TSQ Series

AutoSRM User Guide

with Chromeleon 7.2 support

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Thermo SCIENTIFIC

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For research use only: Not for use in diagnostic procedures.

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,

- recalibration, and

- changes and repairs

have been carried out by authorized personnel and if:

- the local installation complies with local law regulations,

- the instrument is used according to the instructions provided, and

- if its operation is only entrusted to qualified trained personnel.

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

Regulatory Compliance

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

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

EMC and Safety Standards

- ITQ, and Ion Trap Series standards: EMC: EN 61326-1:2006. Safety: IEC 61010-1:2001, IEC 61010-2-081:2001

- Direct Probe Controller (DPC) standards: EMC: EN 61326-1:2013. Safety: IEC 61010-1:2001, IEC 61010-2-081:2001

- ISQ Series standards: EMC: EN 61326-1:2013. Safety: IEC 61010-1:2010 (ed. 3); IEC 61010-2-081:2015 (ed. 2); IEC 61010-2-010:2014 (ed. 3); IECEE CB SCHEME CERT NO. DE 3-30000

- TSQ 8000 Evo and TSQ Duo standards: EMC: EN 61326-1:2013. Safety: IEC 61010-1:2010 (ed. 3); IEC 61010-2-081:2015 (ed. 2); IEC 61010-2-010:2014 (ed. 3); IECEE CB SCHEME CERT NO. DE 3-30034

Low Voltage Safety Compliance

This device complies with Low Voltage Directive 2011/95/EC.

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.

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For manufacturing location, see the label on the instrument.

WEEE Compliance

This product is required to comply with the European Union's Waste Electrical & Electronic Equipment (WEEE) Directive 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.

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 AutoSRM software application for the Thermo Scientific triple-quadrupole mass spectrometers. These systems provide the selectivity and sensitivity of triple-quadrupole GC/MS systems while also functioning as high-performance single quadrupole instruments. AutoSRM helps users new to triple-quadrupole GC/MS/MS systems develop selected reaction monitoring (SRM) methods.

AutoSRM software allows automated creation and optimization of selected reaction monitoring (SRM) methods and greatly simplifies your workflow. Use AutoSRM to develop SRM methods from existing SIM methods and previous MS/MS methods, or use the software to create brand-new SRM methods that you can then export to the TSQ Series method editor and use for your routine needs.

Contents

- About Your System
- Related Documentation
- System Requirements
- Chromeleon User Management Set Up
- Safety and Special Notices
- Hydrogen Safety Precautions
- Hazardous Substances Precautions
- Contacting Us

About Your System

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

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

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

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

Related Documentation

The GC-MS system includes Help and product manuals as PDF files.

❖ **To view product manuals**

Open the **Manuals** folder on your computer desktop or go to **Start > All Programs > Thermo Instruments > Manuals > TSQ Series > AutoSRM**.

❖ **To open Help**

- On the AutoSRM window, click the Help icon in the upper right corner of the window or press the F1 key.

- For information about setting parameters for a specific window or dialog box, click **Help** (if available) or press the F1 key.

For more information, visit www.thermoscientific.com.

System Requirements

Your chromatography data system must meet these minimum requirements.

System	Requirements
PC Hardware	• 4.6 GHz processor with 16GB RAM • DVD/CD-ROM drive • Video card and monitor capable of 1680×1050 resolution • 1000 GB hard drive • Quad core processor
Software	• Microsoft™ Windows™ 7 SP1 Operating System (64-bit) • Thermo Foundation[1] • Thermo Scientific™ Dionex™ Chromeleon™ 7 (release 7.2 SR3 MUa or later)[2]

Chromeleon User Management Set Up

If user management is enabled in Chromeleon, AutoSRM users must log into Chromeleon when they start AutoSRM. To run AutoSRM, logged in users must have the following Chromeleon access and user privileges assigned to them by the Chromeleon Administrator:

Required Access Privileges:

- Access to the instruments

- Access to the data vault and the folder or sequence in which the template instrument method is located

Required User Privileges:

- Create Instrument Method

- Modify Instrument Method

- Copy Instrument Method

- Control Instrument

- Take Over Instrument Control

- Control Queue

- Control Instrument While Queue Is Running*

- Create Folder

- Create Sequence

- Modify Sequence

- Copy Injection

- Add New Injection

- Delete Finished Or Interrupted Injection**

* Necessary if an autosampler is not configured and a system message asks for a confirmation in order to continue after a manual injection.

** Necessary in order to allow the user to run the same study multiple times. If this privilege is not granted, users can run a study only one time.

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.

Symbol	Descriptor
	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.
	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.

Symbol	Descriptor
	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 and an atomic weight of 1.00794, making it the lightest element. 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 the Mass Spectrometer

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

Figure 1. Hydrogen Safety Screws on the Mass Spectrometer

Left Top Cover Screw

Right Top Cover Screw

Front Panel Screw

Before powering on the MS system, ensure that:

- All the covers and panels of the MS system are firmly attached.

- The vent valve is tightly closed if you vented the system.

- All fittings, ferrules, and o-rings are sealed.

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 might 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 might 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.thermo.com/ms for information about our products.

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

Go to www.thermoscientific.com/wps/portal/ts/contactus.

❖ **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.
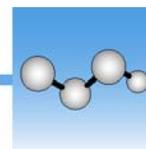
Using AutoSRM

This chapter tells you how to set up and run an AutoSRM discovery study. AutoSRM discovery studies have three sections:

- The precursor ion study

- The product ion study

- SRM optimization

As well as instructions for setting up and running each study, this user guide gives you the steps for importing the resulting transitions into the TSQ Series instrument method and accessing them for routine use.

> **Note** Set up your GC and autosampler methods through the Instrument Method Editor in Chromeleon before developing your AutoSRM study.

Contents

- Determining Precursor Ions

- Conducting the Product Ion Study

- Developing the SRM Optimization Study

- Importing Transitions into the Method Editor

Determining Precursor Ions

The purpose of the first study is to select your precursor ions. During this study, after you name your compounds and enter your vial numbers and retention times, AutoSRM instructs your TSQ Series system to run a full-scan analysis on the compounds.

For precursor ion studies, along with the resulting chromatographic peaks and full-scan spectra, AutoSRM presents you with a table of the most intense product ion masses that you may select. If you wish, however, AutoSRM can automatically pick the product ion masses.

The user interface presents the full-scan peaks and attempts to identify your compounds based on the largest peak at the approximate retention time you provided. You may extract a single ion or perform a baseline subtraction to help you identify potential precursor ions.

> **Note** You will need mid-range concentration standards (500 pg/uL–10 ng/uL) before setting up your AutoSRM method.

❖ **To determine your precursor ions in AutoSRM**

1. Open the TSQ Series Dashboard.

2. Click AutoSRM.

Figure 1. Accessing AutoSRM from the TSQ Series Dashboard
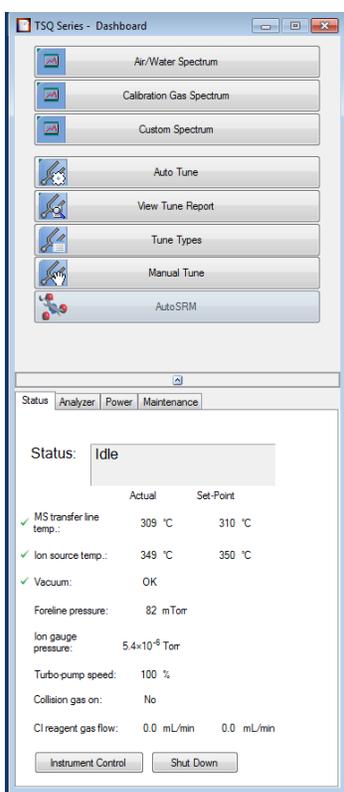
> **Note** If User Management is enabled in Chromeleon, the Chromeleon log on dialog box opens when you start AutoSRM. Enter your Chromeleon User Name and Password to continue.

3. Click the **Create a New Study** icon on the left to create a new study. See Figure 2.

Figure 2.   New AutoSRM Study

Create a New
Study Icon
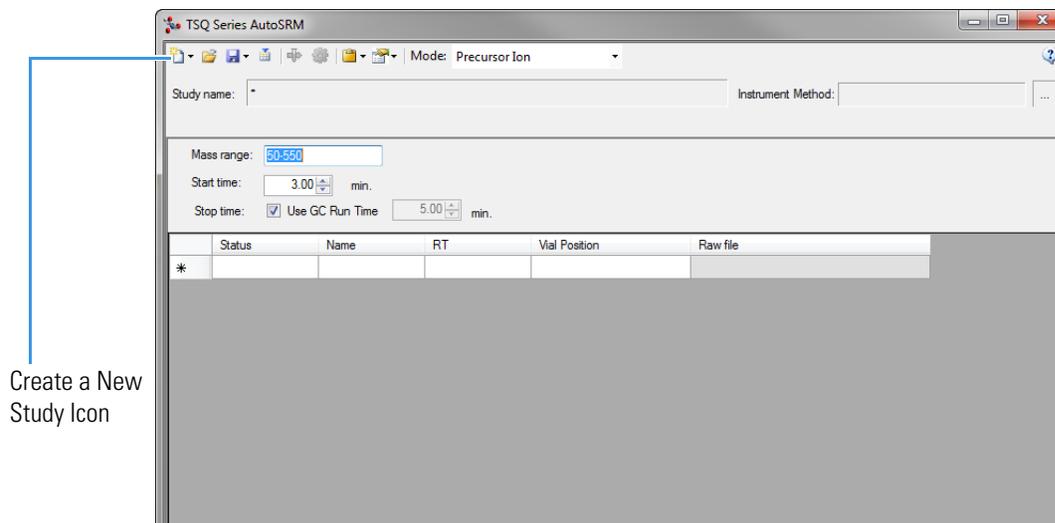
4. A new study window opens.

5. Link to an 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 3.

Figure 3.   Retrieving an Instrument Method

Link to an
Instrument
Method
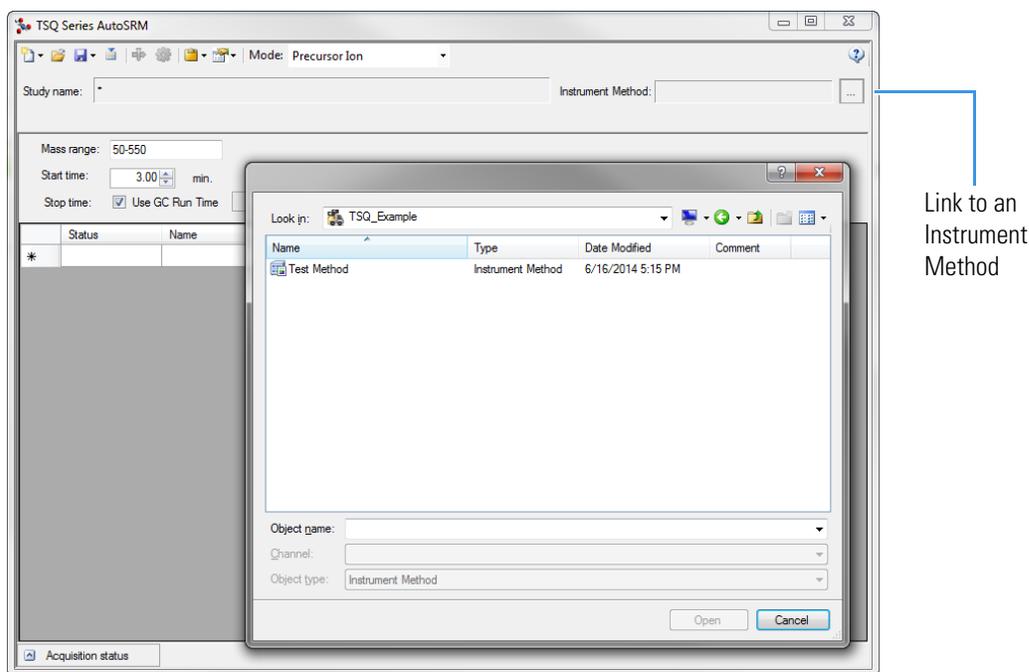
6. If you are importing a file created on another system, click the import icon. See Figure 4.

