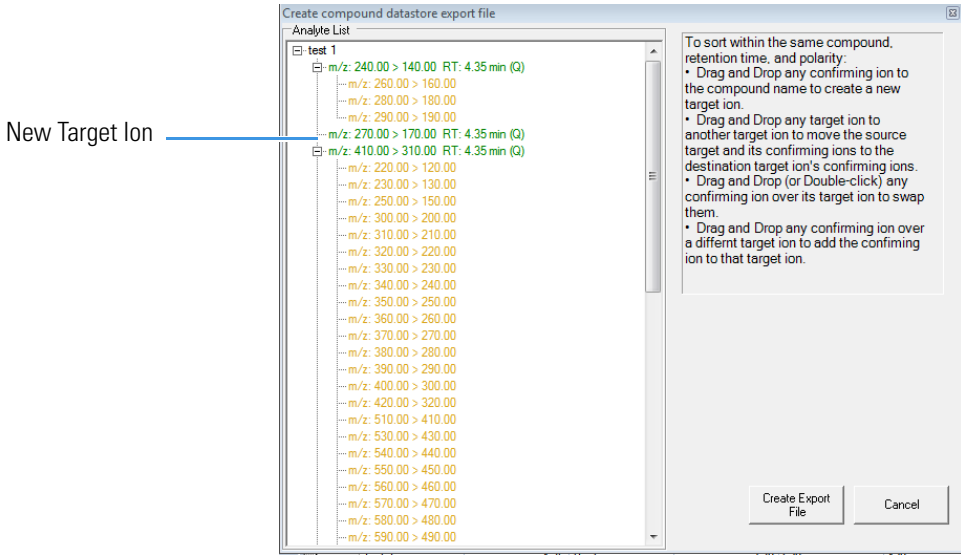
    a. To create a new target ion from the list of confirming ions with identical retention times for a compound, select the confirming ion of interest.



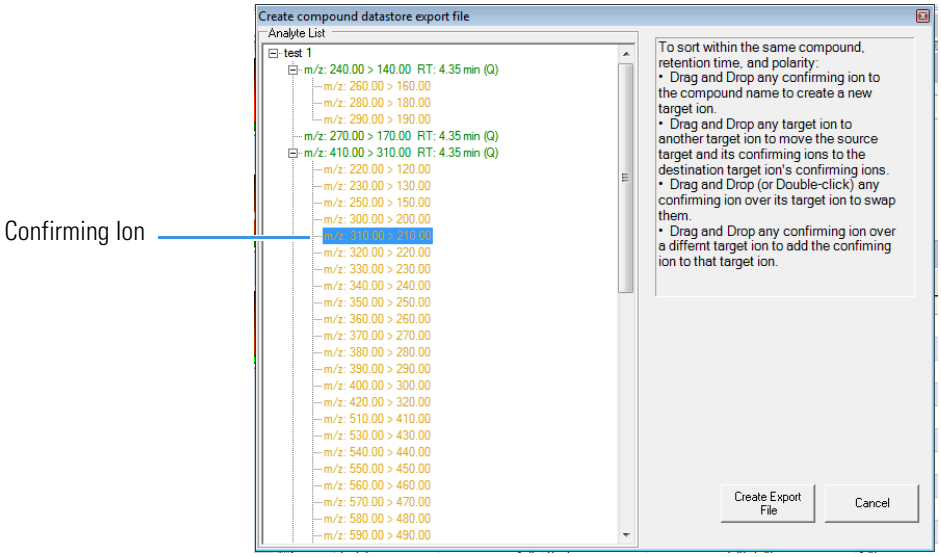
    b. Drag the confirming ion to the compound name at the top of the list.



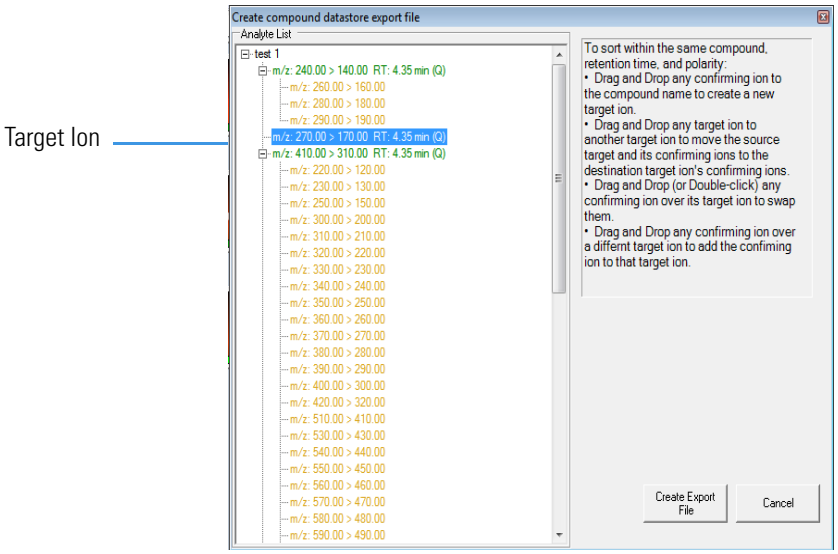
c. The confirming ion now appears in the list as a target ion.



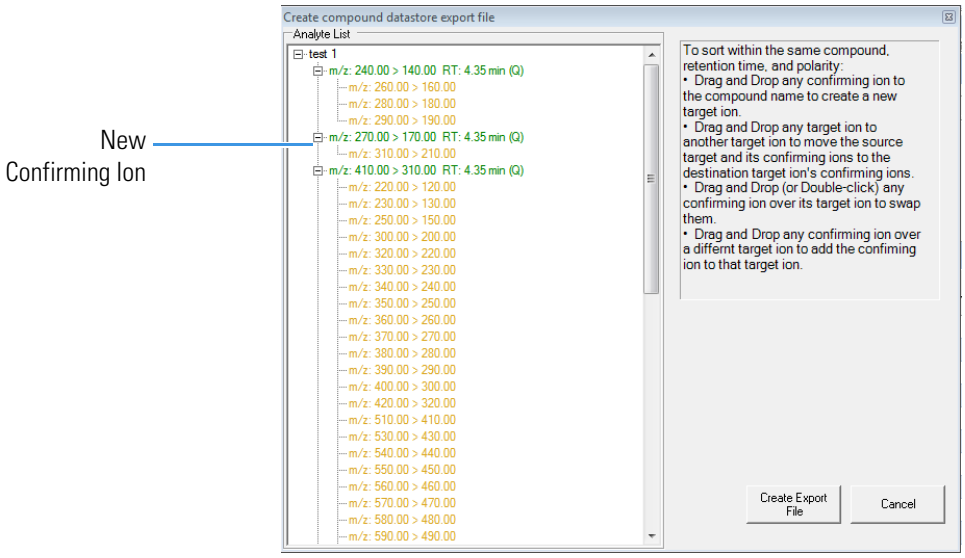
d. To add a new confirming ion to a target ion in the list, select the confirming ion of interest.



e. Drag the confirming ion under the target ion of interest.



f. The confirming ion appears in the list under the selected quantitation ion.

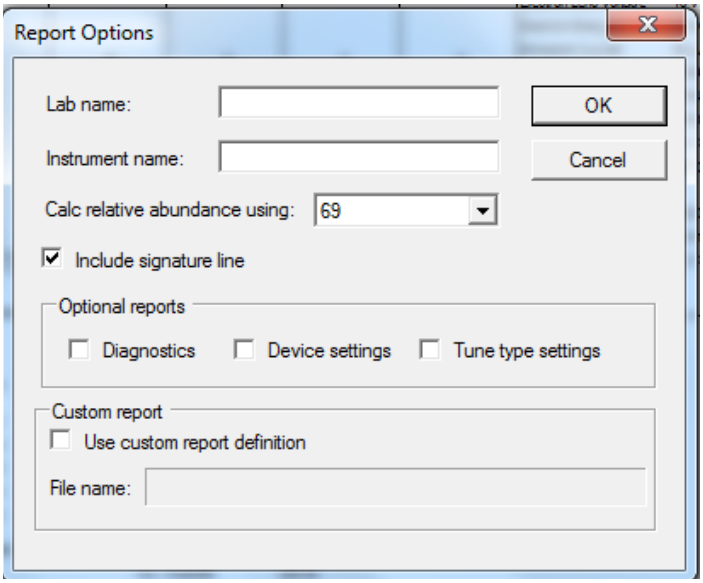


g. Click **Create Export File** when you are through creating your ion list to export the list to the TraceFinder software compound database.

16. Choose **ISQ Series | Create Segment List From Timed Scan List** to import a general acquisition method.

17. Choose **ISQ Series | View Tune Report** to view the latest tune report the method will use. Choose **Report Options** to open the Report Options dialog box (see Figure 52) and add identifying information to the tune report.

Figure 52. Configuring the Tune Report Options



Creating a Method for the GC

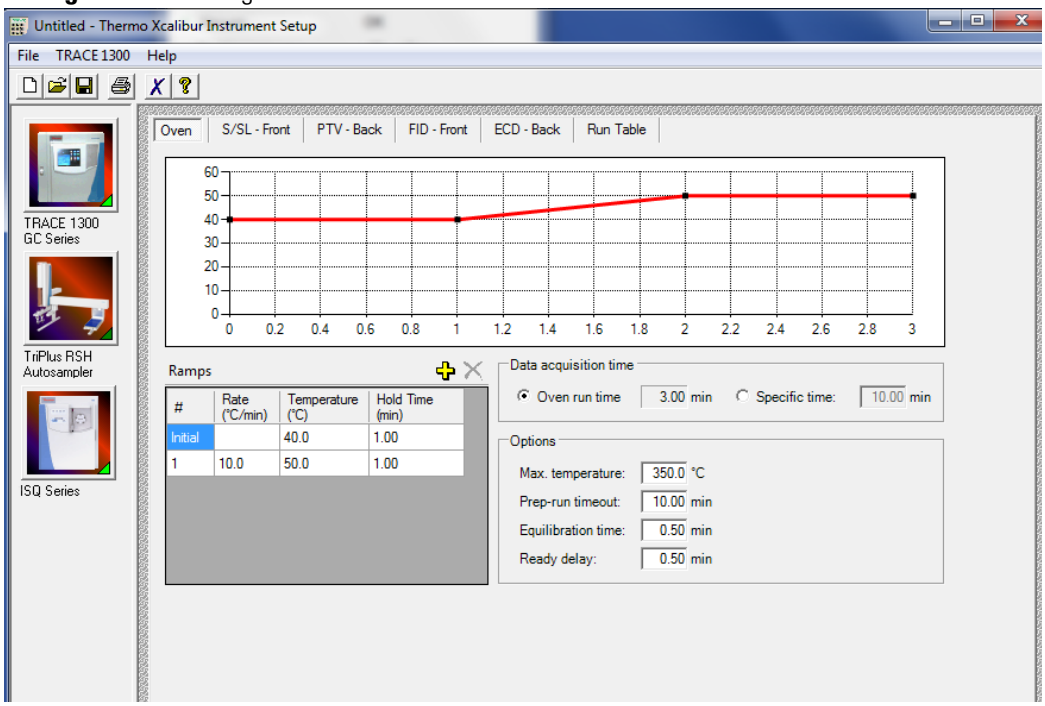
Click the GC icon in the left pane to create a GC method.

Creating a method for the TRACE 1300 or TRACE 1310 GC

❖ To create a method for the TRACE 1300 or TRACE 1310 GC

1. Click the **Oven** tab to set the oven temperatures. There is always at least one temperature and time in any GC temperature program. In the Initial row, enter the initial temperature, which must be 4 °C above room temperature and less than the maximum operating temperature of your GC column. If you set the initial temperature to a value below this limit, the GC will not reach the initial temperature. If you set the temperature above the limit, the GC column will get damaged. You can set the initial hold time to a value between 0 and 999.99 minutes. The typical initial temperature is at least 10 °C above the boiling point of your sample solvent and the initial time is usually long enough for the solvent to move through the column.

Figure 53. Setting the GC Oven Parameters on the TRACE 1300 or TRACE 1310 GC



   a. You can select a maximum of 32 temperature ramps, each with their own ramp rates, final temperatures and hold times. A typical program will have one or two ramps. The GC temperature profile is the primary method for separating your analytes from each other, the solvent, and the matrix. Your temperature profile will have to be optimized for your analysis needs.

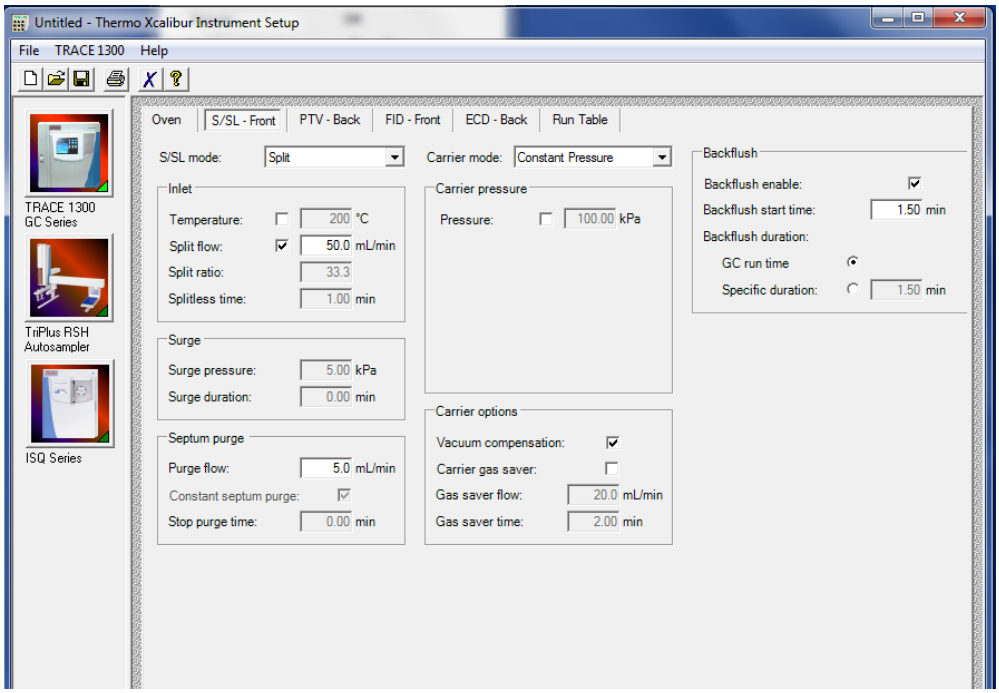
   b. You can also select the maximum allowed oven temperature, which should be set to the maximum temperature allowed by your method, *not* the maximum allowed by your column. The maximum temperature allowed by the GC is 450 °C. This will prevent you from accidentally using a temperature that will damage your column. The prep-run timeout is the maximum amount of time that the GC will wait before it gives up on an injection. As an example, with the default value of 10 minutes, if the GC is ready to receive an injection, but does not receive it after ten minutes, the GC

will stop waiting. This usually occurs in case of an error. The equilibration time is a delay between when the GC is at temperature and when the GC reports as being ready. This delay is typically set to 0.5 minutes.

CAUTION INSTRUMENT DAMAGE. Be sure not to overheat the GC column or it may contaminate the ISQ Series mass spectrometer.

2. The TRACE 1300 and 1310 GC also have the option to enable the use of cryogenics to cool the oven. If this option is selected, then the minimum allowed temperature in a temperature ramp will fall from 0 °C to -99 °C. The GC also allows the use of a post run column cleaning. This is not typically used because the material that is purged from the column in this step would go into the ISQ Series mass spectrometer, which can lead to contamination. If you want to use this feature, set the GC oven temperature, as well as the amount of time to remain at that temperature after the analytical run is complete. You can also set the amount of pressure used to push the carrier gas through the column.
3. If you have a split / splitless inlet (SSL) injector, click the **S/SL-Front** or **S/SL-Back** tab to configure the injector port settings. The inlet should be turned on and set to a temperature that is at least 10 °C higher than the boiling point of your least volatile analyte. The material should be injected into the port to vaporize and move into the GC column quickly. Higher temperatures can lead to thermal decomposition of some analytes, so you will have to optimize the injector temperature for your analysis. The SSL temperature can be set up to 400 °C (a typical value would be 225 °C).

Figure 54. Locating the Settings for the SSL Injector on the TRACE 1300 or TRACE 1310 GC



a. Useful for diluting high concentrations of sample, the split flow is the amount of gas that is swept through the injector to the exhaust port. Higher values will give more dilution. The split flow will reduce the amount of contamination that builds up in your system. The split flow ratio is the ratio of the split flow to the carrier gas flow. It is effectively the dilution ratio of the sample. This setting is typically turned on and set to a flow of 50 mL/min. However, more carrier gas will be used, so for your analysis, lower split flows may be more acceptable. If you set the split ratio, the software will calculate the correct split flow. The reverse is true also.

Tip We recommend turning on the septum purge, which means additional carrier gas will go through the injector. The default purge flow value is 5 mL/min. This reduces the buildup of contaminants in the injector, on the column, and in the ISQ Series instrument. If you perform a splitless injection, even if the split flow is set, the split flow will be turned off for the splitless time. The septum purge will be turned off for the stop purge time. After these times, the split flow and septum purge will be reactivated.

b. You can set the carrier mode to **Constant Flow**, **Constant Pressure**, **Programmed Flow**, or **Programmed Pressure**. The gas flow and the oven temperature work together to determine how well the analytes are separated and how long the analysis will take. If you use constant pressure, as the column is heated in the oven, the flow rate will fall because the hotter column is more resistant to carrier gas flow. If you use constant flow, the carrier gas pressure will increase as the column temperature increases to keep the flow constant. Constant flow is more common. Typical flow rates are 1-3 mL/min. The pressure depends on the column length and internal dimensions, so there is not a typical value. Because the outlet of the column is in the

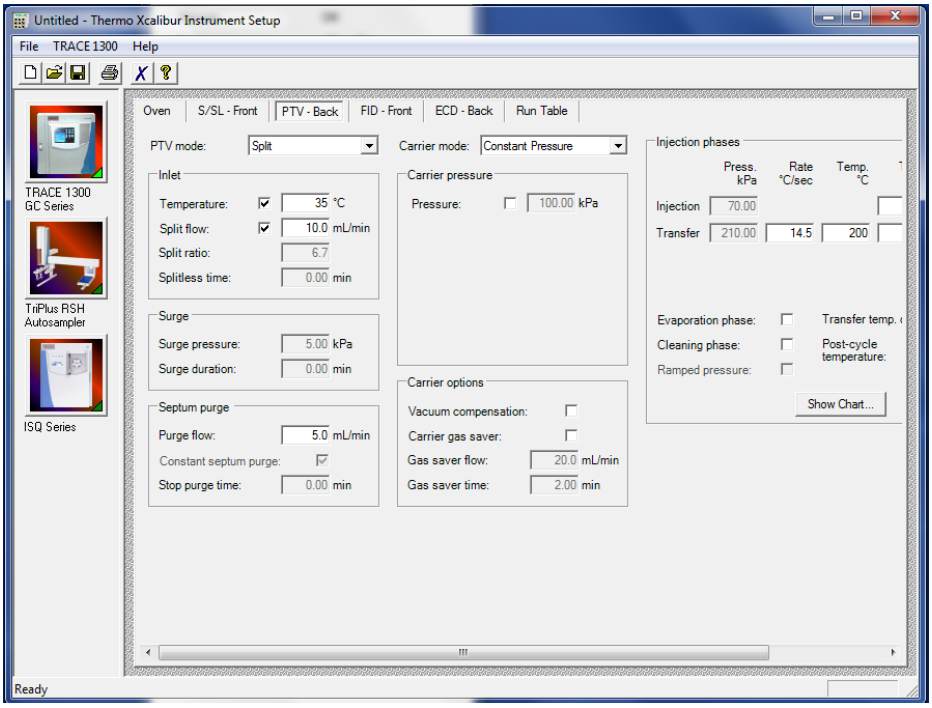
ISQ Series instrument, which is under vacuum, the vacuum compensation *must* be on to ensure accurate flow rates.

c. The flow can also be operated in programmed flow or programmed pressure modes. In these modes, you may have up to three flow rates or pressures to use during an analytical run. This is not commonly used, but may be necessary if you have a particularly challenging separation.

d. In an effort to reduce the amount of carrier gas used, check **Carrier Gas Saver**. When used, the split flow will be reduced to the gas saver flow after the gas saver time. It is not recommended to use a flow of less than 20 mL/min because contaminants can build up in the injector, column, and ISQ Series instrument, which can affect the system performance. It is also possible for air to diffuse back into the column with low split flows when the column head pressure is low.

e. Finally, if your analysis requires a higher flow to quickly sweep the analytes into the column, which may be needed with high temperature injectors and thermally labile compounds, you can use the surge pressure to increase the column flow for the surge duration time.

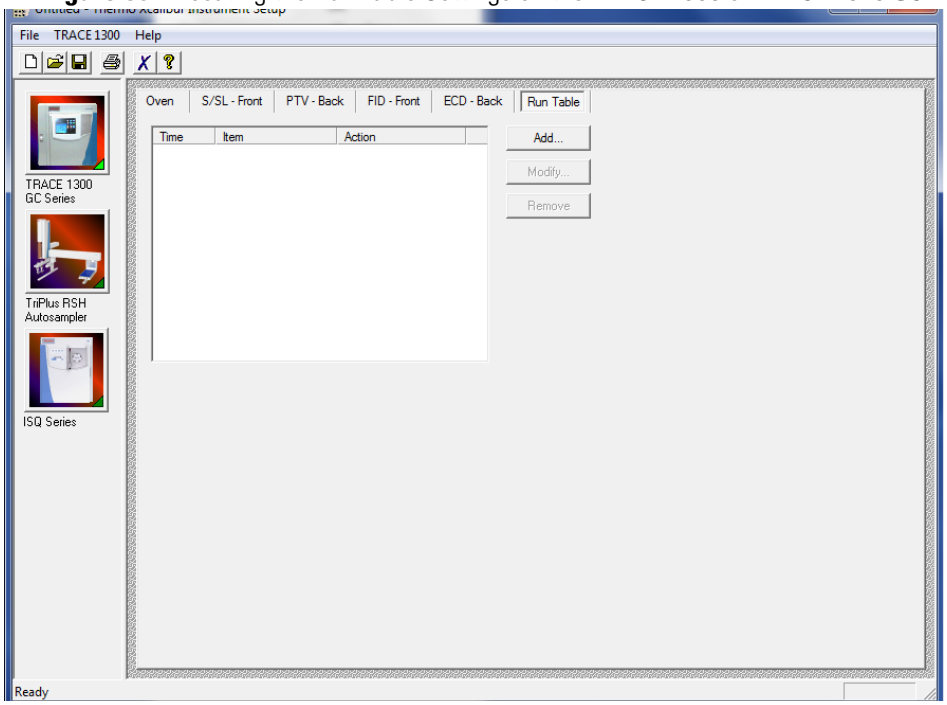
Figure 55. Locating the Carrier Gas Settings for the TRACE 1300 or TRACE 1310 GC



4. If you have a Programmable Temperature Vaporizer (PTV), click the **PTV-Front** or **PTV-Back** tab to configure it. The PTV is a low thermal mass injector that allows the instrument to rapidly heat or cool the inlet. You can use the PTV tab to program the temperature of the injector. See the GC documentation for details about the PTV or other types of injectors.

5. Click the **Run Table** tab to configure how to control external valves and devices. Consult the GC documentation for more detailed information.

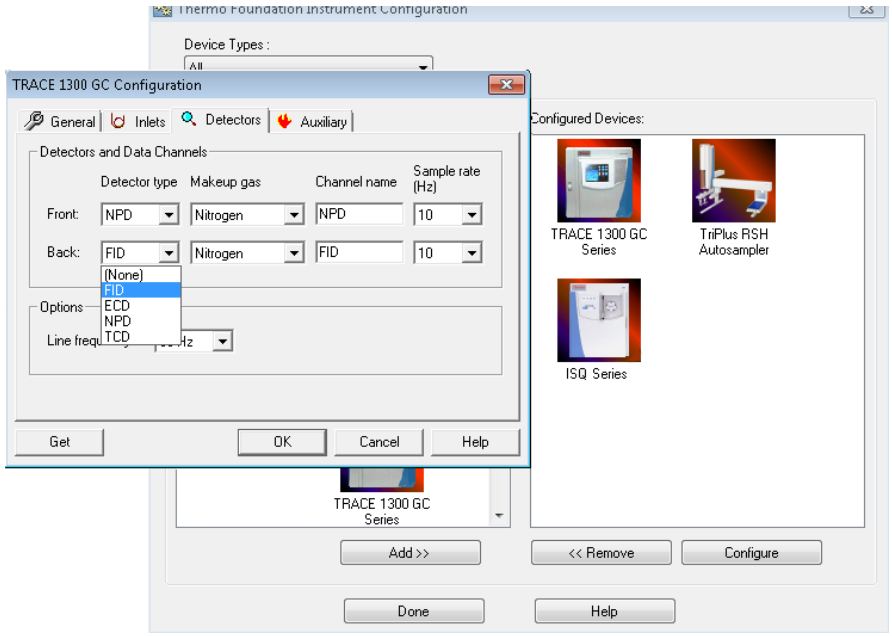
Figure 56. Locating the Run Table Settings on the TRACE 1300 or TRACE 1310 GC .



Note The user interface reflects the current configuration of your GC. If you add, remove, or change inlets or detectors, redo your instrument method according to the new GC configuration.

6. To add an inlet or detector to the method editor user interface:
   a. Attach the inlet or detector to the GC. See the GC documentation for instructions.
   b. Add the inlet or detector to the current instrument configuration. Go to **Start | All Programs | Thermo Foundation 3.0 | Instrument Configuration**.
   c. In the **Configured Devices** panel, click the TRACE 1300 or TRACE 1310 GC icon. Select **Configure**.

Figure 57. Configuring the Inlets or Detectors on the TRACE 1300 or TRACE 1310 GC

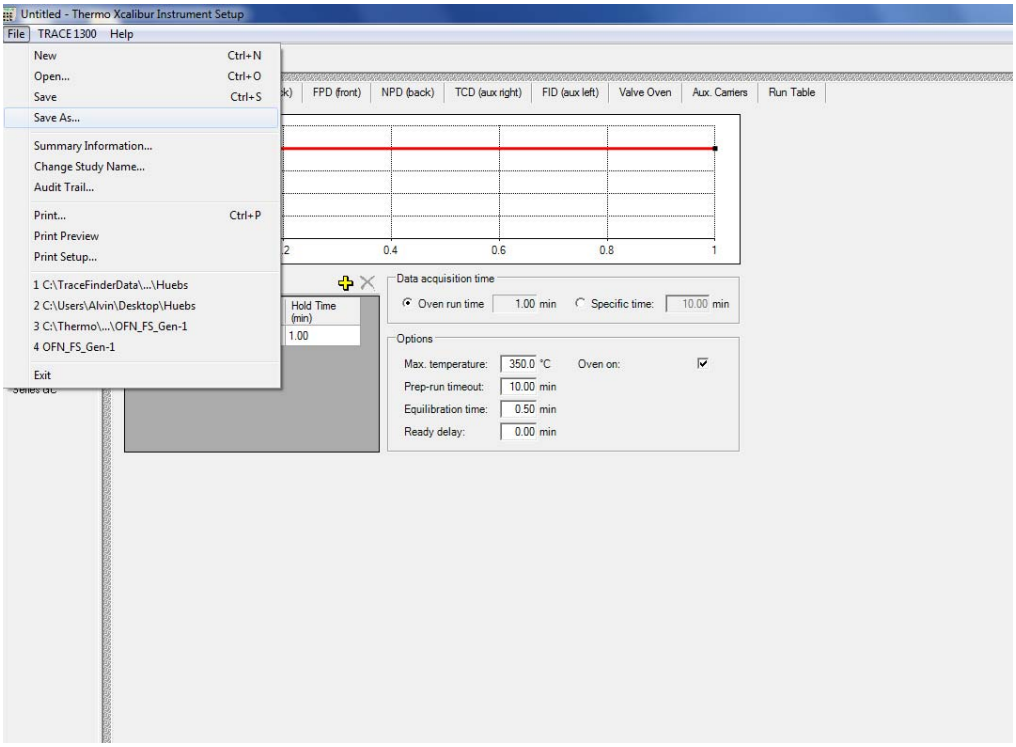


d. Select the **Inlet** or **Detector** tab as appropriate.

e. Click **Get**. The software automatically detects the attached modules. Select the gases as appropriate to your setup. The default Channel names are Channel 1 and Channel 2. You may want to change them to something more descriptive. Your hardware is now configured.

7. When you are finished creating methods for each component in your GC/MS system, select **File | Save As...** from the main menu or click the icon.

Figure 58. Saving a TRACE 1300 or TRACE 1310 GC Method



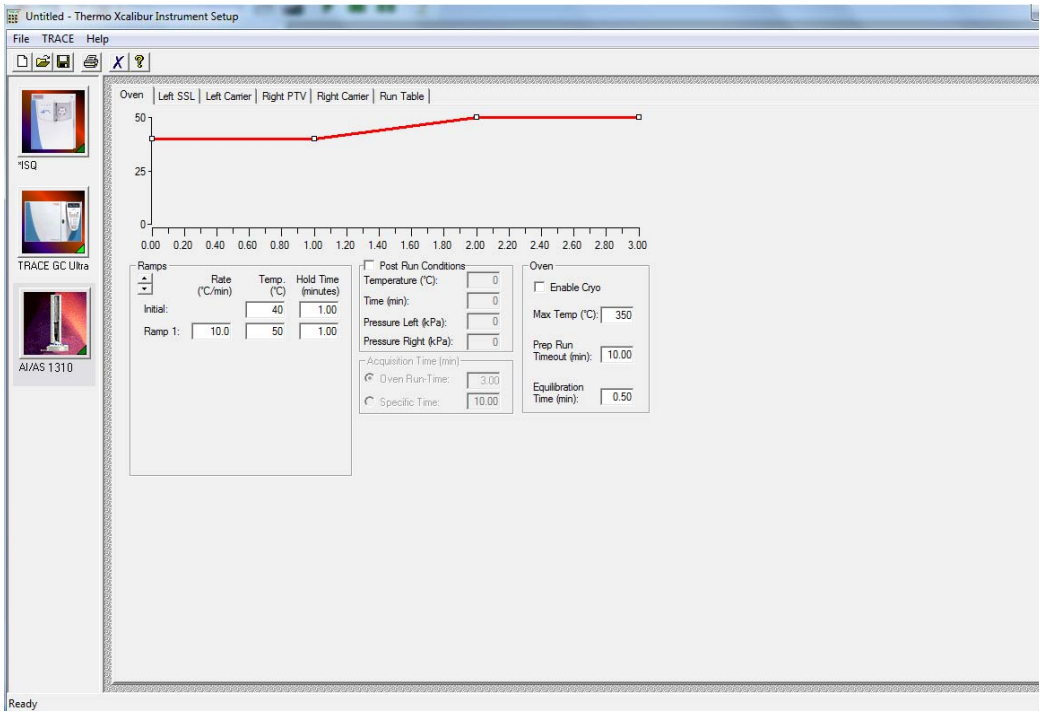
Creating a Method for the TRACE GC Ultra

❖ To create a method for the TRACE GC Ultra

Note This process only covers the left SSL, but the process is the same for the right SSL. For information on how to use the other injector types, consult the TRACE GC Ultra documentation.

1. Click the **Oven** tab to set the oven temperatures. There is always at least one temperature and time in any GC temperature program. In the Initial row, enter the initial temperature, which must be 4 °C above room temperature and less than the maximum operating temperature of your GC column. If you set the initial temperature to a value below this limit, the GC will not reach the initial temperature. If you set the temperature above the limit, the GC column will get damaged. You can set the initial hold time to a value between 0 and 999.99 minutes. The typical initial temperature is at least 10 °C above the boiling point of your sample solvent and the initial time is usually long enough for the solvent to move through the column.

Figure 59. Setting the TRACE GC Ultra Oven Parameters



a. You can select a maximum of seven temperature ramps, each with their own ramp rates, final temperatures and hold times. A typical program will have one or two ramps. However, the GC temperature profile is the primary method for separating your analytes from each other, the solvent, and the matrix. Your temperature profile will have to be optimized for your analysis needs.

b. You can also select the maximum allowed oven temperature, which should be set to the maximum temperature allowed by your column. This will prevent you from accidentally using a temperature that will damage your column. The prep-run timeout is the maximum amount of time that the GC will wait before it gives up on an injection. As an example, with the default value of 10 minutes, if the GC is ready to receive an injection, but does not receive it after ten minutes, the GC will stop waiting. This usually occurs in case of an error. The equilibration time is a delay between when the GC is at temperature and when the GC reports as being ready. This delay is typically set to 0.1 minutes.

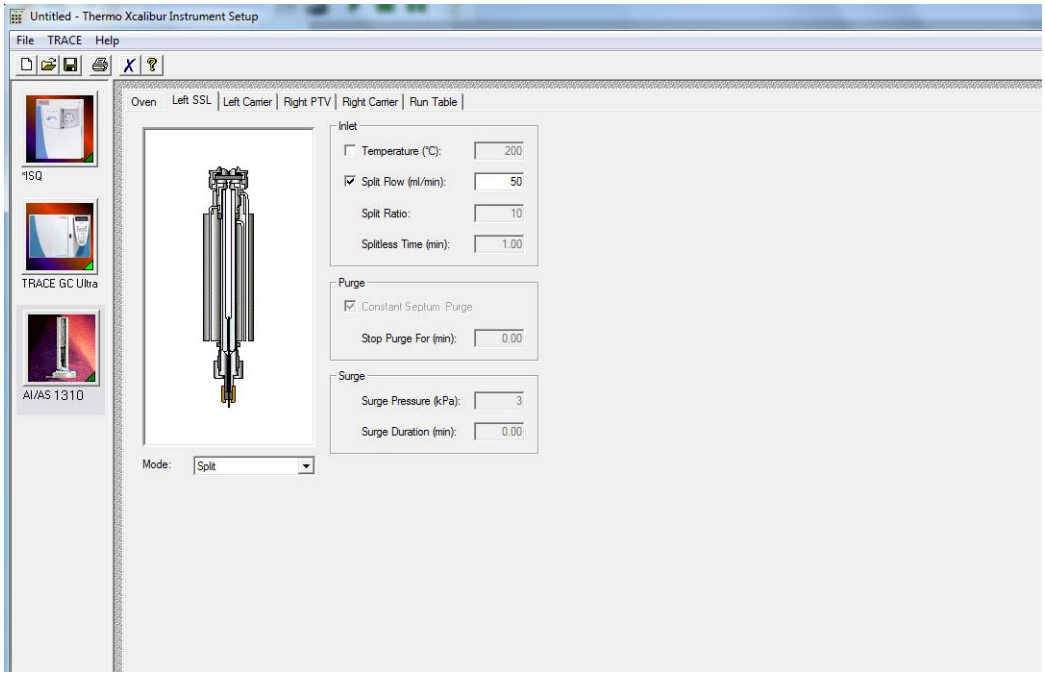
2. The TRACE GC Ultra also has the option to enable the use of cryogenics to cool the oven. If this option is selected, then the minimum allowed temperature in a temperature ramp will fall from 0 °C to -99 °C. The GC also allows the use of a post run column cleaning. This is not typically used because the material that is purged from the column in this step would go into the ISQ Series instrument, which can lead to contamination. If you want to use this feature, set the GC oven temperature, as well as the amount of time

to remain at that temperature after the analytical run is complete. You can also set the amount of pressure used to push the carrier gas through the column.

CAUTION INSTRUMENT DAMAGE. Be sure not to overheat the GC column or it may contaminate the ISQ Series instrument.

3. If you have a split / splitless inlet (SSL) injector, click the **Left SSL** or **Right SSL** tab to configure the injector port settings. The inlet should be turned on and set to a temperature that is at least 10 °C higher than the boiling point of your least volatile analyte. The material should be injected into the port to vaporize and move into the GC column quickly. Higher temperatures can lead to thermal decomposition of some analytes, so you will have to optimize the injector temperature for your analysis. The SSL temperature can be set between 50 and 400 °C (a typical value would be 225 °C).

Figure 60. Setting the TRACE GC Ultra SSL Injector Parameters

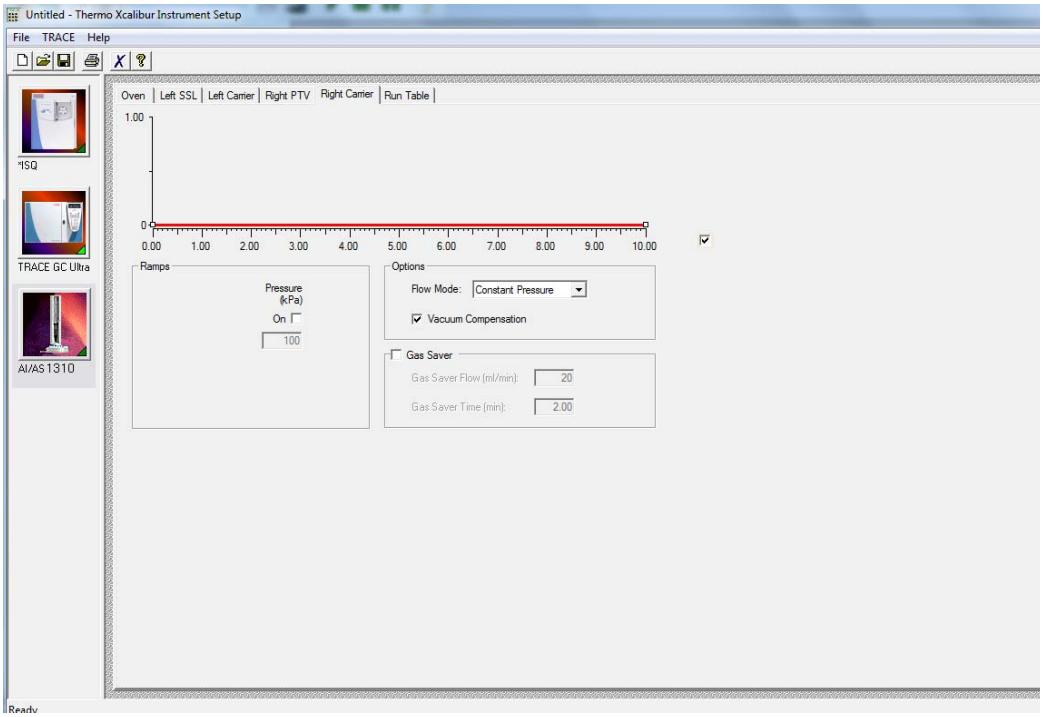


a. Useful for diluting high concentrations of sample, the split flow is the amount of gas that is swept through the injector to the exhaust port. Higher values will give more dilution. The split flow will reduce the amount of contamination that builds up in your system. The split flow ratio is the ratio of the split flow to the carrier gas flow. It is effectively the dilution ratio of the sample. This setting is typically turned on and set to a flow of 50 mL/min. However, more carrier gas will be used, so for your analysis, lower split flows may be more acceptable.

Tip We recommend turning on the septum purge, which means an additional 5 mL/min of carrier gas will go through the injector. This reduces the buildup of contaminants in the injector, on the column, and in the ISQ Series instrument. If you perform a splitless injection, even if the split flow is set, the split flow will be turned off for the splitless time. The septum purge will be turned off for the stop purge time. After these times, the split flow and septum purge will be reactivated.

b. Finally, if your analysis requires a higher flow to quickly sweep the analytes into the column, which may be needed with high temperature injectors and thermally labile compounds, you can use the surge pressure to increase the column flow for the surge duration time.

Figure 61. Setting the Carrier Gas Parameters on the TRACE GC Ultra



4. If you have a Programmable Temperature Vaporizer (PTV), click the **Left PTV** or **Right PTV** tab to configure it. The PTV is a low thermal mass injector that allows the instrument to rapidly heat or cool the inlet. You can use the PTV tab to program the temperature of the injector. See the GC documentation for details about the PTV or other types of injectors.

5. Click the **Left Carrier** or **Right Carrier** tab. The gas flow and the oven temperature work together to determine how well the analytes are separated and how long the analysis will take. You can select constant pressure or constant flow. If you use constant pressure, as the column is heated in the oven, the flow rate will fall because the hotter column is more resistant to carrier gas flow. If you use constant flow, the carrier gas pressure will increase as the column temperature increases to keep the flow constant. Constant flow is more common. Typical flow rates are 1-3 mL/min. The pressure depends on the column length

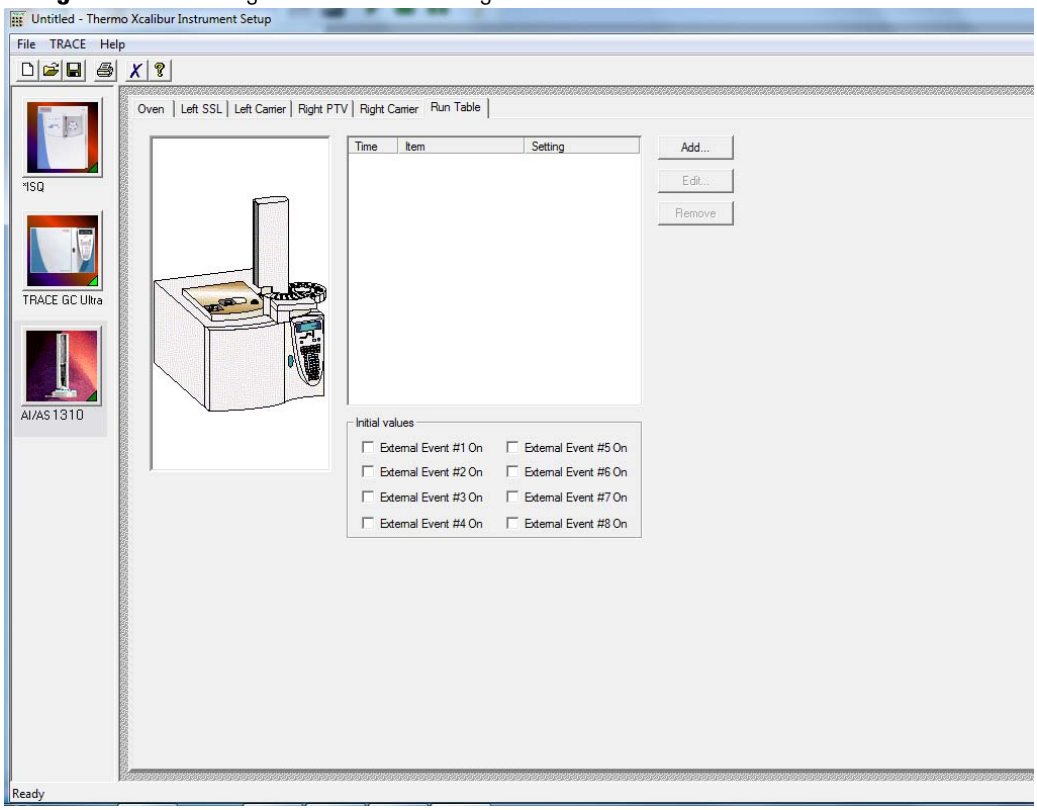
and internal dimensions, so there is not a typical value. Because the outlet of the column is in the ISQ Series instrument, which is under vacuum, the vacuum compensation must be on.

a. The flow can also be operated in programmed flow or programmed pressure modes. In these modes, you may have up to three flow rates or pressures to use during an analytical run. This is not commonly used, but may be necessary if you have a particularly challenging separation.

b. In an effort to reduce the amount of carrier gas used, you can activate the Gas Saver mode. When used, the split flow will be reduced to the gas saver flow after the gas saver time. It is not recommended to use a flow of less than 20 mL/min because contaminants can build up in the injector, column, and ISQ Series instrument, which can affect the system performance.