Figure 4.   Importing Compounds from an External File

Import Icon
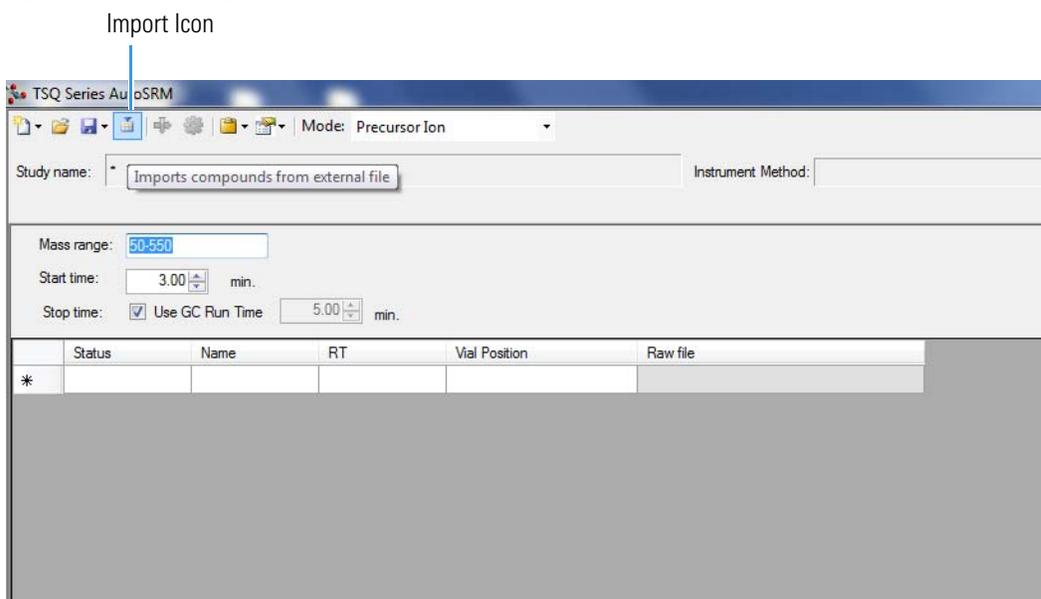
7. The **SIMBridge** dialog box opens. Choose the language of your method file from the **Source Locale** drop-down menu. See Figure 5.

Figure 5.   Setting the Source Language of Method Files using SIMBridge
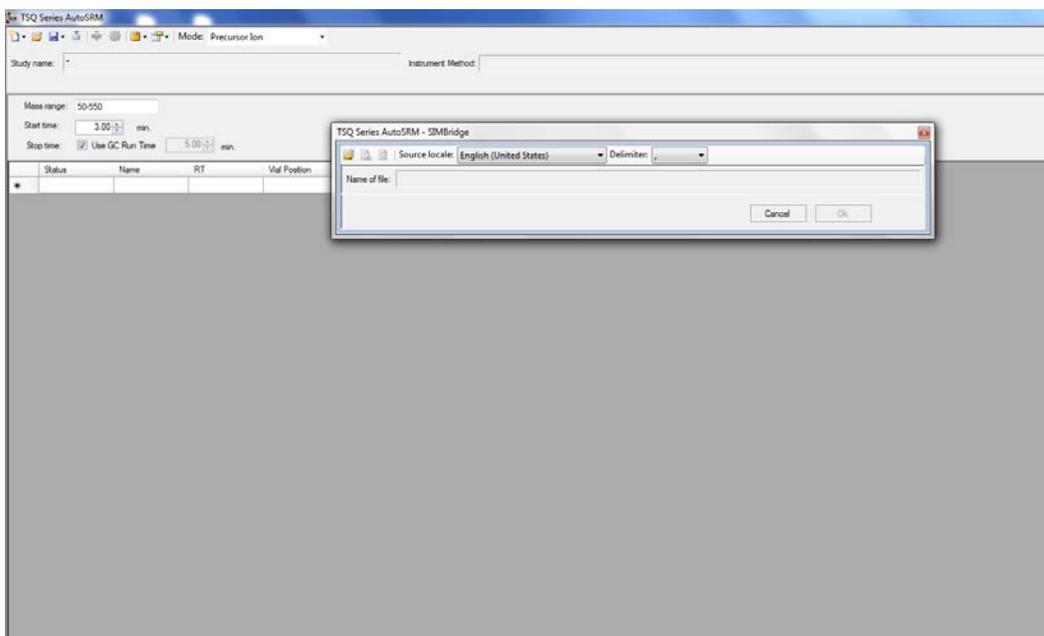
8. Click the File icon. The **Select the File Containing the Scans** dialog box opens. Browse to your file. See Figure 6.

Figure 6. Linking to an External File using SIM Bridge
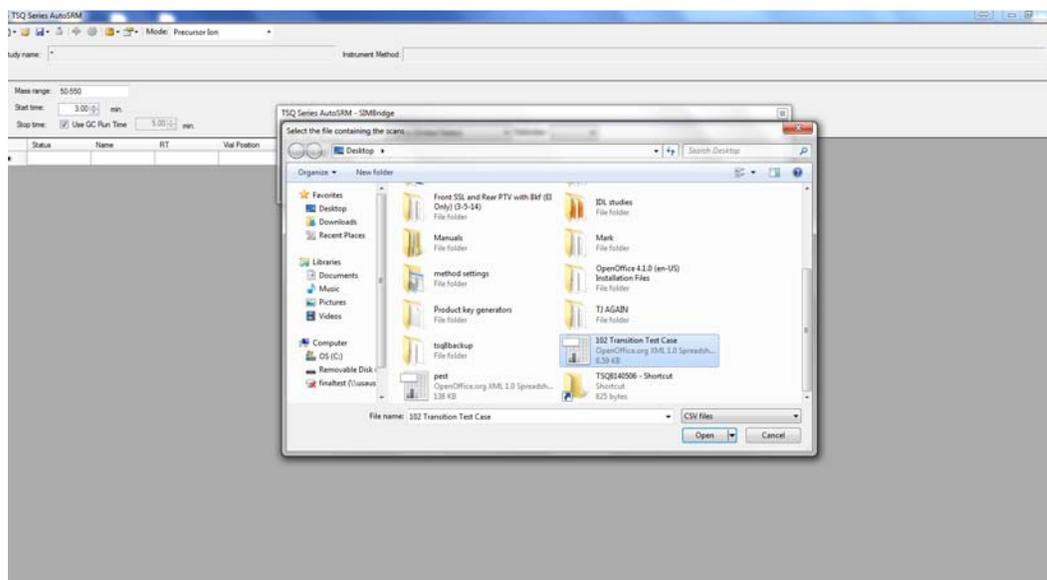
9. Click **Open** to open the method in SIMBridge.

10. 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. See Figure 7.

Figure 7. Changing Method Headings in SIMBridge
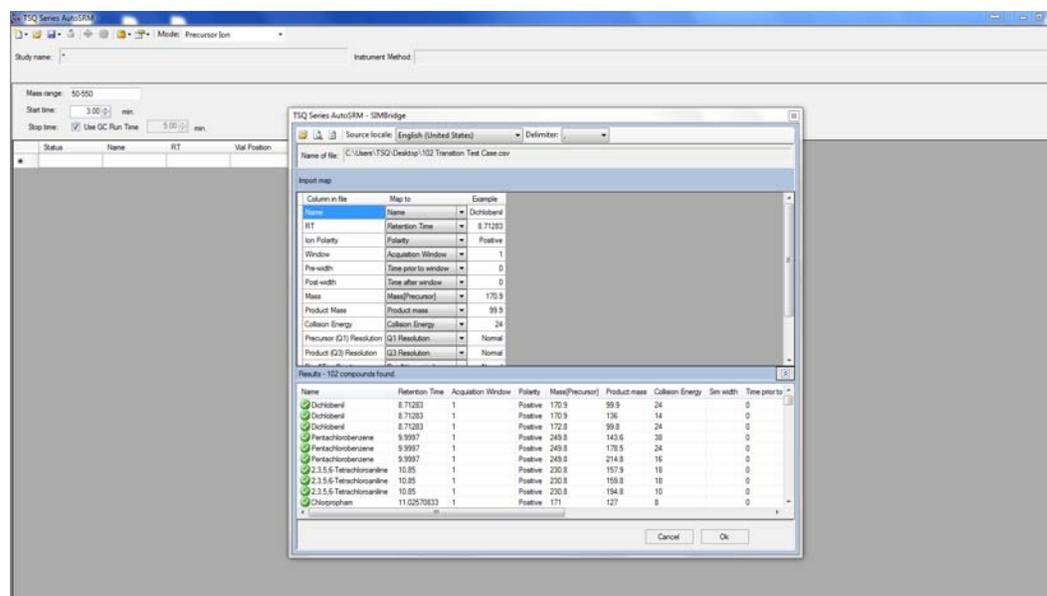
11. Your method opens in AutoSRM. AutoSRM will use the GC and autosampler parameters from this method file. You may set the Mass Range, Start Time, and Stop Time. See Figure 8.

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

Note If the method's start time is reduced, you risk scanning over the solvent front. This might contaminate the source.

Figure 8. Adjusting the Settings
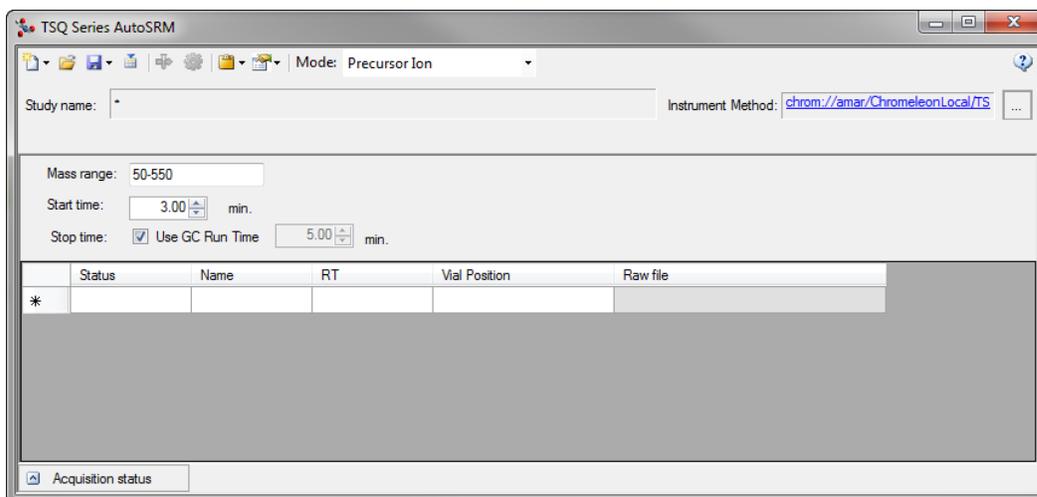
12. Enter the compound name, approximate retention time, and vial number for each compound you wish to optimize.

Tip You can import the compound information from another source by clicking the Import icon. The import file must be in the same .csv format that the TSQ Series instrument method exports through its Export Timed Scans function

13. Click the **Save the Study** icon.

Tip To simplify your workflow, create a folder for your study. Files that AutoSRM generates, including raw data files, will be saved into the same folder in which you save the study file.

14. Give your study a name and save it in the study folder. See Figure 9.

Figure 9.   Saving an AutoSRM Study
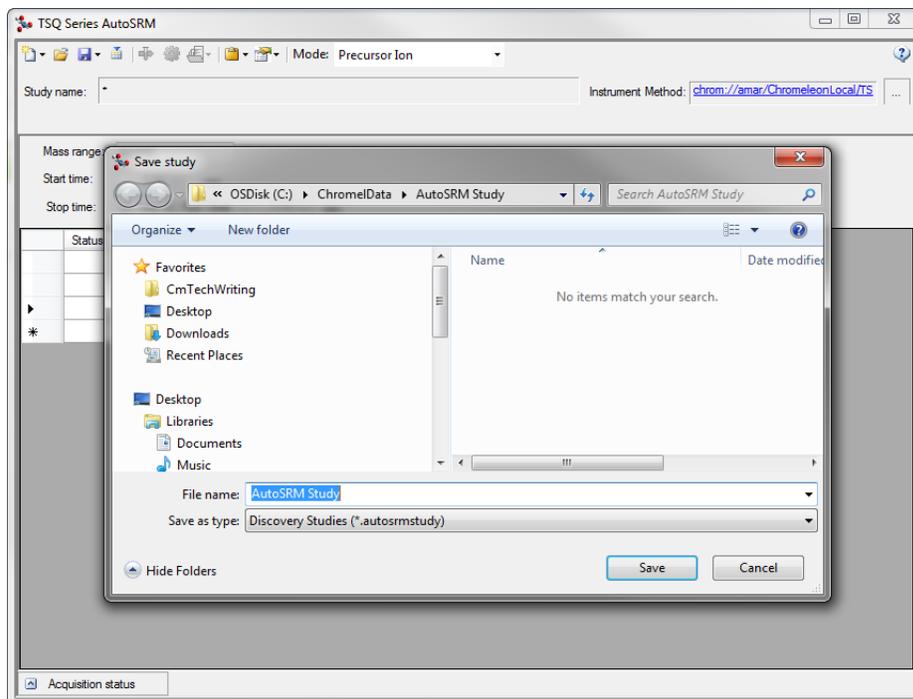
15. The Windows Explorer window closes.

16. The Study Name is the name you assigned. See Figure 10.

Figure 10.   Acquiring Acquisition Data
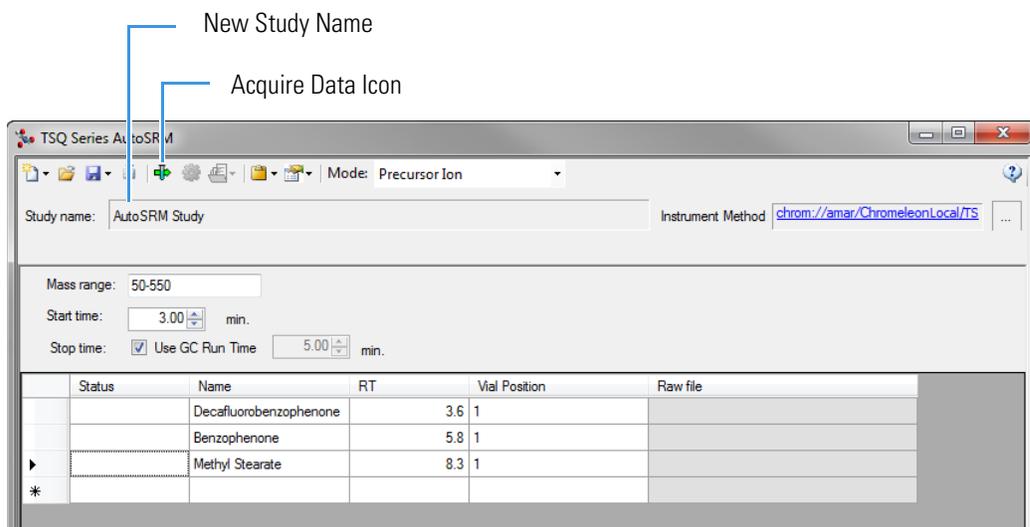
17. (CI Only) If you are running a chemical ionization (CI) study, select **Positive** or **Negative** from the **Ion Polarity** pull-down menu. See Figure 11.

Figure 11. Setting Ion Polarity in a CI Method
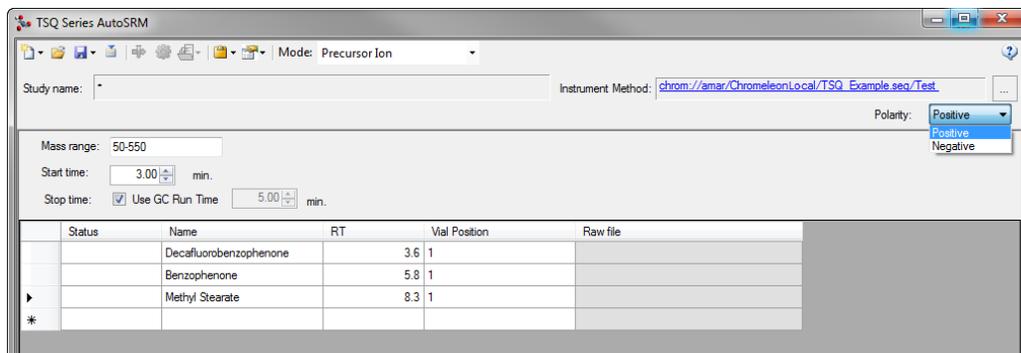
18. Click the **Acquire Data** icon to run your samples. See Figure 10.

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

The Submit Study for Acquisition window opens. See Figure 12.

Figure 12. Submitting a Study for Acquisition
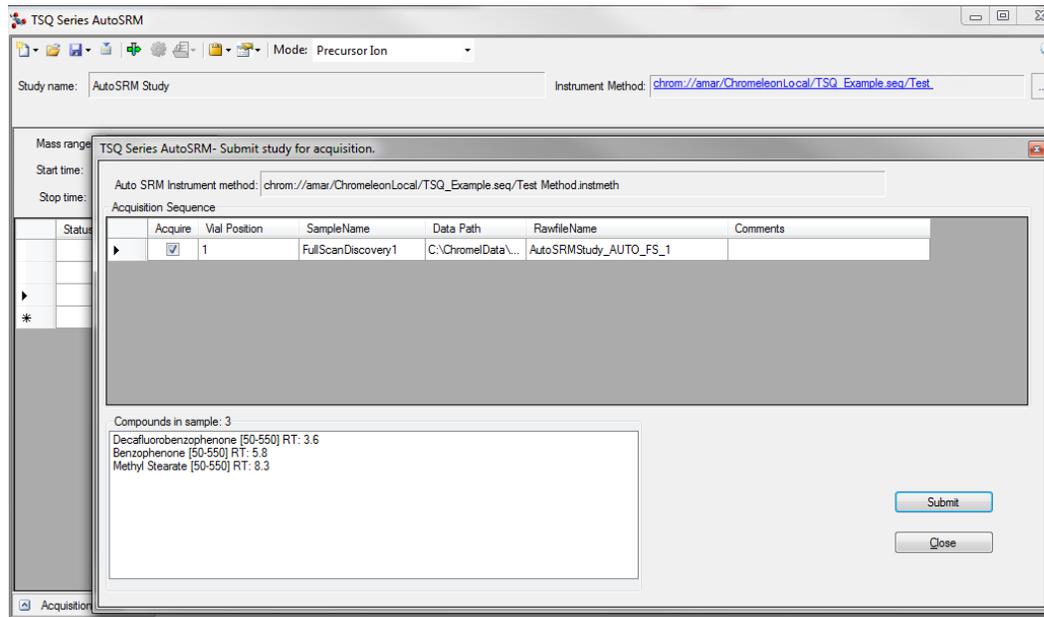
19. Click **Submit** to submit the samples to the instrument.

The AutoSRM 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.

20. 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 13.

Figure 13. Displaying the Acquisition Status Pane
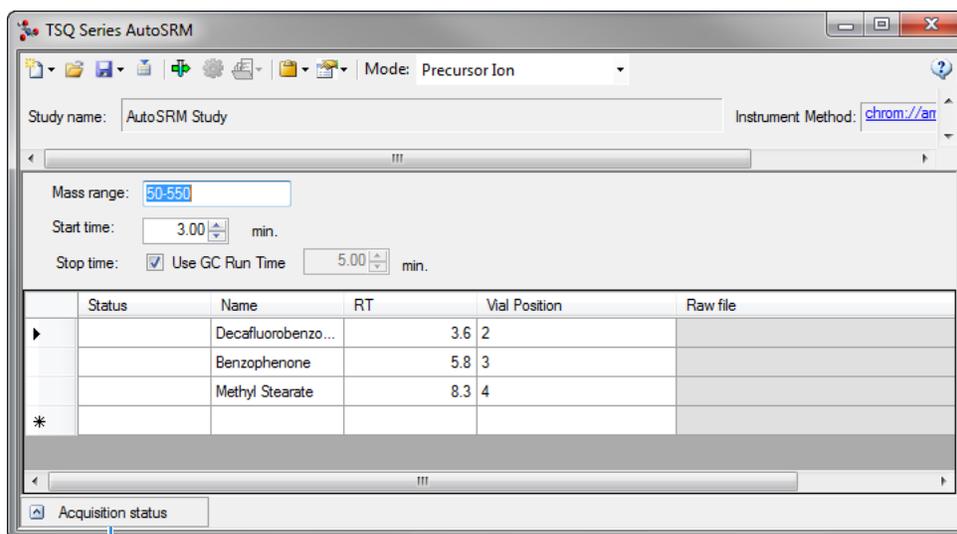
Acquisition Status Button

The pane contains a Chromeleon ePanel Set that you can use to monitor the status of
AutoSRM injections. See Figure 14. 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 14. Viewing Instrument Status on the Home ePanel
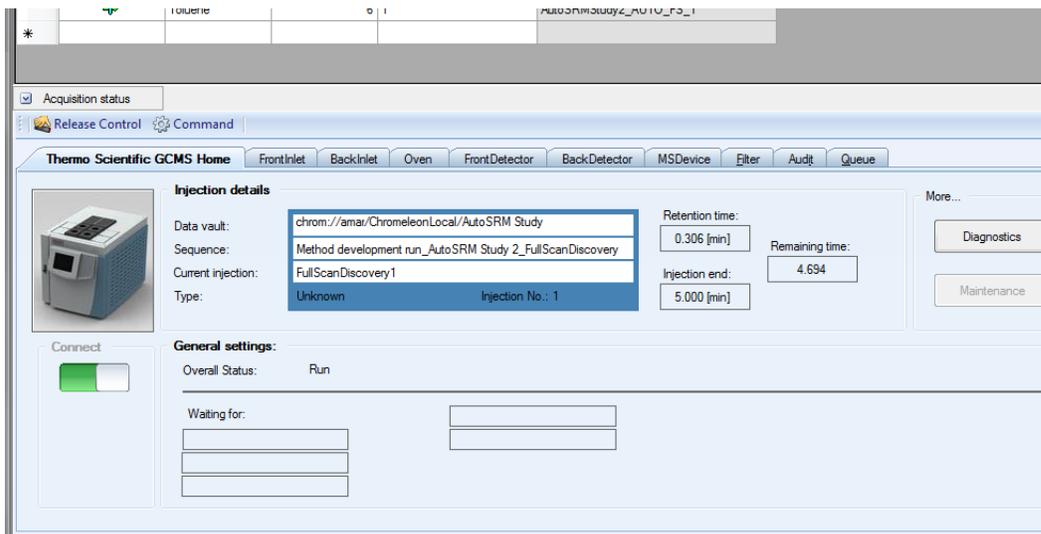
21. For an overview of the status of the run, click the **Home** tab. To monitor or control a configured device (mass spectrometer, detector, oven, etc.) click the tab for the device.