6. Click the **Run Table** tab to configure how to control external valves and devices. Consult the appropriate documentation for more detailed information.

Figure 62. Locating the Run Table Settings on the TRACE GC Ultra



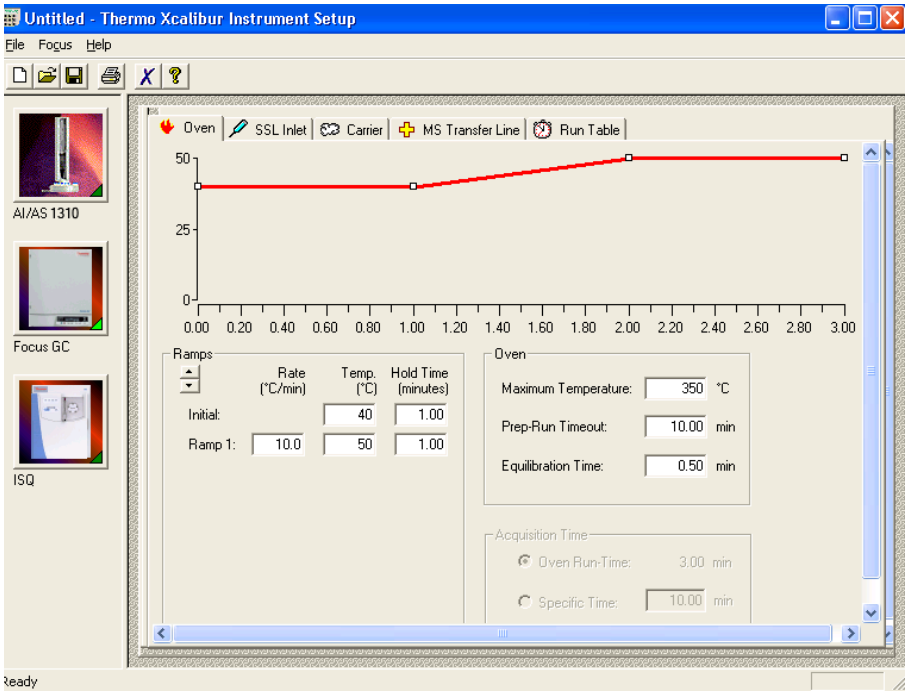
Creating a Method for the FOCUS GC

❖ To create a method for the FOCUS GC

1. Click the **Oven** tab to set the oven temperatures. There is always at least one temperature and time in any GC temperature program. In the Initial row, enter the initial

temperature, which must be 4 °C above room temperature and less than the maximum operating temperature of your GC column. If you set the initial temperature to a value below this limit, the GC will not reach the initial temperature. If you set the temperature above the limit, the GC column will get damaged. You can set the initial hold time to a value between 0 and 600 minutes. The typical initial temperature is at least 10 °C above the boiling point of your sample solvent and the initial time is usually long enough for the solvent to move through the column.

Figure 63. Setting the FOCUS GC Oven Parameters



a. You can select a maximum of seven temperature ramps, each with their own ramp rates, final temperatures and hold times. A typical program will have one or two ramps. However, the GC temperature profile is the primary method for separating your analytes from each other, the solvent, and the matrix. Your temperature profile will have to be optimized for your analysis needs.

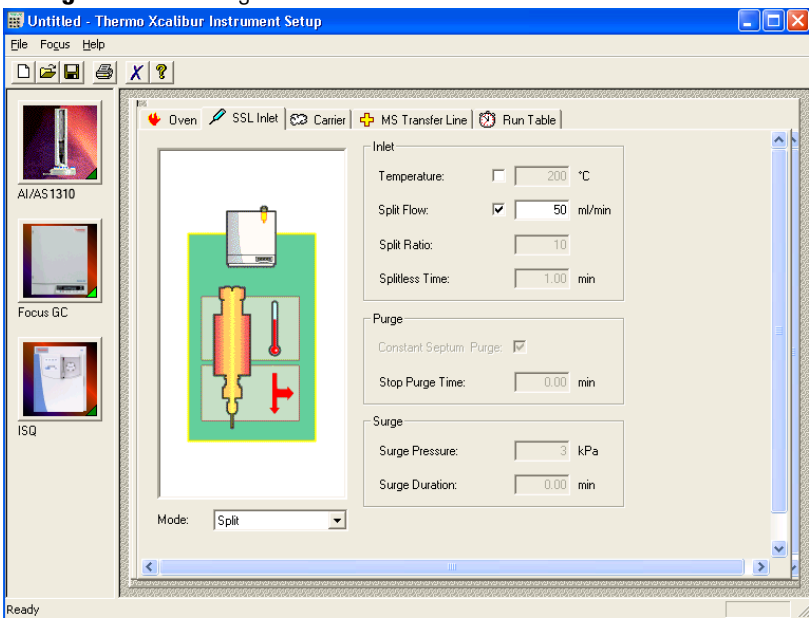
b. You can also select the maximum allowed oven temperature, which should be set to the maximum temperature allowed by your column. This will prevent you from accidentally using a temperature that will damage your column. The prep-run timeout is the maximum amount of time that the GC will wait before it gives up on an injection. As an example, with a default value of 10 minutes, if the GC is ready to receive an injection, but does not receive it after ten minutes, the GC will stop waiting. This usually occurs in case of an error. The equilibration time is a delay

between when the GC is at temperature and when the GC reports as being ready. This delay is typically set to 0.1 minutes.

CAUTION INSTRUMENT DAMAGE. Be sure not to overheat the GC column or it may contaminate the ISQ Series instrument.

2. If you have a split / splitless inlet (SSL) injector, click the **SSL** tab to configure the injector port settings.

Figure 64. Setting the SSL Parameters on the FOCUS GC



a. The inlet should be turned on and set to a temperature that is at least 10 °C higher than the boiling point of your least volatile analyte. The material injected into the port will vaporize and move into the GC column quickly. Higher temperatures can lead to thermal decomposition of some analytes, so you will have to optimize the injector temperature for your analysis. Set the SSL temperature between 50 and 375 °C (a typical value would be 225 °C).

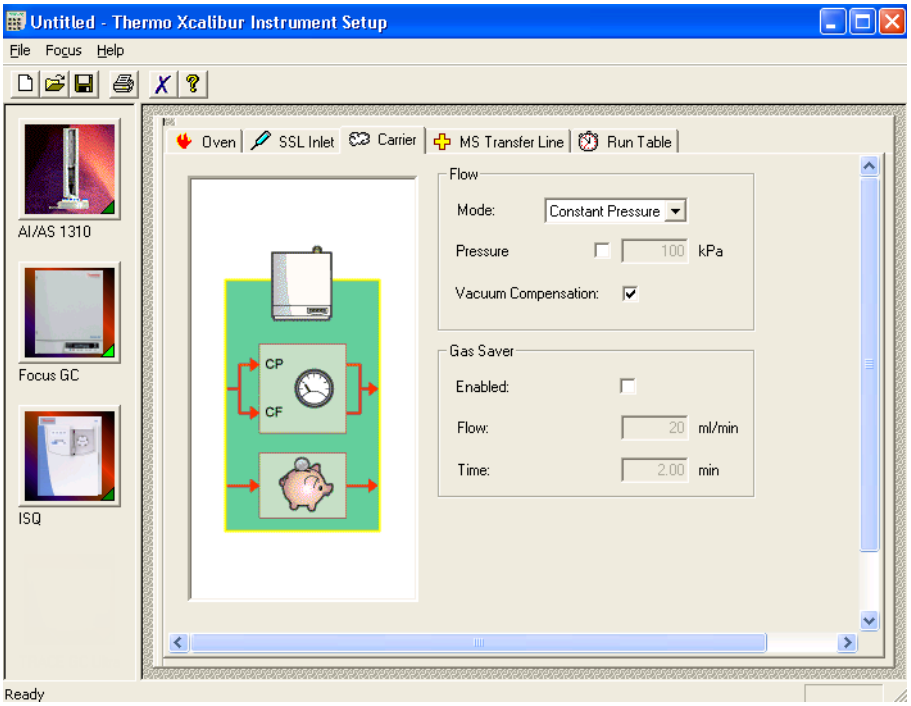
b. Useful for diluting high concentrations of sample, the split flow is the amount of gas that is swept through the injector to the exhaust port. Higher values will give more dilution. The split flow will also reduce the amount of contamination that builds up in your system. The split flow ratio is the ratio of the split flow to the carrier gas flow. It is effectively the dilution ratio of the sample. This is typically turned on and set to a flow of 50 mL/min. However, this increases the carrier gas usage, so for your analysis, lower split flows may be more acceptable.

Tip We recommend turning on the septum purge, which means an additional 5 mL/min of carrier gas will go through the injector. This reduces the buildup of contaminants in the injector, on the column, and in the ISQ Series instrument. If you perform a splitless injection, even if the split flow is set, the split flow will be turned off for the splitless time. The septum purge will be turned off for the stop purge time. After these times, the split flow and septum purge will be reactivated.

c. Finally, if your analysis requires a higher flow to quickly sweep the analytes into the column, which may be needed with high temperature injectors and thermally labile compounds, you can use the surge pressure to increase the column flow for the surge duration time.

3. Click the **Carrier** tab, which is used to set the carrier gas flows. The gas flow and the oven temperature work together to determine how well the analytes are separated and how long the analysis will take. You can select either constant pressure or constant flow. If you use constant pressure, as the column is heated in the oven, the flow rate will fall because a hotter column is more resistant to carrier gas flow. If you use constant flow, the carrier gas pressure will increase as the column temperature increases to keep the flow constant. Constant flow is more common. Typical flow rates are 1-3 mL/min. The pressure depends on the column length and internal dimensions, so there is not a typical value. Because the outlet of the column is in the ISQ Series instrument, which is under vacuum, the vacuum compensation must be on.

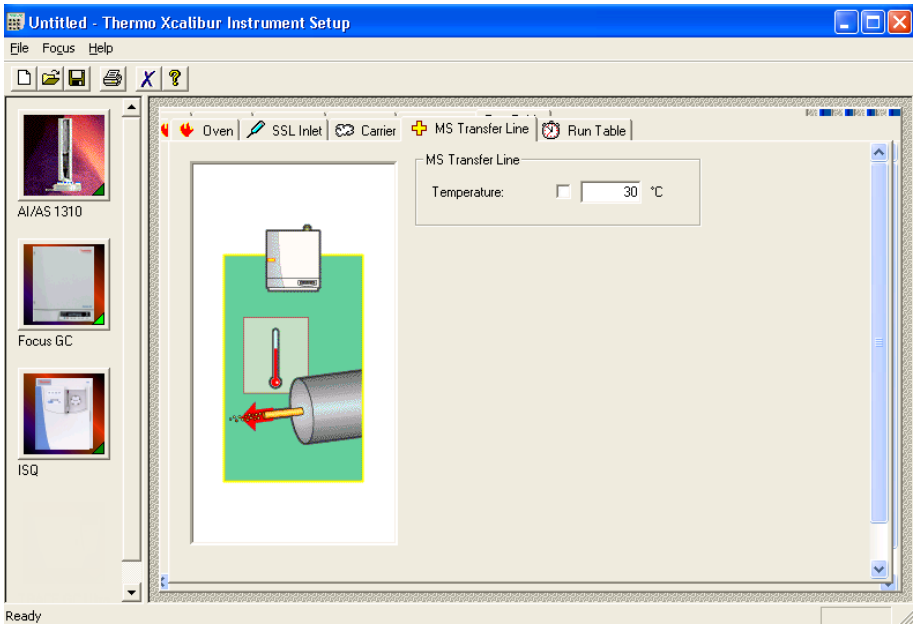
Figure 65. Setting the Carrier Gas Parameters for the FOCUS GC



In an effort to reduce the amount of carrier gas used, you can activate the Gas Saver mode. When used, the split flow will be reduced to the gas saver flow after the gas saver time. It is not recommended to use a flow of less than 20 mL/min because contaminants can build up in the injector, column, and ISQ Series instrument, which can affect system performance.

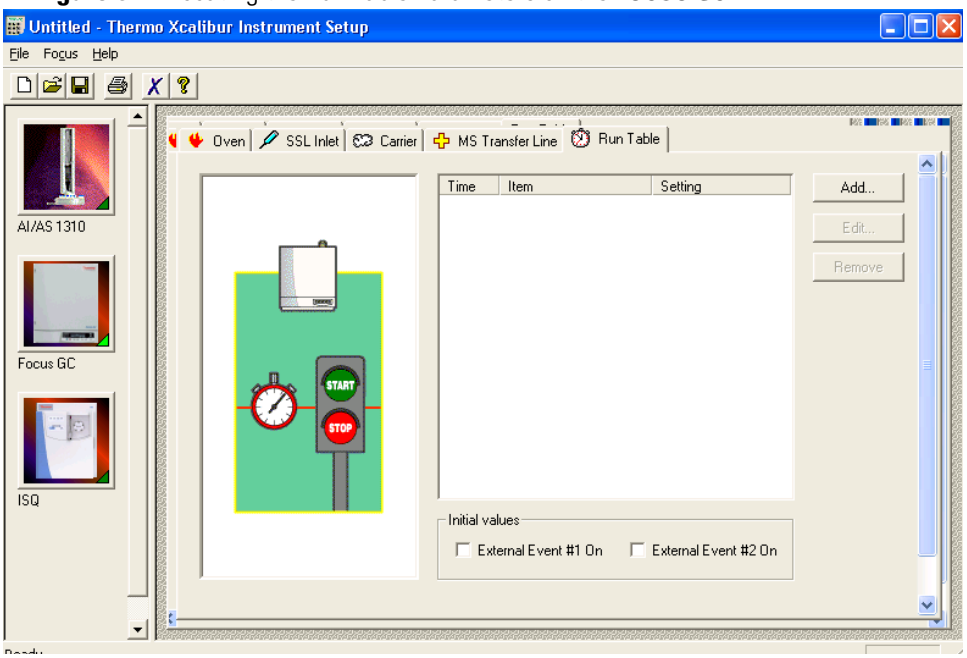
4. Click the **MS Transfer Line** tab to set the temperature of the transfer line for a different instrument. This tab is not used on the ISQ Series mass spectrometer.

Figure 66. Setting the Transfer Line Parameters on the FOCUS GC



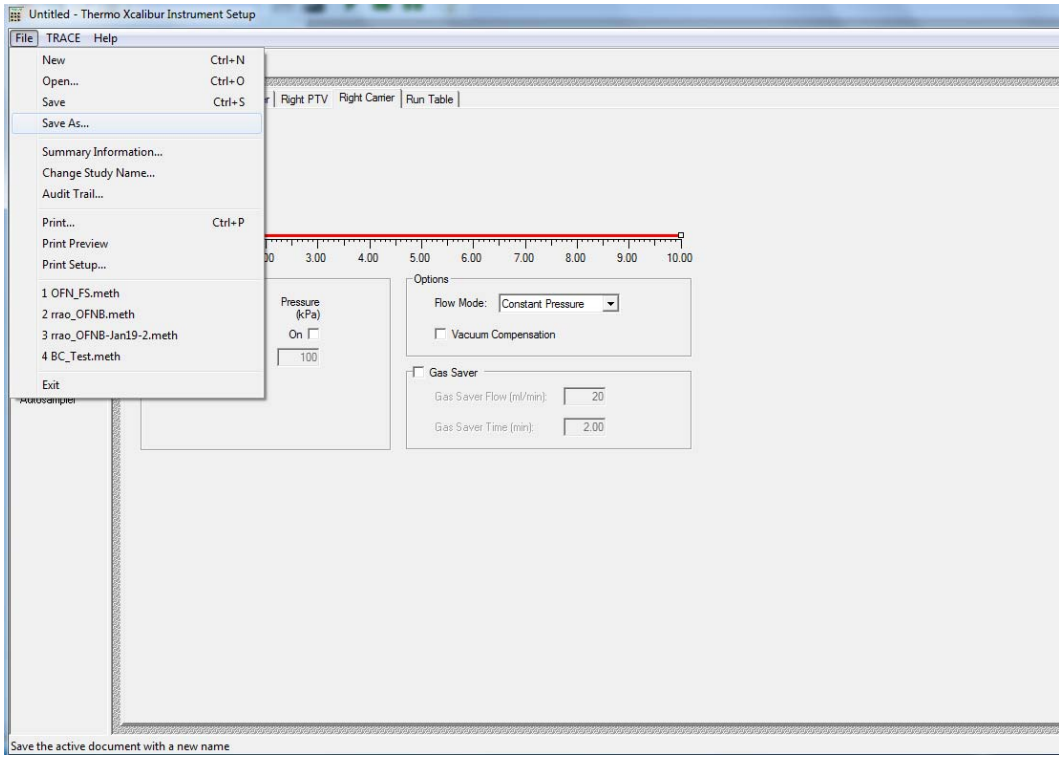
5. Click the **Run Table** tab to configure how to control external valves and other external devices. Consult the appropriate documentation for more detailed information.

Figure 67. Locating the Run Table Parameters on the FOCUS GC



6. When you are finished creating methods for each component in your GC/MS system, select **File | Save As...** from the main menu or click the icon.

Figure 68. Saving Your ISQ Series Instrument Method



5

Using AutoSIM

This chapter will help you use the AutoSIM software utility to set up and run a SIM Ion Study. As well as instructions for setting up and running each study, this chapter gives you the steps for importing the resulting list of SIM ions into the ISQ Series method editor and accessing them for routine use.

Note Set up your GC and autosampler methods through the ISQ Series method editor before developing your AutoSIM method.

Contents

Determining SIM Ions

The purpose of an AutoSIM study is to select your SIM ions. After you name your compounds and enter your vial numbers and retention times, AutoSIM instructs your ISQ Series system to run a full-scan analysis on the compounds.

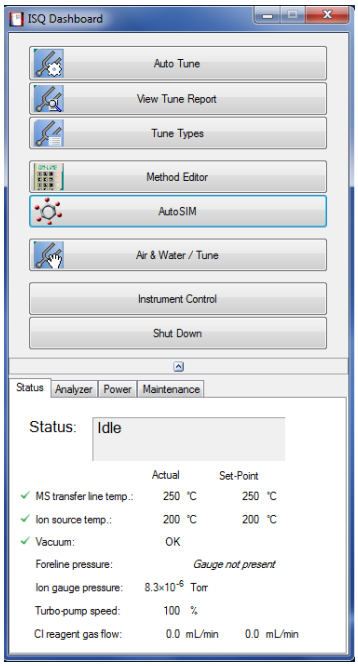
After the full-scan analysis is complete, AutoSIM presents you with the resulting chromatographic peaks and full-scan spectra, and then provides optional setting for sorting the results for your SIM ions.

Note You must have mid-range concentration standards (500 pg/µL–10 ng/µL) before setting up your AutoSIM method.

❖ To determine your SIM ions in AutoSIM

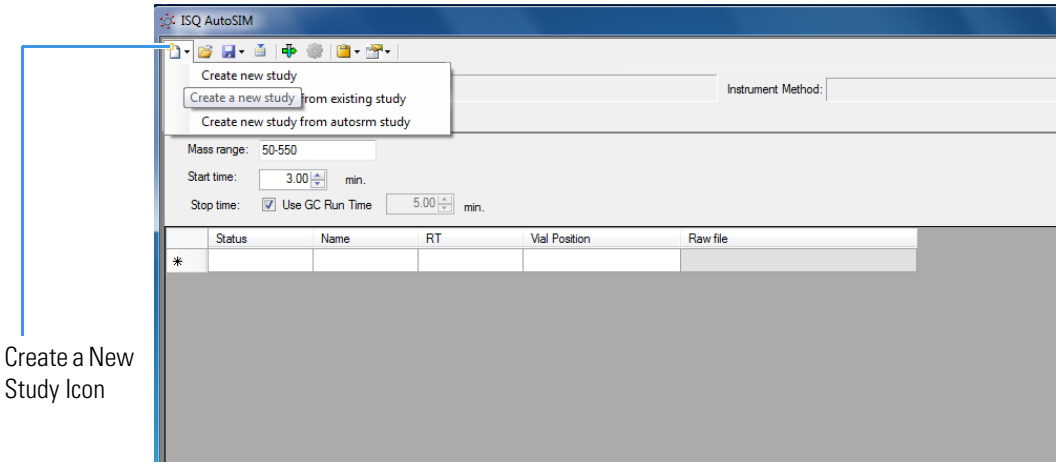
1. Click the AutoSIM button on the ISQ Dashboard to open the AutoSIM utility. See Figure 69.

Figure 69. Accessing AutoSIM on the ISQ Dashboard



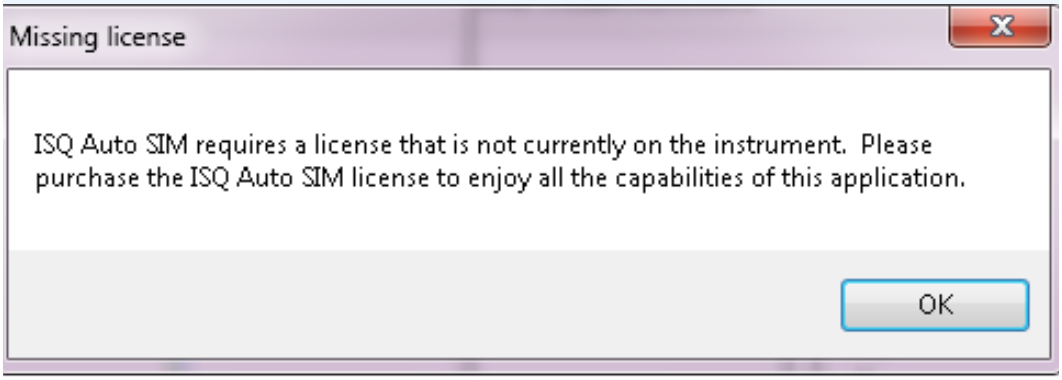
2. Click the Create a New Study icon on the left to create a new study.

Figure 70. New AutoSIM Study



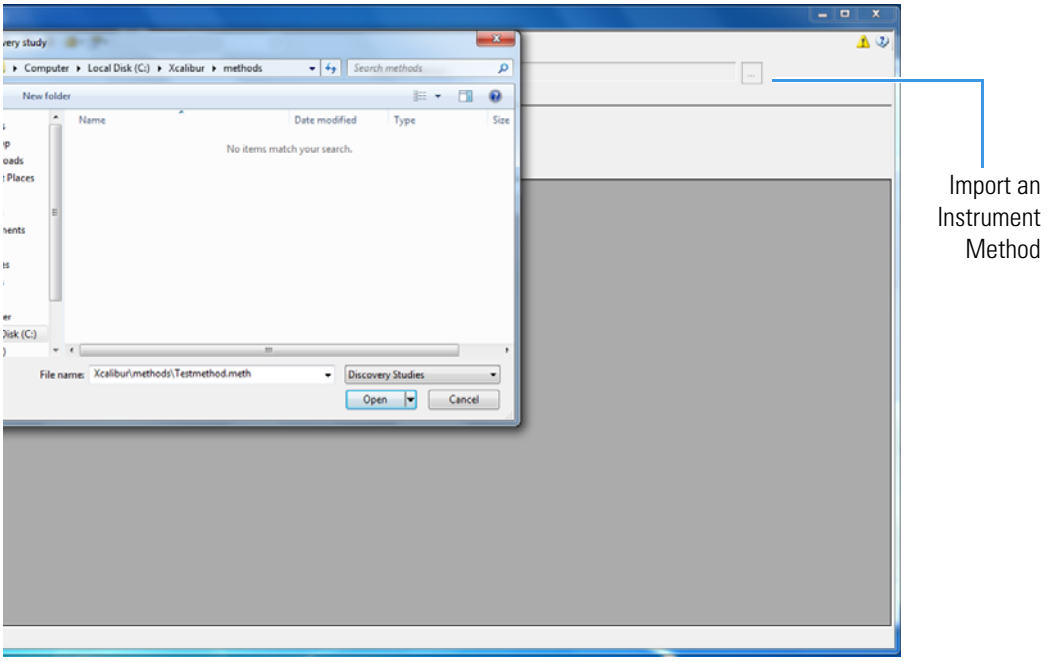
3. A new study window opens.

Note If at any time you see the error message below, go to "Upgrading the Software" on page 184 and follow the instructions for getting your software license.



4. Link to your saved instrument method file (that you created using the method editor) by clicking on the ellipsis icon next to the Instrument Method window. AutoSIM will use the GC and autosampler parameters from this method file. See Figure 71.

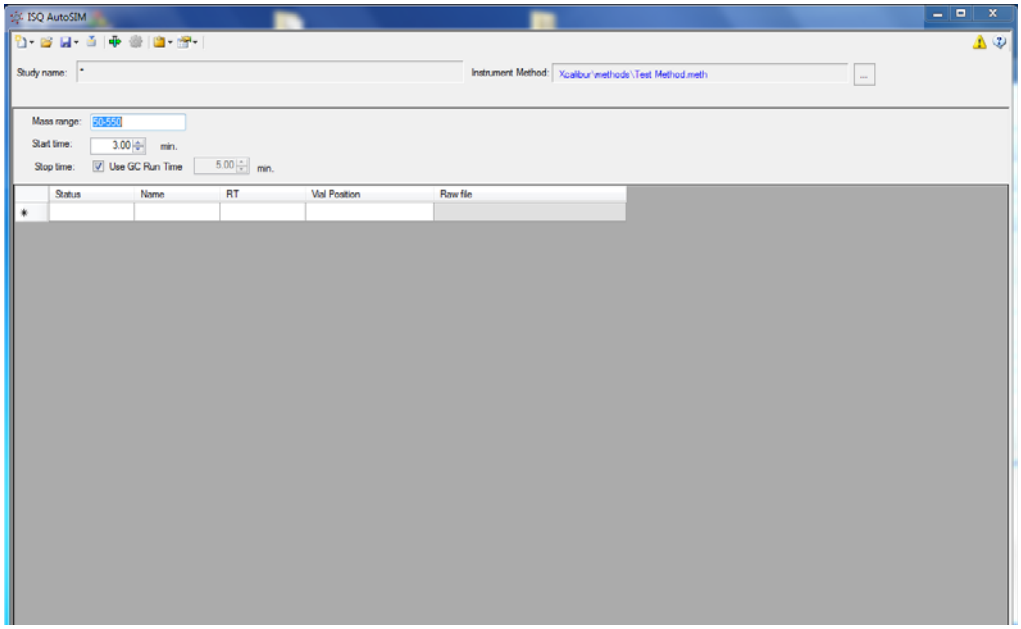
Figure 71. Retrieving an Instrument Method



5. Select an instrument method file and click Open.
6. You may set the Mass Range, Start Time, and Stop Time. See Figure 72.

   Any changes you make to your MS method here will override the method editor settings.

Figure 72. Adjusting the Settings

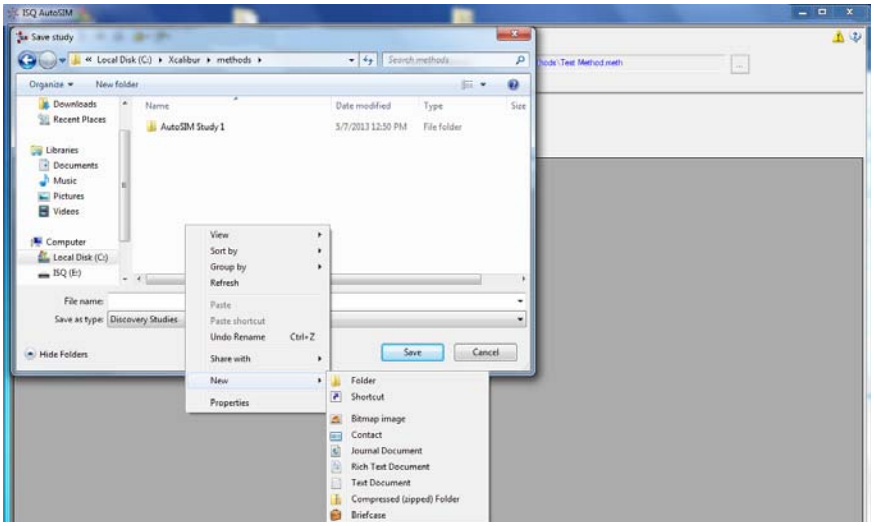


7. Enter the compound name, approximate retention time, and vial number for each compound you wish to optimize. If you already have a method for processing full scan data you can choose to import compounds from an external file. Their names and retention times will fill the compound list and their primary quantitation ion will be displayed in the mass filter box once the full scan data is acquired.

8. Save the study.

Tip Create a folder for all files associated with your AutoSIM study. Otherwise, the study results files will be saved in the general instrument method folder and crowd it. See Figure 73.

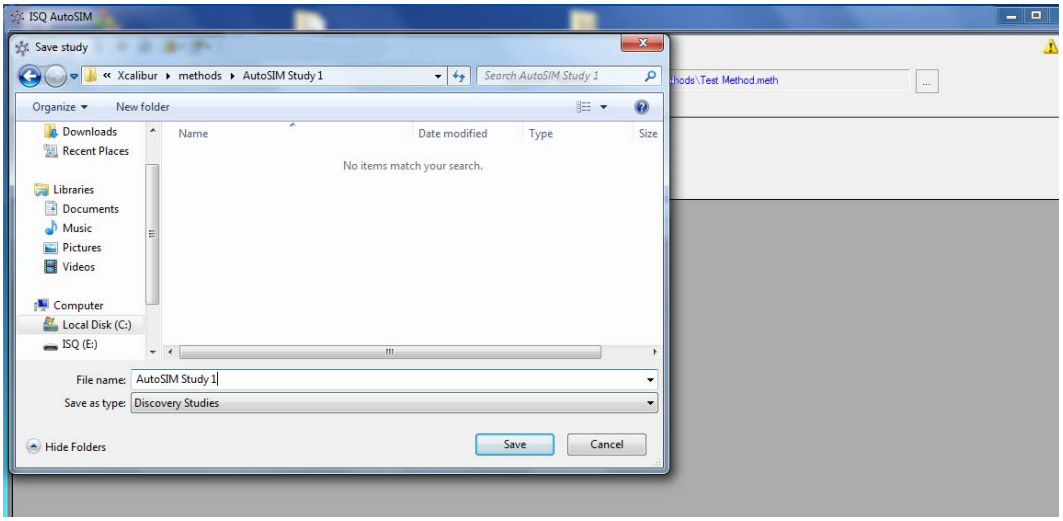
Figure 73. Creating an AutoSIM Study Folder



9. Open the folder.
10. Give your study a file name.
11. Save your study in the Study folder. See Figure 74.

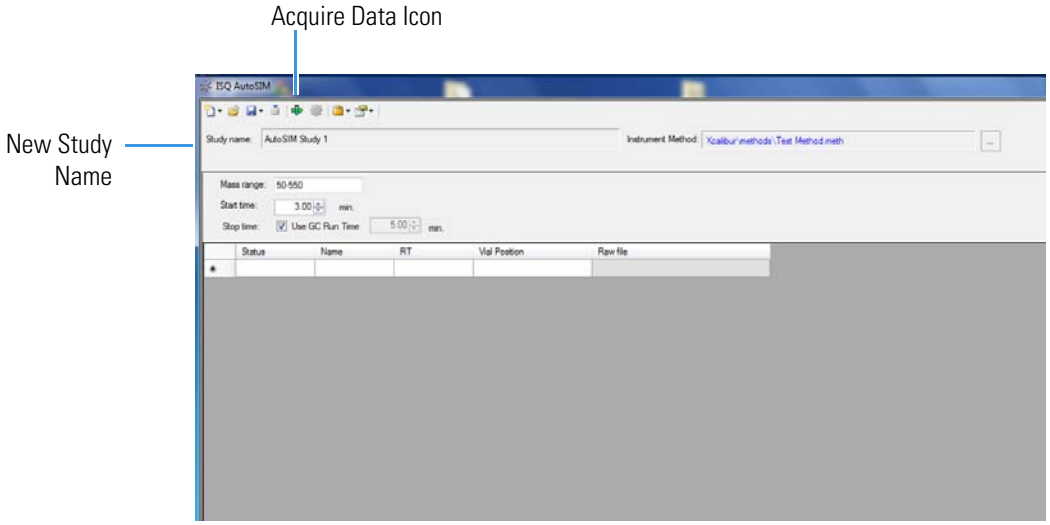
Note All files, including raw data files, that AutoSIM generates will be saved into the same folder that you save the study file. To simplify your workflow, create a folder for your study.

Figure 74. Saving an AutoSIM Study



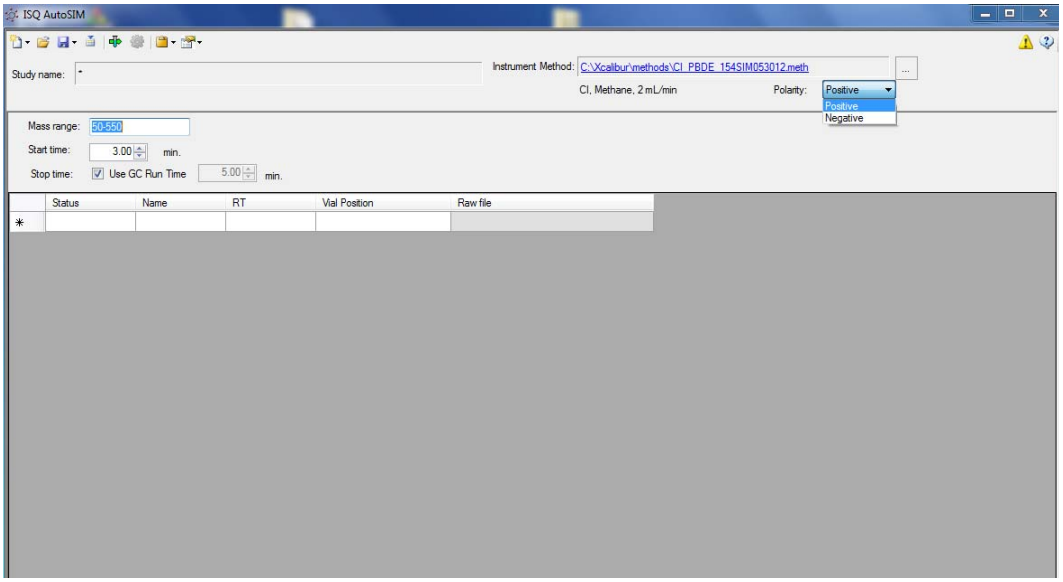
12. The Windows Explorer window closes.
13. The AutoSIM Study Name is the name you assigned. See Figure 75.

Figure 75. Acquiring Acquisition Data



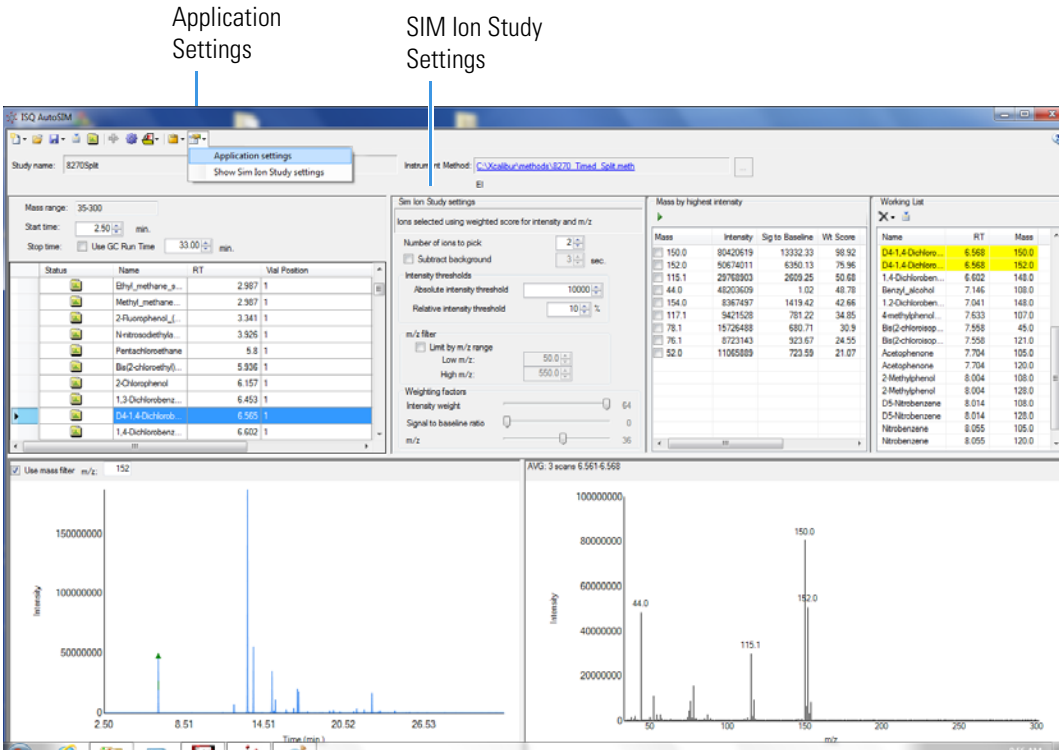
14. (CI Only) If you are running a chemical ionization (CI) study, select **Positive** or **Negative** from the **Ion Polarity** pull-down menu. See Figure 76.

Figure 76. Setting Ion Polarity in a CI Method



15. To access the options for SIM ion settings, click the **Applications Settings** icon and select **SIM Ion Settings** to open the SIM Ion Settings box. See Figure 77.

Figure 77. Application Settings and SIM Ion Study Settings



16. By default, SIM ions are sorted by highest intensity. In the SIM Ion Study Settings box, you may select SIM ions according to the following criteria.

a. **Number of Ions to Pick**: Selects the number of SIM ions picked for each compound.

b. **Subtract Background**: Checking this box subtracts background from the spectrum. Subtracting the background may reduce baseline noise automatically away from the selected peak. This will help identify your target compounds, clarify intensities, and reduce column bleed. If the automatic background subtraction is not ideal (i.e., due to co-eluting peaks), you may select to manually subtract background for individual compounds by right clicking on the chromatogram and then highlighting the scan or scans to use for subtraction.

c. **Intensity Thresholds**: Allows you to choose intensity levels.

   i. **Absolute Intensity Threshold**: Sets the intensity range that all ions must fall into before being selected as SIM ion candidates. All ions must be greater than this intensity to be selected as SIM ion candidates.

   ii. **Minimum Intensity Threshold**: Sets the minimum intensity for an ion to be a candidate for the SIM ion list. Ions must have a relative abundance greater than or equal to this percentage to be selected as SIM ion candidates.

d. **Limit by *m/z* Range**: Check this box and set the low *m/z* and high *m/z* to limit your SIM ion selection list to certain masses within the set scan range.

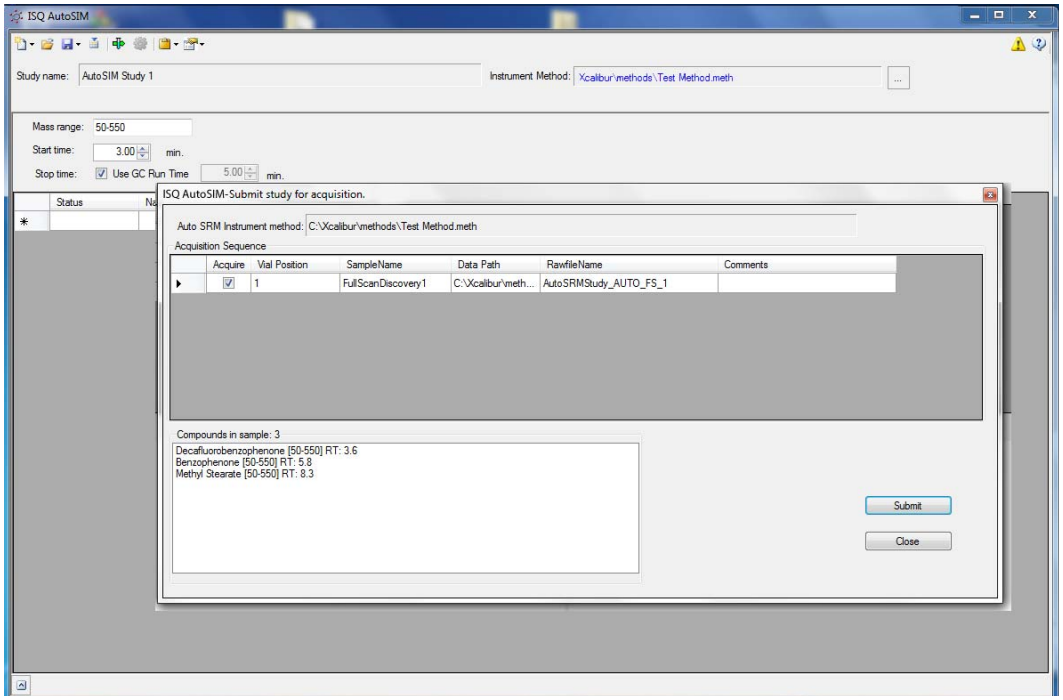
e. **Weighting Factors**: Use the sliding bars and check boxes to set the values you want to give each SIM ion study setting.

17. Click the **Acquire Data** icon to run your samples. See Figure 75.

Note AutoSIM calculates the number of injections needed based on the compound list and vial positions you assigned.

18. The Submit Study for Acquisition window opens. See Figure 78.

Figure 78. Submitting a Study for Acquisition



19. Click **Submit** to submit the samples to the instrument.

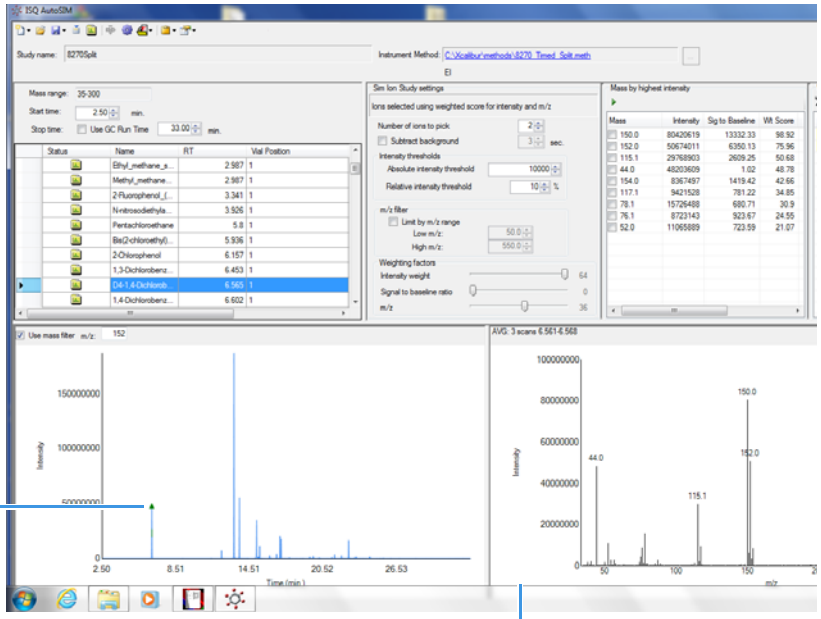
    Once the samples have finished running, the software analyzes the data.

20. The results appear in the AutoSIM window. See Figure 79.

    The results displayed correspond to the peak topped by the green triangle. The Mass by Highest Intensity pane contains a list the highest intensity ions at the indicated retention time.

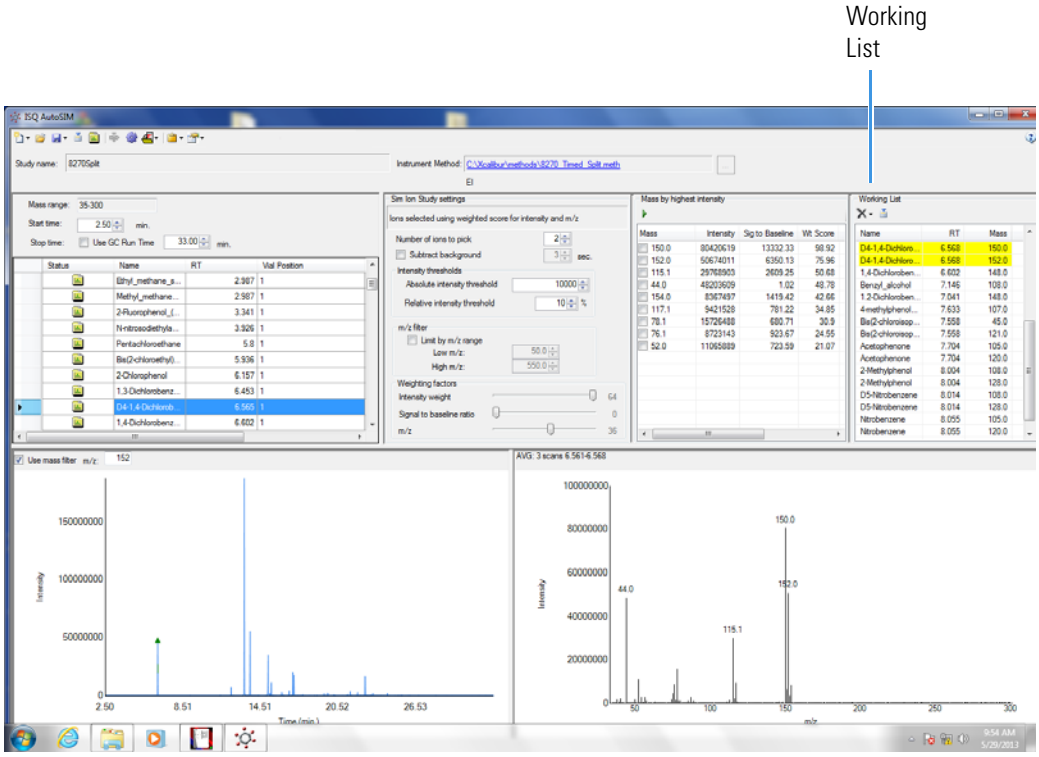
> **Note** Background subtraction updates the ions in the Mass by Highest Intensity pane.

Figure 79. Study Results in AutoSIM



21. Select the check box next to the SIM ions you want to send to the working list.
22. Click the green arrow icon to push the SIM ions you selected to the working list. See Figure 80.

Figure 80. Selecting SIM Ions

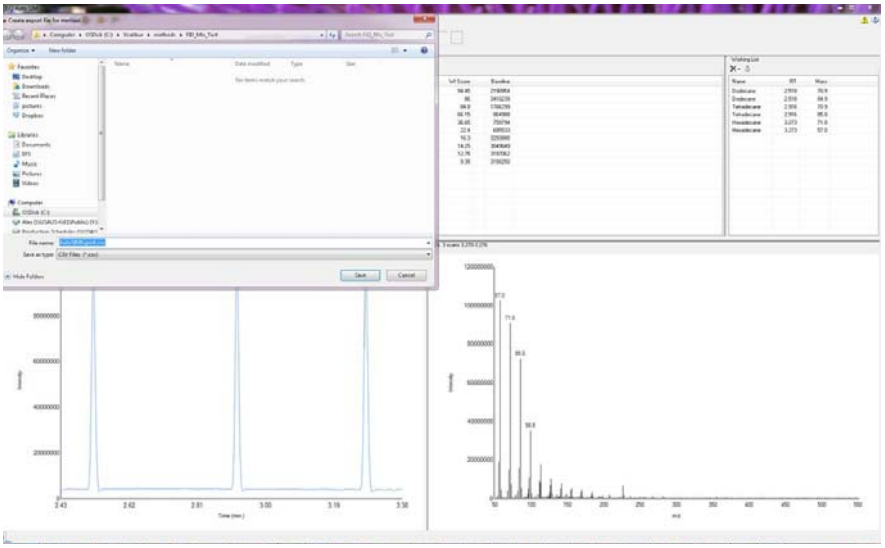


23. Repeat this process for all of your compounds.

Note You can select SIM ions by checking them in the mass list or send them directly to the working list by double-clicking on the ion in the spectra window.

24. Once you have selected all your SIM ions, go to **File | Save As** and export your SIM ion study as a .csv file. See Figure 81.

Figure 81. Exporting your SIM Ion Study

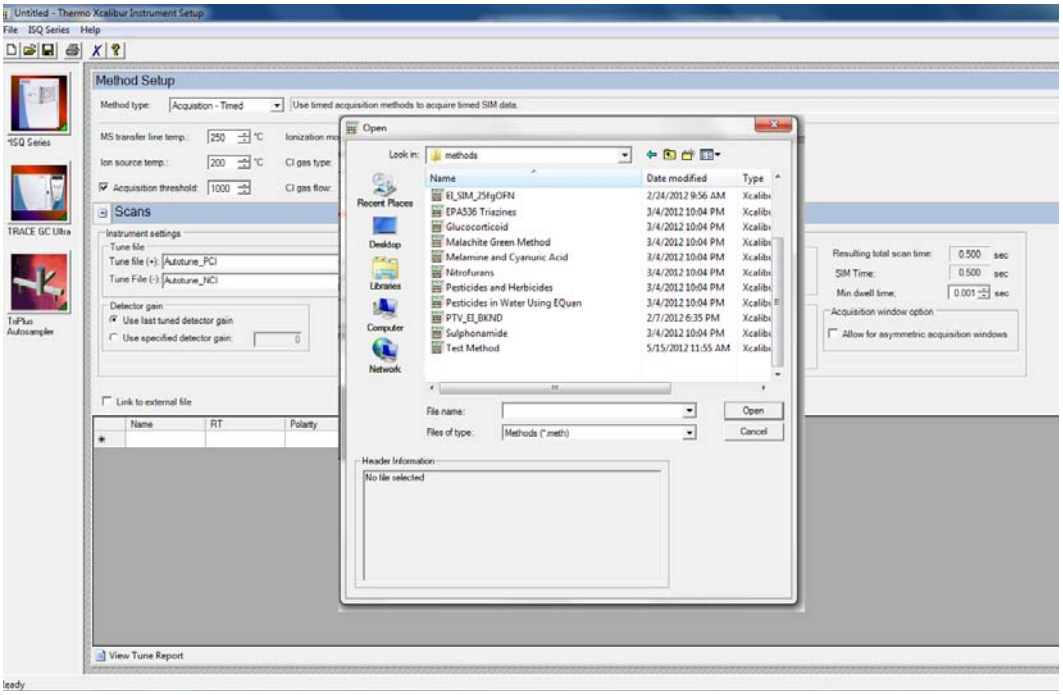


Importing Transitions to the Method Editor

❖ **To import the list of transitions you created in AutoSIM**

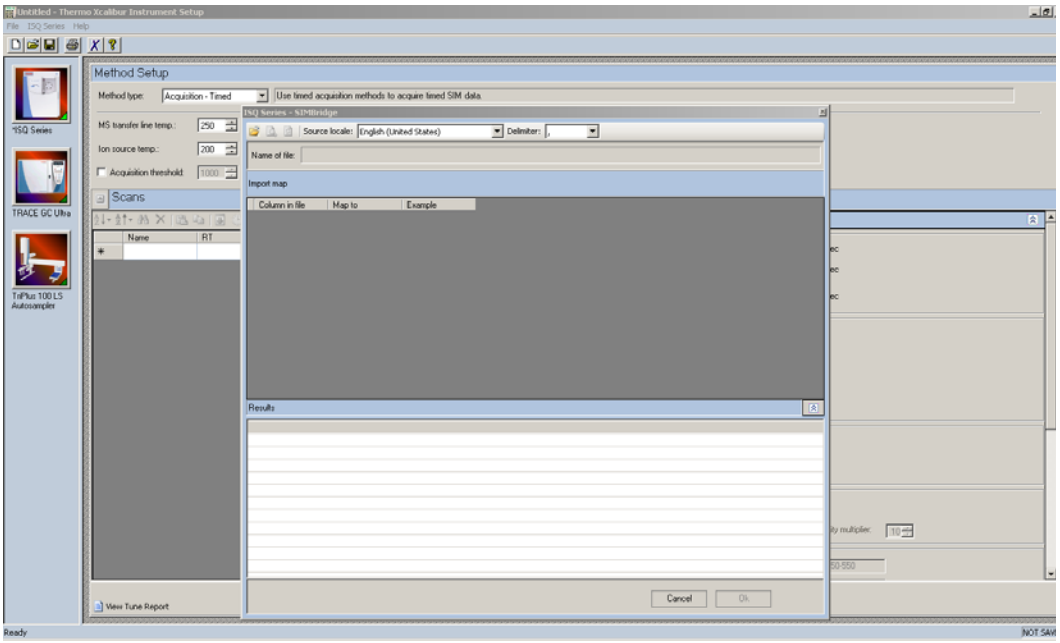
1. Open the method editor on the ISQ Dashboard.
2. Open your method in Xcalibur. See Figure 82.

Figure 82. Opening Instrument Method in Xcalibur



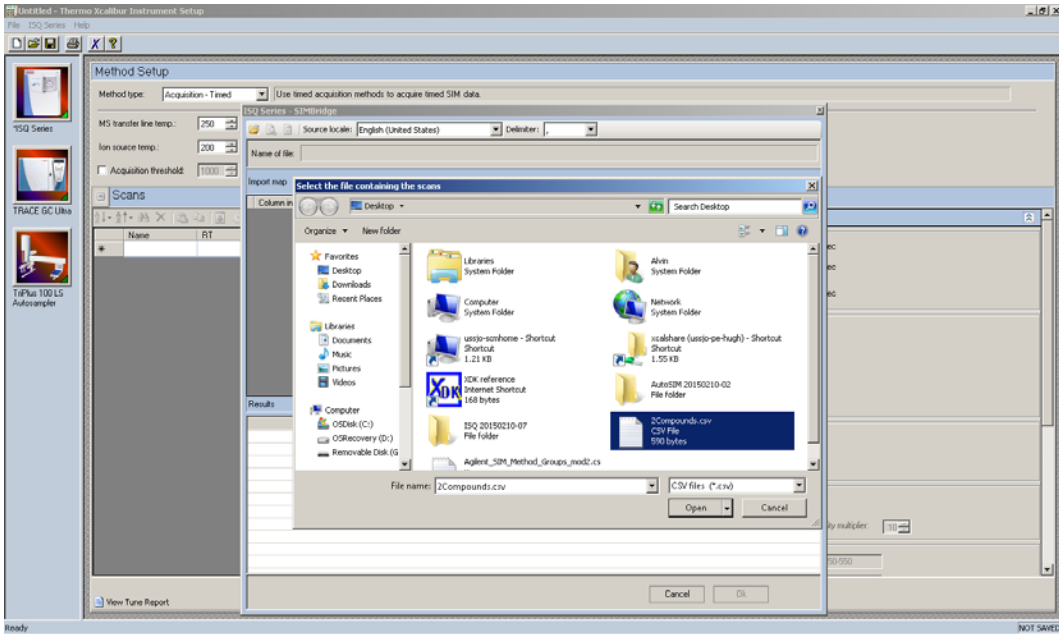
3. If you have the desired method open in AutoSIM, click the **Link to External File** in the ISQ Series method editor to open your method in the ISQ Series method editor
   a. After clicking **Link to External File**, the **SIMBridge** dialog box opens. Choose the language of your method file from the **Source Locale** drop-down menu.

Figure 83. Setting the Source Language of Method Files using SIMBridge



b. Browse to your file.

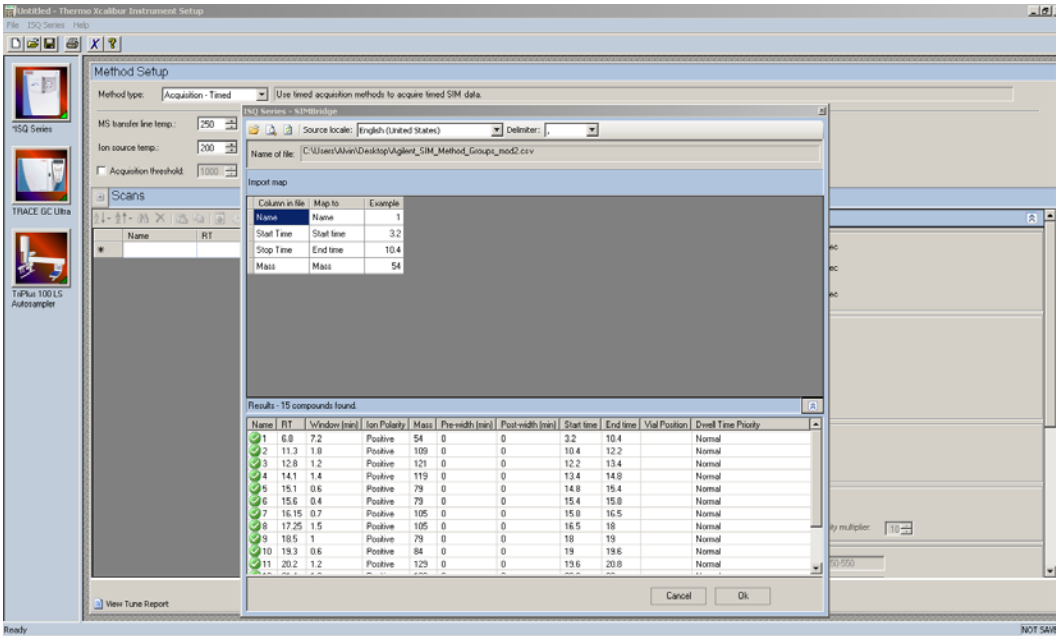
Figure 84. Linking to an External File using SIM Bridge



c. Click **Open** to open the method in SIMBridge.

d. If necessary, change the method headings in your original file to match those in the method editor. A green check mark appears when your method is validated.

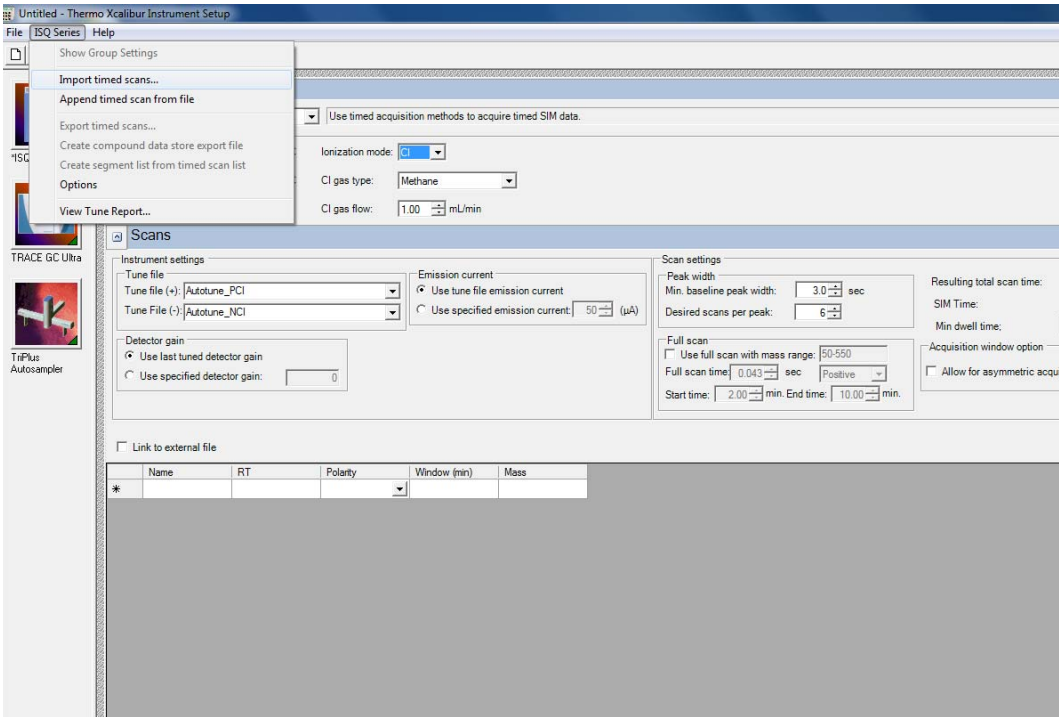
Figure 85. Changing Method Headings in SIMBridge



e. Click **Open** and the external method will be opened in the method editor.

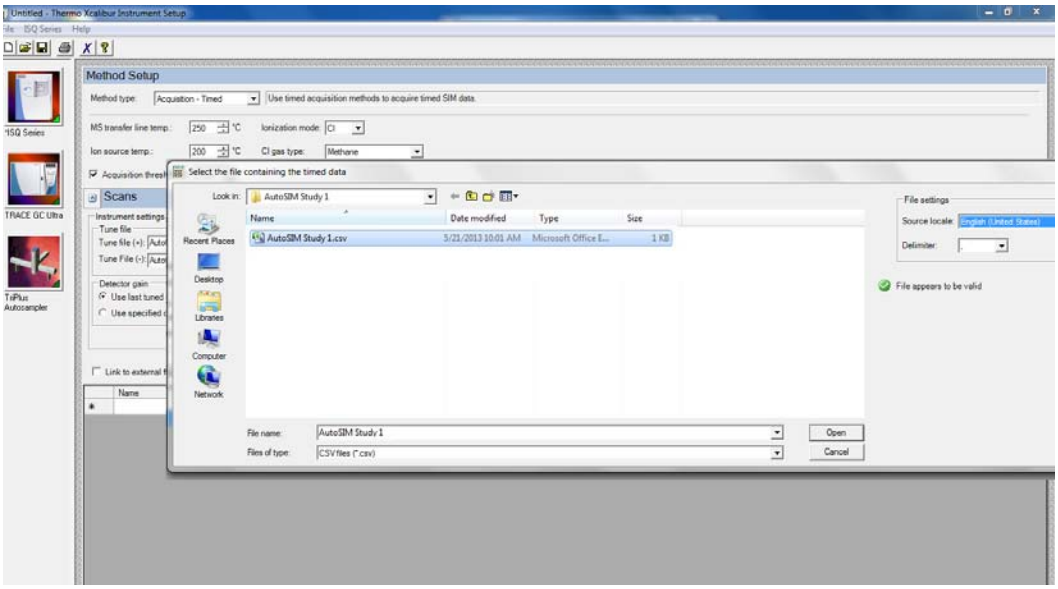
4. Click the ISQ Series icon in the method editor side pane.
5. Select Acquisition-Timed from the Method Type drop-down menu.
6. From the top menu, select **ISQ Series | Import Timed Scans**. See Figure 86.

Figure 86. Selecting Acquisition-Timed Method



7. Browse to the location where you saved the .csv file you created in AutoSIM. The software informs you if your .csv file is valid. See Figure 87.

Figure 87. Linking to an External .csv File



8. Click **Show Analysis** and review the imported list of compounds.
9. Adjust your scan settings as necessary. See "Creating a Method" on page 43 for more information.
10. Once you are satisfied with your method, save it.
11. Run a set of samples to verify that the method meets your needs.

Determining SIM Ions in Chromeleon

The purpose of an AutoSIM study is to select your SIM ions. After you name your compounds and enter your vial numbers and retention times, AutoSIM instructs your ISQ Series system to run a full-scan analysis on the compounds.

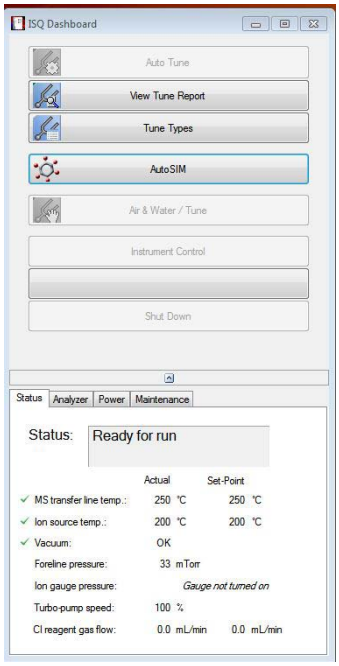
After the full-scan analysis is complete, AutoSIM presents you with the resulting chromatographic peaks and full-scan spectra, and then provides optional setting for sorting the results for your SIM ions.

Note You must have mid-range concentration standards (500 pg/µL–10 ng/µL) before setting up your AutoSIM method.

❖ To determine your SIM ions in AutoSIM

1. Click the AutoSIM button on the ISQ Dashboard to open the AutoSIM utility. See Figure 69.

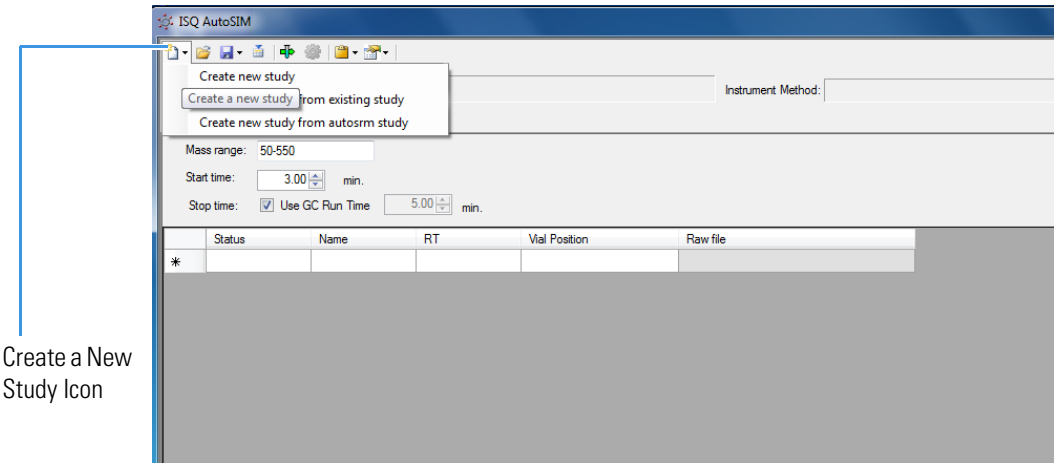
Figure 88. Accessing AutoSIM on the ISQ Dashboard



Note If User Management is enabled in Chromeleon, the Chromeleon log on dialog box opens when you start AutoSIM. Enter your Chromeleon User Name and Password to continue.

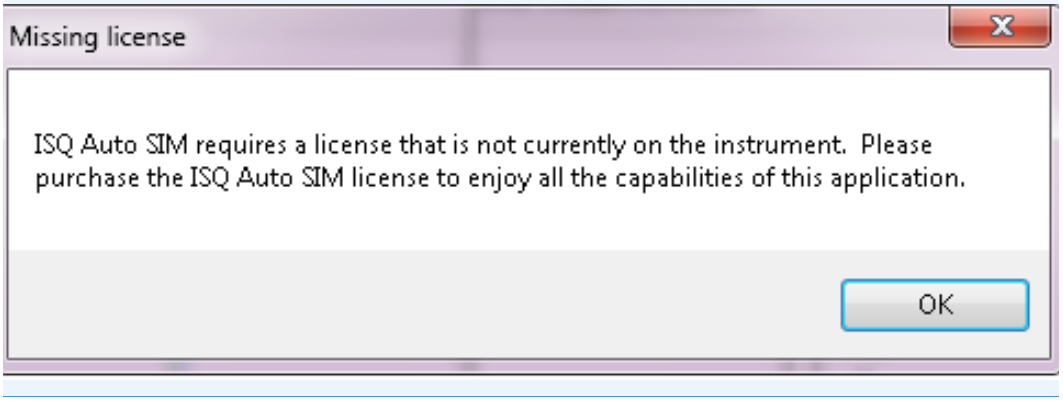
2. Click the Create a New Study icon on the left to create a new study.

Figure 89. New AutoSIM Study



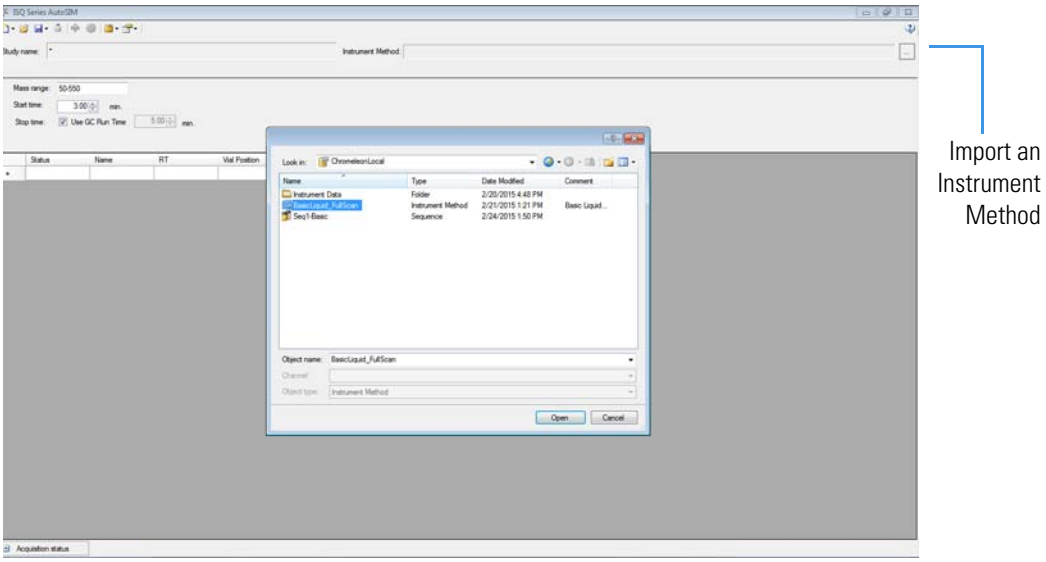
3. A new study window opens.

Note If at any time you see the error message below, go to "Upgrading the Software" on page 184 and follow the instructions for getting your software license.



4. Link to your saved instrument method file (that you created using the Chromeleon Instrument Method Editor) by clicking on the ellipsis icon next to the Instrument Method window. See Figure 71.

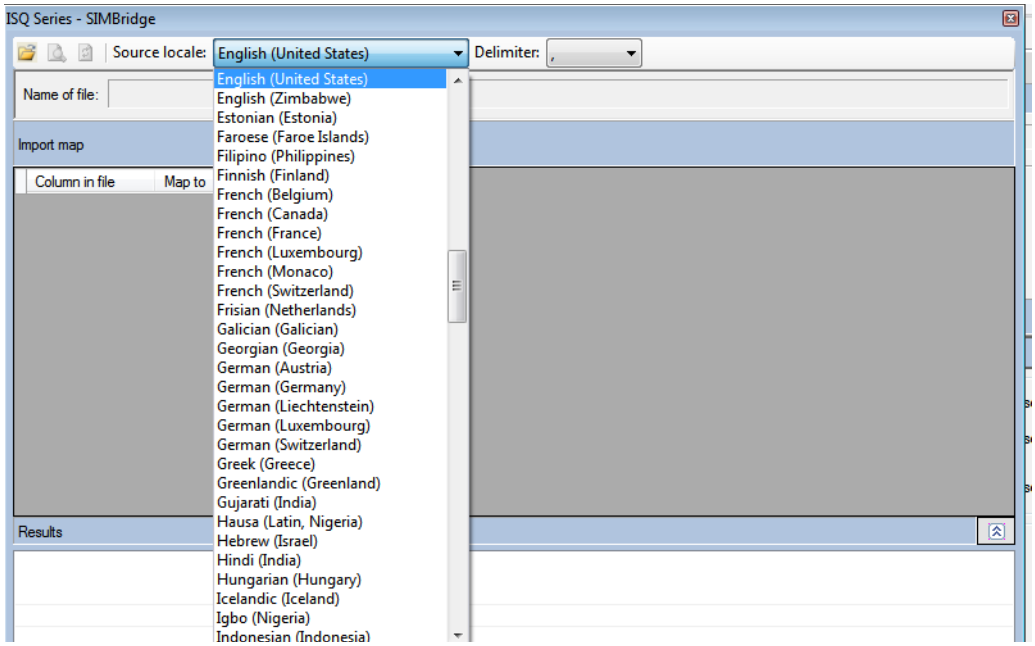
Figure 90. Retrieving an Instrument Method



5. If you are importing a file created on another system, click the import icon. Otherwise, select an instrument method file and click Open.

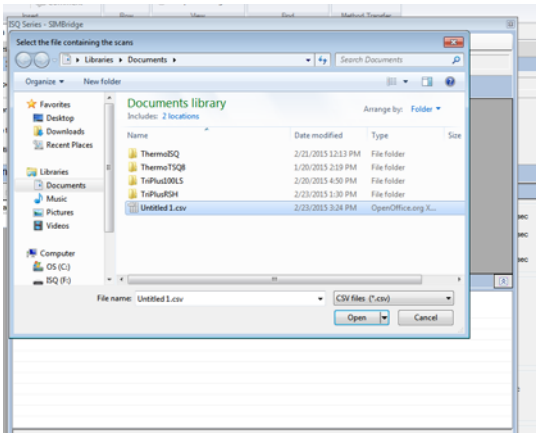
6. The **SIMBridge** dialog box opens. Choose the language of your method file from the **Source Locale** drop-down menu. See Figure 91.

Figure 91. Setting the Source Language of Method Files using SIMBridge



7. Click the File icon. The **Select the File Containing the Scans** dialog box opens. Browse to your file. See Figure 92.

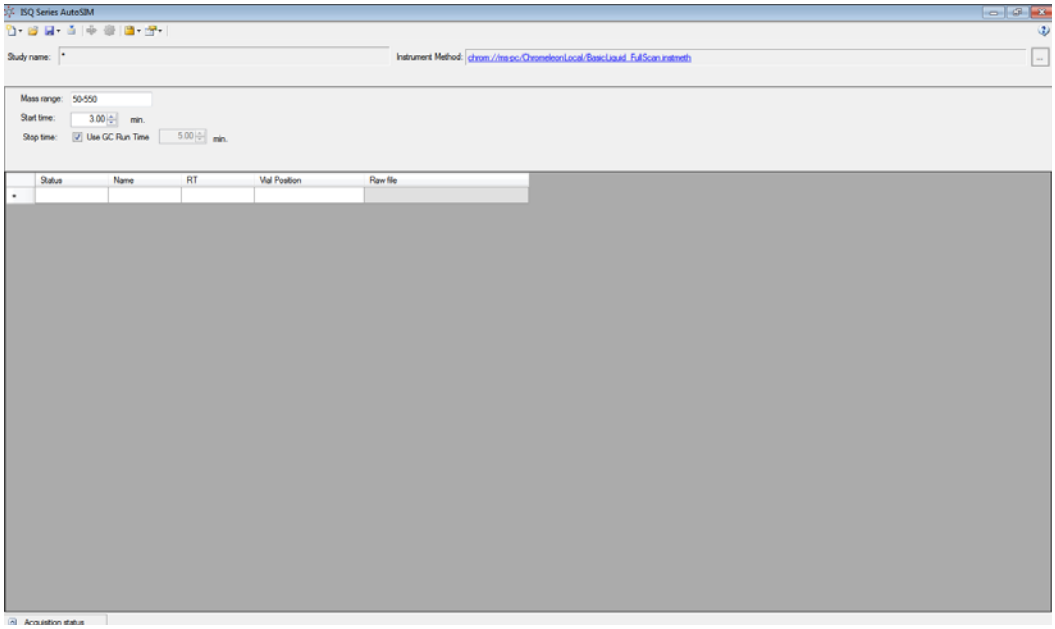
Figure 92. Linking to an External File using SIM Bridge



8. Click **Open** to open the method in SIMBridge.
9. If necessary, change the method headings in your original file to match those in the method editor. A green check mark appears when your method is validated.
10. Your method opens in AutoSIM. AutoSIM uses the GC and autosampler parameters from this method file. You may set the Mass Range, Start Time, and Stop Time. See Figure 72.

    Any changes you make to your MS method here will override the method editor settings.

Figure 93. Adjusting the Settings

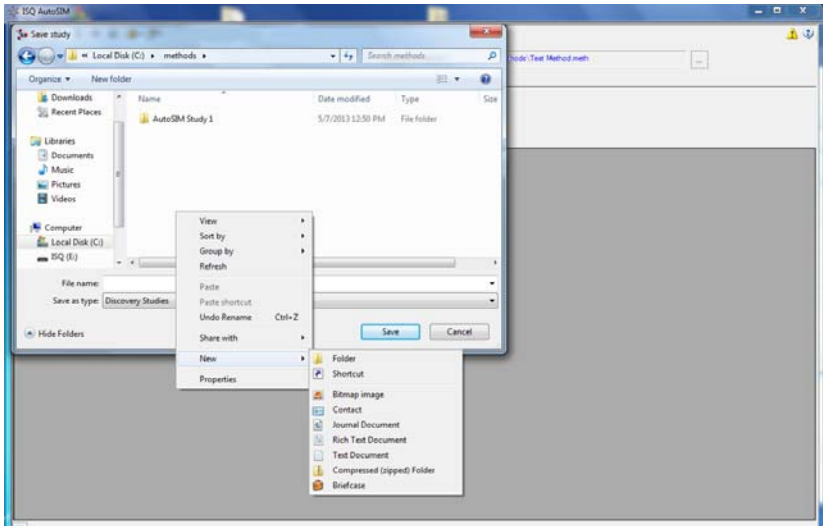


11. Enter the compound name, approximate retention time, and vial number for each compound you wish to optimize. If you already have a method for processing full scan data you can choose to import compounds from an external file. Their names and retention times will fill the compound list and their primary quantitation ion will be displayed in the mass filter box once the full scan data is acquired.

12. Save the study.

Tip Create a folder for all files associated with your AutoSIM study. Otherwise, the study results files will be saved in the general instrument method folder and crowd it. See Figure 73.

Figure 94. Creating an AutoSIM Study Folder



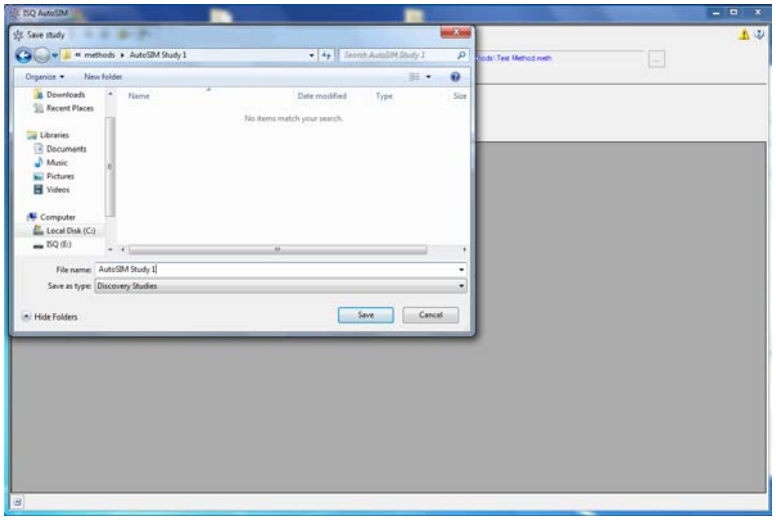
13. Open the folder.

14. Give your study a file name.

15. Save your study in the Study folder. See Figure 74.

Note All files, including raw data files, that AutoSIM generates will be saved into the same folder that you save the study file. To simplify your workflow, create a folder for your study.

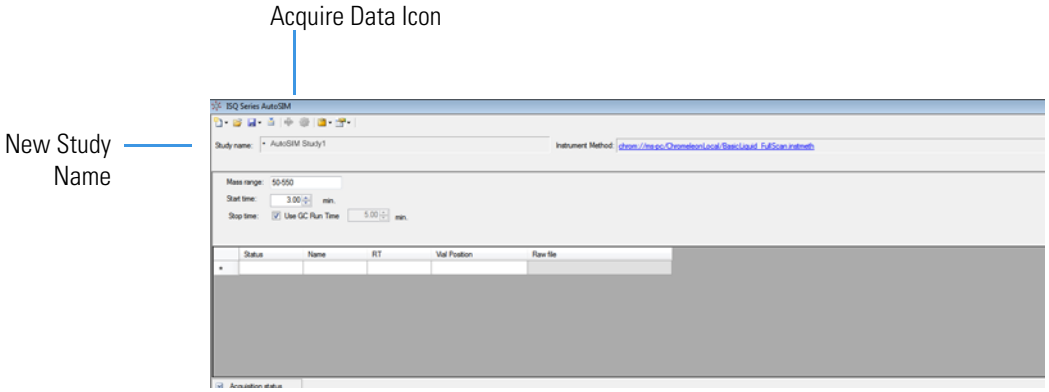
Figure 95. Saving an AutoSIM Study



16. The Windows Explorer window closes.

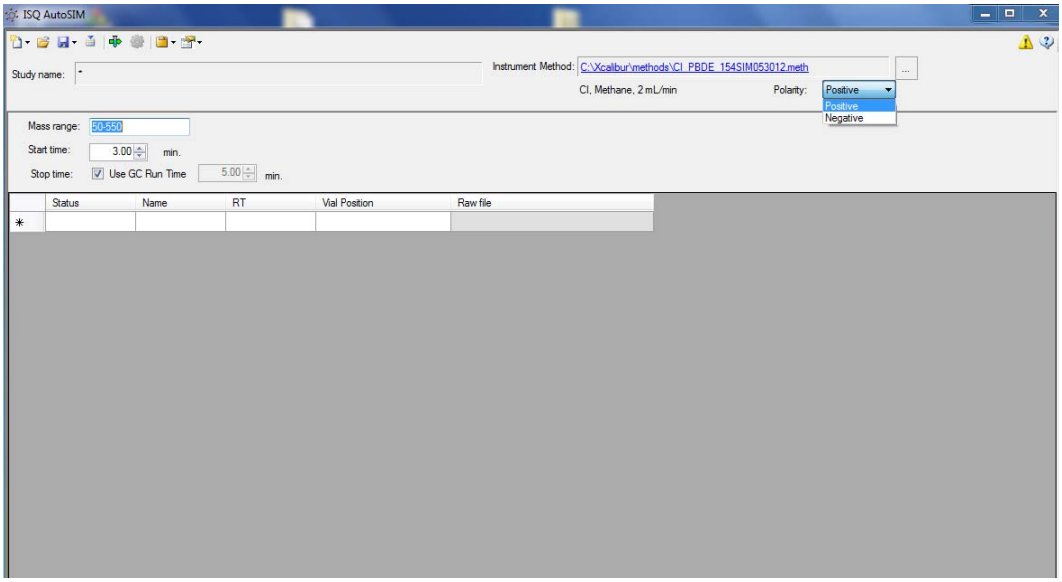
17. The AutoSIM Study Name is the name you assigned. See Figure 75.

Figure 96. Acquiring Acquisition Data



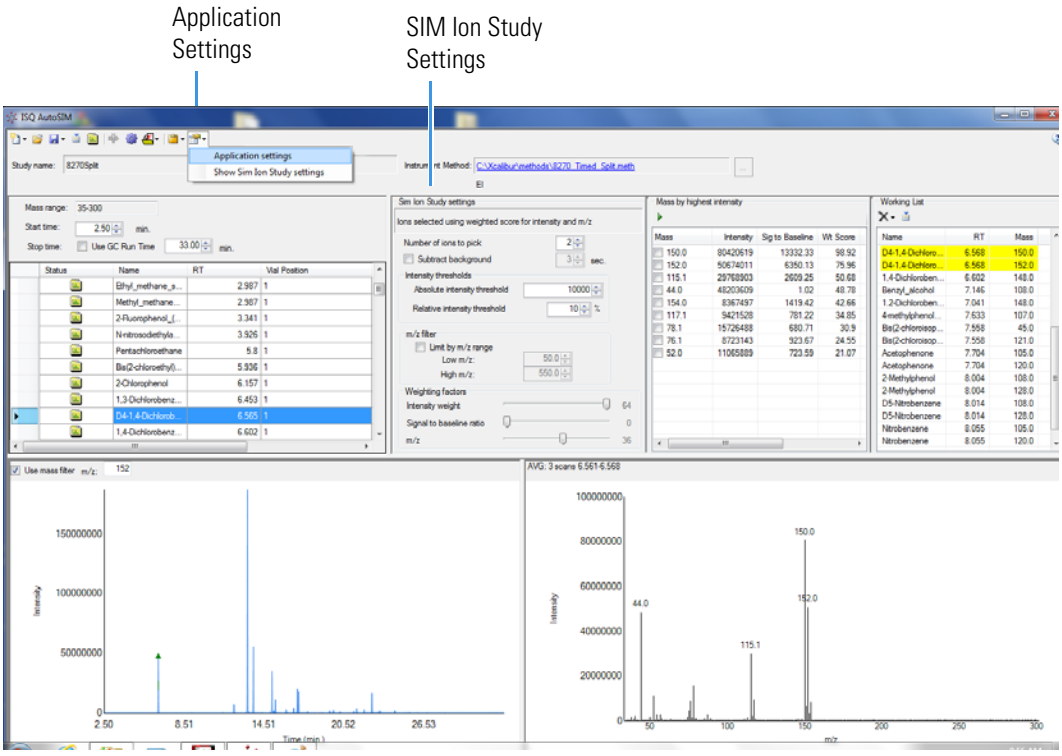
18. (CI Only) If you are running a chemical ionization (CI) study, select **Positive** or **Negative** from the **Ion Polarity** pull-down menu. See Figure 76.

Figure 97. Setting Ion Polarity in a CI Method



19. To access the options for SIM ion settings, click the **Applications Settings** icon and select **SIM Ion Settings** to open the SIM Ion Settings box. See Figure 77.