22. To view the queue status or to stop or start the queue, click the **Queue** tab. See Figure 15.

Figure 15. Viewing Queue Status on the Queue ePanel
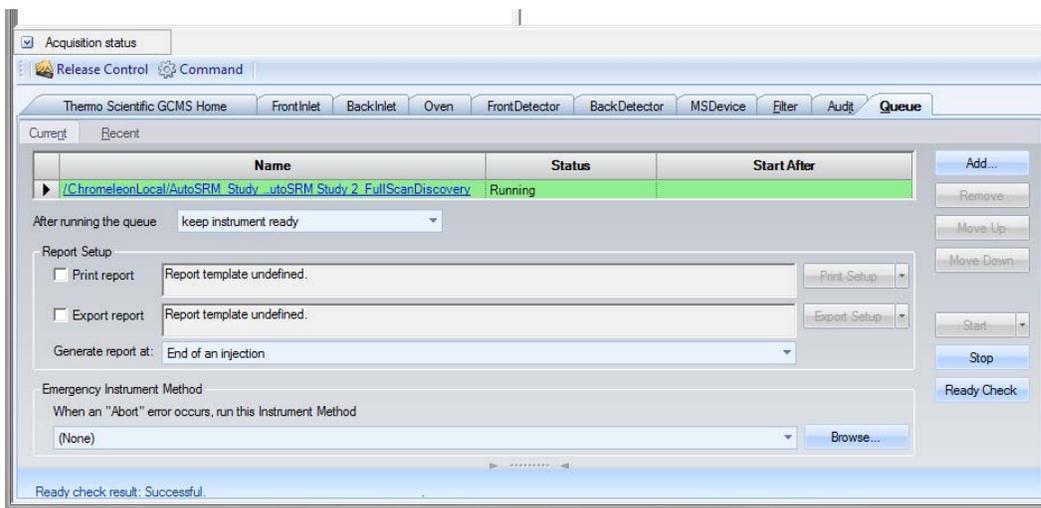
Once the samples have finished running, the software analyzes the data.

23. The results appear in the AutoSRM window. See Figure 16.

Figure 16. Study Results in AutoSRM

Peak Associated with Displayed Results

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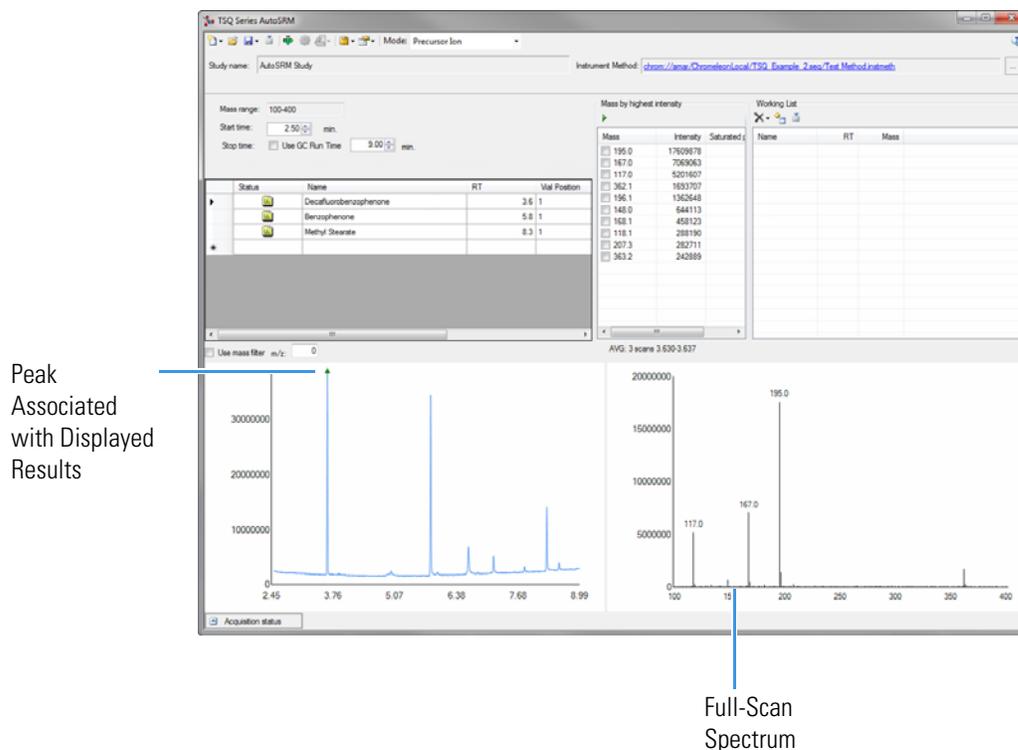
The results displayed correspond to the peak topped by the green triangle. The Mass by Highest Intensity pane contains a list of the highest intensity ions at the indicated retention time.

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

You are now ready to select the precursor ions you want to send to the working list.

> **Tip** To have AutoSRM select the precursor ions for you, click the purple **Auto Process** icon (see Figure 17). AutoSRM selects the highest intensity ions for each compound. The number of ions chosen for each compound can be set in the options window.

Figure 17. Automatically Selecting Precursor Ions

Auto Process Icon
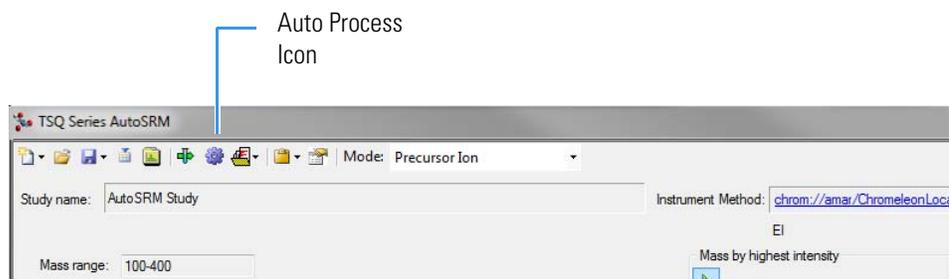
24. Select the check box next to the precursor ions you want to send to the working list, double-click on the ions in the spectra window, or click the **Settings** icon and choose **Show Precursor Ion Settings** to access additional settings for selecting precursor ions. See Figure 18.

Figure 18. Accessing the Precursor Ion Settings
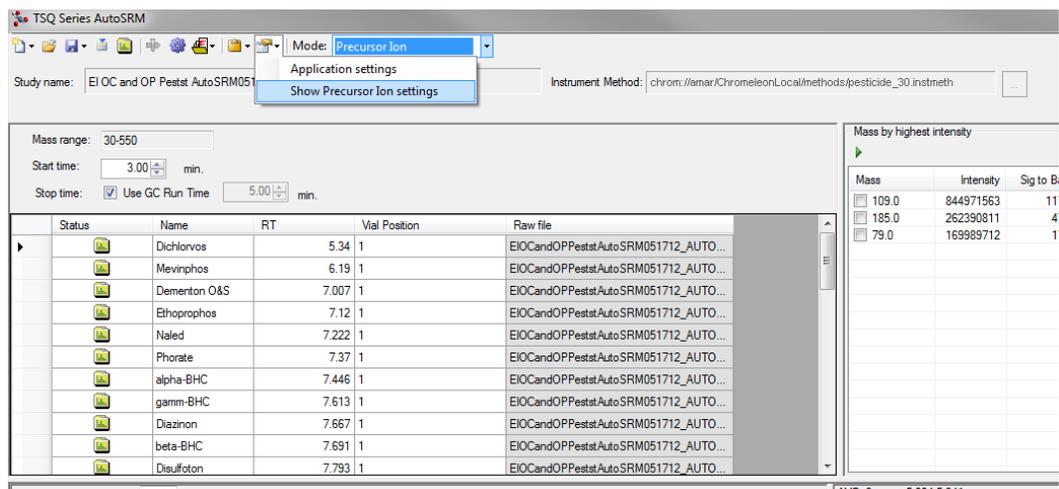
25. The **Precursor Ion Settings** pane opens. See Figure 19.

Figure 19. Precursor Ion Settings
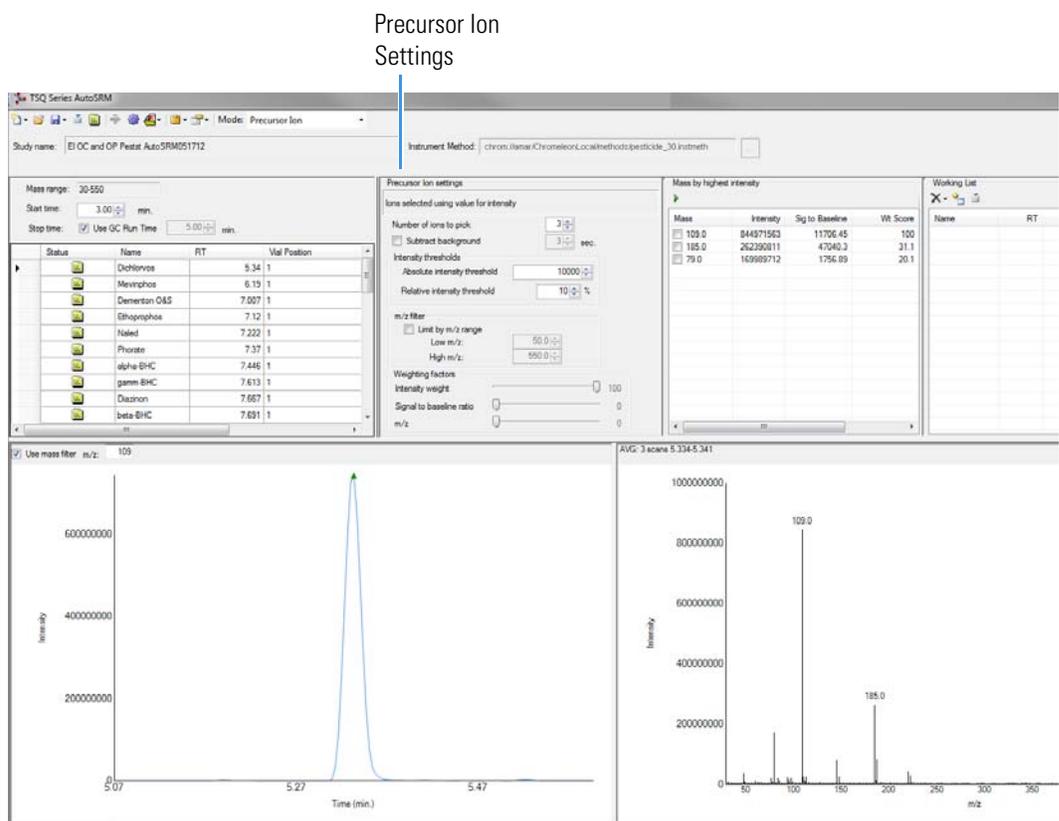
26. By default, precursor ions are sorted by highest intensity. In the Precursor Ion Settings box, you may select precursor ions according to the following criteria.

    a. **Number of Ions to Pick**: Selects the number of precursor 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 may reduce the effects of column bleed. If the automatic background subtraction is not ideal (e.g., 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 select precursor ion candidates based on intensity levels.

        i. **Absolute Intensity Threshold**: Sets the minimum intensity level such that all ions must be greater than this intensity to be selected as precursor ion candidates.