Figure 98. Application Settings and SIM Ion Study Settings



20. By default, SIM ions are sorted by highest intensity. In the SIM Ion Study Settings box, you may select SIM ions according to the following criteria.

a. **Number of Ions to Pick**: Selects the number of SIM ions picked for each compound.

b. **Subtract Background**: Checking this box subtracts background from the spectrum. Subtracting the background may reduce baseline noise automatically away from the selected peak. This will help identify your target compounds, clarify intensities, and reduce column bleed. If the automatic background subtraction is not ideal (i.e., due to co-eluting peaks), you may select to manually subtract background for individual compounds by right clicking on the chromatogram and then highlighting the scan or scans to use for subtraction.

c. **Intensity Thresholds**: Allows you to choose intensity levels.

   i. **Absolute Intensity Threshold**: Sets the intensity range that all ions must fall into before being selected as SIM ion candidates. All ions must be greater than this intensity to be selected as SIM ion candidates.

   ii. **Minimum Intensity Threshold**: Sets the minimum intensity for an ion to be a candidate for the SIM ion list. Ions must have a relative abundance greater than or equal to this percentage to be selected as SIM ion candidates.

d. **Limit by *m/z* Range**: Check this box and set the low *m/z* and high *m/z* to limit your SIM ion selection list to certain masses within the set scan range.

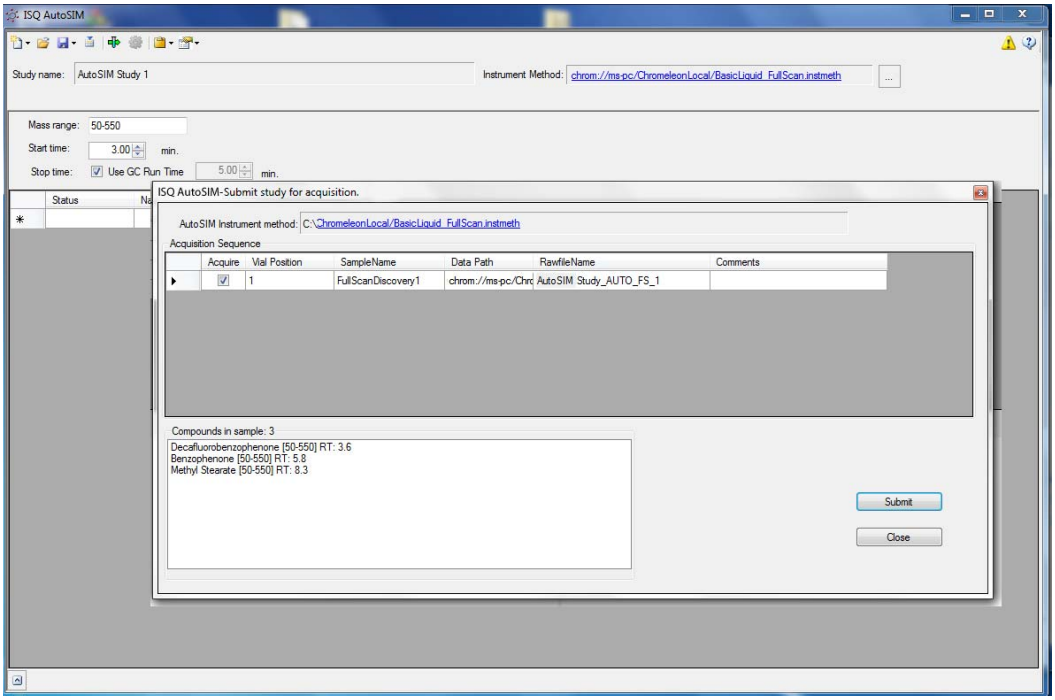
e. **Weighting Factors**: Use the sliding bars and check boxes to set the values you want to give each SIM ion study setting.

21. Click the **Acquire Data** icon to run your samples. See Figure 75.

Note AutoSIM calculates the number of injections needed based on the compound list and vial positions you assigned.

22. The Submit Study for Acquisition window opens. See Figure 78.

Figure 99. Submitting a Study for Acquisition



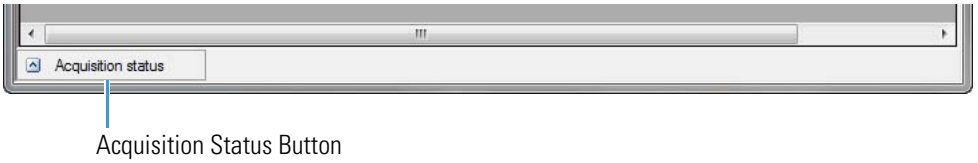
23. Click **Submit** to submit the samples to the instrument.

    The AutoSIM study is added to the Chromeleon sequence queue. Chromeleon performs a Ready Check to verify that the instrument is ready for operation and the instrument method is error-free. If the Ready Check passes, the run starts.

> **Note** If the run does not start automatically after you click Submit, review the Chromeleon Ready Check messages listed in the Acquisition summary pane on the Submit dialog box. Close the Submit dialog box, correct the errors, and then resubmit the study.
>
> If the following Ready Check message appears: "The instrument is currently in 'Hold' condition," follow these steps to release the Hold:
> - On the Acquisition Status pane (described below), click the **Command** button.
> - On the Command window, click the System icon. In the Properties list, select **HoldMode Off** and press ENTER.

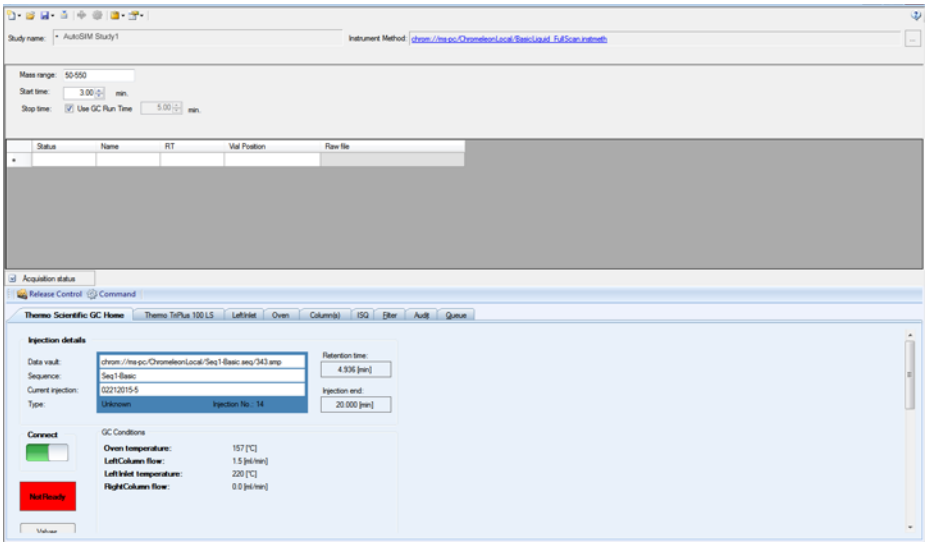
24. The Acquisition Status pane displays status information about the running instrument. If the pane is not currently visible, click the **Acquisition Status** button. See Figure 100.

Figure 100. Displaying the Acquisition Status Pane



The pane contains a Chromeleon ePanel Set that you can use to monitor the status of AutoSIM injections. See Figure 101. You can also use the ePanel Set to monitor the devices configured in the instrument and to see the status of the Chromeleon sequence queue. For details about Chromeleon ePanel Sets, refer to the Chromeleon Help.

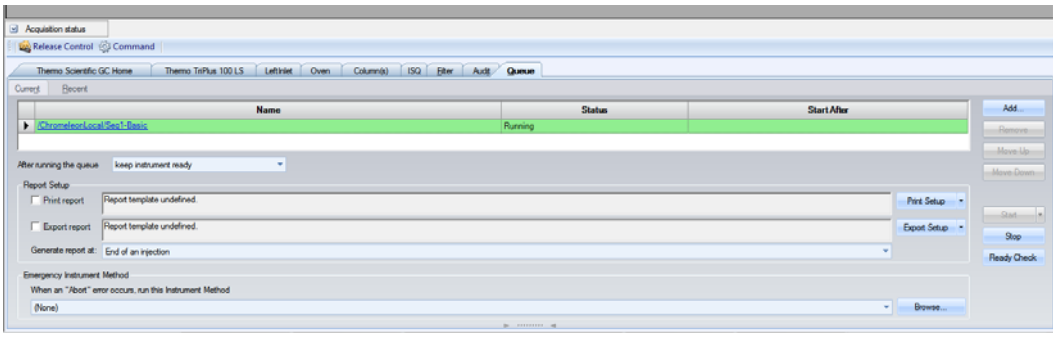
Figure 101. Viewing Instrument Status on the Home ePanel



25. For an overview of the status of the run, click the **Home** tab. To monitor or control a configured device (mass spectrometer, detector, oven, etc.) click the tab for the device.

26. To view the queue status or to stop or start the queue, click the **Queue** tab. See Figure 102.

Figure 102. Viewing Queue Status on the Queue ePanel



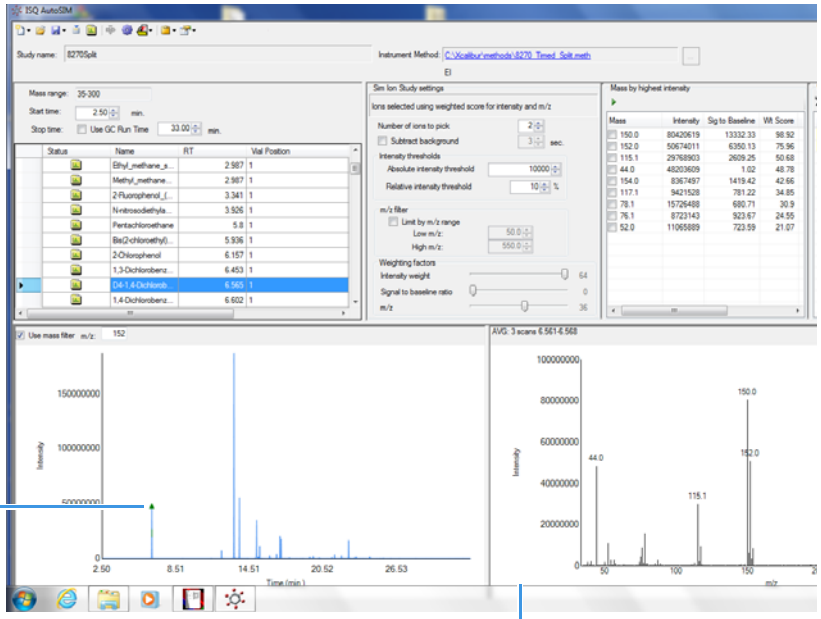
Once the samples have finished running, the software analyzes the data.

27. The results appear in the AutoSIM window. See Figure 79.

The results displayed correspond to the peak topped by the green triangle. The Mass by Highest Intensity pane contains a list the highest intensity ions at the indicated retention time.

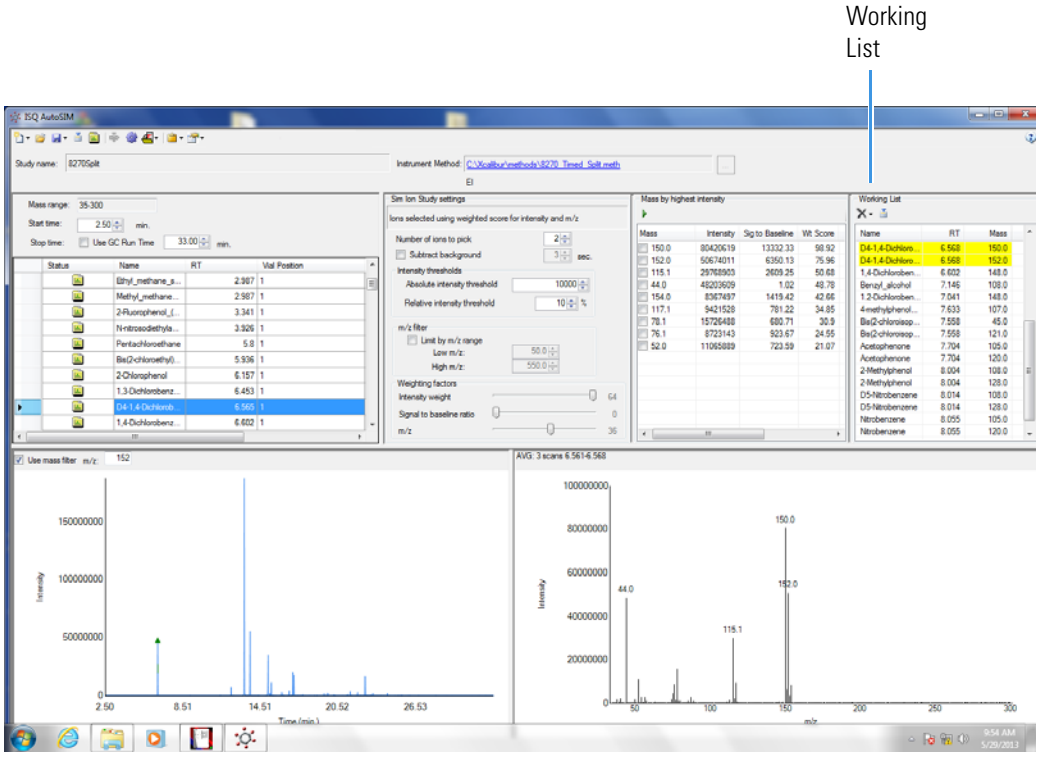
Note Background subtraction updates the ions in the Mass by Highest Intensity pane.

Figure 103. Study Results in AutoSIM



28. Select the check box next to the SIM ions you want to send to the working list.
29. Click the green arrow icon to push the SIM ions you selected to the working list. See Figure 80.

Figure 104. Selecting SIM Ions

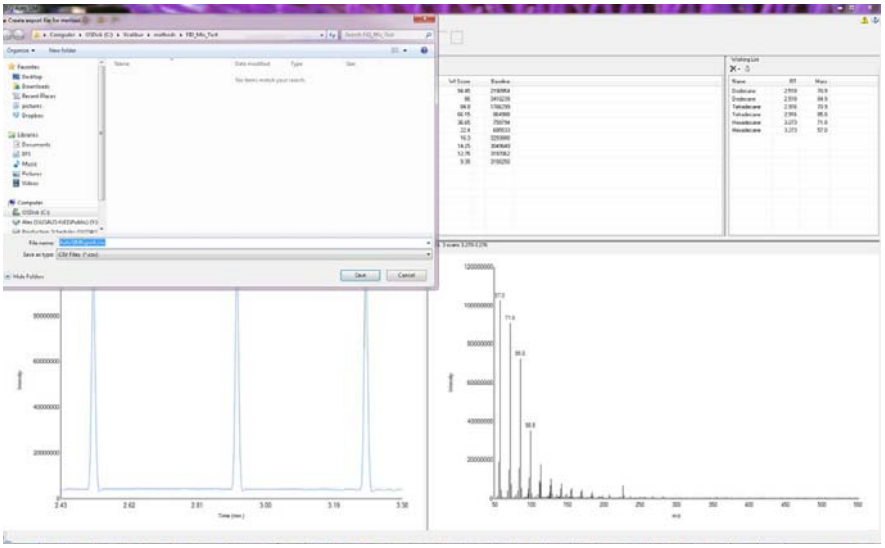


30. Repeat this process for all of your compounds.

Note You can select SIM ions by checking them in the mass list or send them directly to the working list by double-clicking on the ion in the spectra window.

31. Once you have selected all your SIM ions, go to **File | Save As** and export your SIM ion study as a .csv file. See Figure 81.

Figure 105. Exporting your SIM Ion Study



Importing Transitions to the Chromeleon Instrument Method Editor

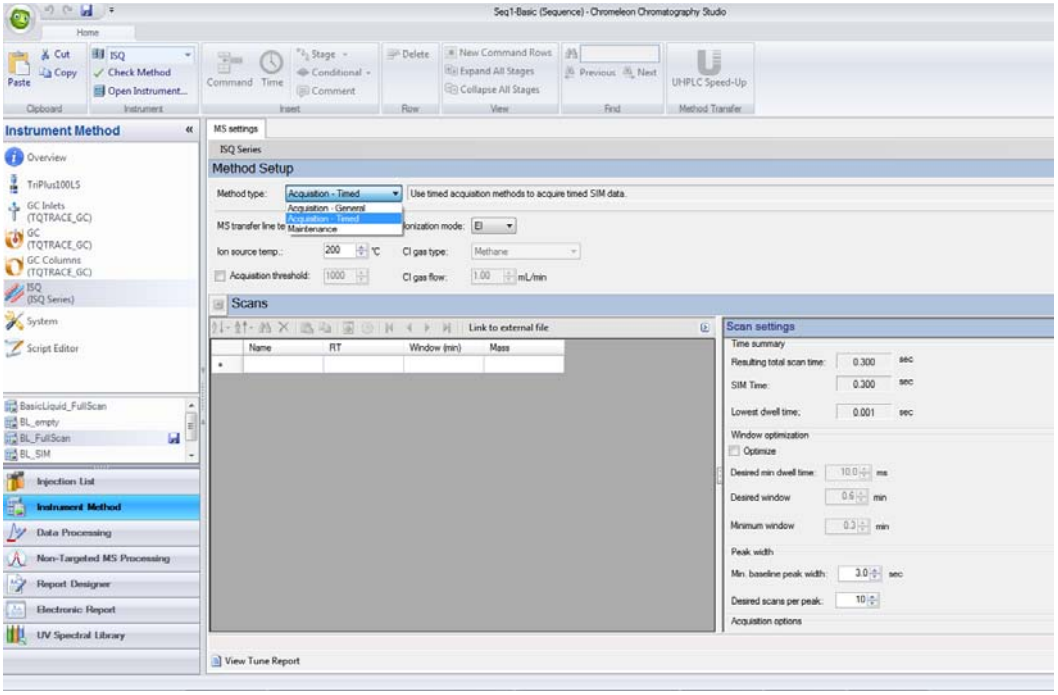
❖ To import the list of transitions you created in AutoSIM

1. Open the Chromeleon Instrument Method Editor.

Tip Click the link in the Instrument Method box in AutoSIM to open Chromeleon.

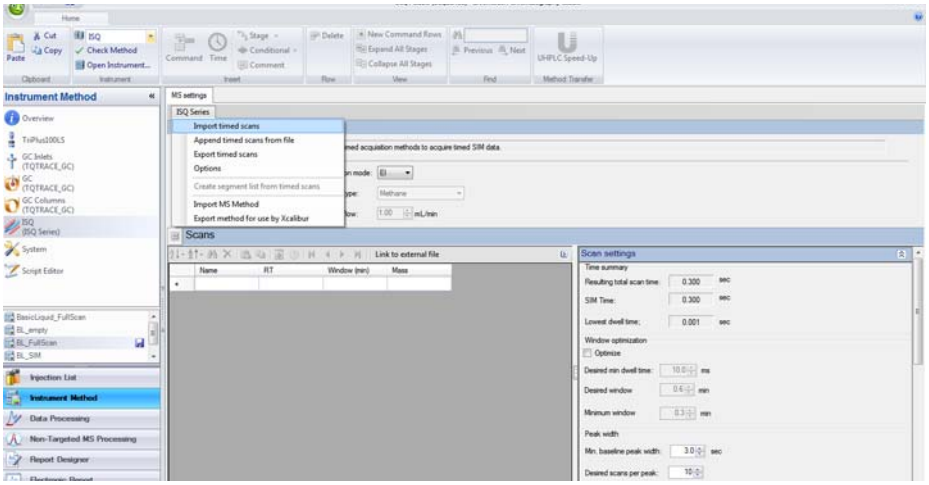
2. Select the **Acquisition – Timed** method type.

Figure 106. Opening Instrument Method in Xcalibur



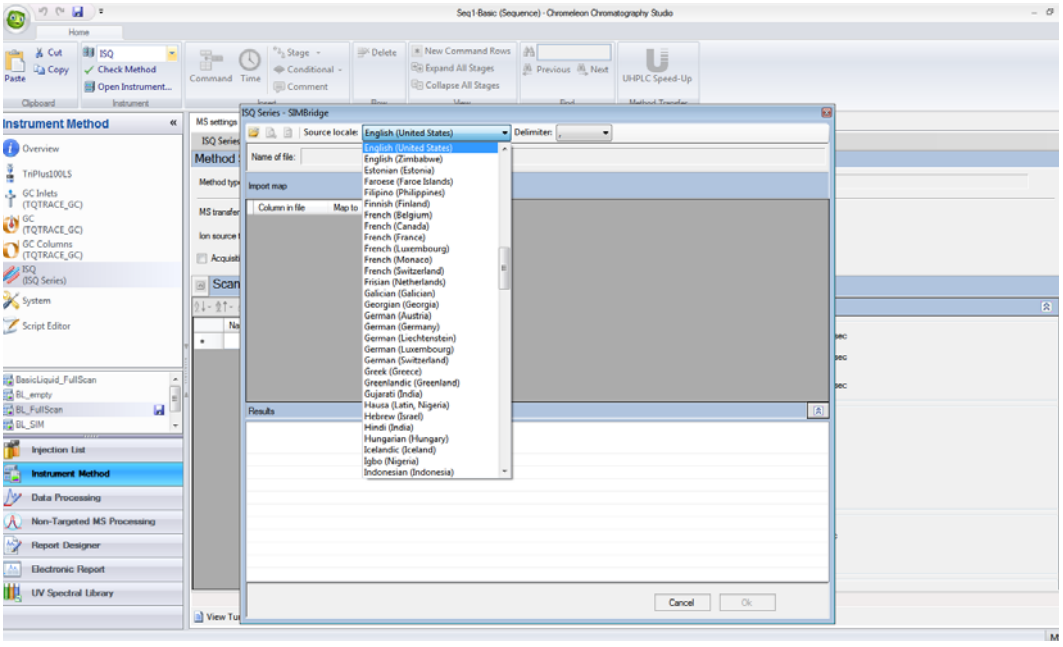
3. In the **MS Settings** pane, select **ISQ Series > Import Timed Scans**.

Figure 107. Importing Timed Scans



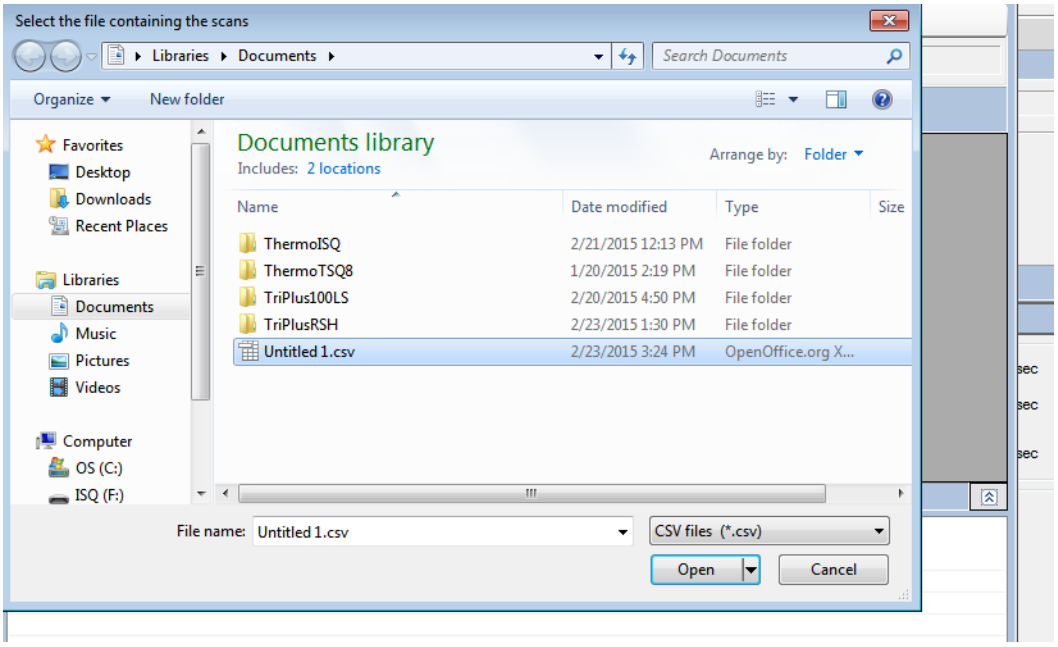
4. The **SIMBridge** dialog box opens. Choose the language of your method file from the **Source Locale** drop-down menu.

Figure 108. Setting the Source Language of Method Files using SIMBridge



5. Browse to your file.

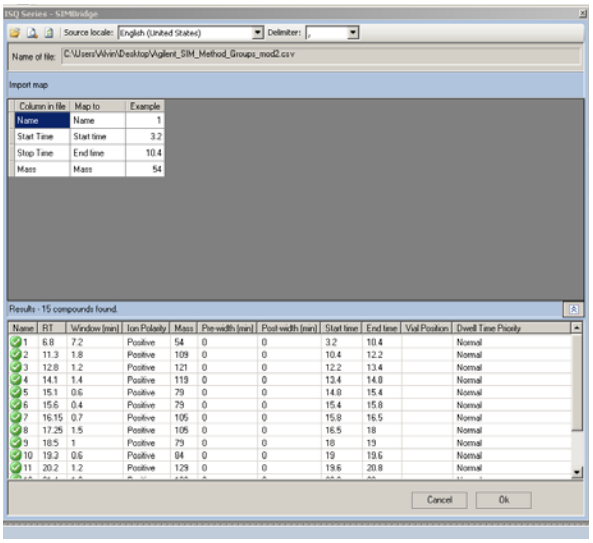
Figure 109. Linking to an External File using SIM Bridge



6. Click **Open** to open the method in SIMBridge.

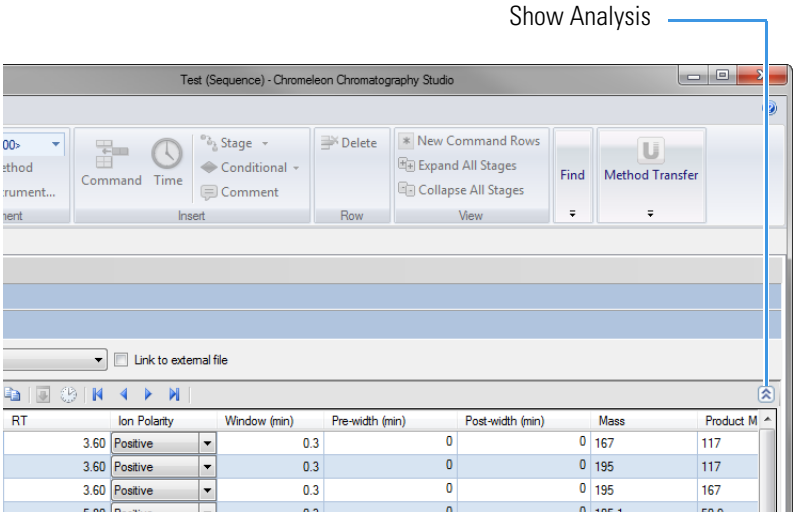
7. If necessary, change the method headings in your original file to match those in the method editor. A green check mark appears when your method is validated.

Figure 110. Changing Method Headings in SIMBridge



8. Click **Open** and the external method will be opened in the Chromeleon Instrument Method editor.
9. Click the ISQ Series icon in the method editor side pane.
10. Click **Show Analysis** and review the imported list of compounds.

Figure 111. Selecting Show Analysis



11. Adjust your scan settings as necessary. See "Creating a Method" on page 43 for more information.
12. Once you are satisfied with your method, save it.
13. Run a set of samples to verify that the method meets your needs.

6

Running a Sample

This chapter describes how to prepare a sample and then run a sequence.

Contents

Preparing Your Sample

The primary goal of sample preparation is to reduce the amount of unwanted contaminant in a sample or to increase the reliability of sample detection. As a result, you should prepare your samples to increase the relative abundance of compounds you want to analyze and decrease the relative abundance of the compounds you aren't interested in. Refer to your lab's standard operating procedure (SOP) for your particular sample preparation method.

Different solvents are used to dissolve different compounds. Be sure to choose a solvent that will dissolve the compounds you want to analyze. The solvent should be compatible with the stationary phase of your GC column when making sample dilutions.

Once your sample is prepared, you can transfer it to a sealed vial so that you can inject it into the GC. It is important to use a sealed vial because you do not want the concentration to be altered, which is what happens when the solvent evaporates.

Running a Sequence

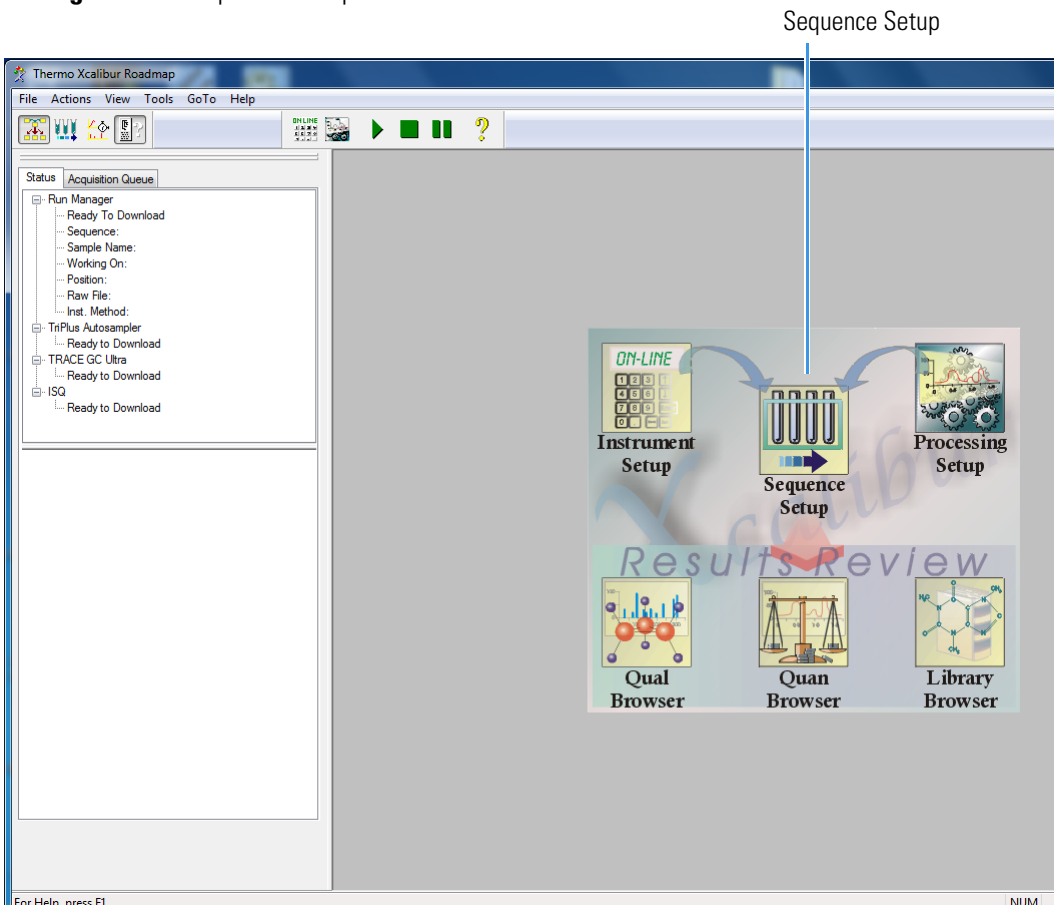
Once you've prepared your sample, you can use Xcalibur to run your sample(s). Xcalibur uses a sequence (series of tasks) to prepare the instruments for data acquisition, as well as monitor the injection and collection of data from the GC/MS.

A sequence can be used to acquire data from a sample or prepare the system for maintenance.

❖ To run a new sequence of samples

1. Double-click the Xcalibur software icon on your computer desktop.
2. In the Xcalibur Roadmap window, click the **Sequence Setup** icon.

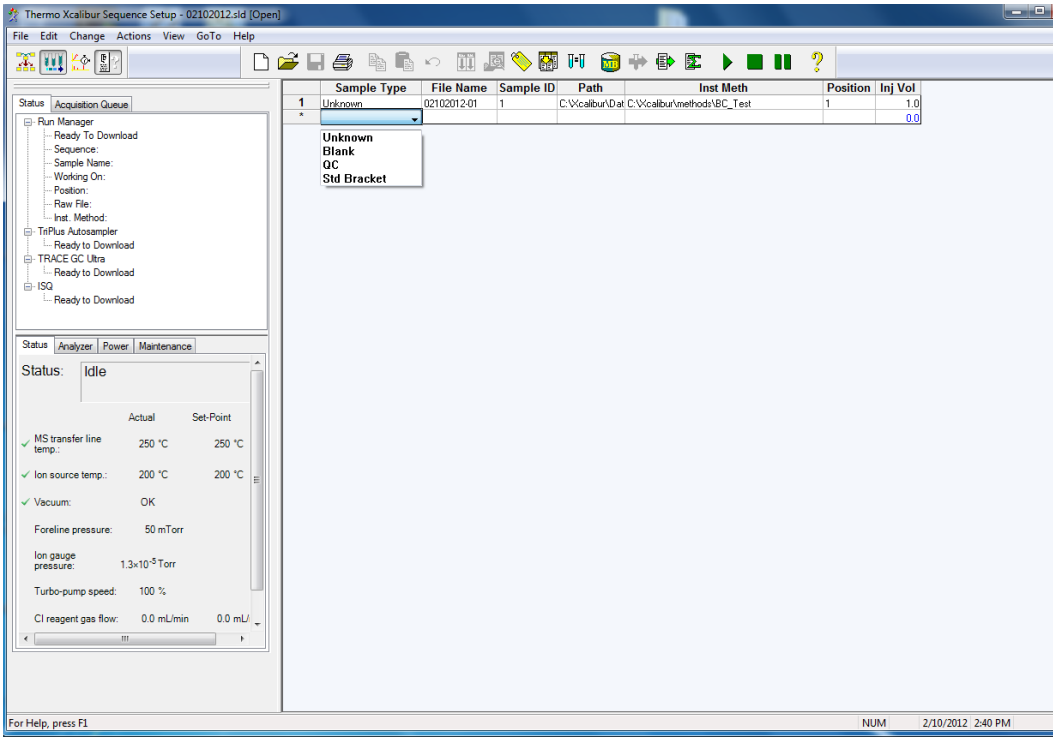
Figure 112. Sequence Setup in Xcalibur



3. 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 113. Entering the Sample Type



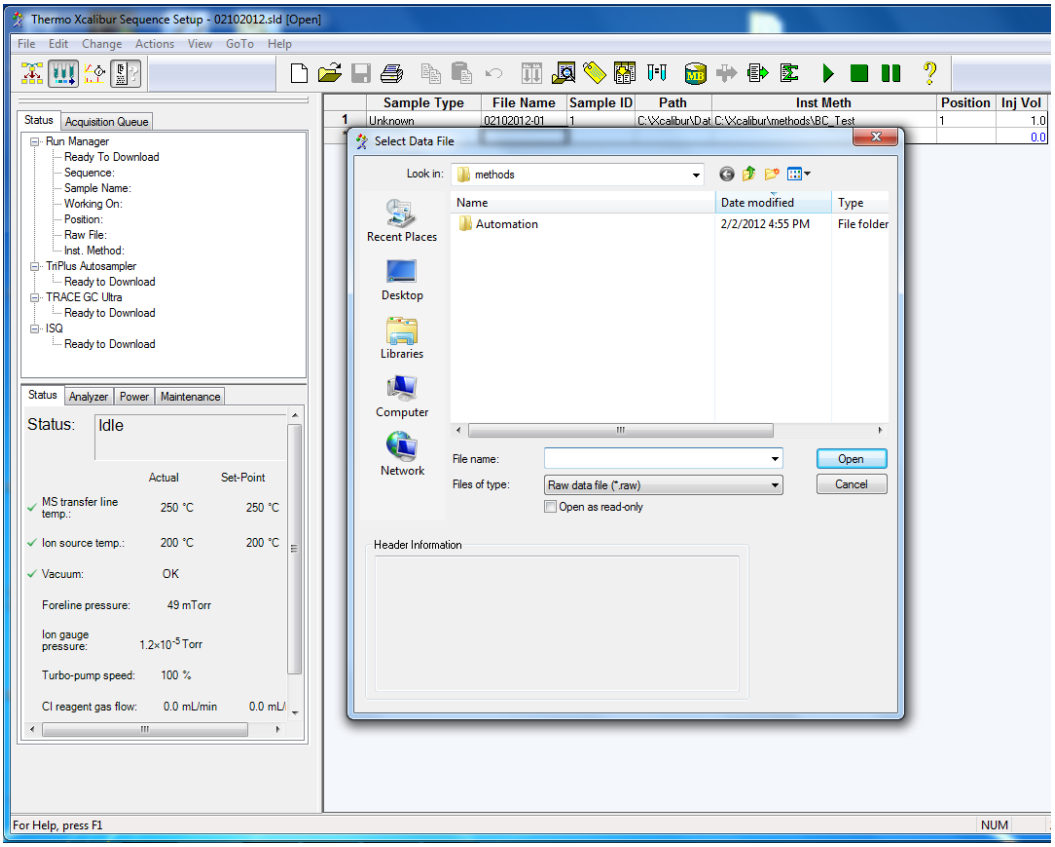
Once you select a sample type, default information automatically appears in some of the other columns. You can also right-click on the field to clear, copy, or paste into the field, as well as add and delete rows.

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.

4. Click the first row in the **File Name** column and enter a file name for your first data file. If you double-click the field, you can browse to a raw data file on your computer. You can also right-click on the field to clear, copy, or paste into the field, browse to the raw data file, or add and delete rows.

Note Your file name cannot contain spaces.

Figure 114. Naming a File

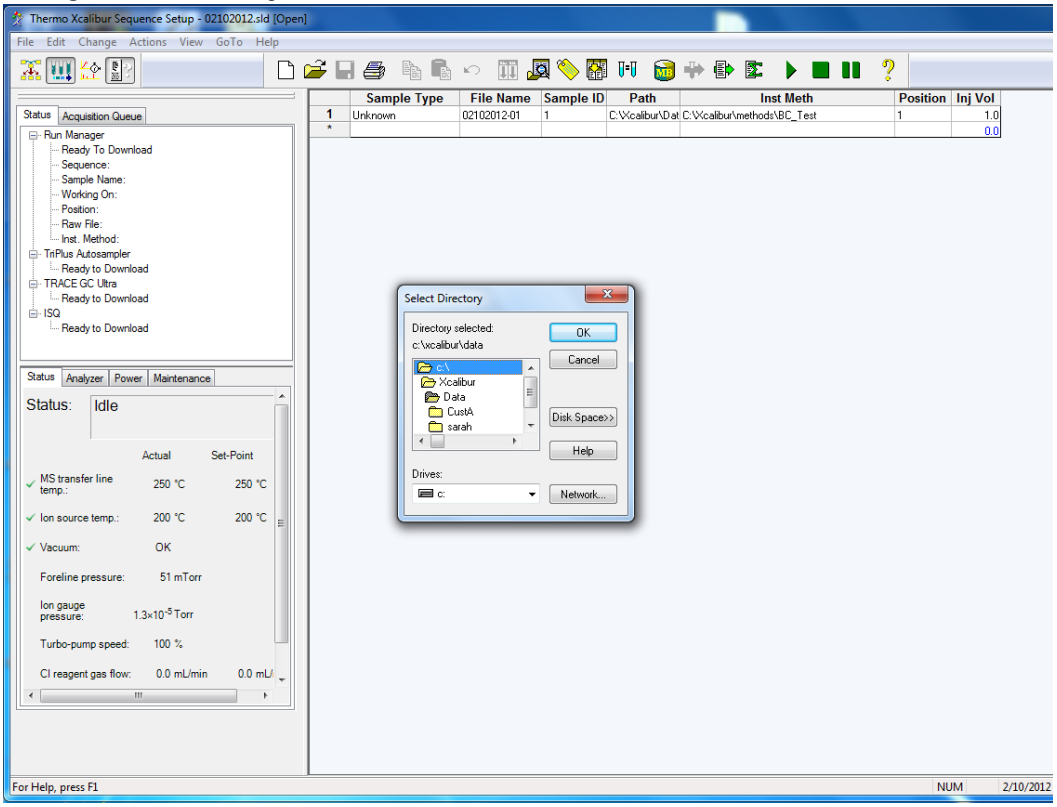


Tip You can double-click in the **File Name** field and browse to a raw file on your computer.

5. 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 don't have that many samples so you don't 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.

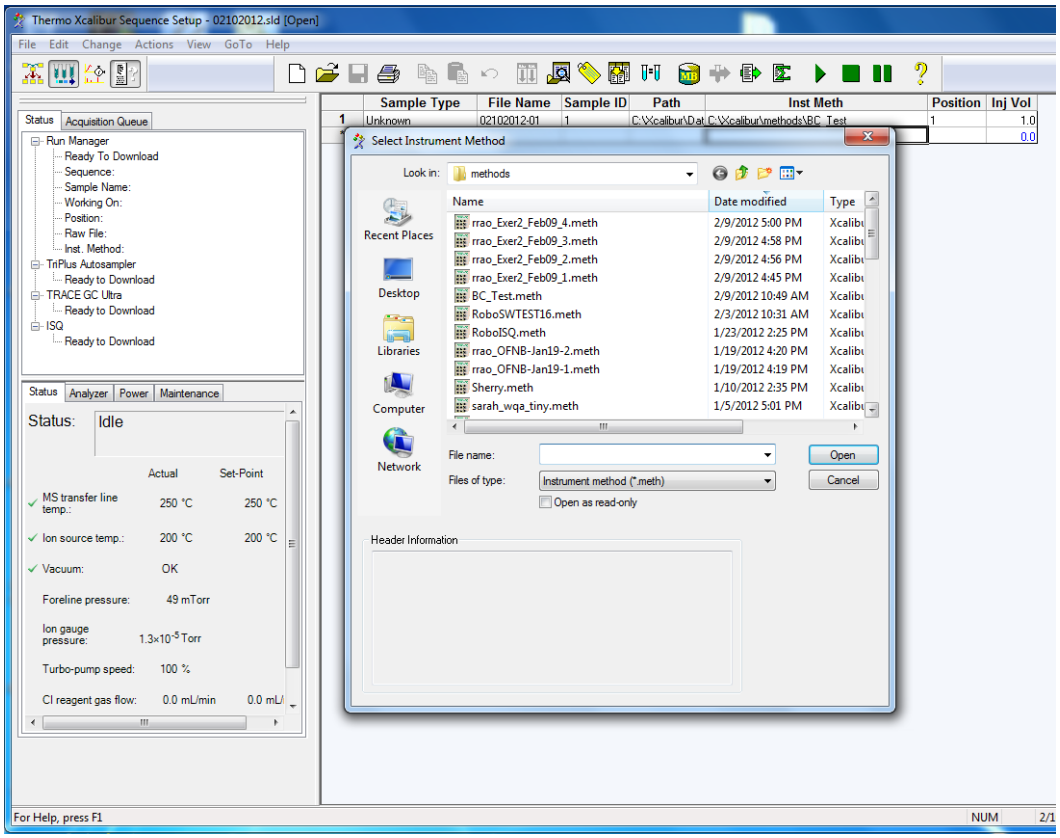
6. Double-click the first row in the **Path** column and select the folder in which to store your data files. You can also right-click on the field to clear, copy, or paste into the field, browse to a folder on your computer, or add and delete rows.