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

    d. **Limit by *m/z* Range**: Check this box and set the low *m/z* and high *m/z* to limit your precursor 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 precursor ion study setting. The available factors are **Intensity Weight** (peak height), **Signal to Baseline Ratio** (peak height/baseline height), and ***m/z***. Set the weights of each from 0 to 100, with a total weight of 100.

    > **Tip** When selecting precursor ions, you often get better results by weighting *m/z* higher than intensity weight.

27. Click the green arrow icon to push the precursor ions you selected to the working list. See Figure 20.

Figure 20. Selecting Precursor Ions for Product Ion Study
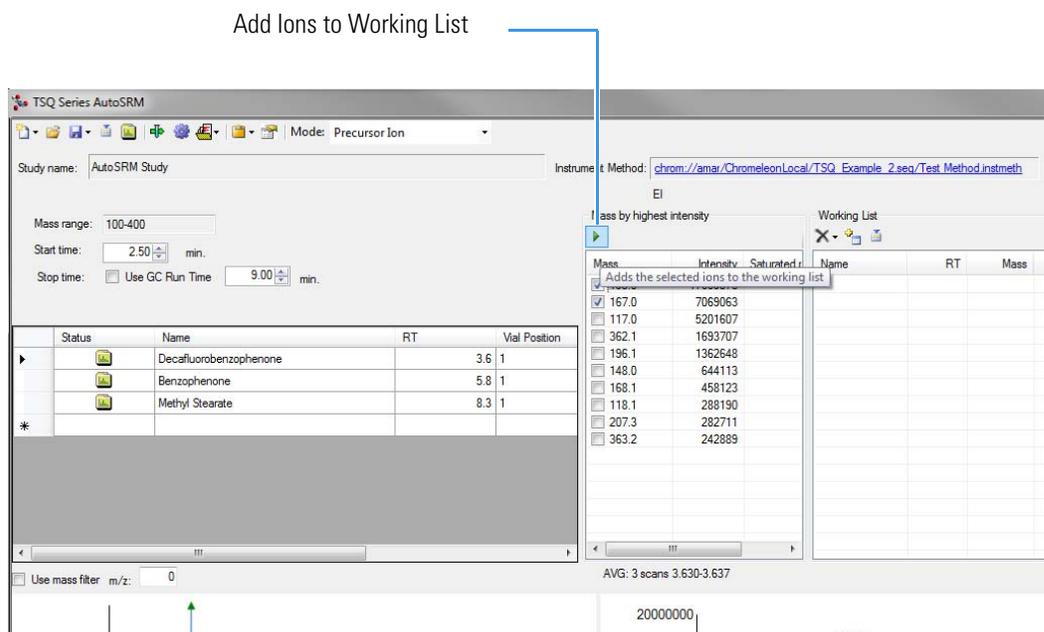
28. Repeat this process for all of your compounds.

> **Note** You can select precursor ions by checking them in the mass list or double-clicking on the ion in the spectra window.

You have obtained your list of precursor ions and are now ready to conduct the product ion study.

29. When you have reviewed all the compounds and selected the precursor ions, click the **Push** icon in the working list to push the transitions in the working list to the Product Ion Study. See Figure 21.

Figure 21. Pushing Compounds in Working List to Product Ion Study
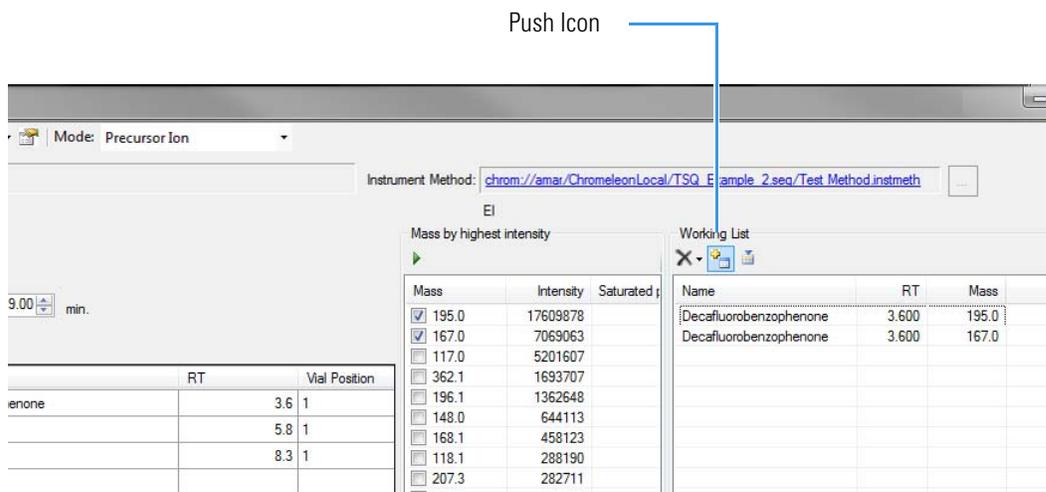
Conducting the Product Ion Study

Now that you have selected your precursor masses, you can find the product masses. AutoSRM instructs the TSQ Series system to acquire product ion scans of your precursor masses at three collision energies. You do not have to set up any methods or sequences; AutoSRM automatically does these for you. Also, the software instructs the autosampler to make multiple injections if compound retention times are too close or if multiple precursor ions are selected for a single compound.

Along with your chromatographic peak and your product ion spectra, AutoSRM presents you with a table of the most intense product ion masses from which to choose. If you prefer, AutoSRM can select the product ion masses for you.

Tip You can import a SIM method directly into this step by clicking the Import icon. The import file must be in the same .csv format that the TSQ Series instrument method exports through its Export Timed Scans function.

❖ **To conduct a product ion study**

1. The AutoSRM window displays the list of precursor ions you selected for the Product Ion study. See Figure 22.

Figure 22. Precursor Ions Selected for Product Ion Study
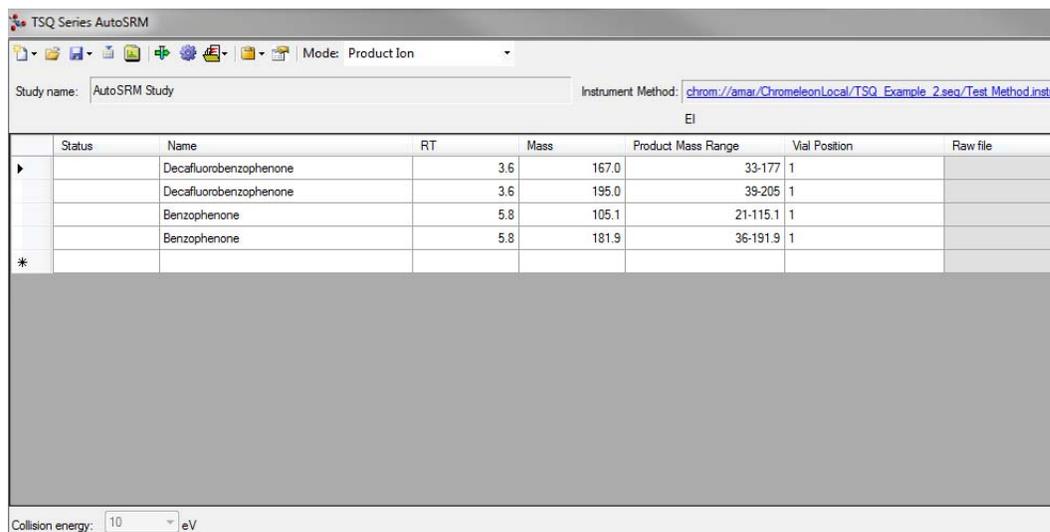
2. Click the **Settings** icon to access the product ion settings. See Figure 23.

Figure 23. Accessing Product Ion Settings

3. The Product Ion Settings box opens. See Figure 24.

Figure 24. Product Ion Settings
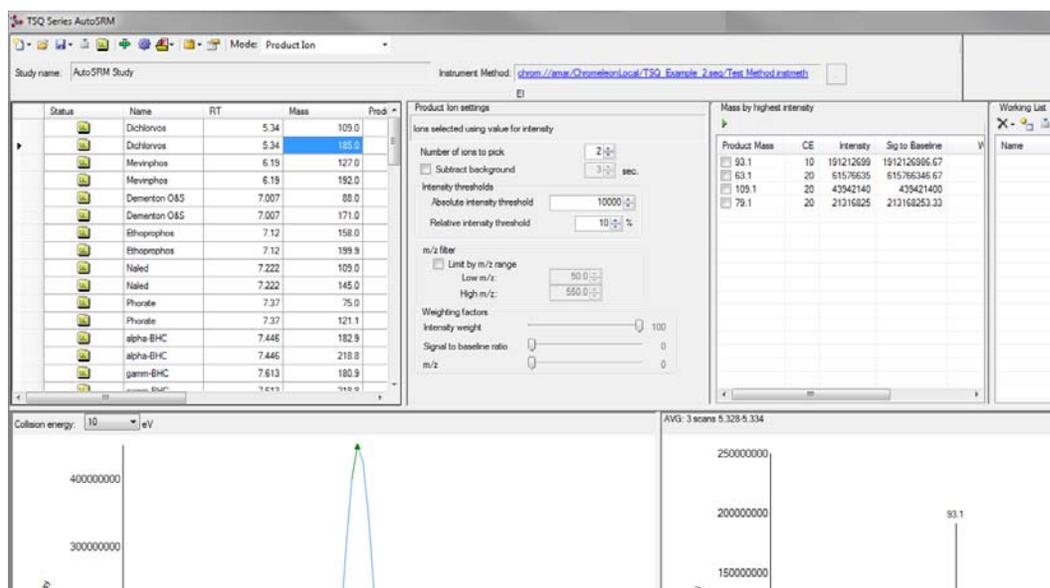
4. By default, product ions are sorted by highest intensity. In the Product Ion Settings box, you may select product ions according to the following criteria.

   a. **Number of Ions to Pick**: Selects the number of product 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 the effects of column bleed. If the automatic background subtraction is not ideal (e.g., 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 use intensity levels.

      i. **Absolute Intensity Threshold**: Sets the minimum intensity level such that all ions must be greater than this intensity to be selected as precursor ion candidates.

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

   d. **Limit by *m/z* Range**: Check this box and set the low *m/z* and high *m/z* to limit your product 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 product ion study setting. The available factors are **Intensity Weight** (peak height), **Signal to Baseline Ratio** (peak height/baseline height), and ***m/z***. Set the weights of each from 0 to 100, with a total weight of 100.

   > **Tip** When selecting product ions, you often get better results by weighting intensity weight higher than *m/z*.