Figure 115. Selecting a Folder to Store Data Files



7. Double-click the first row in the **Instrument Method** column, 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.

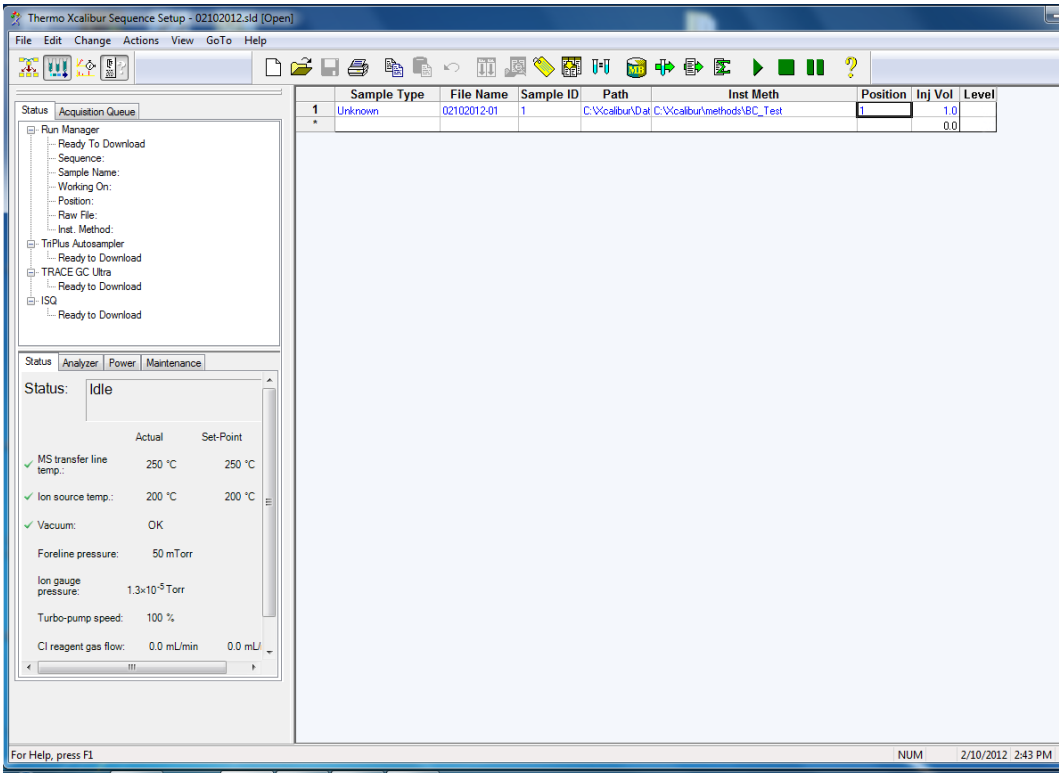
Figure 116. Opening an Instrument Method Folder



8. 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.

9. Click the first row in the **Position** column and enter the sample's vial number if you are using an autosampler. You can also right-click on the field to clear, copy, or paste into the field, as well as add and delete rows.

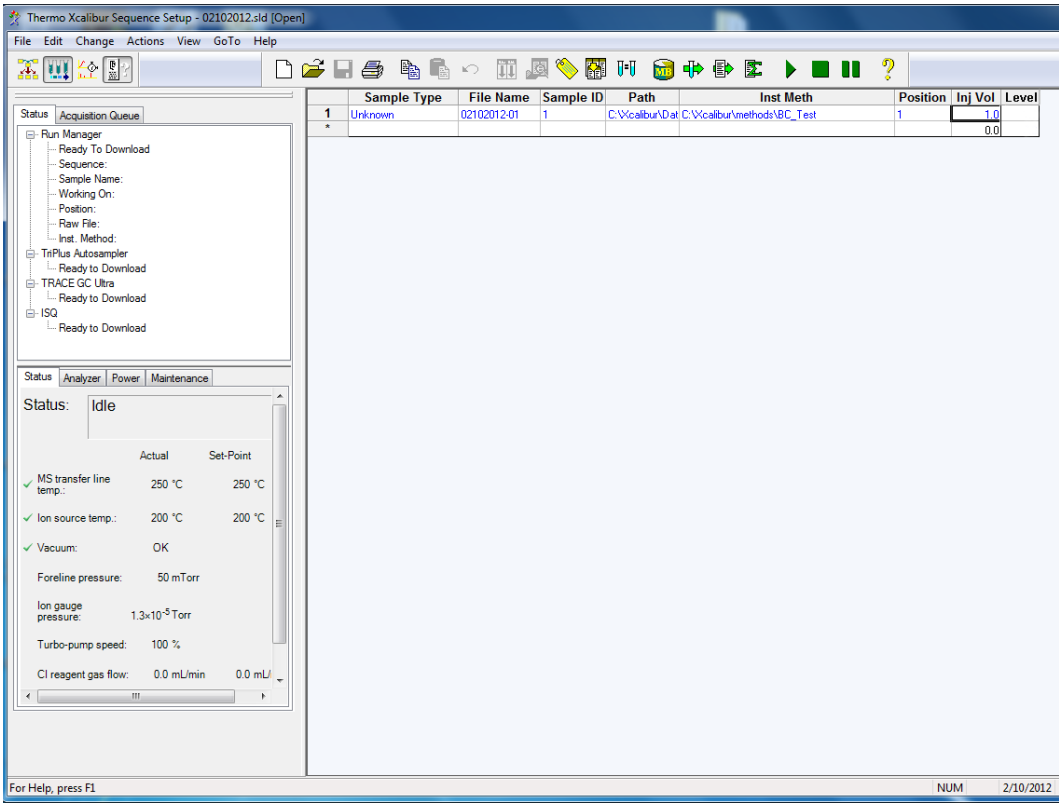
Figure 117. Entering the Sample Vial Position



10. Click the first row of the **Injection Volume** column and enter the amount of sample you are injecting if you are using an autosampler. You can also right-click on the field to clear, copy, or paste into the field, as well as add and delete rows.

> **Note** This field is not used by the AI/AS 3000 or AI/AS 1300, which use an injection volume from the instrument method. The TriPlus and TriPlus RSH autosamplers must be configured to read this value.

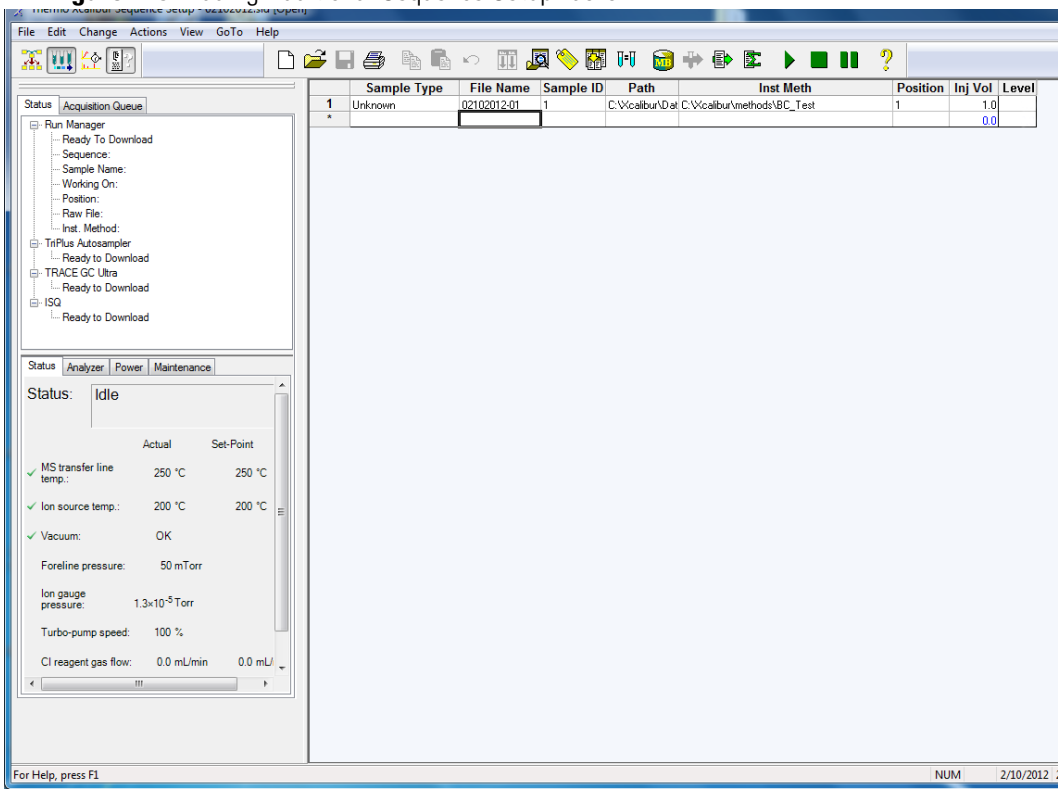
Figure 118. Setting the Injection Volume



11. 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.

12. Now that you've 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.

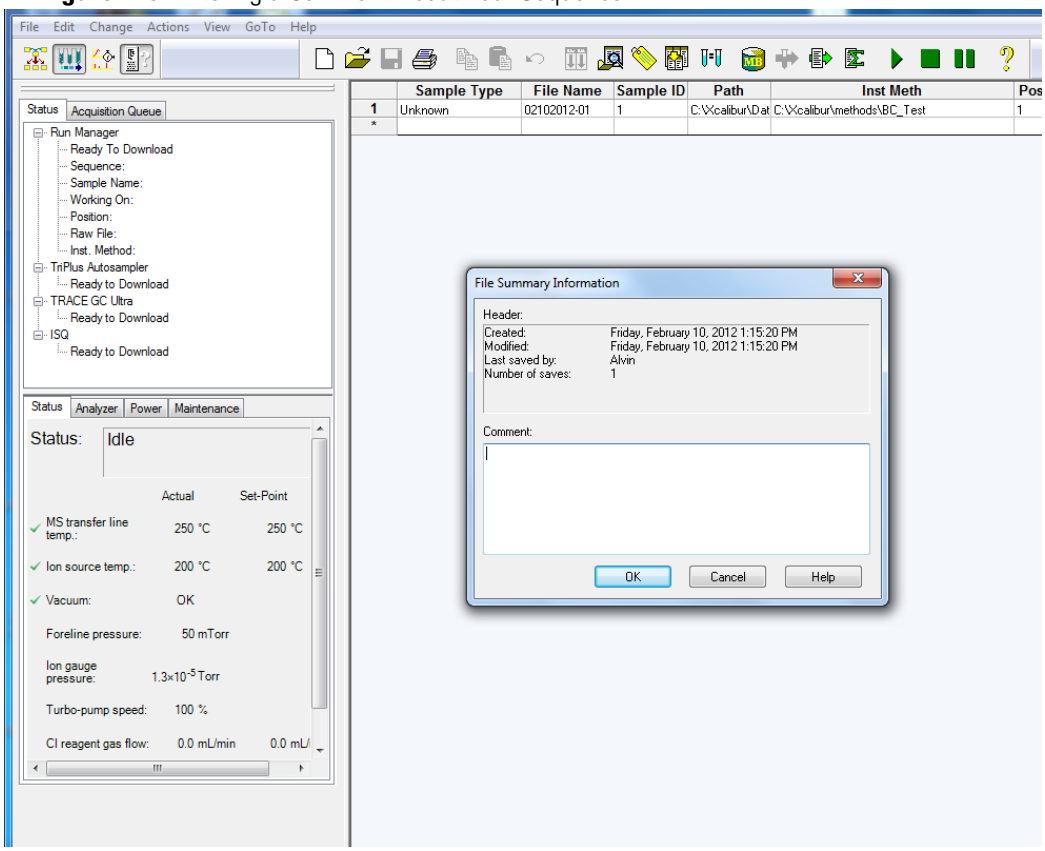
Figure 119. Adding Additional Sequence Setup Tasks



13. Once you've created a sequence of all your samples, select **File | Save** or click the icon to save the sequence.

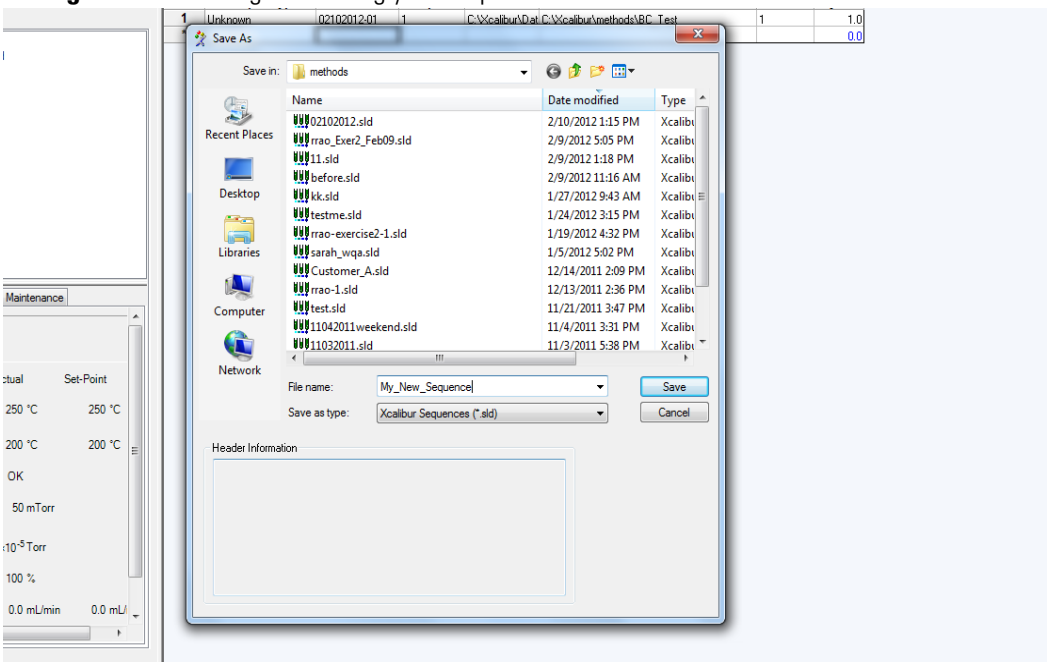
14. Enter a comment and click **OK**.

Figure 120. Entering a Comment About Your Sequence



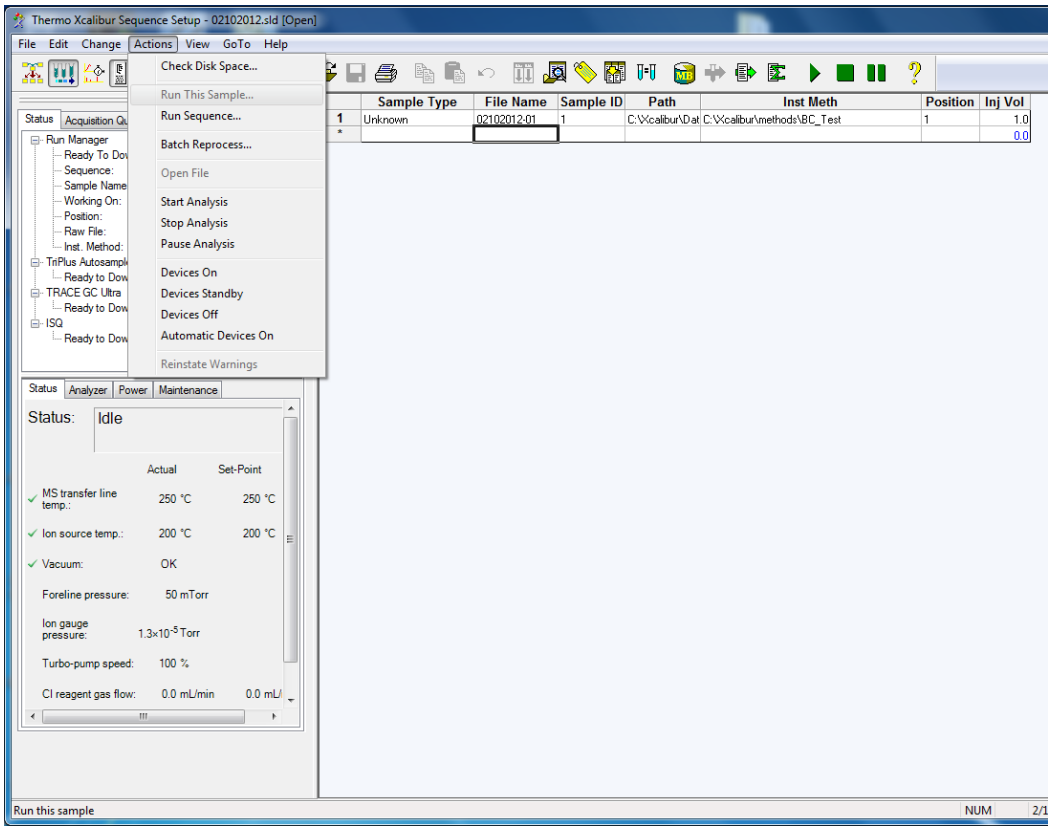
15. Enter the name of your sequence and click the **Save** button.

Figure 121. Naming and Saving your Sequence



16. 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 tool bar.

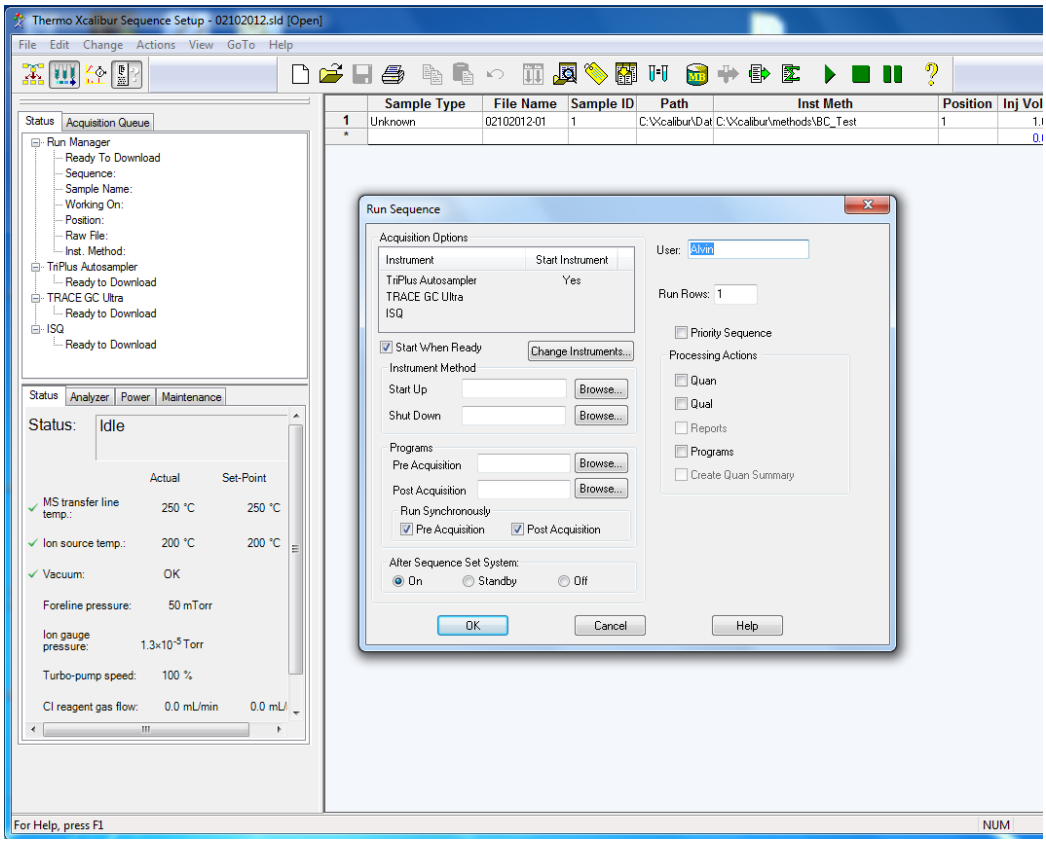
Figure 122. Selecting a Row of Samples to Run



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

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

Figure 123. Customizing a Sequence Run



a. Select the **Start When Ready** checkbox to run the sequence when the instruments are ready.

b. Click the **Change Instruments** button and use the **In Use** and **Start Instrument** columns to define the instruments that are in use and the instrument that will define the start of the sequence. Then click the **OK** button.

> **Note** If you are making a manual injection, deselect the autosampler in the **Start Instrument** and **In Use** columns. Once you make the injection, press the Start button on the GC.

> **Note** Even if the autosampler or GC are not in use, they can prevent the sequence from starting if they are not ready. If the autosampler or GC is not powered on or not in the ready state, remove its device driver from instrument configuration before starting a sequence that does not use the device.

c. In the **Instrument Method** group, you can browse to the sequence to be used when the instrument starts up or shuts down.

d. In the **Programs** group, you can browse to the executables for **Pre-Acquisition** and **Post-Acquisition**. These fields are used to automatically run processing methods after the data acquisition is complete. Because you are developing your method, you haven't defined a processing method for this field.

e. Configure the **Run Synchronously** group:

- **Pre-Acquisition**—Select this option to enable the executable to run before the data is acquired or deselect it to run the executable in parallel with data acquisition.
- **Post-Acquisition**—Select this option to enable the executable to run after the data is acquired or deselect it to run the executable in parallel with data acquisition.

f. 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 turn off the ISQ Series mass spectrometer by turning off the heaters, turbo pump and foreline pump.

IMPORTANT If you are using hydrogen as a carrier gas, manually turn off the GC after the sequence completes if you are using this setting.

g. 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.

h. In the **Run Rows** field, enter the rows to be run in the sequence. Each value must be separated by a hyphen.

i. Check 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.

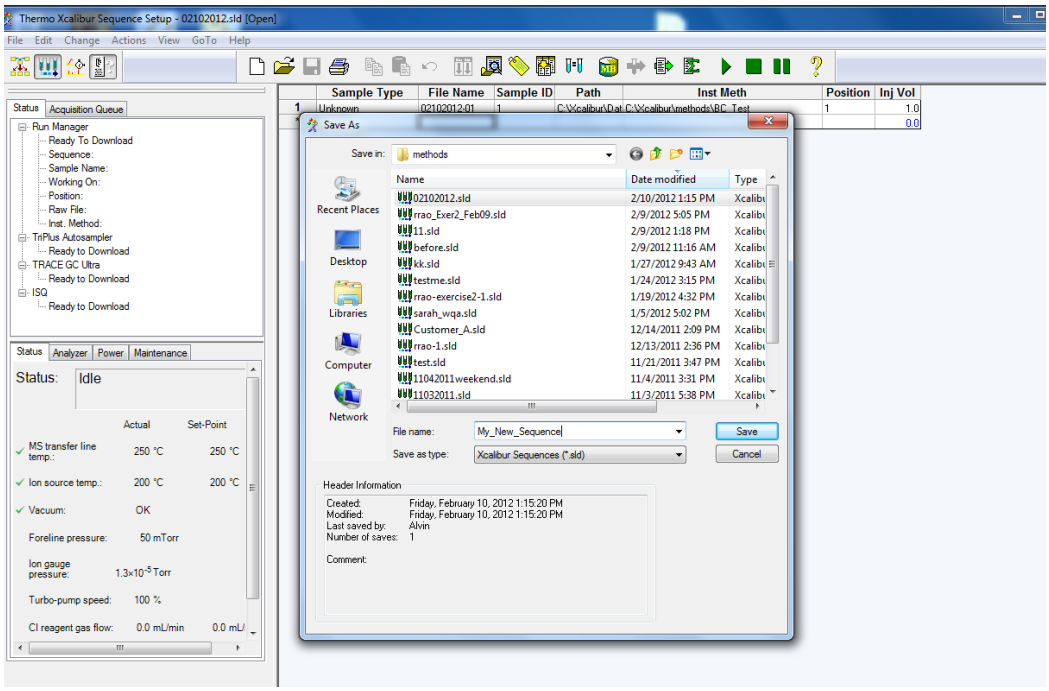
j. In the Processing Actions group, select **Quan**, **Qual**, **Reports**, **Programs** or **Create Quan Summary**. Since you are developing your method, you can skip this setting.

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.

18. Click **OK** to run the sample or sequence. and store your data on the computer.

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

Figure 124. Saving a Sequence to the Computer



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

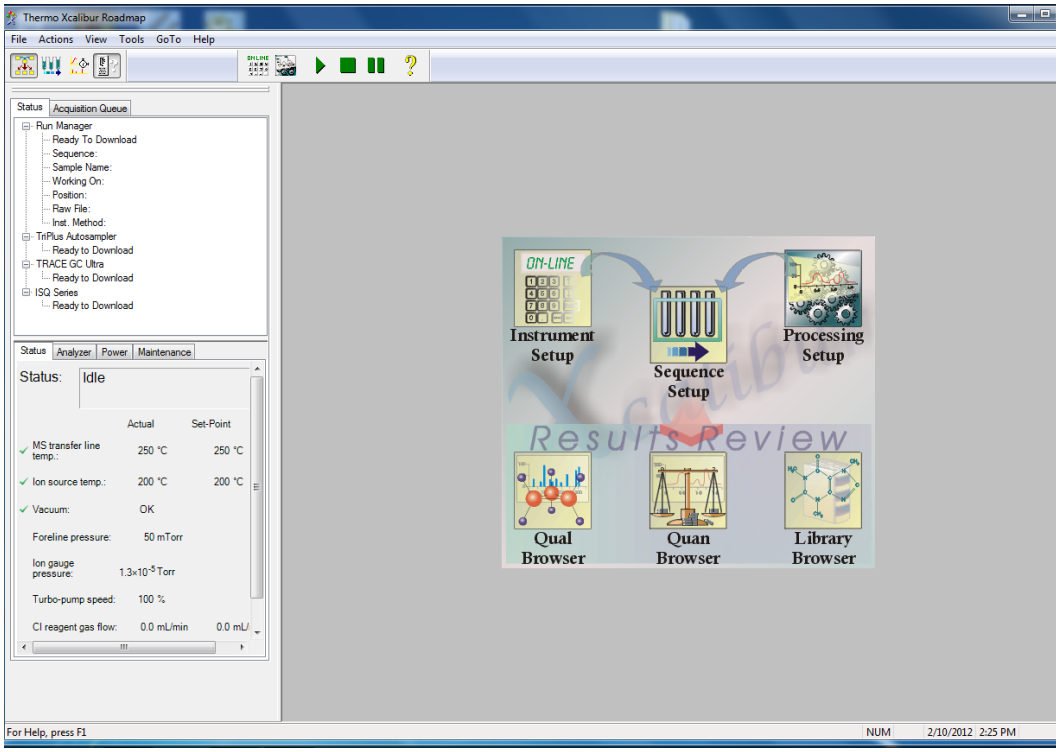
Exploring Your Data

Once you have acquired your data, you need to look at your data to make sure the peaks have a good shape and that the peak area is large enough for your needs. The Qual Browser utility of Xcalibur allows you to view chromatograms and spectra from raw files or qualitative processing result files. The data in this section refers to an instrument qualification run that was performed in the factory. This same run, using 1pg octafluoronaphthalene, was also performed by a Field Service Engineer at your laboratory. Other compounds will have different retention times and ions.

To view your data in Qual Browser:

1. Double-click the Xcalibur software icon on your computer desktop.
2. In the Xcalibur Roadmap window, click the Qual Browser icon.

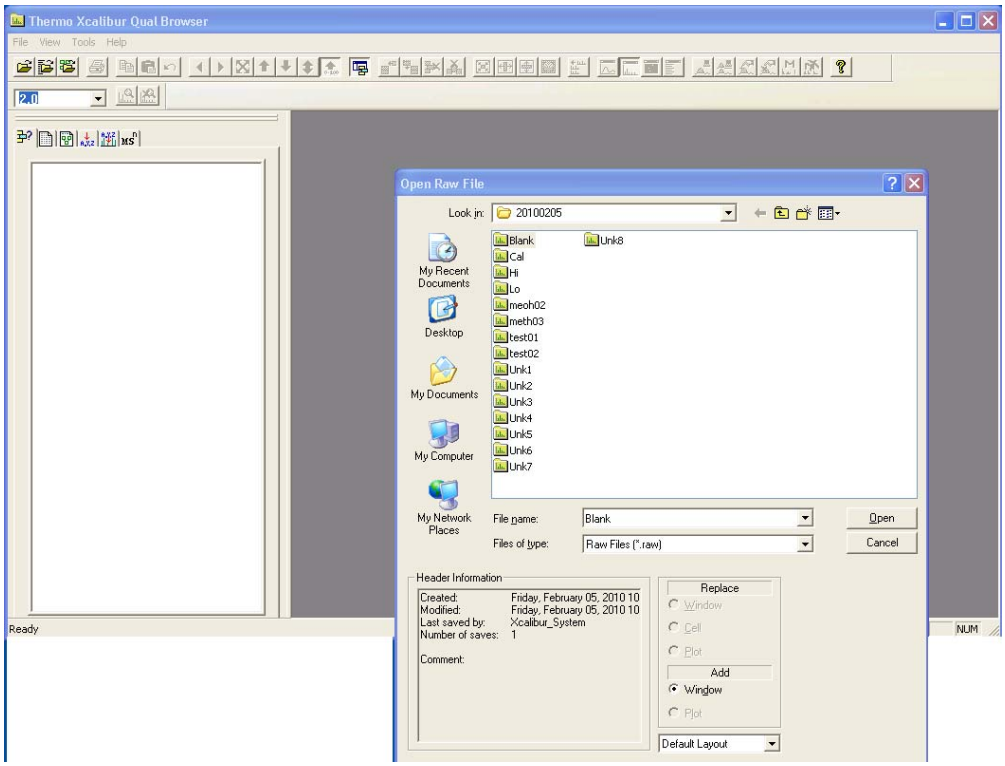
Figure 125. Xcalibur Roadmap



3. In the Qual Browser window, select **File | Open** from the main menu or click the icon.

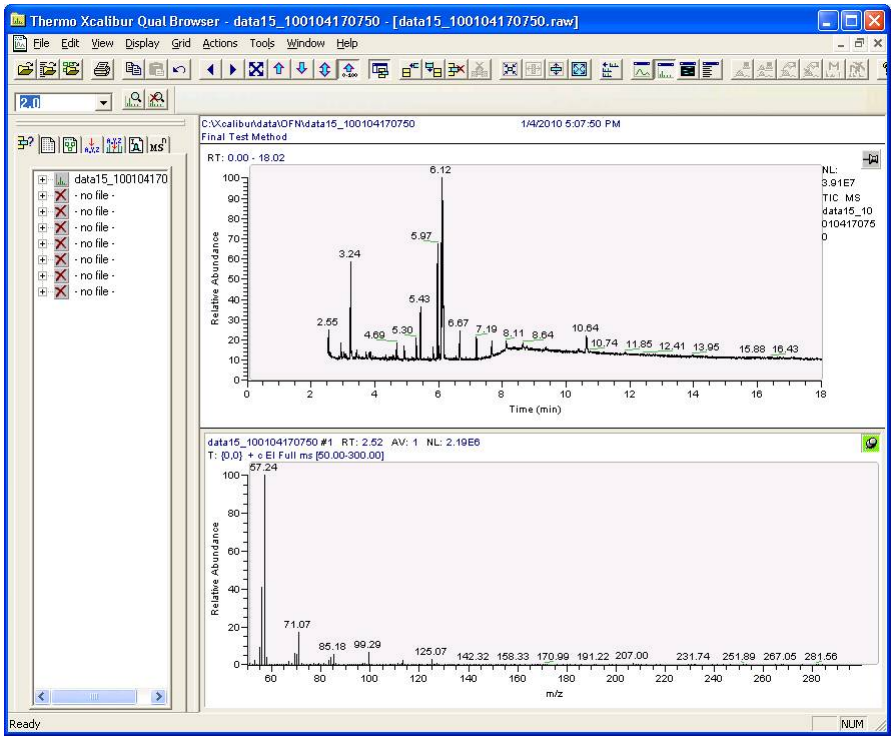
4. Select the raw file(s) you ran in your sequence and click **Open**.

Figure 126. Selecting the Raw File



5. The chromatogram is shown in the upper pane of the window and the spectra in the bottom pane.

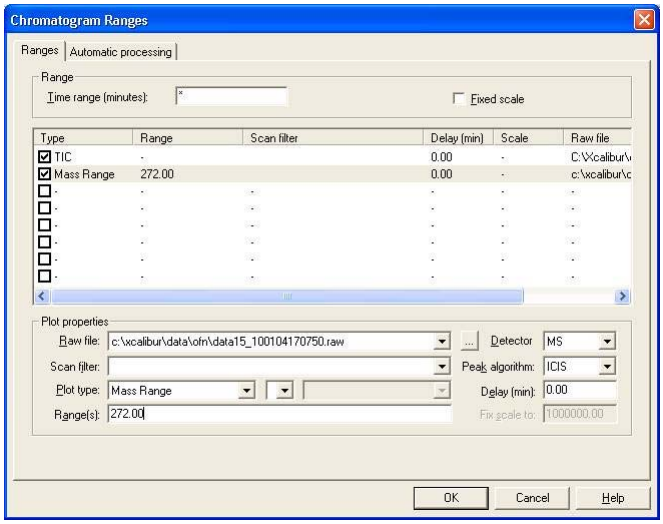
Figure 127. Viewing the Chromatogram



6. Right-click on the pinned chromatogram window and select **Ranges** from the pull-down menu.

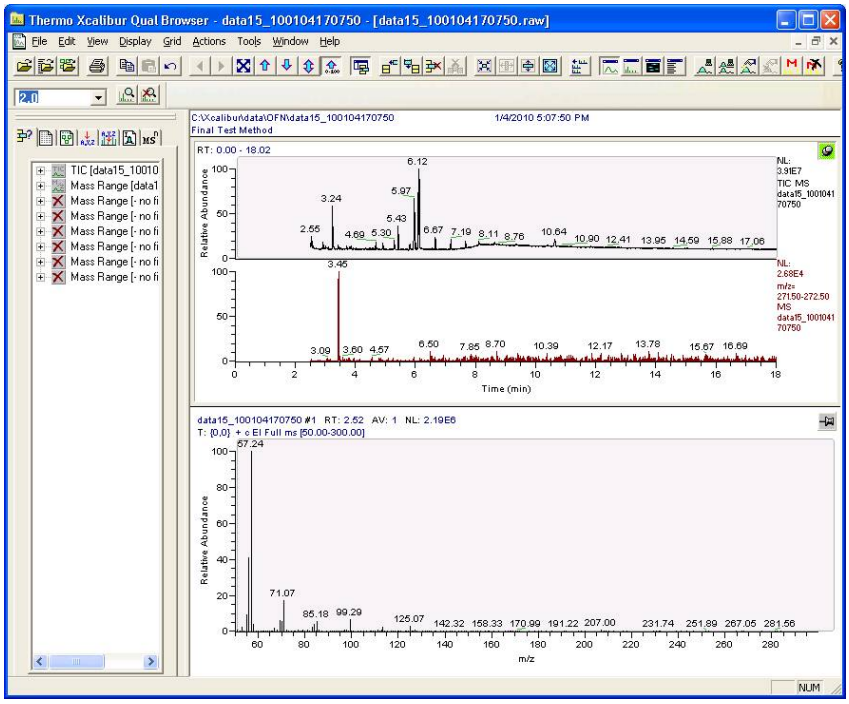
7. Specify multiple sub-panes in the chromatogram window. For OFN, which has a dominant ion at *m/z* 272, select a mass range that contains only this ion. By eliminating all the masses that are not generated by your compound, it is much easier to find and quantify the GC peak area.

Figure 128. Selecting the Mass Range



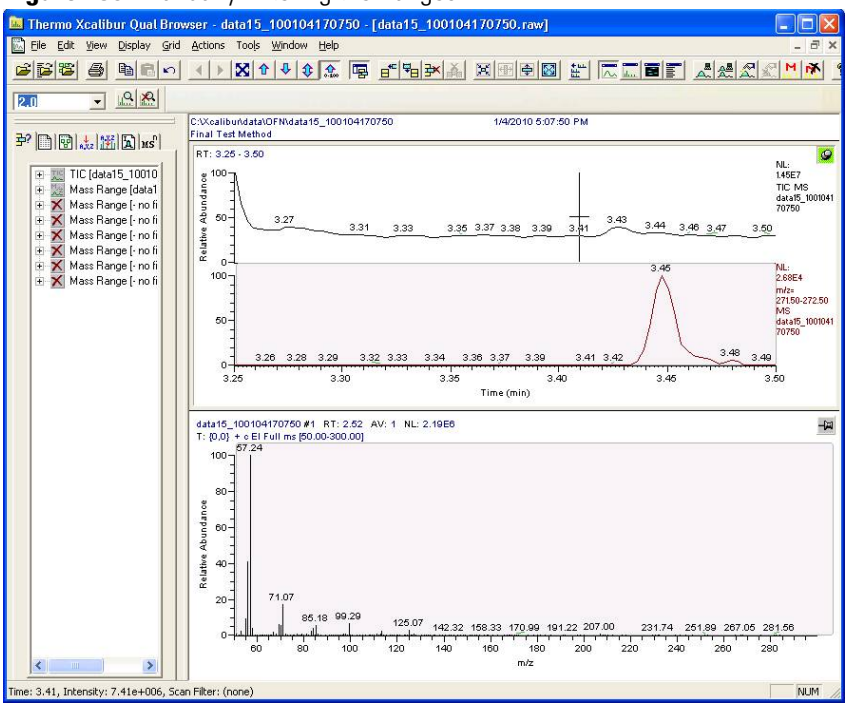
With only the *m/z* 272 ion shown, it is apparent that the OFN is around a retention time of 3.45 minutes.

Figure 129. Determining the Retention Times



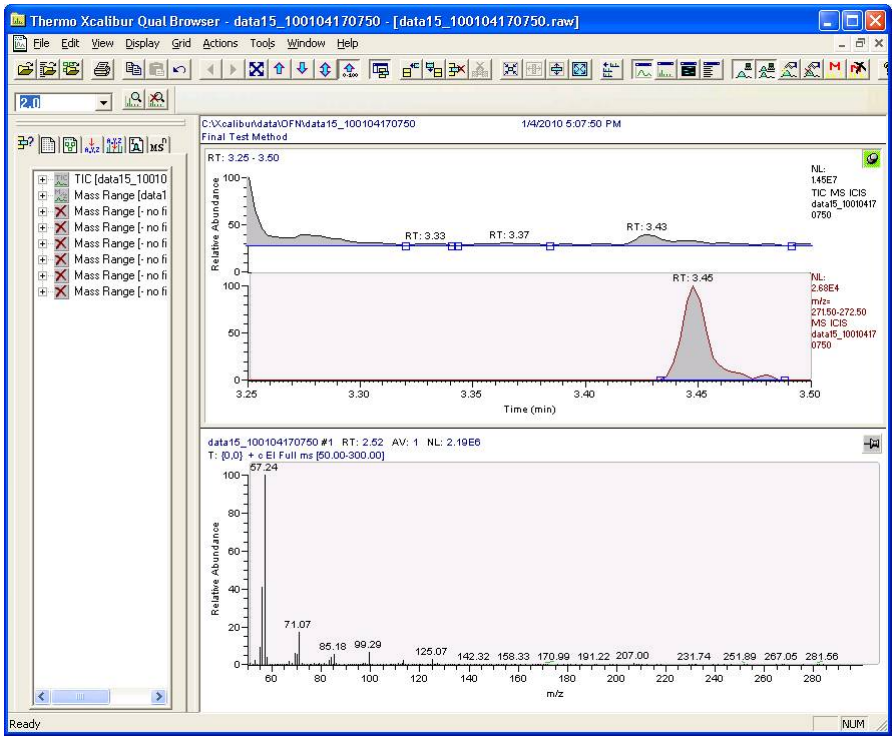
To look more closely at your data, left-click on the peak to zoom in on it. You can also hold your mouse down over the peak and draw a line over it to zoom in. Another way to zoom in is to return to the **Ranges** tab and manually enter the time range to display.

Figure 130. Manually Entering the Ranges



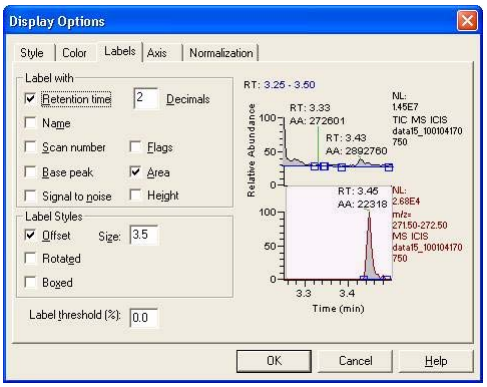
8. Right-click in the Chromatogram pane and select **Peak Detection | Toggle Detection in All Plots** to enable Qual Browser to find the peaks and define their edges.

Figure 131. Finding the Peaks in Qual Browser



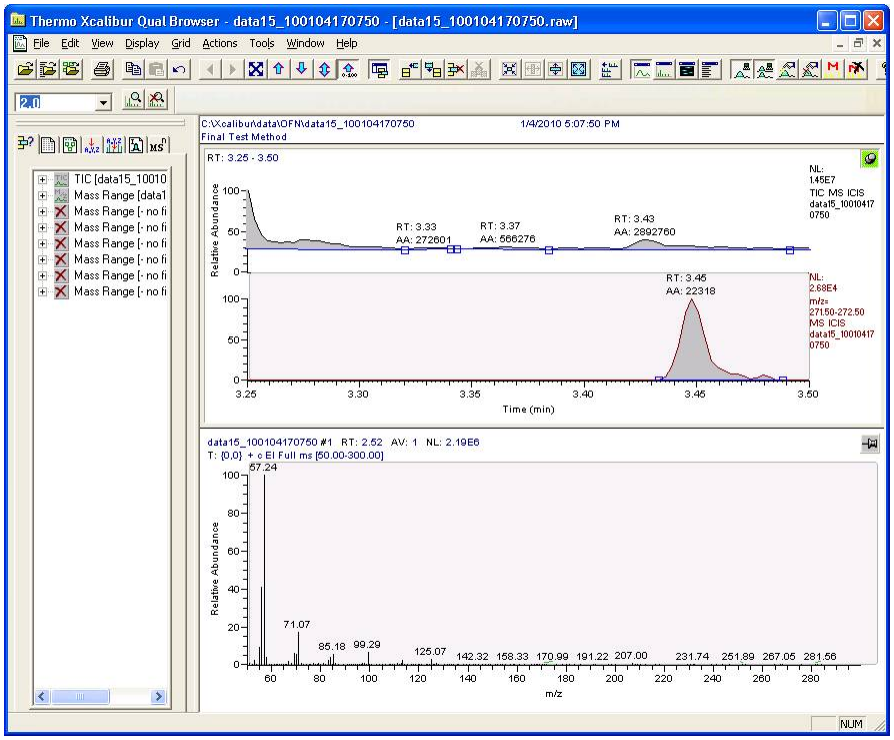
9. Right-click in the Chromatogram pane and select **Display Options**.