5. Review the product ion list and update the vial numbers if necessary.

6. Save the study by clicking the **Save** icon.

7. Click the green **Acquire Data** icon to submit the study for acquisition.

8. Click **Submit**. See Figure 25.

Figure 25. Submitting Precursor Ions to Product Ion Study
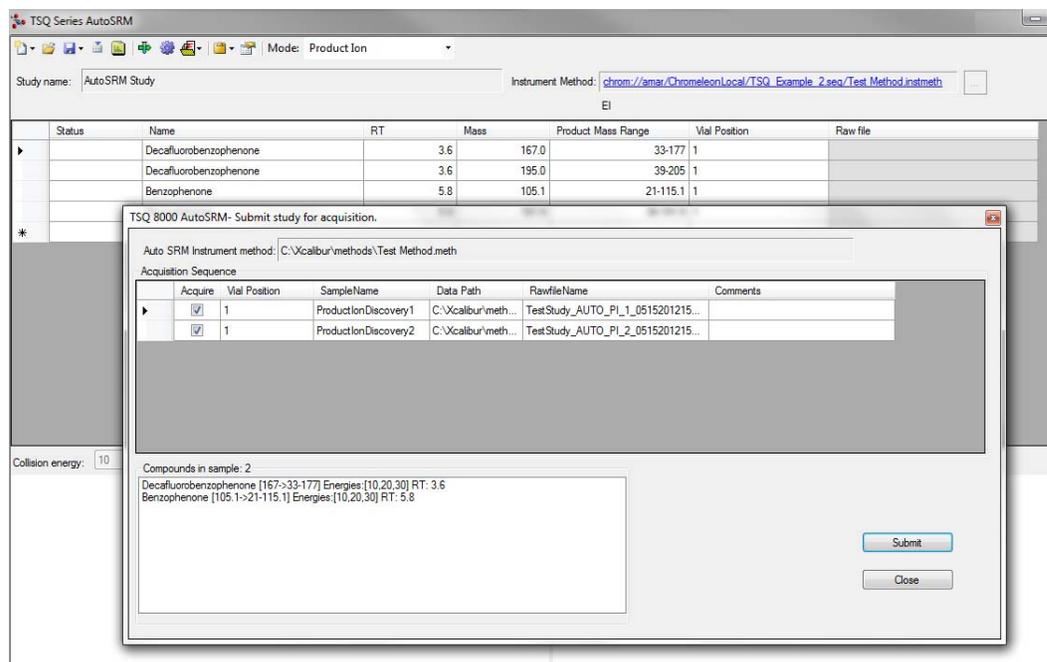
AutoSRM calculates the number of injections needed based on the compound list and vial positions you assigned.

During the Product Ion study, each product ion scans at 10, 20, and 30 eV collision energies to get a good collision energy range over which to search for the best product ions.

9. Select the different collision energies from the drop-down box to view the product ions produced at different collision energies. See Figure 26.

Figure 26. Selecting Collision Energies

Collision Energy
Drop-down Box
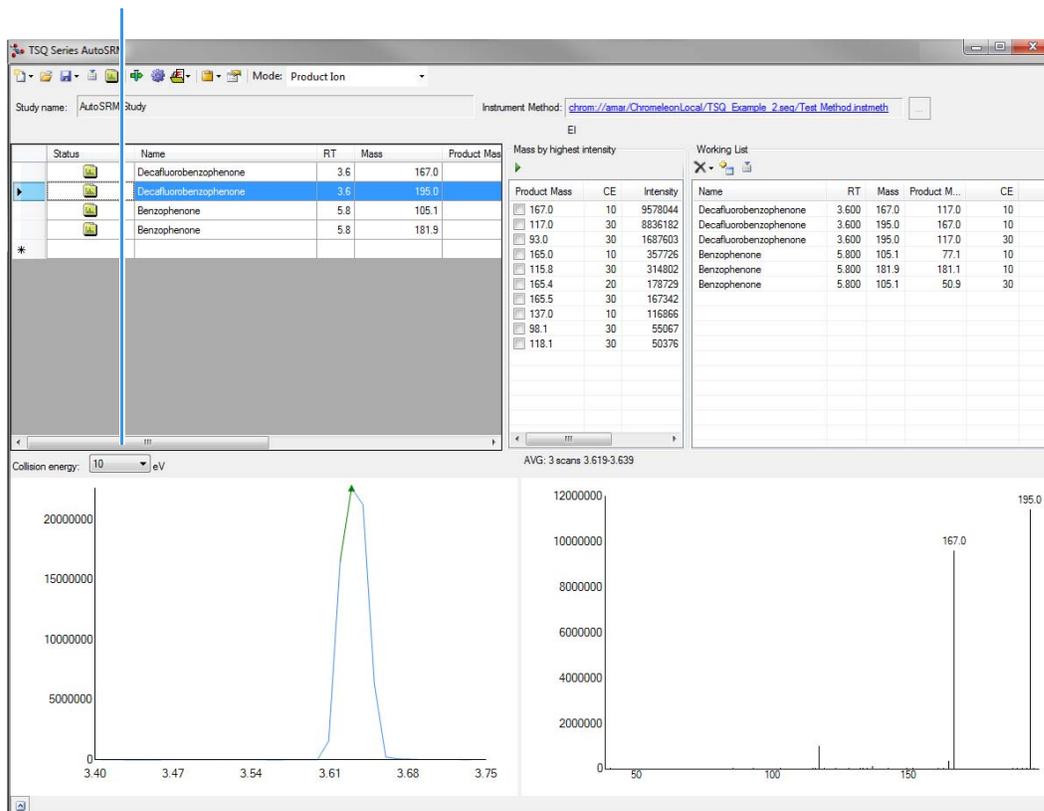
10. Choose the product ions and collision energies you want for SRM optimization and push them to the working list. See Figure 27.

> **Note** AutoSRM lists product ions in order of highest energy intensity and at the optimal collision energy for each product ion.

> **Tip** AutoSRM automatically processes the data for you and suggests transitions if you click the purple **Auto Process** icon.

Figure 27. Pushing Product Ions to Working List for SRM Optimization
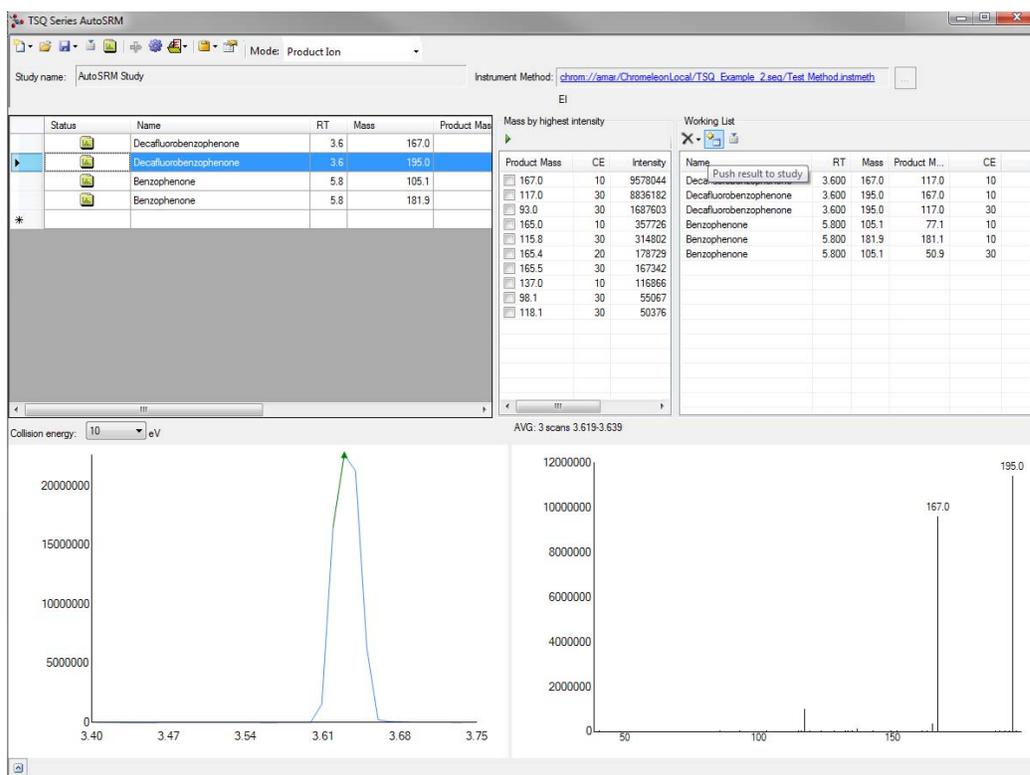
From this point you may export your transition list to your instrument method, or you may send your selected transitions to an SRM optimization study for further optimization.

Developing the SRM Optimization Study

The final step in SRM development is the SRM optimization step. Now that you have selected precursor and product masses, AutoSRM acquires those transitions at multiple collision energies. Because of its fast scanning capabilities, the TSQ Series GC-MS system has the ability to analyze three transitions per compound, each at 10 unique collision energies, in a single injection.

❖ **To develop an SRM optimization study**

1. Push the working list of precursor ions to the SRM optimization study.

> **Tip** You can import an SRM method directly into this step by clicking the Import icon. The import file must be in the same .csv format that the TSQ Series instrument method exports through its Export Timed Scans function.

2. Select Targeted or Full Range study in the SRM Energy Range drop-down box. See Figure 28.

   Targeted optimizations use a collision energy range that is 10 eV on either side of the target energy in 2 eV steps. Full Range optimizations use collision energies from 5 to 50 eV in 5 eV steps.

> **Note** A single study cannot perform both Full Range and Targeted studies. If you want to do both, you must create a separate study. This can be accomplished through the Create New Study from Existing Study function.

Figure 28. Selecting SRM Energy Range

3. Save the study and submit the samples for analysis. See Figure 29.

Figure 29. Submitting SRM Optimization Study for Analysis
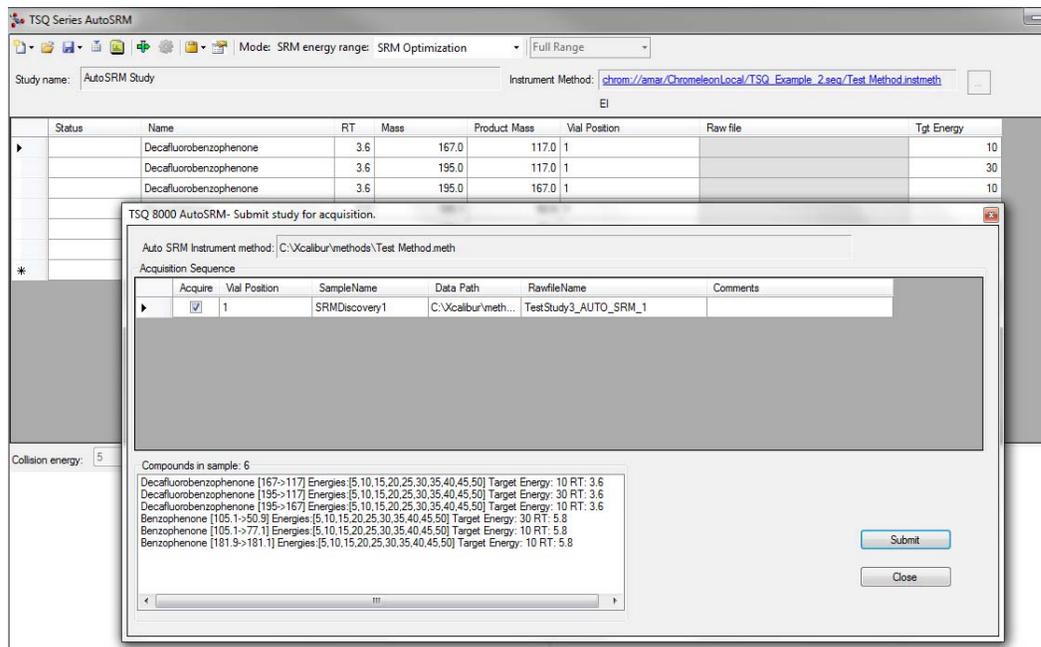
4. Once the analysis is complete, the software displays the data for you to perform a final review. See Figure 30.