Figure 132. Selecting the Display Options



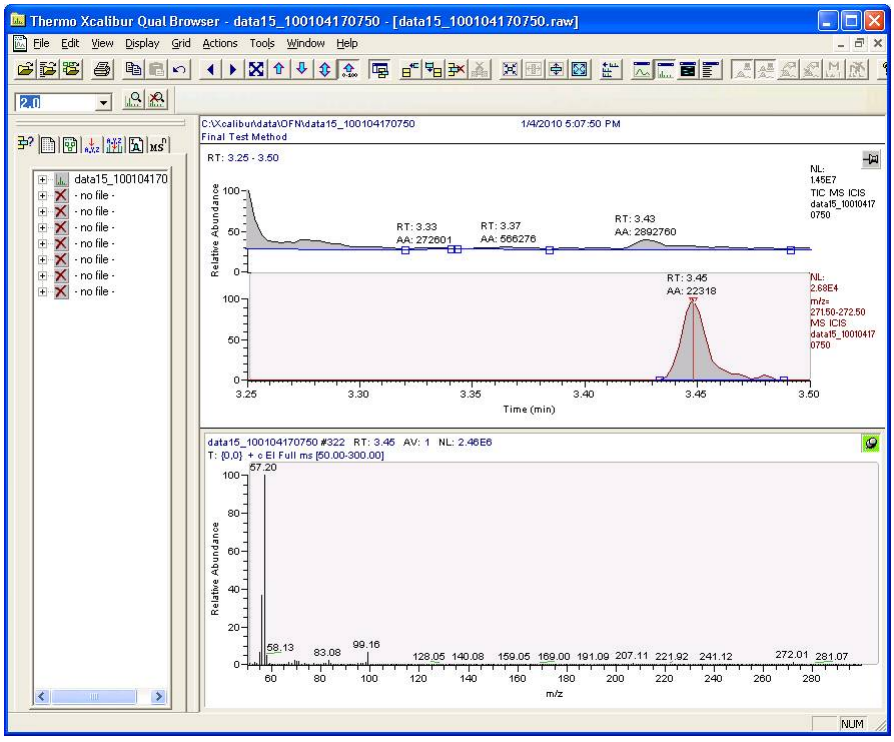
10. Click the **Labels** tab and select **Retention Time and Area** in the Label With group.

Figure 133. Labeling the Display



11. With the MS window pinned, click on the peak to open the mass spectrum at the point you clicked.

Figure 134. Opening the Mass Spectrum



12. In this spectrum, there are large peaks at *m/z* 57 and 99. These are very common ions from hydrocarbons. The OFN peak is near *m/z* 272.

Figure 135. Setting the Spectrum Range

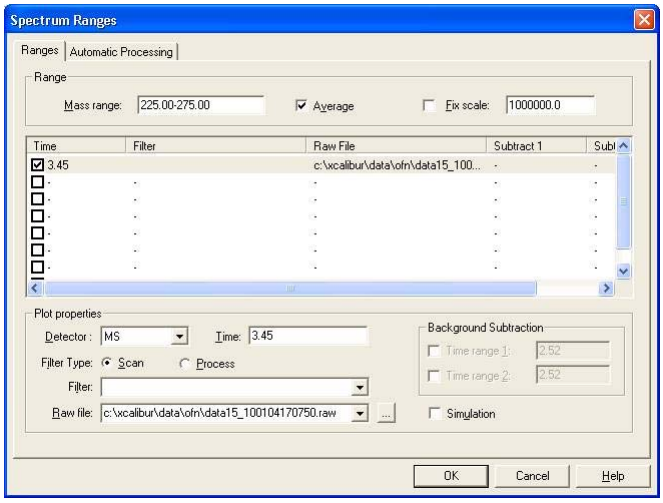
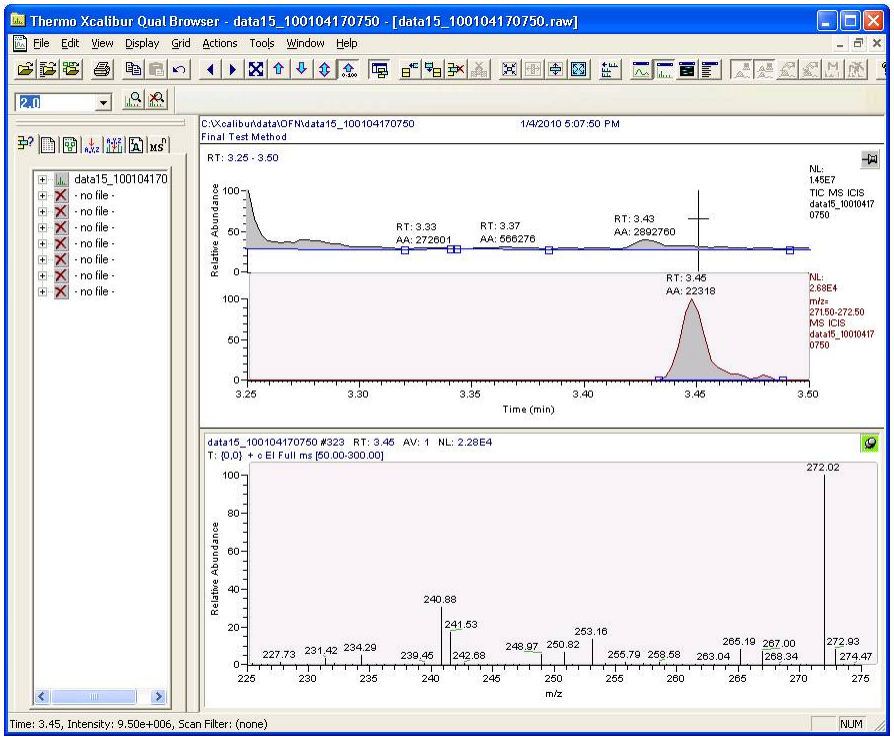


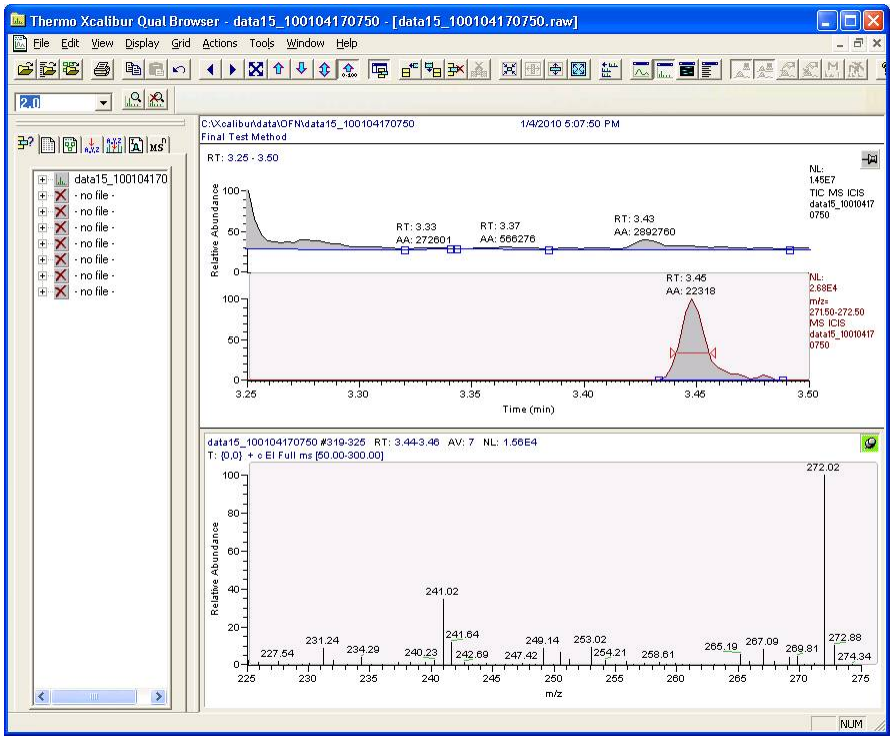
Figure 136. Finding the OFN Peak



13. Left-click on the GC peak and drag a line across it to average several points across the GC peak.

Note If the chromatogram window is pinned, this action will zoom in on the peak, but if the mass spectrum window is pinned, this action will average the spectrum.

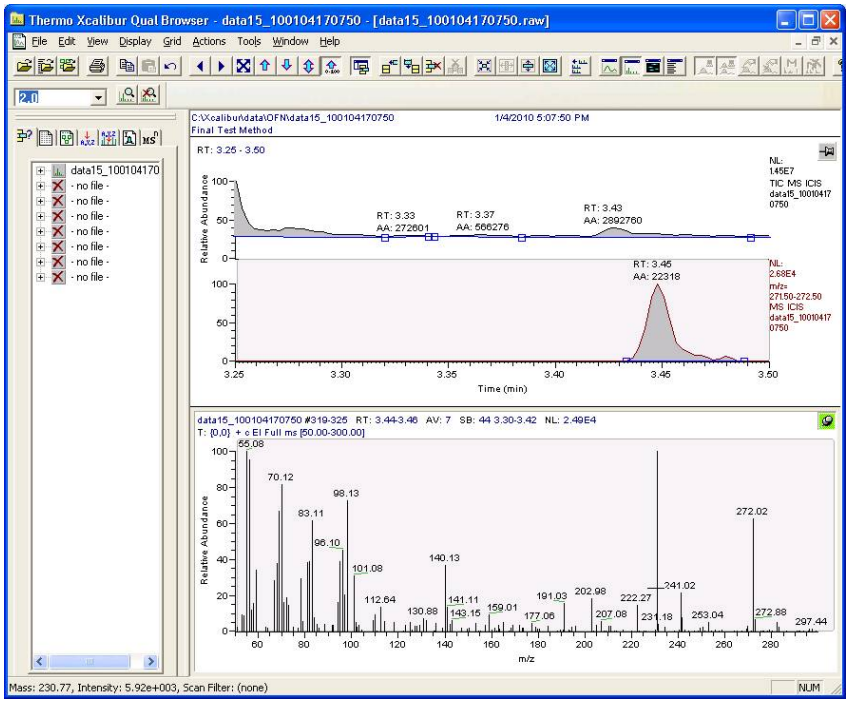
Figure 137. Averaging the Spectrum



To perform a background subtraction and improve the spectral library searching, right-click in the MS pane, select subtract spectra and then subtract one range or two. Usually, a one range subtraction is performed before the peak eluted. The range to be subtracted out should not contain any of your chromatographic peak.

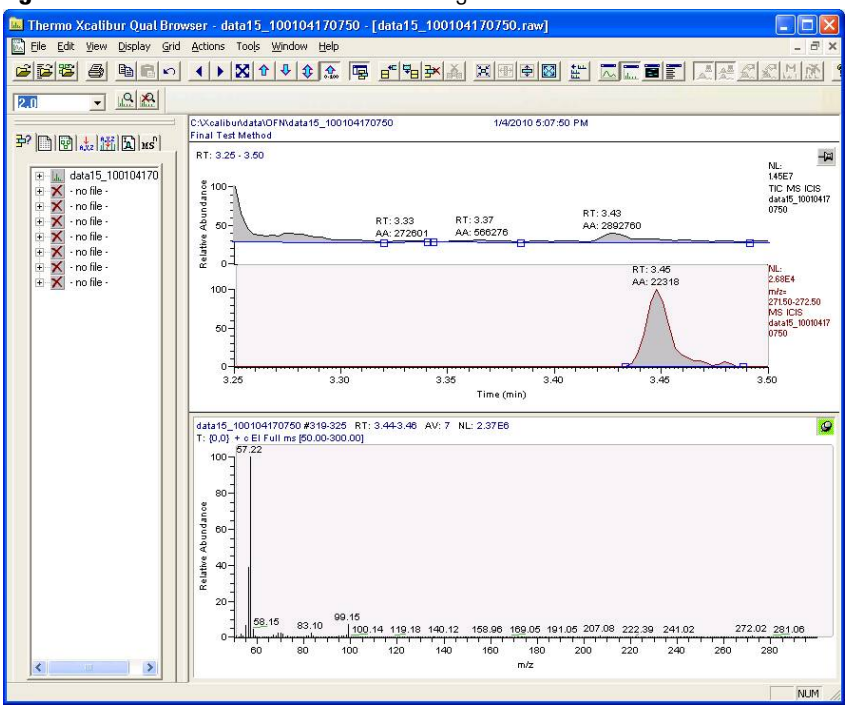
The full scan shows the ions from OFN (at *m/z* 222, 241, and 272) much more clearly than without the background subtraction.

Figure 138. OFN in Full Scan With Background Subtraction



Without background subtraction, the full scan would look like the graphic below.

Figure 139. OFN in Full Scan Without Background Subtraction



14. There are many other ways to change the way you data displays in *Qual Browser*. See the *Qual Browser* online help for details.

8

Optimizing Your Method

If you did not quite get the results you were expecting from your method, use the suggestions in this chapter to modify it to obtain better results.

Contents

Changing the Chromatographic Separation

Peak shapes are defined by the chromatographic conditions. If your peak is too wide, too narrow or not symmetrical enough, then changing the chromatographic conditions are the best way to improve your method. You should begin by changing the GC carrier gas flow or oven temperatures (the initial temperature, initial hold time, ramp temperature, final temperature for that ramp, and the hold time at that final temperature). These temperatures can be adjusted for each ramp.

It is important to keep in mind that oven changes are strongly dependent on the nature of the compounds you are analyzing. At some point, the GC oven has to be above the boiling point of the compounds you are looking for. If the GC oven is not at the boiling point, the compounds will not volatilize and they will become immobilized. Changing ramp rates is usually used to separate coeluting peaks.

Finding the Best Way to Make an Injection

Adjusting the way you get the sample from the needle into the column can sometimes improve the results of your data. Try modifying the autosampler method, injecting a different amount of liquid, adjusting the injector port temperature or flow, or changing the speed of your injection. You can also try using a hot or cold needle injection. In some cases adjusting your injection port liners may give you better results. (For detailed instructions, see the user guide for your autosampler.)

Improving the Way You Prepare Samples

Although sample preparation adds time and expense to the overall analysis, a more focused method can give you better results. Try extracting your sample in a solvent that increases the solubility of the analytes of interest, but does not increase the solubility of the other compounds. Try switching solvents if your method will allow it.

You can also use or change the phase of a solid phase extraction cartridge, which gives you similar results as changing a solvent. You can affect the way you prepare samples by changing the type of cartridge you are using.

Changing the Scan Rate

The precision of your data depends on how well you define your chromatographic peak. Typically, you get good precision when sampling ten times across the chromatographic peak. Increasing the scan rate increases the number of times you've sampled across the peak. However, increasing the scan rate too much results in mass spectral noise, which decreases your analytical precision. To optimize your scan rate, select a rate that will give you 8-12 points across a chromatographic peak.

Narrowing the Mass Range

By narrowing your mass range, you can look directly at the compounds of interest. However, if you're looking at a large number of compounds that have a broad range of mass fragments, a wide mass range makes sense. To narrow the mass range, refine your scan parameters to a smaller number. A narrower mass range will also allow you to decrease the scan rate and get the same chromatographic peak sampling. Breaking your MS method into groups allows you to create compound-specific MS settings to optimize your data.

Adjusting the Transfer Line Temperature

If your transfer line temperature is set too low, the less volatile compounds may get stuck in the transfer line and never make it into the ion source. On the other hand, if your transfer line is too hot, you could damage the column or cause a thermal breakdown in the compounds you are analyzing. Typically, the transfer line temperature should be 10 °C over the highest boiling point of the compounds of interest, but no more than the maximum safe operating temperature of the column. Increase the source temperature as well to improve response for high-boiling compounds and prevent column bleed and matrix compounds from dirtying the ion source.

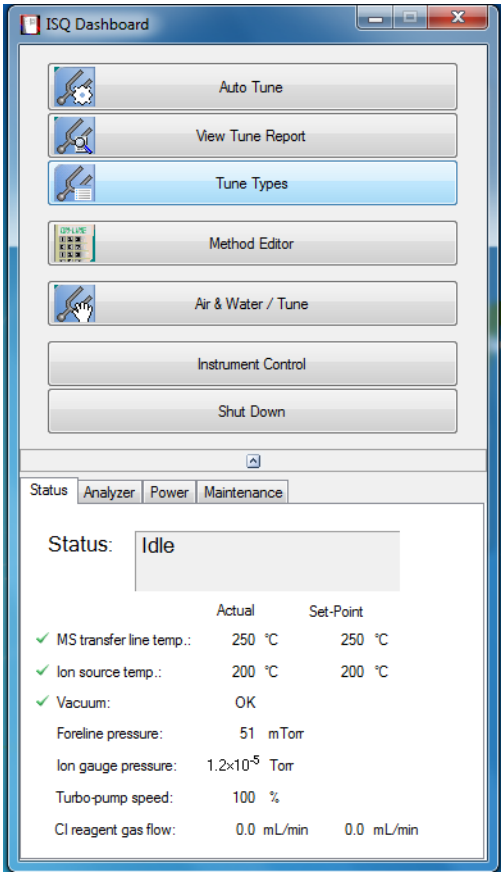
Note The transfer line temperature and ion source temperature should be similar to avoid contaminating the ion source.

Modifying an Automatic Tune

ISQ Series Autotune is a utility that uses certain parameters in the tune types to optimize system performance when generating a tune file. To modify an automatic tune:

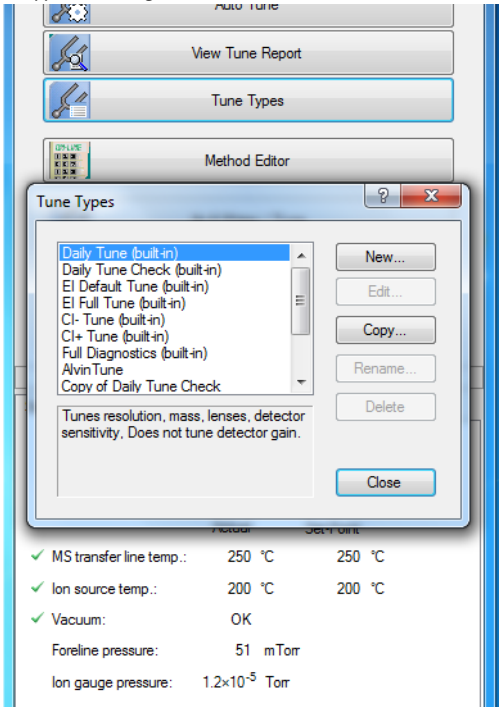
1. Click **Tune Types** on the ISQ Series Dashboard to the Xcalibur software icon on your computer desktop.

   Figure 140. Accessing the Tune Type Editor from the ISQ Series Dashboard



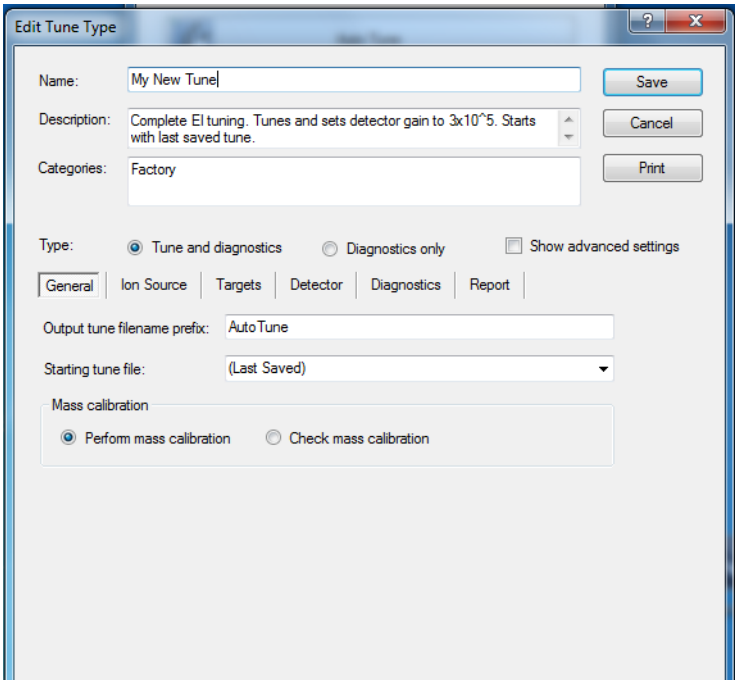
2. In the Tune Types dialog box, select a tune type to edit and click the **Copy** button.

Figure 141. Using the Tune Types Dialog Box



3. Configure the options under the **General** tab.

Figure 142. Configuring the General Options



- **Name**—Use this field to enter a name for your tune type.
- **Description**—Use this field to enter details or notes about your tune type.

- **Type**—Select **Tune and Diagnostics** to run a lens tune with diagnostics or select **Diagnostics Only** if you are creating a diagnostics test.
- **Output Tune Filename Prefix**—Use this field to enter a prefix to be added to the title of your tune report. For example, if you are always running BFB reports, you could enter BFB here to distinguish it from other reports you are generating.
- **Starting Tune File**—Use this pull-down menu to select a tune file that your tune type will be based on:

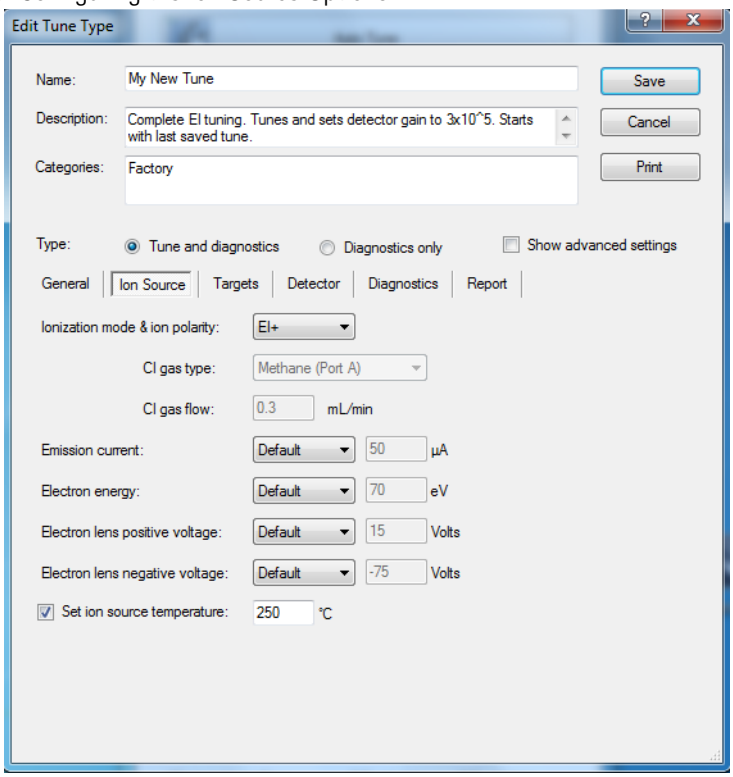
Note If you select a tune file with a prefix-only name rather than a tune file with a date-time stamp, the most recent tune file of with that prefix name will be loaded at the start of each tune.

  - Select **Factory** to use a default factory-made file on the instrument that can be used to begin tuning an instrument with a clean ion source.
  - Select **Last Saved** to use a tune file saved on the instrument by the most recent successful automatic tune. A tune file may also be loaded and saved to the instrument for the Last Saved tune file using *ISQ Series Manual Tune*.
  - Select a specific tune file if you have a reliable tune file you want to use as a test for the new tune file.

- **Perform Mass Calibration**—Select this button to enable the system to recalibrate all of the masses during a tune.
- **Check Mass Calibration**—Select this button to enable the system to confirm that your mass calibration is correct rather than performing a mass calibration.

4. Configure the options under the **Ion Source** tab.

Figure 143. Configuring the Ion Source Options



- **Ionization Mode and Ion Polarity**—Use this pull-down menu to select a mode:
  - Select **EI+** to run a tune in Electron Ionization (EI) mode.
  - Select **CI+** to run a tune in Chemical Ionization (CI) mode and positive ions.
  - Select **CI-** to run a tune in CI mode and negative ions.
- **CI Gas Type** (only enabled if you select CI+ or CI-)—Use this pull-down menu to select a gas type, but make sure your selection is the reagent gas attached to your system and that the correct gas port is selected. Methane is commonly used for CI+. Other gases will change the efficiency and energy of the ionization process.

Note When changing CI gas types or gas port, there is a two minute delay while the reagent gas is purging. During this time, the Busy light will be lit and you have to wait until the light goes off before using the ISQ Series system's software.

- **CI Gas Flow** (enabled when you select a CI Gas Type)—Use this field to enter the flow rate of your CI gas.
- **Emission Current**—Use this field to define the emission current you use to run subsequent tunes, but not the emission current that is used for data acquisition.
  - **Tune File**—Select this option to use the value in the tune file you selected in the General tab.
  - **Default**—Select this option to use the default emission current, which is 50 µA.

  - **Custom**—Select this option if you want to use a value other than 50 µA when increasing or decreasing the sensitivity of the instrument. For the emission current, the default is 50 µA. You should tune with the same value you are planning to use for your analysis. The use of emission currents above 100 µA may lead to the generation of too many ions in the source, lower filament lifetime, and cause unnecessary source degradation.

- **Electron Energy**—Use this field to indicate the energy of the electrons that come off the filament and to extend the lifetime of your filament.
  - **Default**—Select this option to use the default electron energy, which is 70 V.
  - **Tune File**—Select this option to use the value in the tune file you selected in the General tab.
  - **Custom**—Select this option to set the energy of the electrons emitted by the filament. For example, you could change the voltage if you wanted to change the ionization efficiency and fragmentation of the sample. This is typically set to 70 V because the standard EI libraries are based on 70 eV electron beams.

Note Reducing the electron energy to less than 70 eV is not recommended. The calibration compound will not be sufficiently ionized for tuning or calibrating at low electron energies.

- **Electron Lens Positive Voltage**—Use this field to allow the electrons to enter the ion volume.
  - **Default**—Select this option to use the default electron lens positive voltage, which is 15 V.
  - **Tune File**—Select this option to use the value used in the tune file you selected in the General tab.
  - **Custom**—Select this option if you do not want to use the default value for the tune. For the electron lens positive voltage, the default is 15 V. You should tune with the same value you are planning to use for your analysis. This value affects the focusing of the electron beam into the source. If you change your electron energy from 70 V, this value will also change. This voltage must always be at least 45 V above the voltage applied to the filament. The voltage applied to the filament is the same number, but the opposite sign, of the electron energy.
- **Electron Lens Negative Voltage**. This field is used to allow the electrons to enter the ion volume.
  - **Default**—Select this option to use the default electron lens negative voltage, which is -75 V.
  - **Tune File**—Select this option to use the value used in the tune file you selected in the General tab.

– **Custom**—Select this option if you do not want to use the default value for the tune. For the electron lens negative voltage, the default is -75 V. You should tune with the same value you are planning to use for your analysis. This value affects how well the electrons are kept from entering the ion source when they are not supposed to. If you change your electron energy from 70 V, this value will also change. This voltage should always be at least 5V below the voltage applied to the filament. The voltage applied to the filament is the same number, but the opposite sign, of the electron energy. If you set this value to be smaller than the electron energy, for example, if the EE is 70 eV, and the negative voltage is set to -50 V, then electrons can not be stopped from entering the ionization region. If the negative voltage is set to -75 V, though, the electrons will be blocked from entering the source. This field is not used at this time.

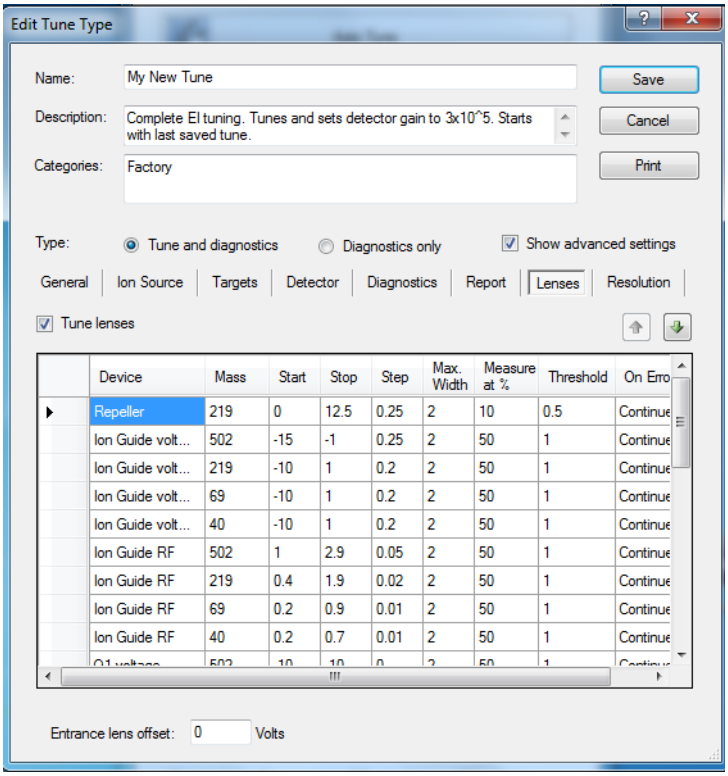
- **Set Ion Source Temperature**—Select this checkbox to enable the temperature field. Then enter a value between 0 and 350 °C (default is 200 °C). The optimal temperature is determined by the molecular structure and weight of the compounds you are analyzing. Heavier compounds require a higher temperature. You should set the temperature as high as possible to keep the ion source clean and maintain the right amount of sensitivity.

Tip If you will be tuning regularly between running sample sets, you can save time waiting for the temperatures to equilibrate by setting the tune temperatures to the same temperature used in your acquisition method.

5. Configure the options under the **Lenses** tab. The lens tune is the main portion of the tune algorithm. In this section you can choose which components to tune, which mass to optimize for the tune, what the range of allowed values are, how to move through the range, how much the signal must change for a new value to be selected, and what (if anything) should be done about the resolution of the peak and any errors that occur.

Note Click the **Advanced Settings** check box to access the **Lenses** tab.

Figure 144. Configuring the Lenses Options



- **Device**—Use this pull-down menu to select the component you want to tune according to the settings in the other columns.
  - **Repeller**—Select this option to control how the repeller pushes the ions out of the ionization region. The voltage applied to this component will have a very strong effect on the energy of the ion beam, which will have a strong effect on the resolution and the intensity. The lower the voltage, the better the resolution. However, higher voltages will prevent ions from striking the repeller surface, which leads to better robustness. Typical values are 0 to 5 V, although a dirty system may have the repeller climb as high as 12.5 V. We do not recommend setting the repeller any higher.
  - **Lens 1**—Select this option to control Lens 1, which is the first of three lenses that the ions see as they leave the ionization region. These three lenses act as a focusing element to maximize the ion beam intensity that is entering the ion guide. This field is typically set between -35 and -50 V.
  - **Lens 2**—Select this option to control Lens 2, which is the second of three lenses that the ions see as they leave the ionization region. These three lenses act as a focusing element to maximize the ion beam intensity that is entering the ion guide. This field is typically set between 0 and -15 V in EI mode. In CI, the optimal voltage may be between 0 and -30 V.

  - **Lens 3**—Select this option to control Lens 3, which is the last of three lenses that the ions see as they leave the ionization region. These three lenses act as a focusing element to maximize the ion beam intensity that is entering the ion guide. This field is typically set near the same voltage as lens 1.
  - **Ion Guide DC**—Select this option to control the ion guide's DC offset voltage. It can potentially help focus the ions into the quadrupole while ensuring that neutral noise is eliminated. The voltage on this component is mass dependent and should be set at several different masses. This field is typically set between +1 and -15 V, depending on the mass of the ion.
  - **Ion Guide RF**—Select this option to control the ion guide's RF voltage. It can potentially help focus the ions into the quadrupole while ensuring that neutral noise is eliminated. The voltage on this component is mass dependent and should be set at several different masses. This field is typically set between 0 and +5 V, depending on the mass of the ion.
  - **Q1**—Select this option to control the voltage that pulls the ions into the quadrupole. The voltage applied to this component will have a very strong effect on the energy of the ion beam, which will have a strong effect on the resolution and the intensity. The lower the voltage, the better the resolution. However, higher voltages will pull more ions into the quads, which leads to better signal. This field is typically set between 0 and -5 V.
  - **Resolution**—Select this option to adjust the ratio of the quadrupole DC and RF voltages to create the resolution required for your analysis. You can set the desired peak width at a given mass and whether you measure the width at 10% or 50% of the peak height. Because there is no static DC voltage involved, the start, stop, and step values are not used.

- **Mass**—Use this pull-down menu to select the ion to be used for tuning.
- **Start**—Use this field to enter the starting voltage for the tune. The start voltage must always be less than the stop voltage. For example, -35 is smaller than 0.
- **Stop**—Use this field to enter the final voltage for the tune.
- **Step**—Use this field to enter the increment for the tuning range. For example, if you tune from 0-50 in increments of 10 V, then you would set the **Step** field to 10.

Note If the start and stop values are the same value, the device will be set to that value and ignore the step size. You may notice that in some tunes devices are set to a value before tuning them in another line. This is done because ramping devices will not allow the tune to run at values higher than those chosen for higher masses for the same device.

- **Max Width**—Use this field to enter the maximum allowable width of the ion during the tune.
- **Measure at %**—Use this pull-down menu to select the location on the peak at which you want to measure the maximum width.
  - **10**—Select this option to measure the width at 10% of the peak height.

  - **50**—Select this option to measure the width at 50% of the peak height.

- **Threshold**—Use this field to enter the change in intensity that has to occur when the tune to select a new voltage. For example, if you set this field to 1.1, the tune will not select a new voltage for that component, unless the intensity is 110% of the old intensity. If you set this field to 1, anytime the new voltage has an intensity larger than the old intensity, the tune will select the new intensity.

Note If a value less than 1 is chosen for ramped devices, the tune will step back in energy until the lower intensity is detected. For example, if 0.8 is chosen, the device value lower in energy than the device value with the maximum response which gives an intensity closest to 80% of the maximum intensity will be chosen.

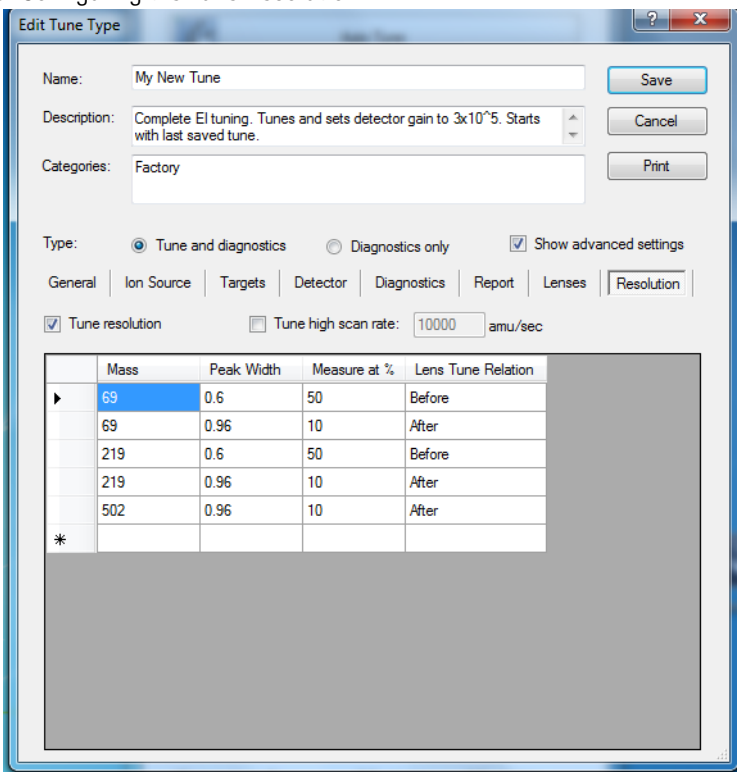
- **On Error**—Use this pull-down menu to select how to handle an error in the tune.
  - **Fail**—Select this option to stop the tune when an error occurs or if no tune points are found meeting the tune criteria.
  - **Continue**—Select this option to allow the tune to continue on to the next device when an error occurs.

6. Click the **Resolution** tab to configure the resolution of your tune. You can tune the resolution during a lens tune or you can tune the resolution by itself.

Note Click the **Advanced Settings** check box to access the **Resolution** tab.

- **Tune Resolution**—Select this option to tune the resolution by itself. This resolution will tune the system at 100 and 1000 amu/s scan rates. You may also tune at a higher scan rate.

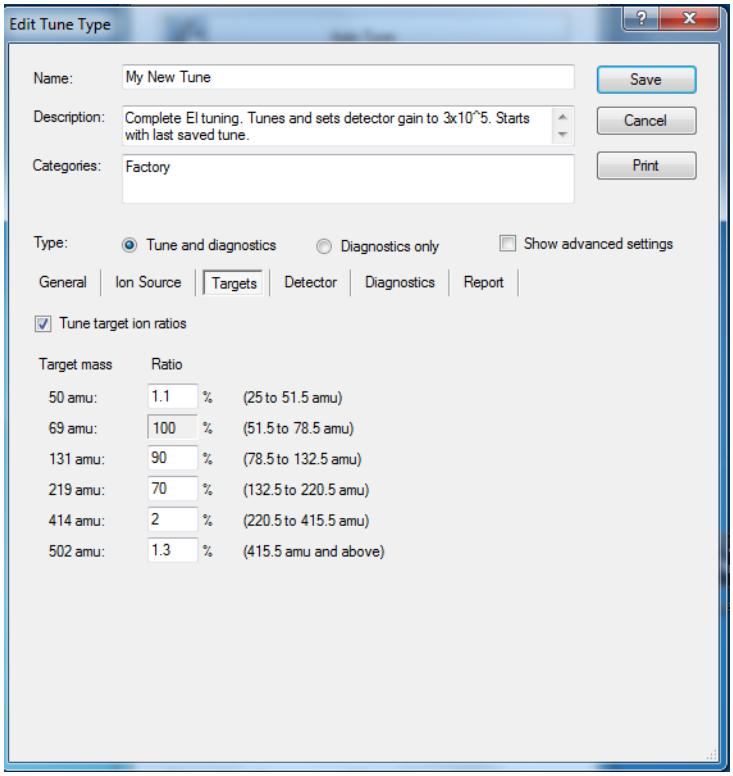
Figure 145. Configuring the Tune Resolution



- **Mass**—Use this pull-down menu to select the ion to be used for tuning.
- **Peak Width**—Use this field to enter the target peak width.
- **Measure at %**—Use this pull-down menu to select the location on the peak at which you want to measure the target peak width.
  - **10**—Select this option to measure the width at 10% of the peak height.
  - **50**—Select this option to measure the width at 50% of the peak height.
- **Lens Tune Relation**—Use this pull-down menu to set the occurrence of the resolution tune parameters before or after a lens tune.
  - **Before and After**—Select this option to use the same resolution parameters before and after a lens tune.
  - **Before**—Select this option to use the resolution parameters before a lens tune.
  - **After**—Select this option to use the resolution parameters after a lens tune.
- **Tune High Scan Rate**—Select this checkbox to tune the resolution at a scan rate in addition to the 100 and 1000 amu/s default scan rates.

7. Click the **Targets** tab to configure how you want to tune your targets. Target tuning is used to adjust the way the ISQ Series instrument tunes to meet regulatory requirements.

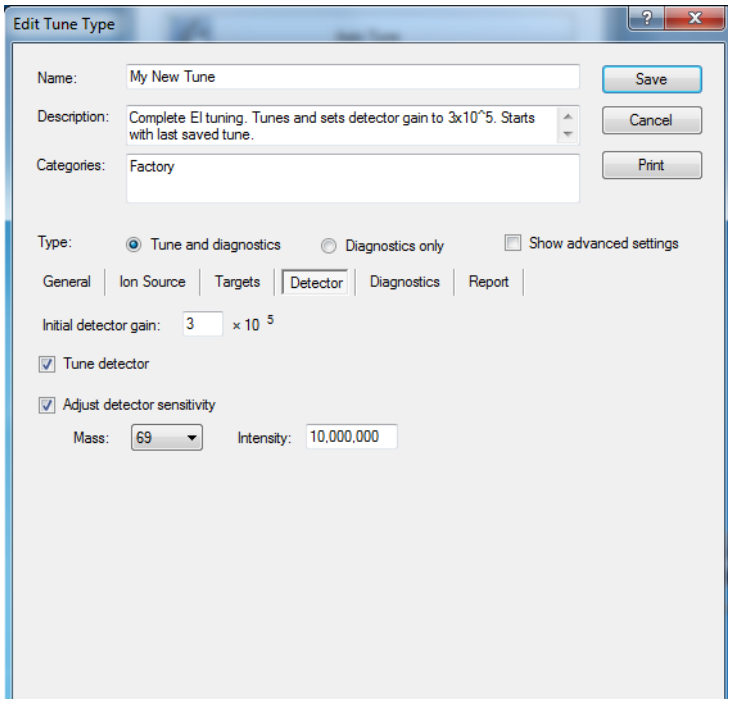
Figure 146. Configuring the Target Ion Ratios



- **Tune Target Ion Ratios**—Select this checkbox to adjust the ratios based on the results from an injection of tuning compound.

8. Configure the options under the **Detector** tab.

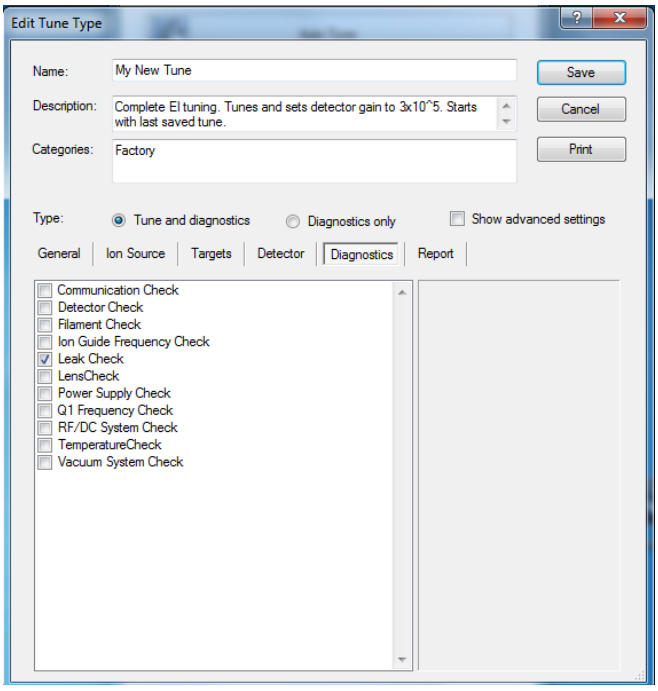
Figure 147. Configuring the Detector Options



- **Initial Detector Gain**—Select this option to set the true gain of the electron multiplier. The gain is the number of electrons generated for every ion that strikes the detector. This is typically set between 1 x10^5 and 3x10^5 electrons per ion. Gains larger than this will generate more electrons per ion, but both the analyte ion and the noise ion signals will be larger. You can also tune to lower gain values, which decreases the signal strength. Lower values also increase the chance that an ion will not be detected. As the electron multiplier ages, the voltage required for a given gain will increase. Depending on your sample load and if your system is leak tight (oxygen is bad for the detector), you should not have to perform this tune very often.
- **Tune Detector**—Select this checkbox to tune the detector.
- **Adjust Detector Sensitivity**—Select this checkbox to tune the detector to generate a consistent area count of a calibration gas ion for the tune report. Because the intensity of the cal gas varies depending on the atmospheric pressure and temperature of the lab, this option will result in larger variation in the analytical runs as compared with using a fixed detector gain.
  - **Mass**—Use this pull-down menu to select the calibration gas mass you want to use.
  - **Intensity**—Use this field to enter the intensity you want to see on the tune report.