Figure 30. Reviewing your SRM Optimization Data
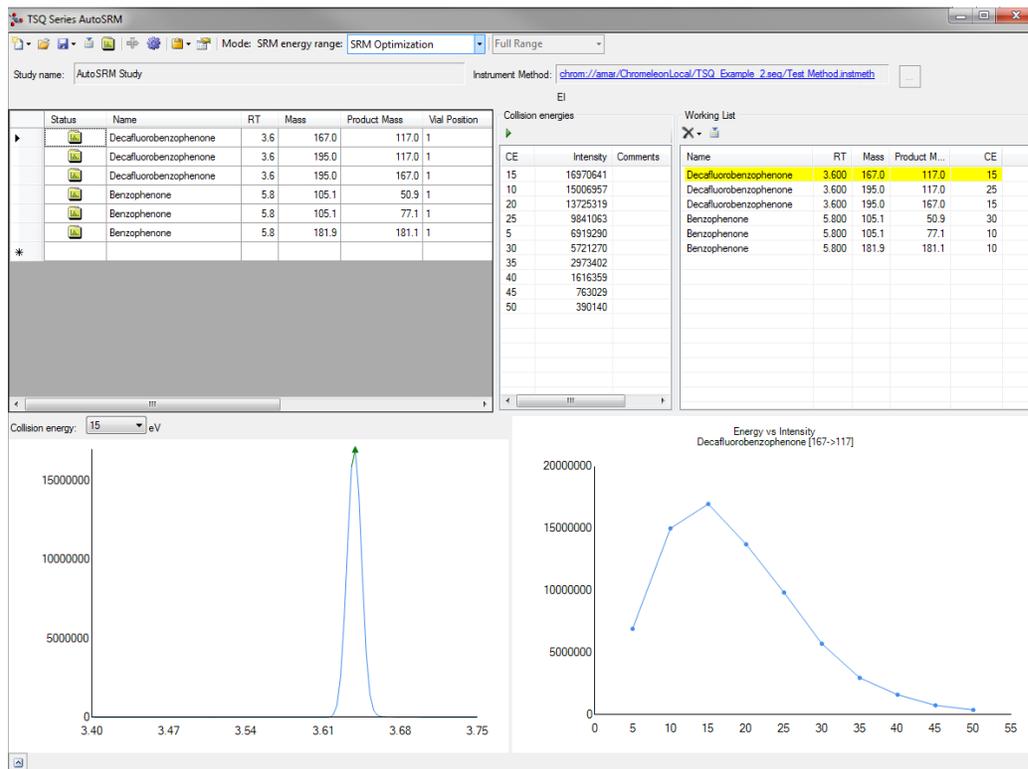
5. Review each transition and push the best collision energy to the working list.

> **Note** The software will choose the peak with the highest intensity. If compounds with similar retention times share transitions, you might need to select the appropriate peak using the green triangle.

6. Once you have reviewed the data and chosen the transitions you want to use, save the study and create a .csv file to transfer the transitions to your instrument method. See Figure 31.

Figure 31. Creating File to Transfer Transitions
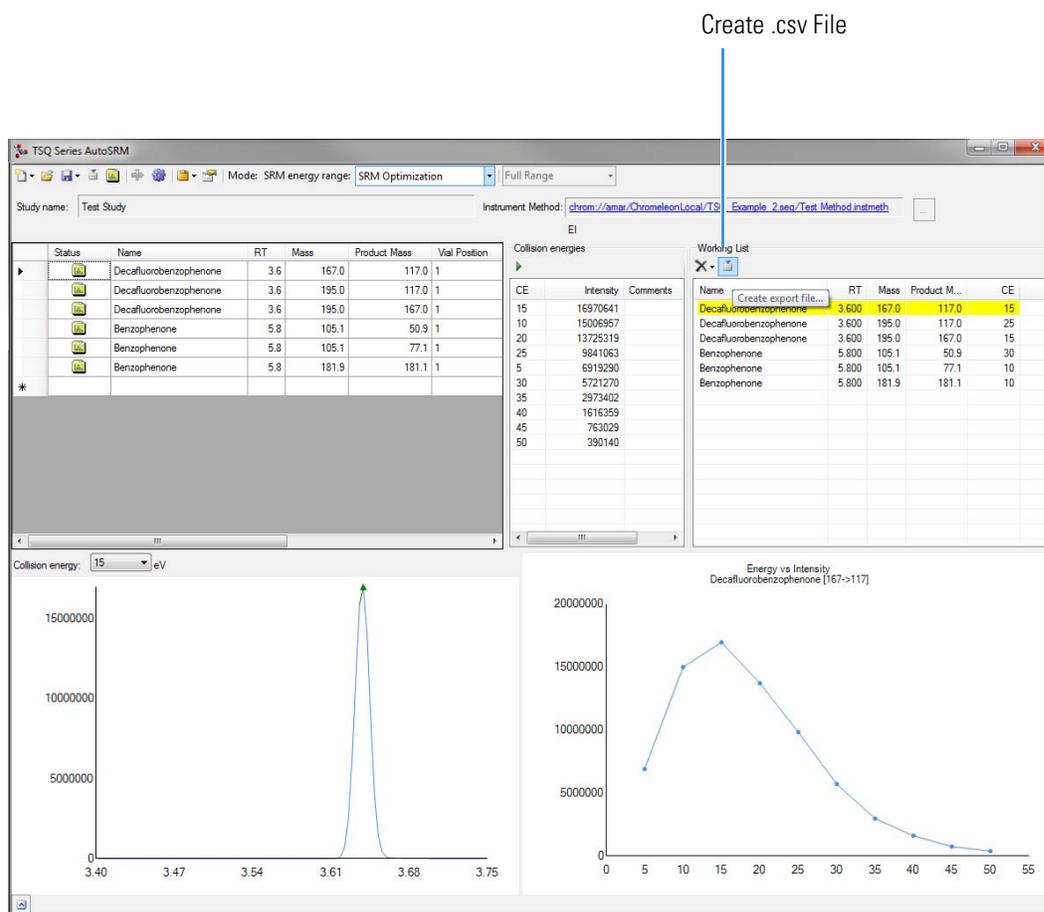
Once complete, AutoSRM allows the simple creation of a TSQ Series instrument method to be ready for routine analysis.

Importing Transitions into the Method Editor

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

1. Open your instrument method in Chromeleon.

> **Tip** Click the link in the Instrument Method box in AutoSRM to open Chromeleon.

2. Click the mass spectrometer icon in the method editor side pane. See Figure 32.

Figure 32. Viewing Instrument Method in Chromeleon
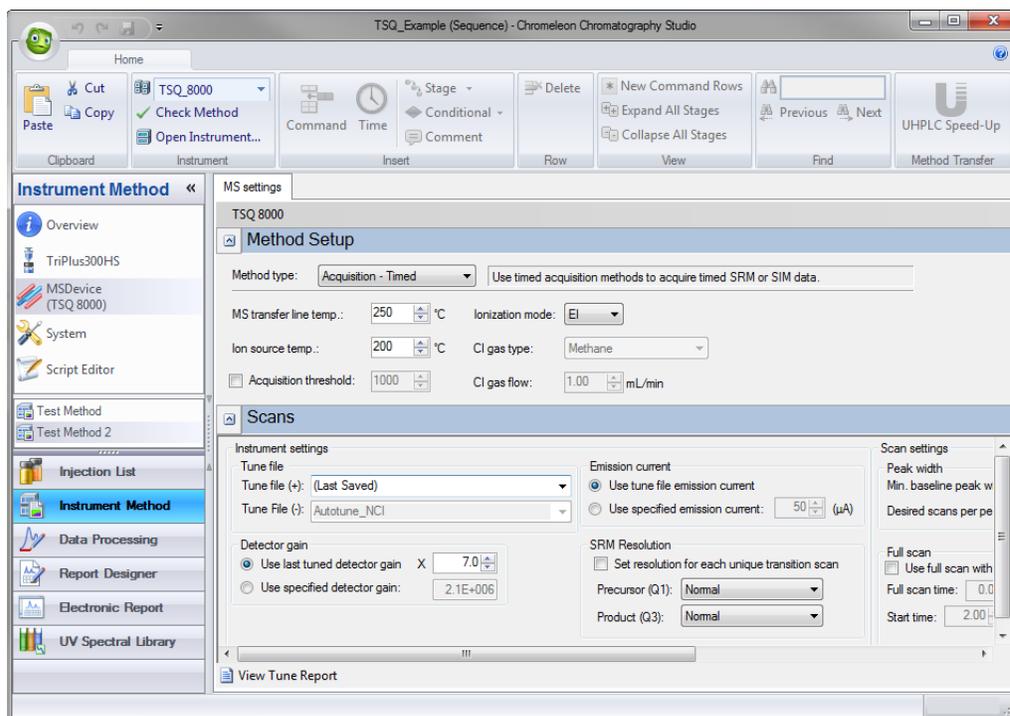
3. From the Method Type drop-down menu, select **Acquisition-Timed**.

4. Under the MS Settings tab, click **TSQ 8000** and select **Import timed scans**. See Figure 33.

Figure 33. Selecting Import Timed Scans
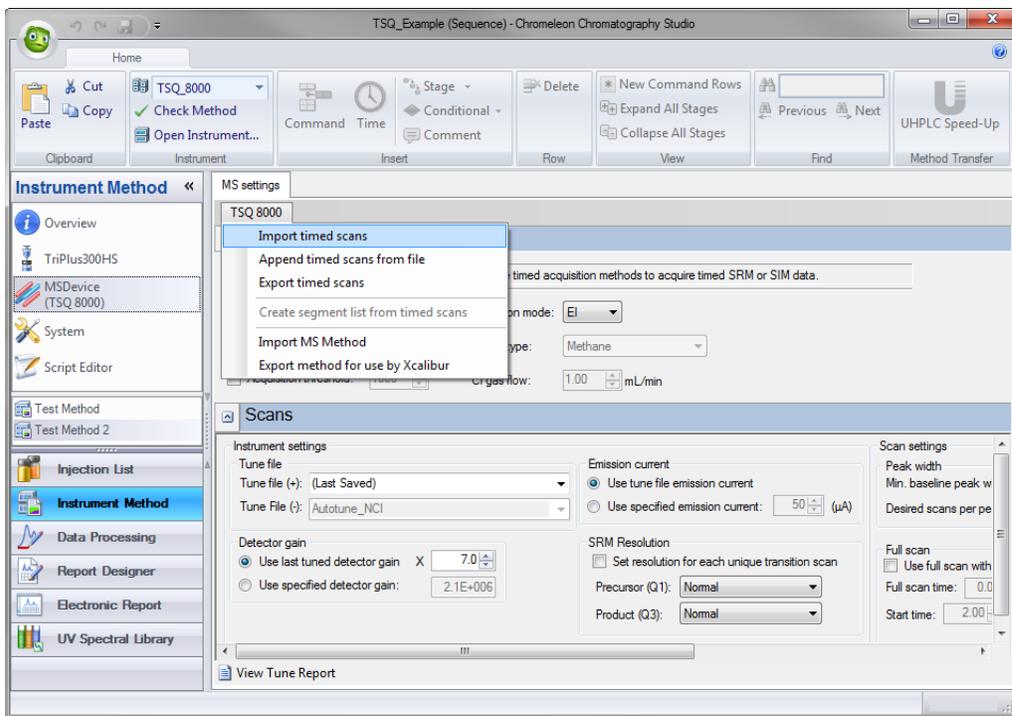
5. Browse to the location where you saved the .csv file you created in AutoSRM. The software informs you if your .csv file is valid. See Figure 34. Click **Open**.