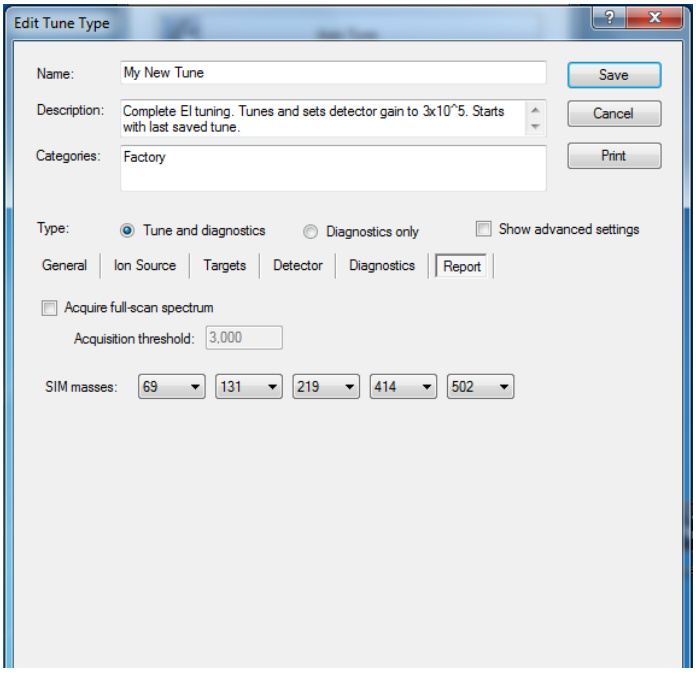
9. Click the **Diagnostics** tab and select a test to confirm the operational ability of the ISQ Series system.

Figure 148. Accessing the ISQ Series System Diagnostics



10. Click the **Report** tab configure how you want to view your data.

Figure 149. Configuring the Report Options

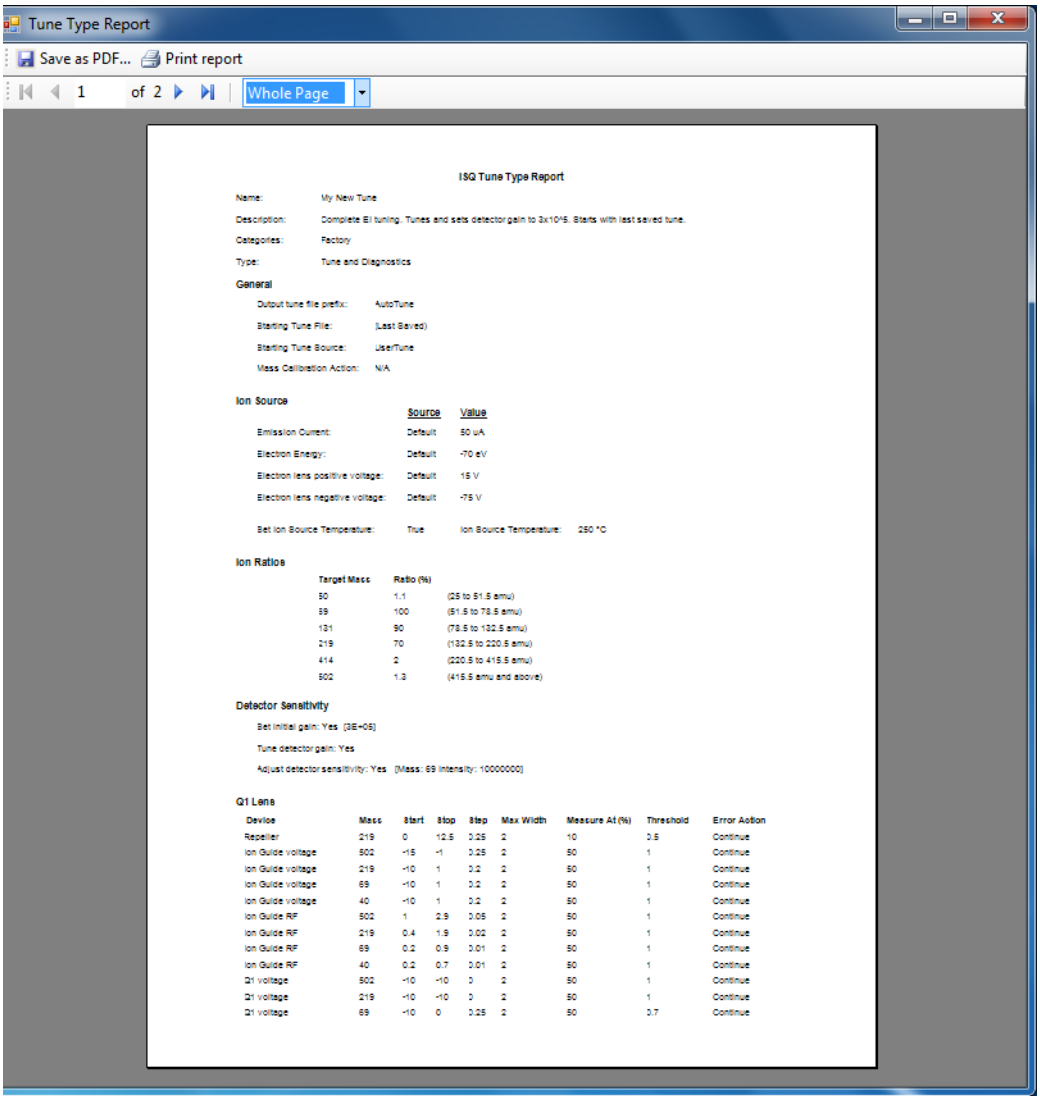


- **Acquire Full-Scan Spectrum**—Select this checkbox to display the full spectrum on your tune report.

- **Acquisition Threshold**—Use this field to enter a minimum peak height for the data file. If your peak has an intensity that is below this threshold, it will not be stored.
- **SIM Masses**—Use these pull-down menus to select masses to be displayed on your tune report. You can select a maximum of five masses, one from each menu.

11. Once you are finished configuring all the tabs, click the **Save** button to save the tune type. Your new tune type is now in the Tune Types window.
12. Click **Print** to save your tune type information as a PDF or to print a hard copy of the information.

Figure 150. Printing the Tune Type Information



13. Click **Close** to return to the Method Setup window. Now the tune type can be selected during an automatic tune.

Troubleshooting

This section contains information to help you diagnose problems with your data. A lot of times, your experience as a scientist will enable you to look at your data and detect that something is wrong either with the instrument or your sample. This chapter describes the most common indications of a problem with a baseline, peak or result.

Contents

As you review your data, you may notice issues with the baseline, peaks, or results. Use the information in this section to troubleshoot and resolve the issue. If there is an issue with the hardware, see the *Troubleshooting* section of the *ISQ Series Hardware Manual*.

WARNING - ELECTRICAL SHOCK HAZARD: When troubleshooting any issue that requires removing a cover on the ISQ Series MS, you should power-off and vent the instrument to avoid any harm to yourself.

A good first step for troubleshooting is to run a tune on the ISQ Series system. If you have good ion intensities, good peak shapes, and no air leak, you might want to look first at the GC, autosampler, or carrier gas.

If you have an air leak, locate and address them. Pay particular attention to the transfer line ferrule, vent valve knob, front panel, and vacuum interlock on the ISQ Series instrument, as well as the inlet on the GC.

If your intensities are too low, make sure carrier vacuum compensation is turned on.

IMPORTANT When inserting a cold ion source cartridge such as after cleaning or when switching between EI and CI modes, the ion source and lens stack will expand as the source cartridge heats, often pushing the ion volume and lenses away from the rear of the instrument where they are firmly held by the RF Lens spring contacts. To avoid intermittent electrical contacts to the lenses, you should insert the ion source cartridge, wait 30 min for it to get to temperature, then remove and reinsert it. See the *ISQ Series Hardware Manual* for instructions on cleaning and inserting the ion source cartridge.

Setting Instrument Conditions for Troubleshooting

Before troubleshooting the ISQ Series system, set the instrument to the conditions in this section in order to compare your system more accurately to the values in the section. All troubleshooting should be performed in EI mode. Once EI mode is working, check CI conditions if relevant.

IMPORTANT Use only Nitrile Cleanroom gloves when touching ion source components. Other types of gloves deposit contaminants on the source components that affect system performance. See the *ISQ Series Spare Parts Guide* for ordering information.

- Clean the ion source cartridge. See the *ISQ Series Hardware Manual* for instructions.
- Install a 15 m × 0.25 mm × 0.25 mm GC column (If using a different column, pressure readings may vary.)
- Ion Source Temperature — 200 °C
- Transfer Line Temperature — 250 °C
- Vacuum Compensation — On
- Column Flow Rate — 1.2 mL/min
- Foreline Pressure — < 100 mTorr

  Note Foreline Pressure is a function of how long the interior of the manifold has been exposed to the atmosphere, the pumping capacity of the turbo pump, length of the foreline hose, and other criteria. As an example, if the system has been recently exposed to atmosphere, the foreline pressure will be above the expected value.

- Pump down the system. Pump down time varies depending on the size of the turbomolecular pump installed.

Note There are no vacuum readings on a standard ISQ QD instrument as it lacks a convectron gauge.

- Air/Water Check — Water (*m/z* 18/69) < 240%

Once you have applied the settings in this section, and have allowed the ISQ Series system to equilibrate, run an EI diagnostic tune even if you cannot see any ion intensities.

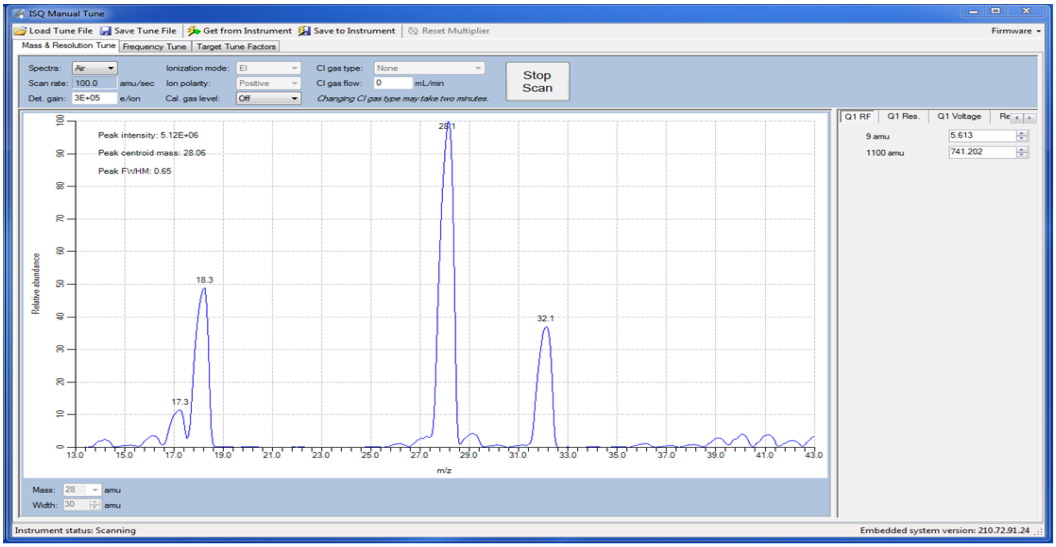
Checking Air/Water Spectra

Before running additional diagnostics, check the air/water spectra of your ISQ Series system in the ISQ Manual Tune utility and use the information in this section to troubleshoot your system.

❖ To check Air/Water spectra on the ISQ Series system

1. Insert the EI ion source if it is not already in place.
2. Open the ISQ Dashboard. Check that all the Status indicators are green and that the turbo pump is set to 100%.
3. Select **Air & Water/Tune** and check the air/water spectrum. See Figure 151.

Figure 151. Typical ISQ Series Air/Water Spectrum for a System with Helium Carrier Gas



The correct settings should be:

- Detector Gain = 3×10^5
- Peak Intensity ≥ 3e6

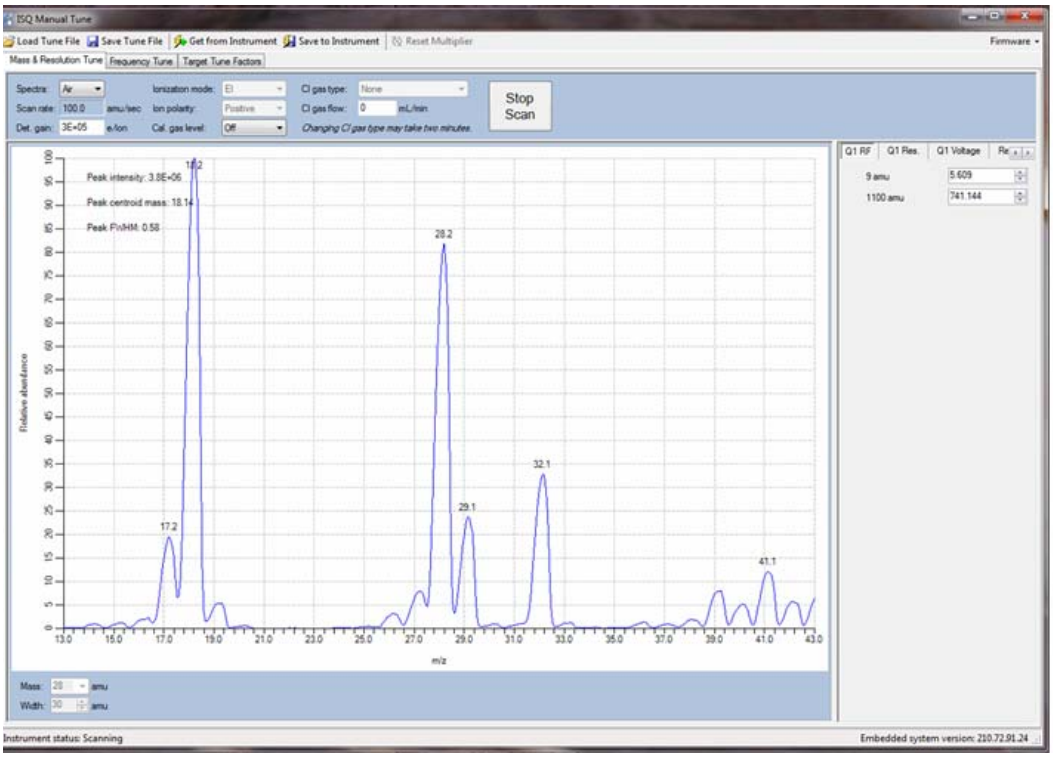
Note Peak intensity varies depending on variables such as the amount of water and nitrogen in the helium gas supply and the column flow.

- Normal ions are present and in acceptable ratios:
  - 18 (Water) — 20–300% of N_2
  - 28 (N_2) — Reference (base) Peak
  - 32 (O_2) — 10–40% of N_2
  - 40 (Argon) — <10% of N_2

> **Note** Do not expect air and water ions to be at relative abundances from atmospheric air. Nitrogen is a common contaminant within the carrier gas and is not removed with most filters, while oxygen is typically removed from helium by most gas filters.

4. Using hydrogen as a carrier gas changes the air/water spectrum on the ISQ Series system. It general more background peaks due to the increased reactivity of the hydrogen gas with the components inside of the sample path. See Figure 152 for an image of a typical ISQ Series air/water spectrum when hydrogen is used as a carrier gas.

Figure 152. Typical ISQ Series Air/Water Spectrum for a System with Hydrogen Carrier Gas



The following conditions can cause changes in the air/water spectrum on the ISQ Series instrument.

1. Standard detector gain is equal to 3e5, but this can vary depending on customer defined tunes.

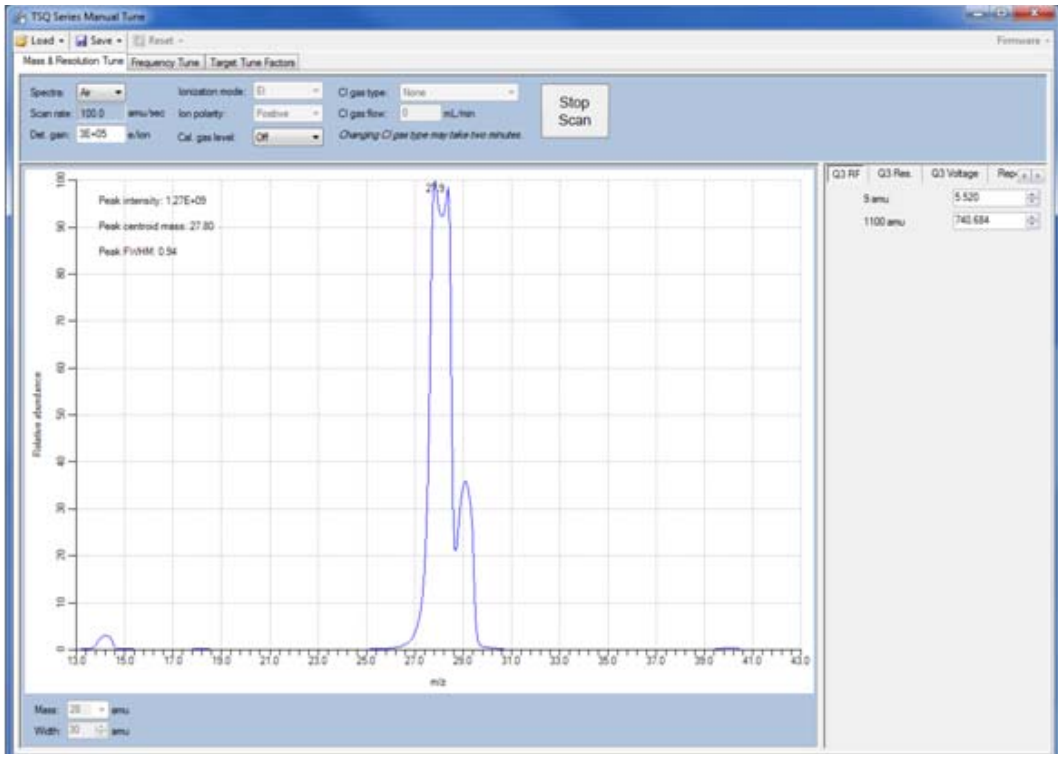
2. As the instrument pumps down over time, the ratio of 18/28 will change as *m/z* 18 decreases with *m/z* 28 remaining constant. This eventually changes *m/z* 28 to the base peak.
3. Changing components of the system such as the column, ion source, or gas supply affects the different masses present in the air/water spectra.
4. Maximum intensity may vary based on different instrument parameters, such as changing the column flow, or accessories.

If any of the previous conditions are not met and a leak is suspected as the root cause, follow "An air leak has been detected" in the *"Investigating Vacuum Issues"* section of the **ISQ Series Hardware Manual** or "How to Know When Your System Needs Maintenance" on page 163.

The next several images show a typical air/water spectrum for several common problems.

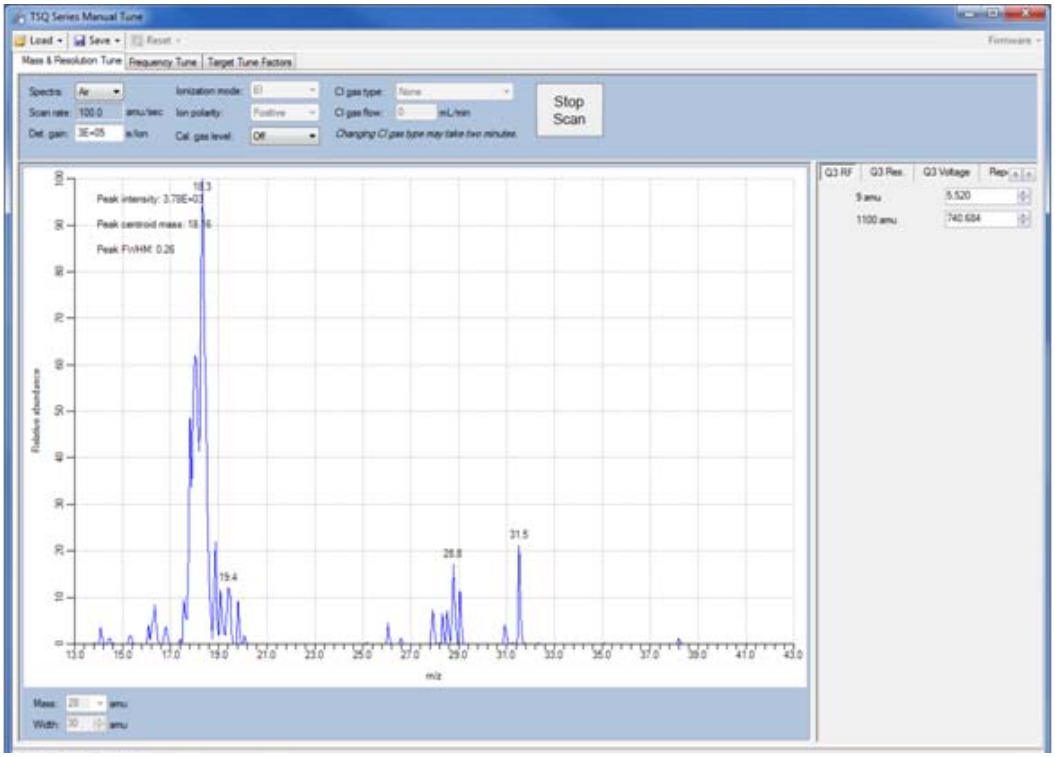
1. Figure 153 is an example of an air/water spectrum of a system with a potential air leak.

Figure 153. Typical Air/Water Spectrum of an ISQ Series System with an Air Leak



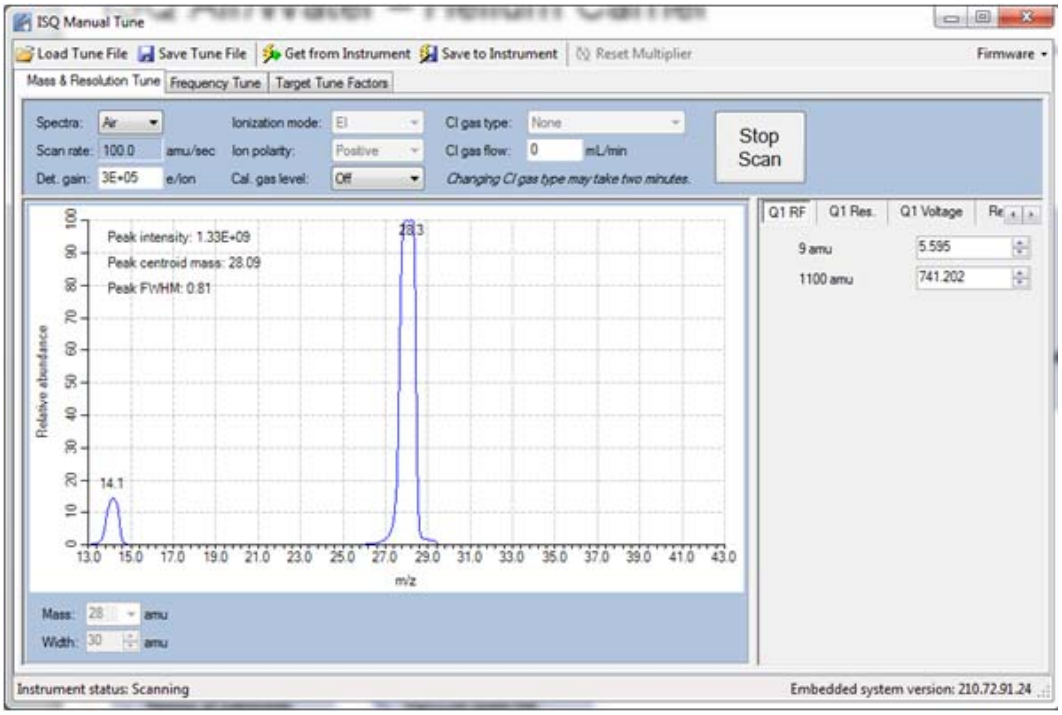
2. Figure 154 is an example of a system with an incorrectly installed ion source.

Figure 154. Typical Air/Water Spectrum of an ISQ Series System with an Incorrectly Installed Ion Source Cartridge



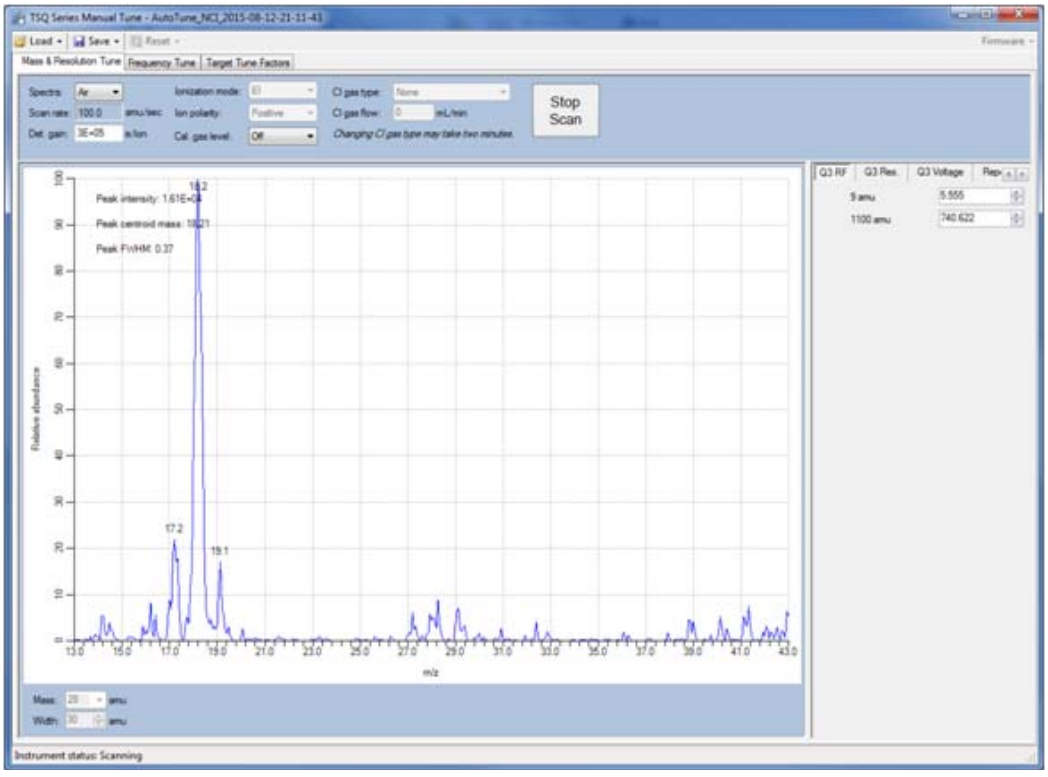
3. Figure 155 is an example of a system with no column flow.

Figure 155. Typical Air/Water Spectrum of an ISQ Series System with no Column Flow



4. Figure 156 is an example of a spectrum with a CI tune file run with an EI ion source.

Figure 156. Typical Air/Water Spectrum of an ISQ Series System with an EI Ion Source Run with a CI Tune File



No Ions Present in Scans

Lacking ions in scans or having low response can be caused by many different issues. The most common causes are instrument settings, improper tune files, or a recent change to the system. When troubleshooting, always go back to the last change made to the system and examine the delta.

❖ **Try the following solutions if you cannot see ions in any scans**

1. Run diagnostic checks (diagnostics only tune type).
2. Remove and reinstall the ion source cartridge.
3. Switch filaments.
4. Confirm the starting tune file is appropriate for ionization mode or source cleanliness level.
5. Confirm the correct ion volume is inserted (EI or CI).
6. Clean the ion source.

❖ **Try the following if you do not see ions in CI mode**

1. Confirm that the CI ion volume is installed.
2. Confirm that the reagent gas is connected and turned on.
3. Confirm an appropriate tune file is being used.
4. Switch filaments.

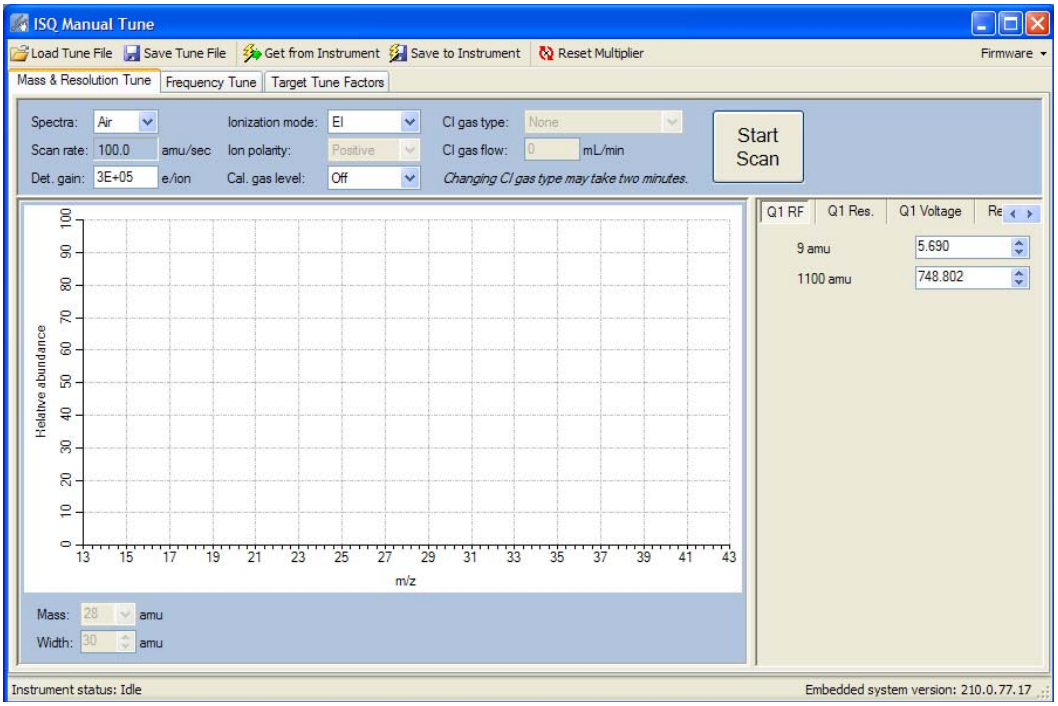
Checking the ISQ Series System Firmware

Confirm that you are running the correct version of the ISQ Series instrument firmware.

❖ **To find the version of firmware installed on your ISQ Series system**

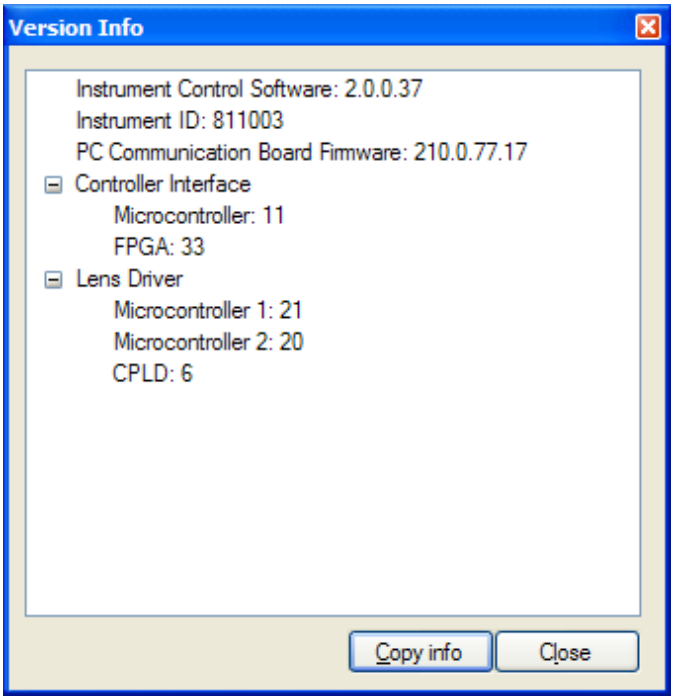
1. On the ISQ Dashboard, click **Air & Water/Tune**. This opens ISQ Manual Tune.

Figure 157. The ISQ Manual Tune Utility



2. Click **Firmware** in the upper right-hand corner of the home screen in ISQ Manual Tune.
3. A new menu opens. Click **Version Info**.
4. A dialog box containing opens that lists the firmware versions for the ISQ Series instrument control drivers, the lens driver board, and the controller interface board. See Figure 158.

Figure 158. Determining the Firmware Versions



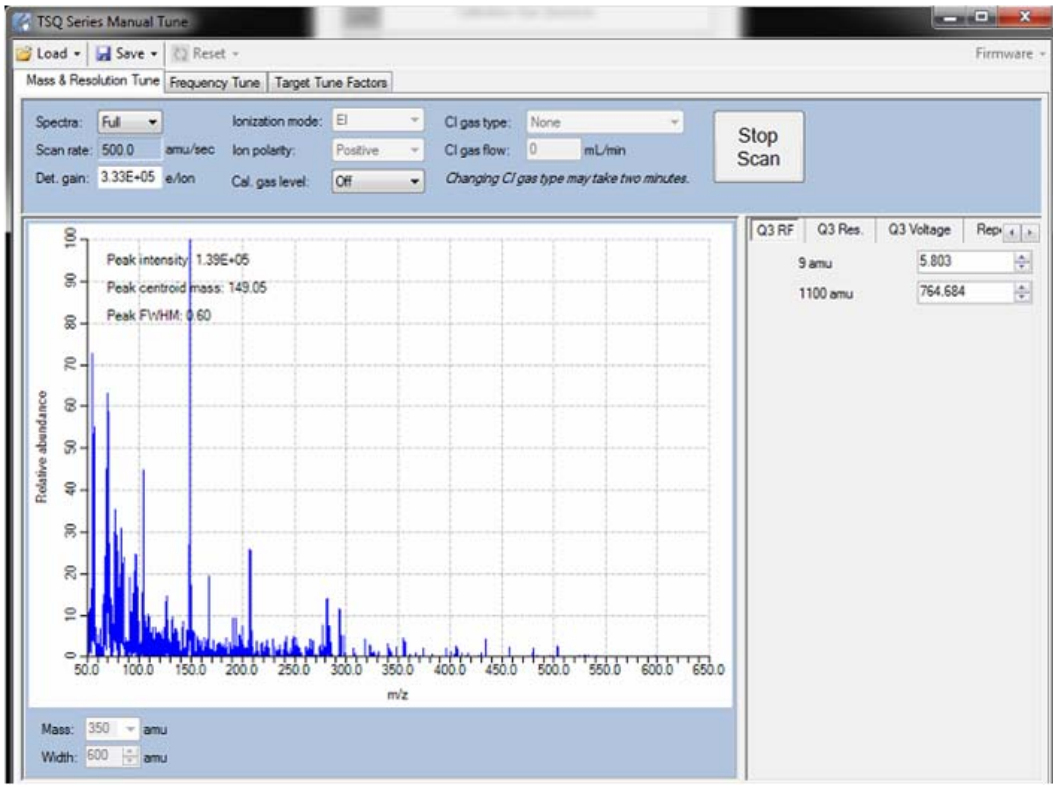
Note Depending on the version of ISQ Series instrument control software you are running, the controller interface board and lens driver board firmware may not be listed.

5. Click **Copy Info** to copy the information to the clipboard on your PC.
6. Save this information. It may be useful to your Thermo Fisher Scientific Field Service Engineer if you need a service call.

How to Know When Your System Needs Maintenance

Typically, you will notice that your instrument needs maintenance when you are analyzing your data on the computer. Figure 159 shows a normal full scan background on an ISQ Series system.

Figure 159. Normal Full Scan Spectrum of an ISQ Series System



A normal system should have the following conditions met;

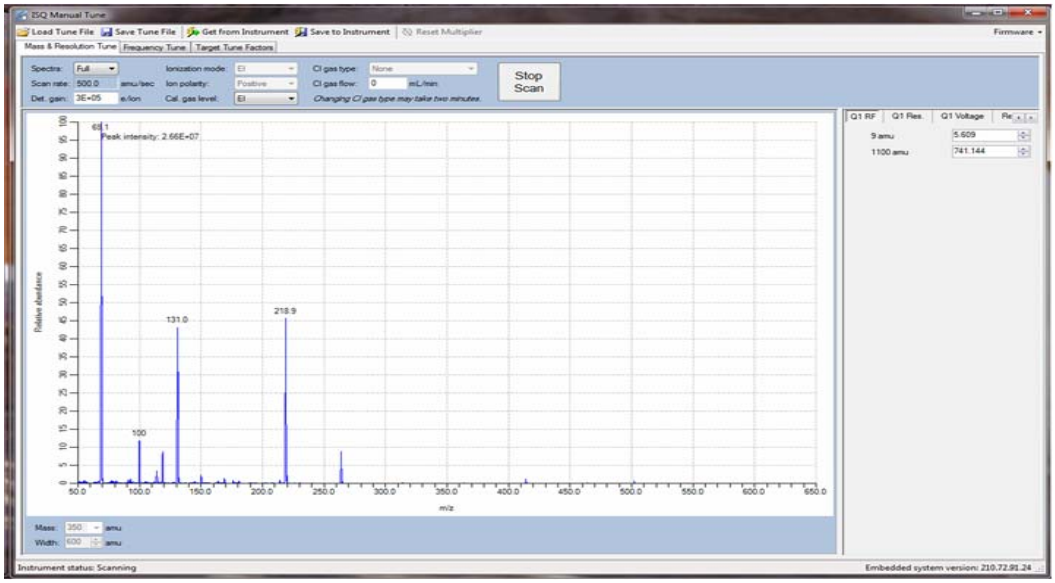
- Detector gain — 3×10^5
- Maximum intensity — $< 1 \times 10^6$
- There should be exponential decay for background noise.

> **Note** Intensity of background ions (chemical noise) should decrease with an increase in *m/z*.

- There should be no extraneous peaks indicating contamination.

If you run a sample with Perfluorotributylamine (PFTBA), the tuning compound, the spectrum should look like Figure 160 below.

Figure 160. Normal PFTBA Profile Spectrum



A normal PFTBA profile spectrum should meet the conditions below.

- Intensity >3 × 10^6
- The ions *m/z* 69, 131, 219, 414, and 502 are all present and in the correct relative ratios.
  - *m/z* 69 or *m/z* 219 is the base peak
  - *m/z* 131 and *m/z* 219 between 60-100%
  - *m/z* 414 and *m/z* 502 between 1-10%

Note High mass ions decrease in relative abundance when ion source temperature increases.

  - *m/z* 100, 119, and 264 are also present and cleanly separated from any noise.
- Mass assignments are correct. Review the tune report for true mass assignment values.
- No extraneous peaks indicating contamination are present.

Some of the most common reasons and indications your instrument needs maintenance are as follows:

- **Contamination**—If you notice excessive background in your mass spectra, it is usually an indication that your instrument is contaminated. Use the mass spectrum in the table below to understand the origin of the contamination. If you notice cleaning solvent peaks, it is usually an indication that your ion source cartridge was installed before it was completely dried. Table 1 shows a list of common contaminants.

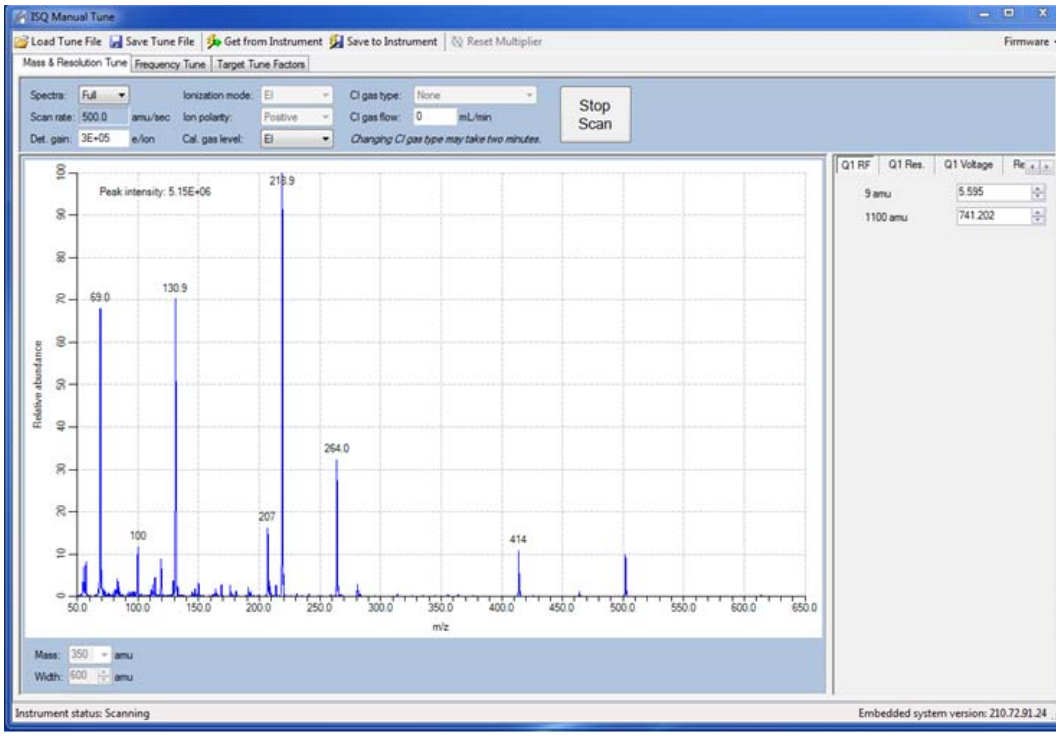
- **Fingerprints**—If you notice a series of mass peaks in your data that are 14 amu apart, it is usually an indication of fingerprint or other hydrocarbon contamination. To avoid fingerprints, you should wear clean, lint-free gloves when performing any type of maintenance on a component in the vacuum manifold of the ISQ.
- **Air Leaks**—If you notice a higher than normal vacuum pressure or poor sensitivity, it is usually an indication of an air leak. Check the last o-ring or ferrule you installed.

Table 1. Common Contaminants

Ions (*m/z*) To Monitor	Compound	Possible Source
13, 14, 15, 16, 17, 29, 41, 57	Methane	CI gas
18, 28, 32, 44 or 14, 16, 19	H_2O, N_2, O_2, CO_2 or N, O	Residual air and water, air leaks, outgassing from Vespel ferrules
69, 100, 119, 131, 169, 181, 214, 219, 264, 376, 414, 426, 464, 502, 576, 614	PFTBA and related ions	PFTBA (tuning compound)
31	Methanol	Cleaning solvent
43, 58	Acetone	Cleaning solvent
78	Benzene	Cleaning solvent
91, 92	Toluene or xylene	Cleaning solvent
105, 106	Xylene	Cleaning solvent
151, 153	Trichloroethane	Cleaning solvent
149	Plasticizer (phthalates)	Use of vinyl or plastic gloves
Peaks spaced 14 amu apart	Hydrocarbons	Fingerprints, foreline pump oil, or other hydrocarbons

Figure 161 shows an abnormal PFTBA profile spectrum.

Figure 161. Abnormal PFTBA Spectrum



In Figure 161, the abnormal results are as follows:

- Maximum intensity < 1.5×10^7 (15,000,0000)
- *m/z* 207 is prominent, indicating column bleed
- Many small contamination peaks present

Investigating Baseline Issues

Table 2. Troubleshooting Baseline Issues in Your Data (Sheet 1 of 2)

Behavior	Characteristic	Cause	Remedy
Drifting Baseline	General	Stationary phase has accumulated in column	Replace the column or cut off the end of the column.
		Chromatographic baseline is high	Replace the column or cut off the end of the column.
		Carrier gas pressure is too low	Check for leaks in injector or flow path. Replace the carrier gas cylinder if it is empty or low. Increase the pressure if maximum injector pressure in method is greater than carrier line pressure set by regulator. Make sure the vacuum compensation is on.
		Carrier gas flow is drifting	Check for leaks in injector or flow path. Check the carrier gas tank.
		Impurities have accumulated in column	Run solvent blank to remove impurities. If impurities persistent after multiple solvent blanks: Inject solvent from a different source. Change syringe, liner and septum. Clean injector. Check impurity levels in your gas. Use the correct gas purity and filter.
	Falling	Carrier gas leak in the system	Perform a leak test and tighten the connections to the carrier gas line if leak is found.
		Column is baking out	Wait for the column to stabilize.
	Rising	Impurities have accumulated in column	Check impurity levels in the gas source. Use correct gas purity.
	Abnormal rise in baseline when oven temperature is high	Impurities have accumulated in column	Recondition or replace the column.

Table 2. Troubleshooting Baseline Issues in Your Data, continued (Sheet 2 of 2)

Behavior	Characteristic	Cause	Remedy
Noisy Chromatographic Peaks	General	Excessive column bleed at high oven temperatures	Reduce the column temperature. Bake out the column. Install a high-temperature column.
			Install oxygen filters in carrier gas line. Check pneumatic and inlet systems for leaks. Use correct gas purity with low oxygen content.
		Column is contaminated or damaged	Condition or replace the column.
		Oven temperature is higher than column's maximum allowable temperature	Reduce oven temperature to the maximum allowable temperature of the column.
		Leak at column fittings	Find leak. Tighten fittings if loose. Replace ferrule if overtightened.
			Transfer line temperature is not set too low.

Investigating Peak Issues

Table 3. Troubleshooting Peak Issues in Your Data (Sheet 1 of 3)

Behavior	Characteristic	Cause	Remedy
Broadening	General	Column higher than optimum of column	Reduce the flow. Make sure vacuum compensation is turned on.
		Column flow lower than optimum of column	Increase the flow.
		Split flow is too low for split injection	Increase the flow to 40-50 ml/min.
		Performance of the column has degraded	Test the column at the optimum flow rate.
		Injector is dirty	Clean or replace the liner.
		Ion source is dirty	Clean the ion source and tune the instrument.
		Column is not far enough into the transferline	The GC column does not extend into the MS source. Use the column measuring tool to confirm column length. If the end of the column is inside the transfer line, an excessive amount of GC effluent will contact the inside wall.
Fronting	General	Column is overloaded	Decrease the injected amount and/or analyte concentrations. Increase the split ratio. Use a column with a thicker film.

Table 3. Troubleshooting Peak Issues in Your Data, continued (Sheet 2 of 3)

Behavior	Characteristic	Cause	Remedy
Tailing	Sample peaks	Column degradation is causing activity	Inject a test mixture and evaluate the column. Replace column if necessary.
		Liner is dirty	Clean or replace the liner.
		Ion source is dirty	Clean the ion source. Run the method with higher source temperature, making certain not to start running samples in matrix until the source has had several hours to reach thermal equilibrium.
		Glass wool or inlet liner is causing activity	Replace wool with fresh silanized wool and install a clean inlet liner.
		Inlet temperature is too low	Increase the temperature of the inlet.
		Column connections are poor or obstructed	Reconnect the column inlet.
		Stationary phase is not appropriate for your target compounds	Replace the column and choose a more appropriate phase for your analysis.
		Final hold oven temperature is too low	Increase the column/oven temperature. Do not exceed the recommended maximum temperature for the stationary phase.
		Transferline temperature is too low	If tailing occurs on late eluting compounds, it is likely the source or transferline temperature is too low.
		Source temperature is too low	If tailing occurs on late eluting compounds, it is likely the source or transferline temperature is too low.
		Poor column characterization	See Changing the Column for information about checking for leaks and column flow.

Table 3. Troubleshooting Peak Issues in Your Data, continued (Sheet 3 of 3)

Behavior	Characteristic	Cause	Remedy
Ghost Peaks	General	Incomplete elution of previous sample	Increase the final oven program temperature or total run time. Increase the column flow rate.
		Carrier gas is contaminated	Replace the gas cylinder or filter.
		Laboratory glassware has caused contamination	Ensure the glassware is clean and contaminant-free.
		Injected sample has decomposed	Decrease the injection port temperature. Use the on-column injection technique.
		Injection solution has matrix present	Adequately clean up your sample prior to injection.
		Inlet or pneumatics are contaminated	Remove the column and bake out the inlet. Use a high-quality septum. Replace the split vent filter. Install an in-line filter between the pneumatics and the inlet.
Missing Peaks	Baseline or background present	Column is broken	Replace the column.
		Column flow is incorrect	Make sure the septa are sealing. Make sure vacuum compensation is turned on.
		Backflush settings are incorrect	Set backflush to **off** until after injection.
		Column position in S/SL injector is incorrect (too high)	Check the position of the column.
	No baseline or background present	Poor or missing electrical connection	Check the cable connections.
		ISQ Series instrument is not collecting data	Make sure the tune file is correct. Verify that the Busy light is on during acquisition. Close Xcalibur, open Instrument Configuration, press the **Reset** button on the ISQ Series instrument, wait ten seconds, close Instrument Configuration.

Investigating Results Issues

Table 4. Troubleshooting Results Issues in Your Data (Sheet 1 of 2)

Behavior	Characteristic	Cause	Remedy
Low Reproducibility of Peaks Area	General	Detector gain is set too low	Retune the gain. Increase the electron multiplier voltage. Increase the target ion count.
		Concentration is not compatible with the dynamic range of the detection system	Verify that the sample concentration is suitable for the MS.
		Chromatogram and spectrum are blank	Make sure the tune file is correct. Verify that the Busy light is on during acquisition. Make sure the filament is not burned out.
		Injection technique is not appropriate	Use a different injection technique.
		Injection parameters are not appropriate	Verify the injection temperature and flow rates.
		Sample injection technique is not reproducible	Evaluate the sample preparation sequences. Compare the results with a series of standard injections.
		Syringe or septum is leaking	Check and replace the syringe and/or septum at regular intervals.
		Injection port is leaking	Check the column connections. Run a leak check.
		Injection technique is not suitable	Carefully meter the injected amount. Use a clean, good-quality syringe.
		Ion source is dirty	Clean the ion source and retune the instrument.
		Split flow or ratio control is inadequate	Monitor the flow. Replace the in-line filter.

Table 4. Troubleshooting Results Issues in Your Data, continued (Sheet 2 of 2)

Behavior	Characteristic	Cause	Remedy
Poor Sensitivity	With increased retention time	Carrier gas flow rate is too low	Increase the carrier gas flow rate. Locate and remove possible obstructions in the carrier gas line. Check the septum for leaks. Check the injector/column ferrules for leaks.
	With normal retention time	GC carrier gas line has leaks	Run a leak test and correct leaks.
		Syringe is leaking during injection	Replace syringe or piston seals, if necessary.
		Split injection temperature is too low	Increase the temperature of the injector.
		Voltage is not reaching the lens.	Replace the lens plate and springs if damaged. Remove debris or broken pieces in the manifold. Run a lens check diagnostic. Check the connection by removing or inserting the ion source.
Retention Times	Low reproducibility	DCC is drifting or unstable	Monitor the column pressure or flow. Check and replace the controller, if necessary.
		Injection technique is inadequate	Pick injection technique suitable for the injector and liner you are using.
		Vaporization size of sample inject larger than volume of liner	Reduce the injected amount and/or volume.
		Handshaking is not configured correctly	Be sure the GC is inhibited by the MS and waits for contact closure by the autosampler.
		Column temperature is unstable	Check the main oven door and cooling flap. Monitor the column temperature.

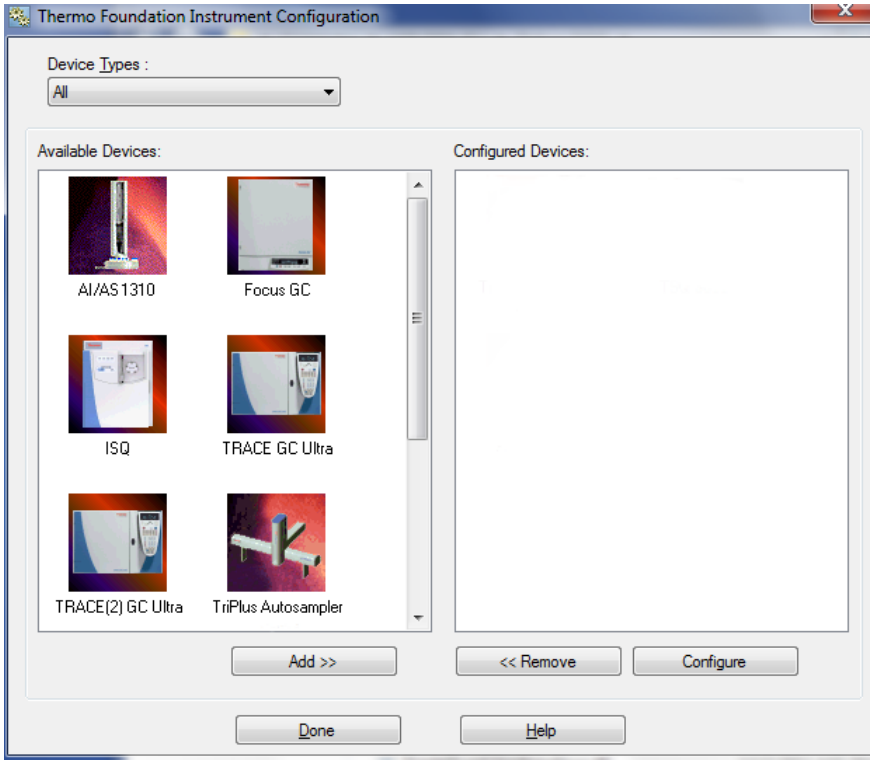
Reconfiguring Your Instrument

If your instrument loses communication with the computer, you have reinstalled Xcalibur, or you have a new computer, you may need to reconfigure the ISQ Series instrument.

❖ To reconfigure your ISQ Series instrument

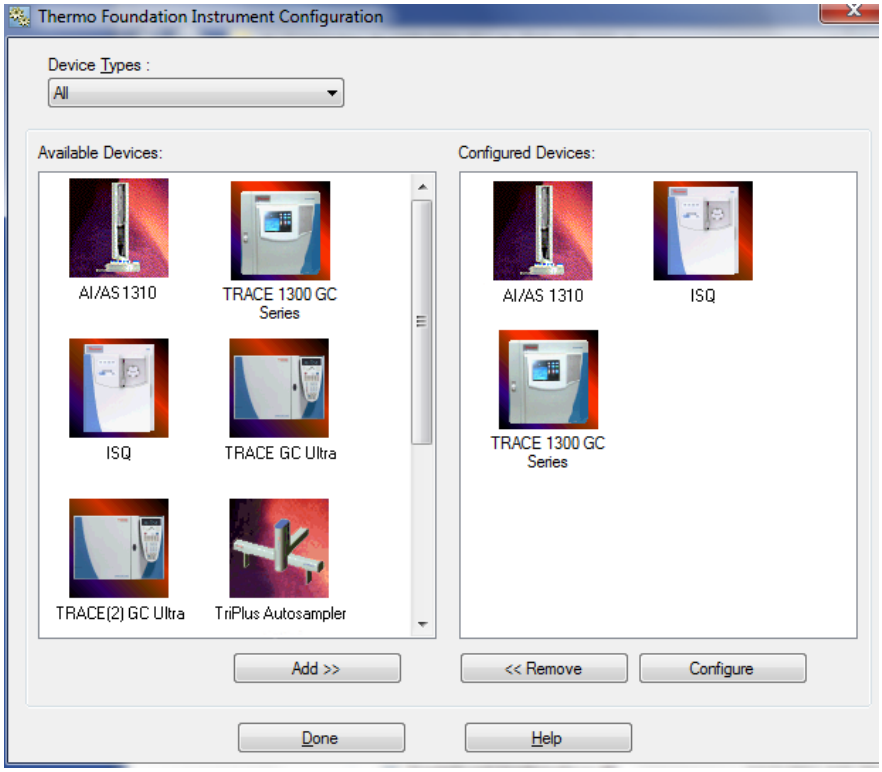
1. From the Start menu on your computer desktop, browse to **Thermo Foundation 3.1 | Instrument Configuration**. When the Instrument Configuration utility opens, you can see an icon of the instruments you have connected.
2. Click the ISQ Series (and other instruments) icon in the Available Devices column and click **Add** to move it into the Configured Devices column.

Figure 162. Finding Available Devices



3. Click the each instrument icon you want to configure and click **Add**.

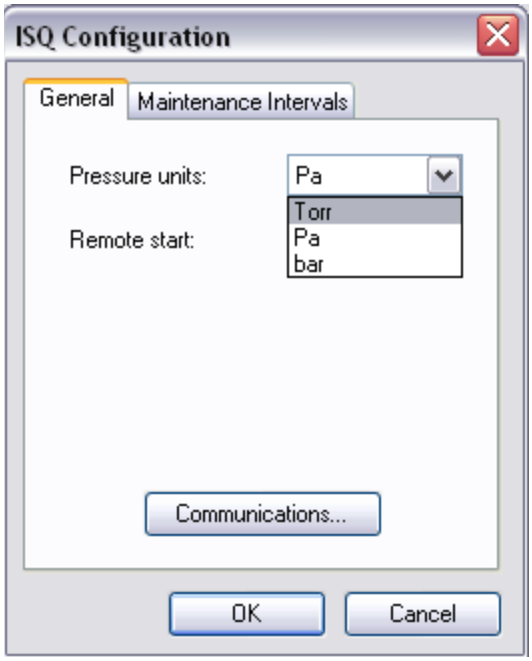
Figure 163. Adding a a Device to be Configured



4. Click **Configure**.

5. In the **General** tab, set the pressure units.

Figure 164. Setting the Pressure Unites

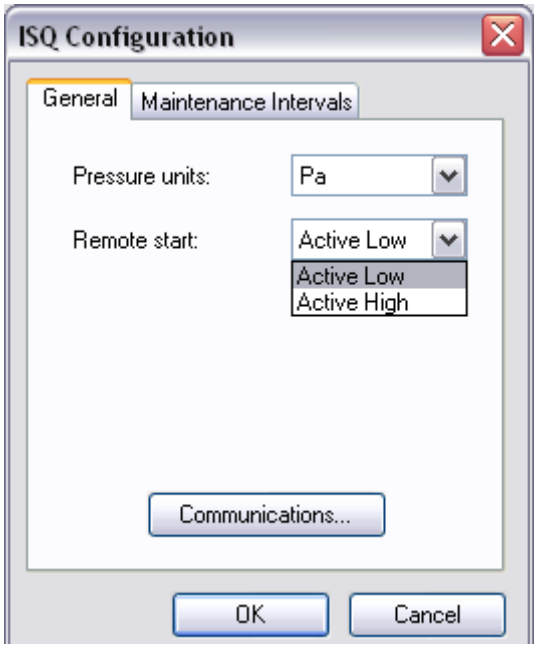


Note You only need to set up the pressure units if you have an ion gauge or convectron gauge installed on your system. The readbacks from these components will display in the units set in this window.

6. Set the remote start. It is used to let the ISQ Series MS know when the GC has started a run. When you configure the GC, you can tell it what to send out to the ISQ Series

system. In this window, you need to make sure the value matches what you set on the GC. The default is **Active Low**.

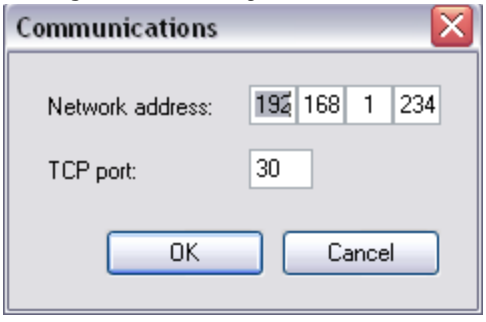
Figure 165. Setting the Remote Start



7. Click the **Communications** button to set the network IP address of the instrument's PC communication board and assign a TCP port.The default settings are entered by the factory and should only be changed after consulting you local IT department and obtaining special software from customer support necessary to reprogram the IP address on your instrument.

Note The instrument must be connected to a dedicated Ethernet port on the PC. The instrument cannot be connected through a LAN.

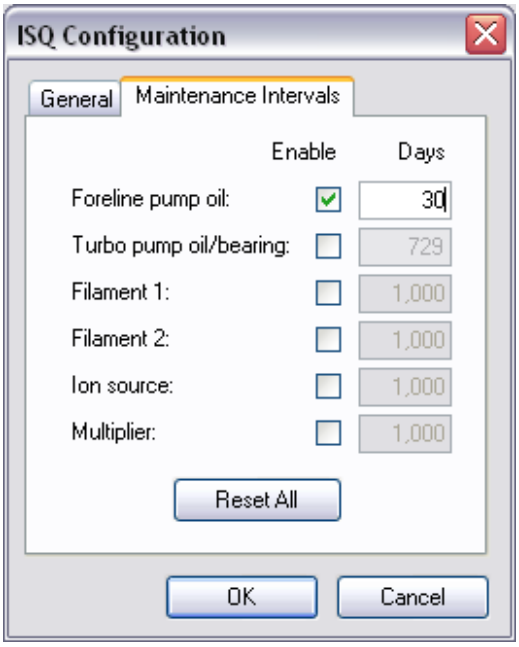
Figure 166. Setting the Network IP Address and the TCP Port



8. Click the **Maintenance Intervals** tab to set the number of days until you plan to perform maintenance on certain components of your GC/MS system. As a default, the foreline pump and turbo pump oil are automatically enabled. You can monitor the progress of these settings in the Status pane of Xcalibur.
9. Select the **Foreline pump oil** checkbox to enable the maintenance intervals. Then set the

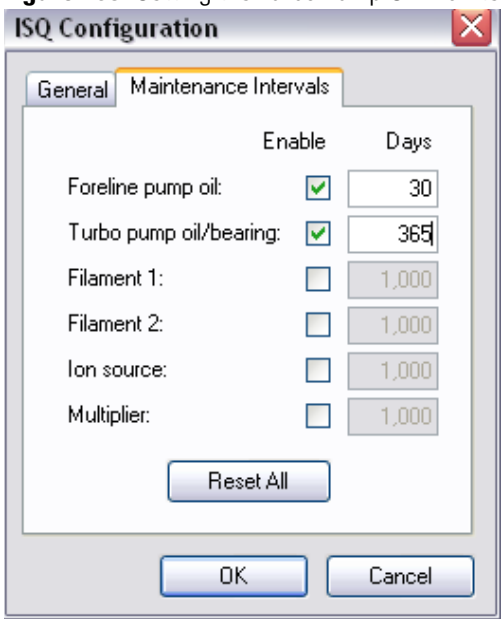
number of days in which you want to be reminded to check the oil. The manufacturer recommends changing the oil every 125 days. If you are using corrosive gases, such as ammonia, you should change the oil every 30 days.

Figure 167. Setting the Foreline Pump Oil Maintenance Interval



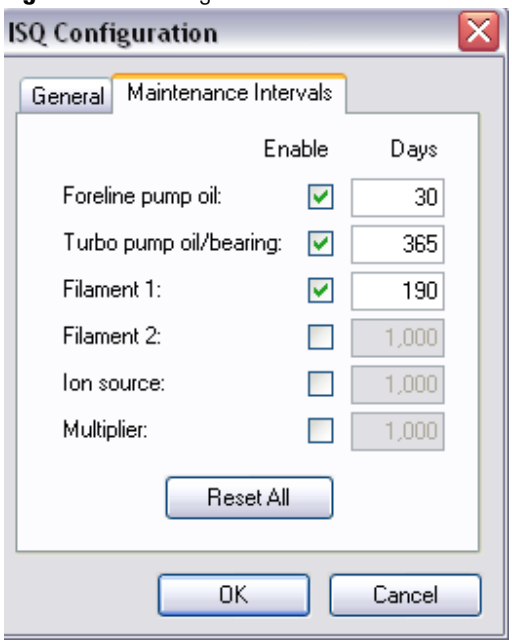
10. Select the **Turbo pump oil/bearing** checkbox to enable the maintenance reminder. Then set the number of days in which you want to be reminded to check the oil. The manufacturer recommends changing the oil cartridge every 730 days and the bearing every 1,460 days.

Figure 168. Setting the Turbo Pump Oil Maintenance Reminder



11. Select the **Filament 1** checkbox to enable the maintenance reminder. In a leak-free system, the filament should last between 30-360 days, depending on usage.

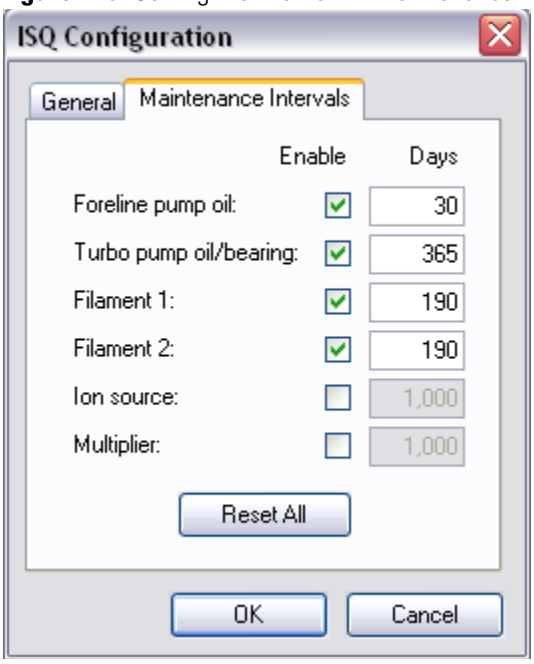
Figure 169. Setting the Filament 1 Maintenance Reminder



12. Select the **Filament 2** checkbox to enable the maintenance reminder. Then set the number of days in which you want to be reminded to check filament 2. In a leak-free

system, the filament should last between 30-360 days, depending on usage.

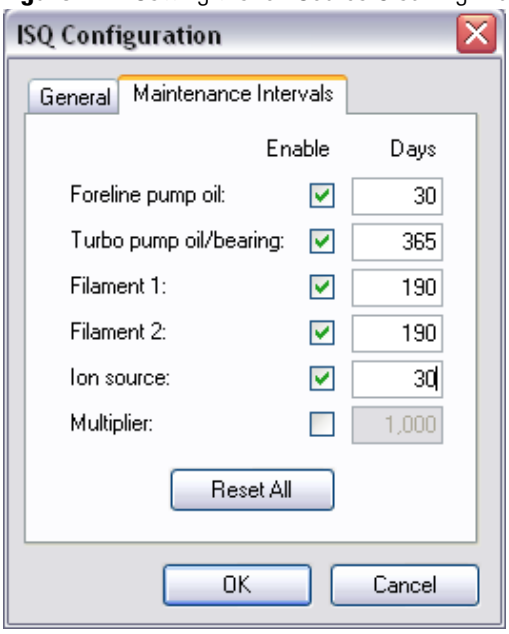
Figure 170. Setting the Filament 2 Maintenance Reminder



13. Select the **Ion source** checkbox to enable the maintenance reminder. Then set the number of days in which you want to be reminded to check the ion source. The time

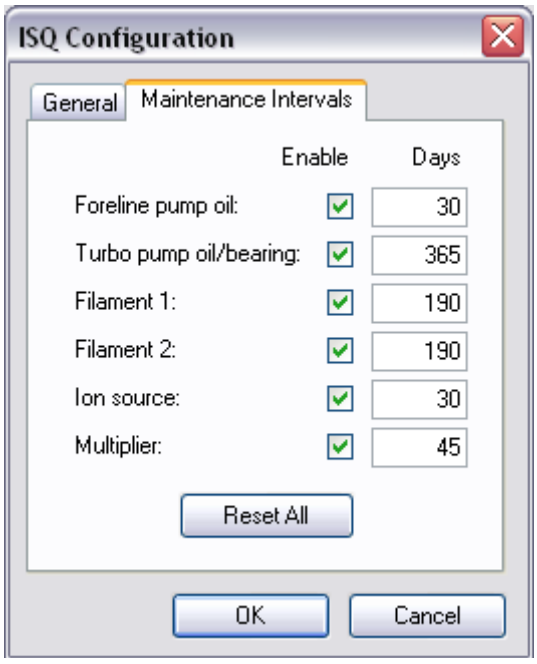
between cleaning depends very strongly on your analysis. You will have to determine the correct length of time between source cleanings.

Figure 171. Setting the Ion Source Cleaning Maintenance Reminder



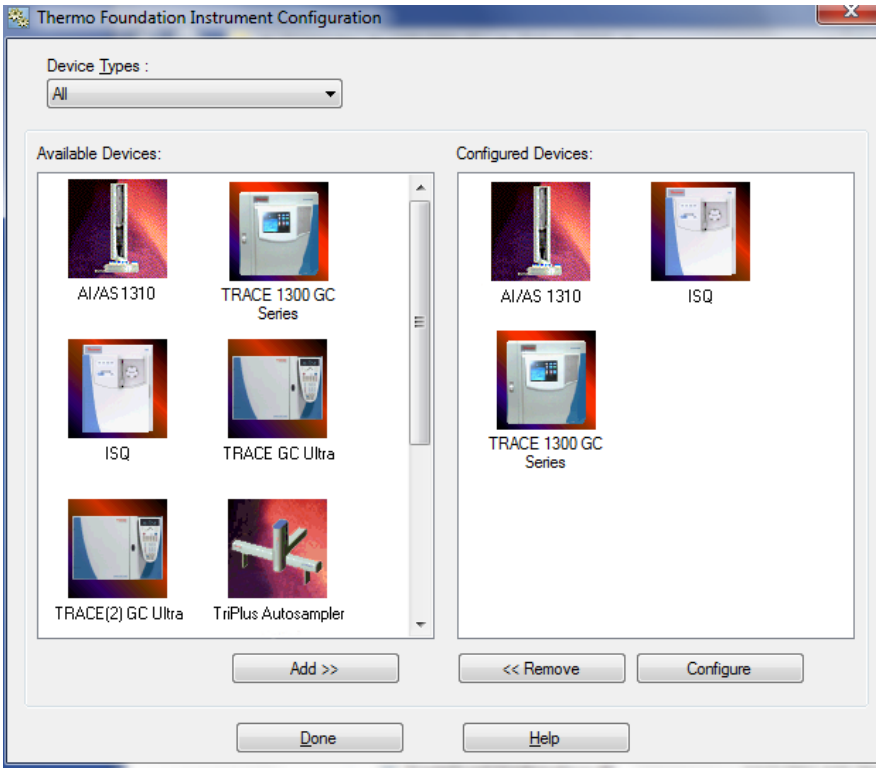
14. Select the **Multiplier** checkbox to enable the maintenance reminder. Then set the number of days in which you want to be reminded to check the electron multiplier.

Figure 172. Setting the Electron Multiplier Maintenance Reminder



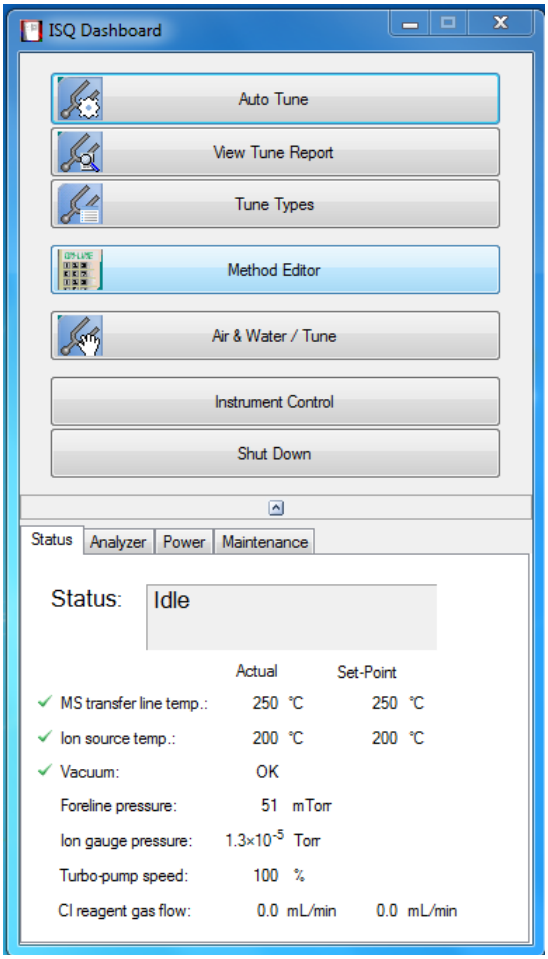
15. Click **OK** to return to the main Instrument Configuration window.

Figure 173. Returning to the Configuration Window



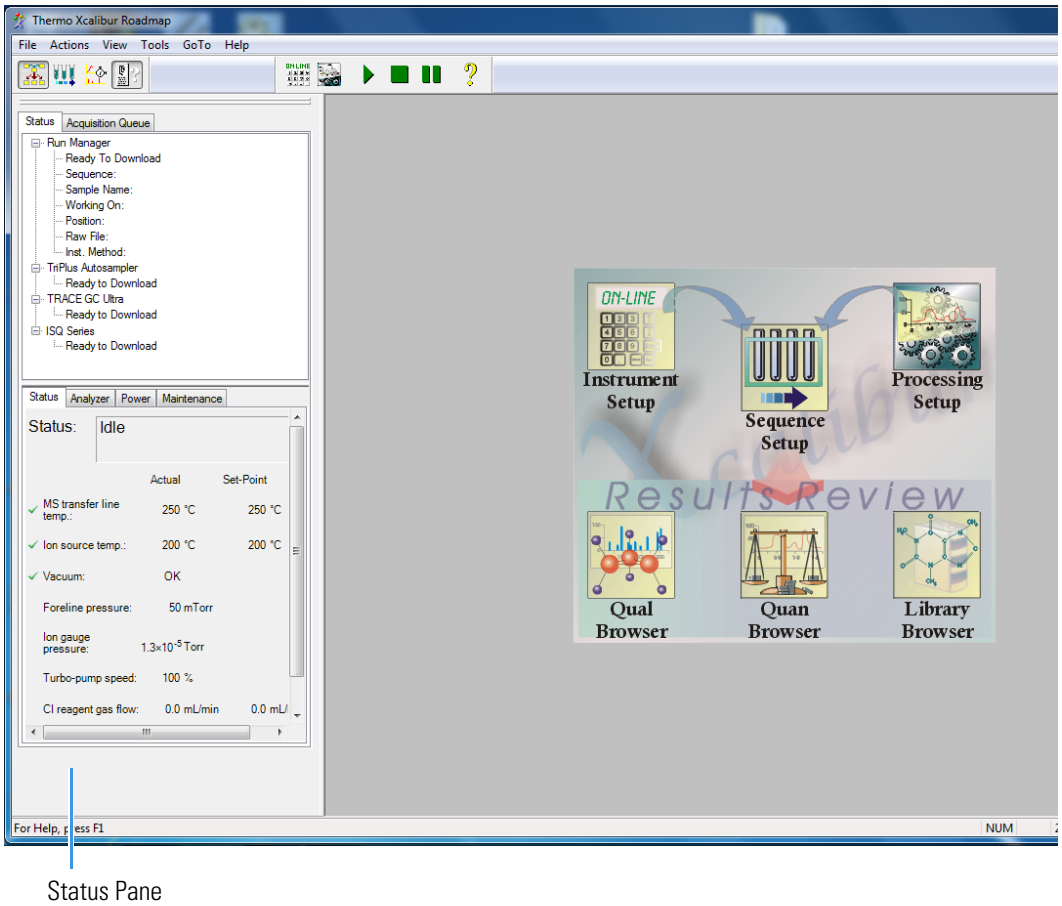
16. Click the **Done** button to close the Instrument Configuration utility.
17. You can check the status of your ISQ Series instrument in the Status tab of the ISQ Dashboard.

Figure 174. Checking the ISQ Series Instrument Status on the ISQ Dashboard



18. In the **Status Pane** of the Xcalibur Roadmap window, you can check the status of all your instruments. See Figure 175.

Figure 175. Checking All Instrument Statuses in Xcalibur



Status Pane

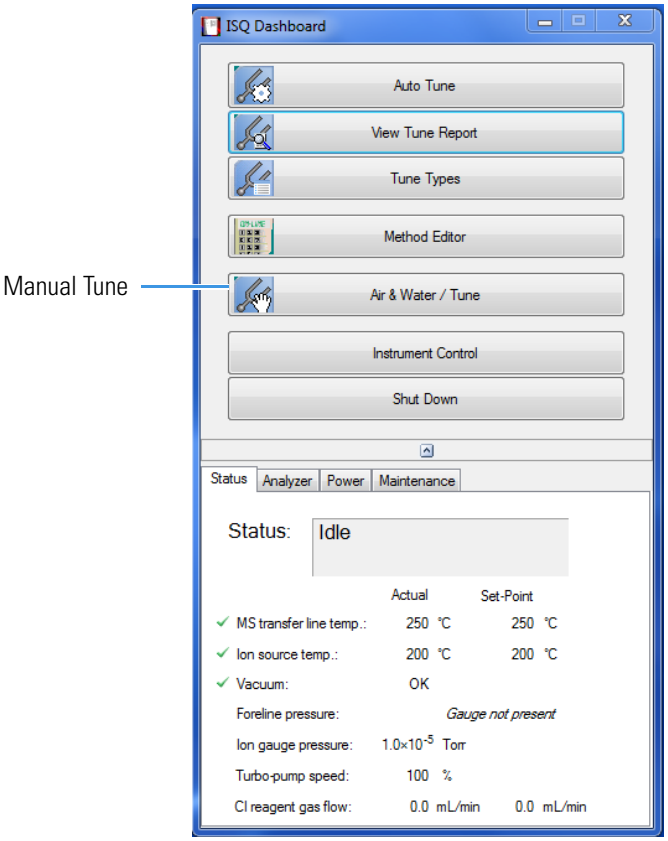
Upgrading the Software

Once you purchase optional software for your ISQ QD system, you will receive a product key from the factory. This product key is required to license your software. Follow the instructions below to enter the product key and activate your software.

Note See the *ISQ Series Spare Parts Guide* for information about ordering software upgrades.

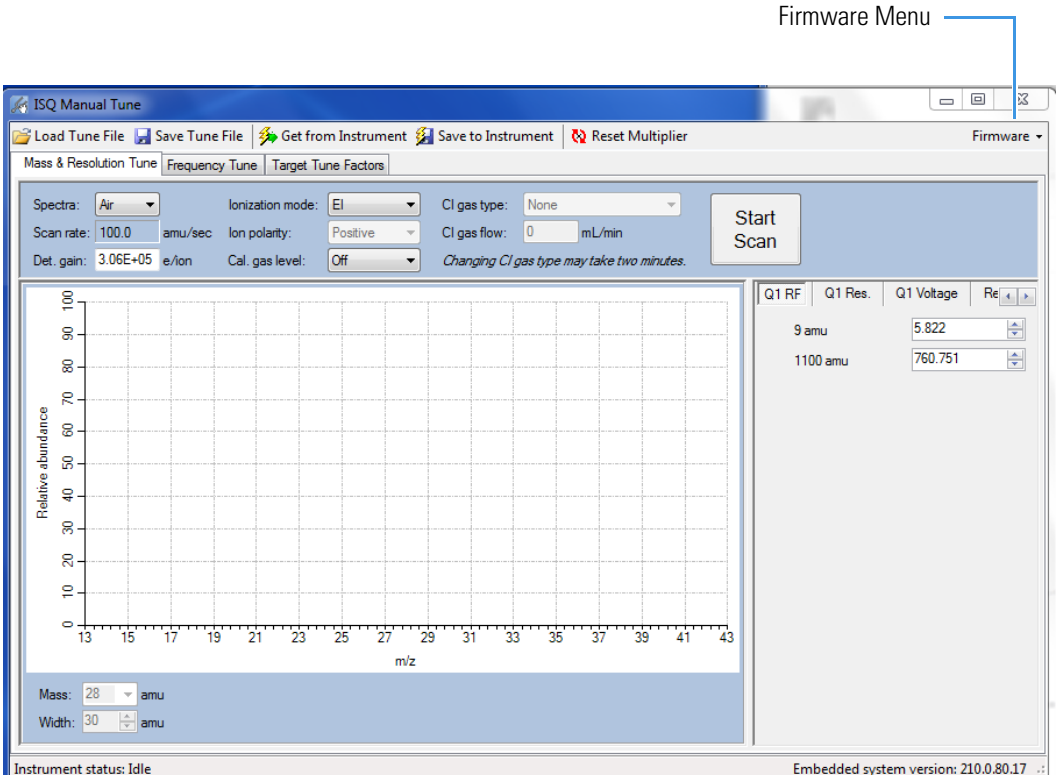
1. Click **Air & Water/Spectrum** on the ISQ Dashboard to open the manual tune utility. See Figure 176.

Figure 176. ISQ Dashboard



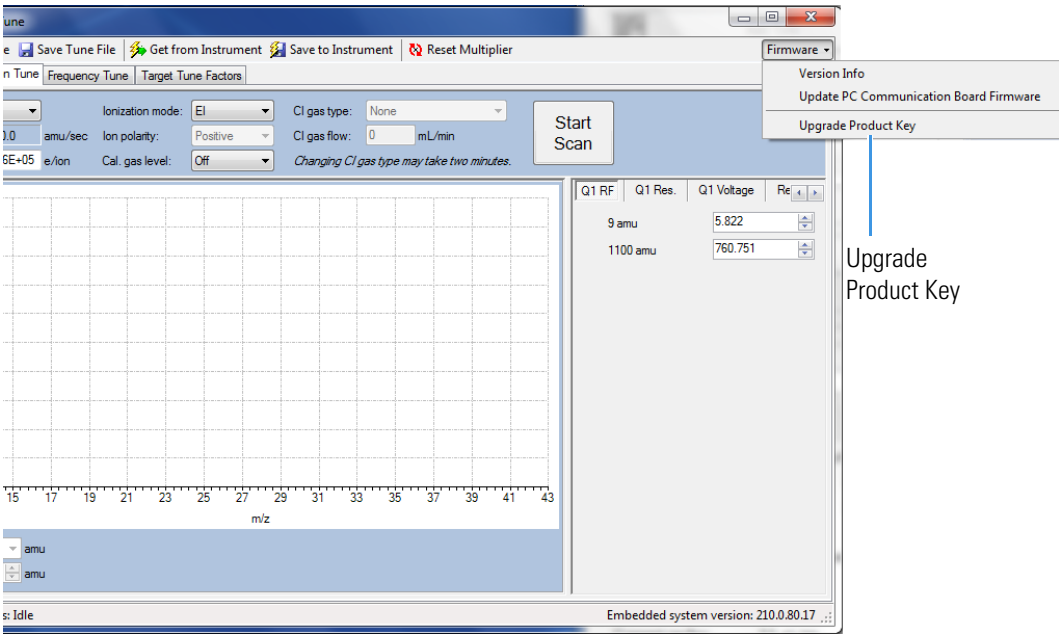
2. The ISQ Manual Tune utility opens. Click **Firmware** on the upper right-hand side of the screen to open the firmware menu. See Figure 177.

Figure 177. ISQ Manual Tune Home



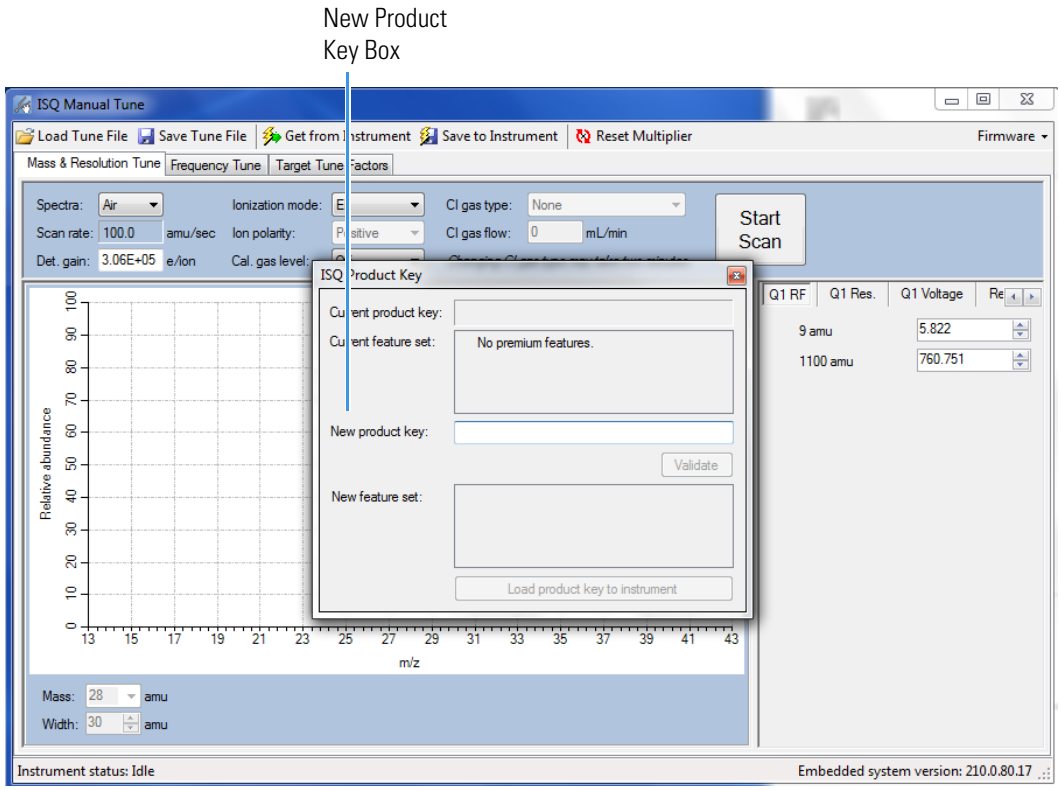
3. The firmware menu opens. Select **Update Product Key** as shown in Figure 178.

Figure 178. Selecting the Upgrade Product Key Option



4. The **ISQ Product Key** window opens. Enter your product key into the **New Product Key** box as shown in Figure 179.

Figure 179. Entering the Product Key



Index

U

V

W