Figure 34. Linking to an External .csv File
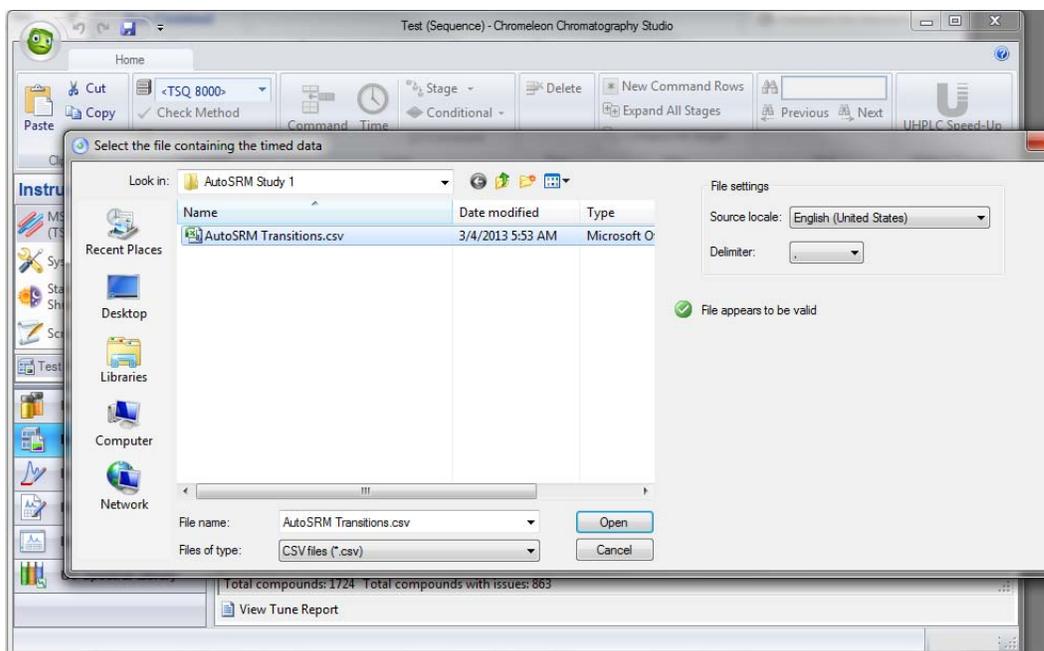
6. Browse to the location where you saved the .csv file you created in AutoSRM. The **SIMBridge** dialog box opens. Choose the language of your method file from the **Source Locale** drop-down menu. See Figure 35.

Figure 35. Setting the Source Language of Method Files using SIMBridge
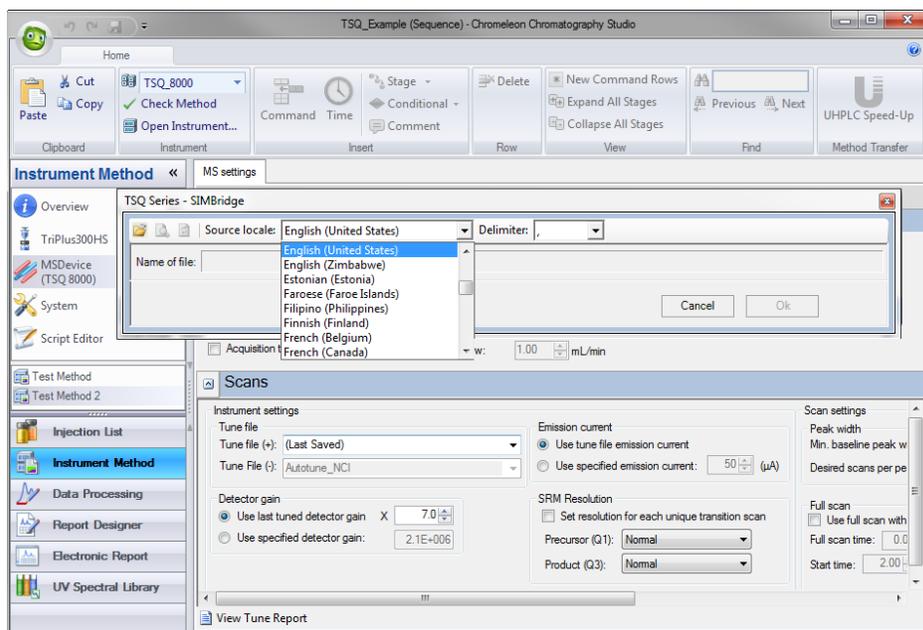
7. Browse to your file. See Figure 36.

Figure 36. Linking to an External File using SIM Bridge
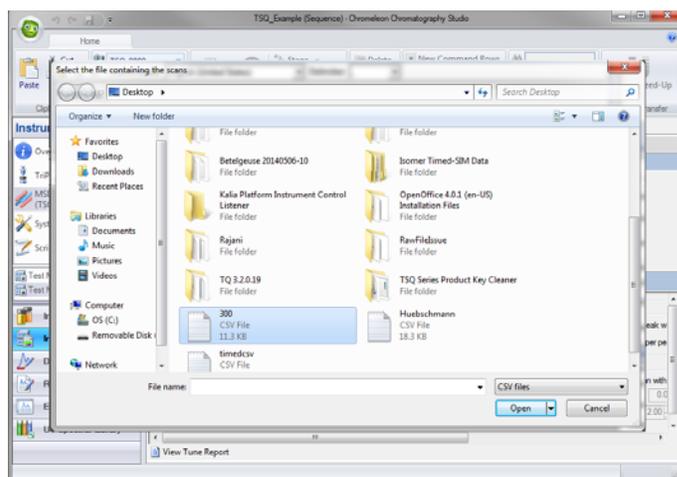
8. Click **Open** to open the method in SIMBridge.

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. See Figure 37.

Figure 37. Changing Method Headings in SIMBridge
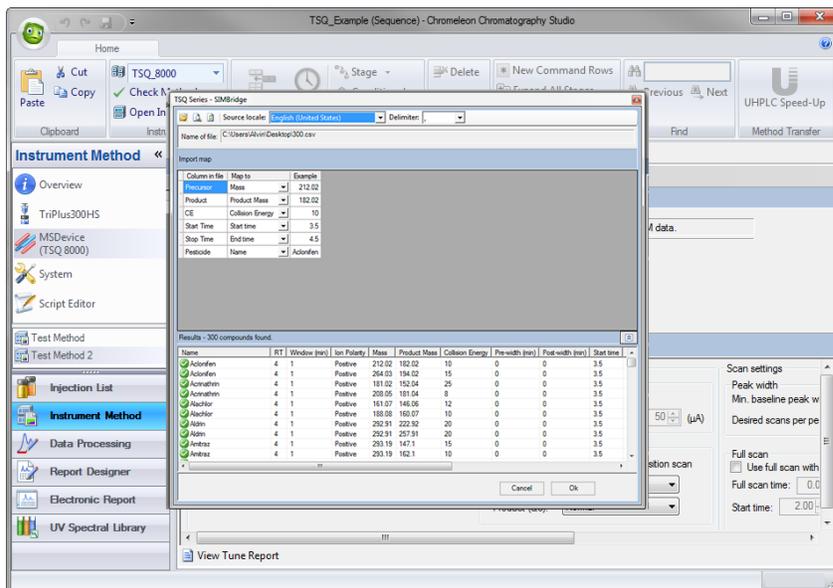
10. Click **Show Analysis** (see Figure 38) and review the imported list of compounds. See Figure 39.

Figure 38. Selecting Show Analysis
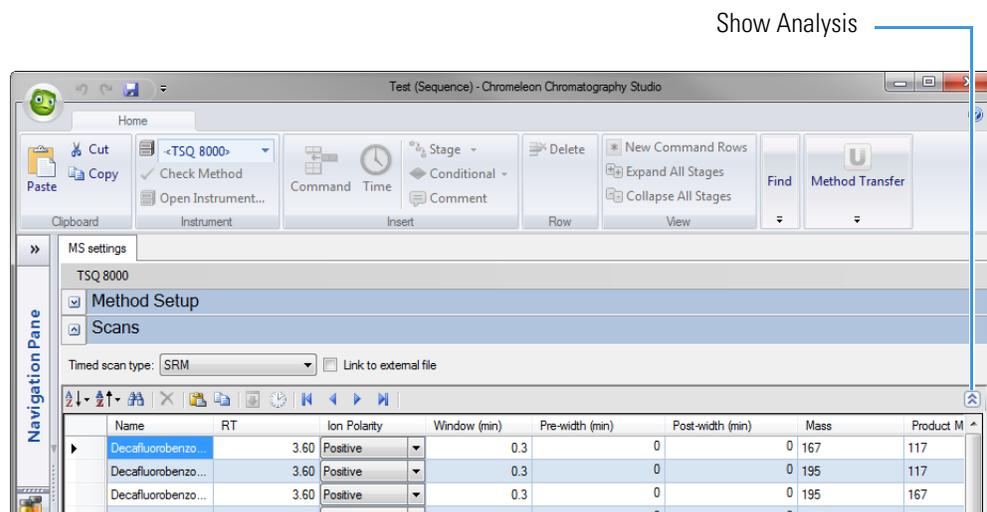
Figure 39. Reviewing the Imported List
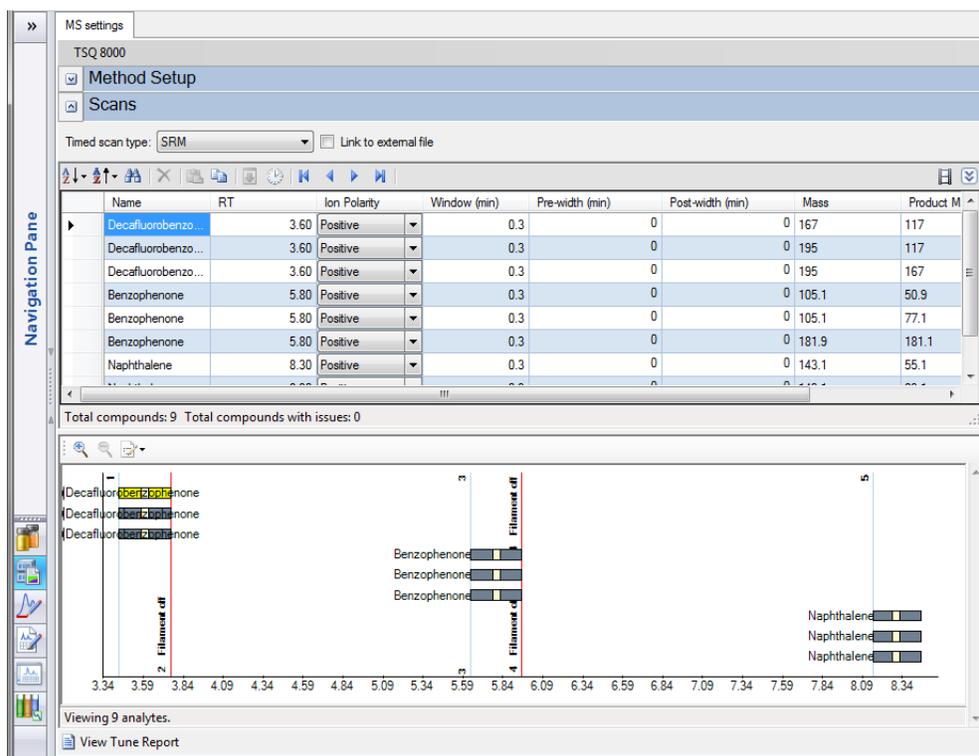
11. Adjust your scan settings as necessary. See the user guide for your instrument for more information.

12. Once you are satisfied with your method, save it.

13. Run a set of samples to verify that the method meets your needs.