AB SCIEX 4500 Series of Instruments

System User Guide

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

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AB Sciex LP is ISO 9001 registered.

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Contents

Foreword

This foreword contains general safety-related information, describes the symbols and conventions used in the documentation, and provides regulatory compliance information. It also describes potential hazards and associated warnings for the system, and the precautions that should be taken to minimize the hazards. In addition to this foreword, refer to the *Site Planning Guide* for site requirements, including mains supply, source exhaust, ventilation, compressed air, nitrogen and the roughing pump.

General Safety Information

Before operating any instrument, become familiar with its operation and with the potential hazards. To prevent personal injury or instrument damage, read, understand, and obey all safety precautions. Warnings in this document and labels on the mass spectrometer are shown with international symbols. Failure to heed these warnings could result in serious injury.

This safety information is intended to supplement federal, state or provincial, and local environmental health and safety (EHS) regulations. The information provided covers instrument-related safety with regard to the operation of the mass spectrometer. It does not cover every safety procedure that should be practised. Ultimately, you and your organization are responsible for compliance with federal, state or provincial, and local EHS regulations and for maintaining a safe laboratory environment.

For more information, refer to the appropriate laboratory reference material and standard operating procedures.

Symbols and Conventions

The following conventions are used throughout the guide.

DANGER! Danger signifies an action which leads to severe injury or death.

WARNING! Personal Injury Hazard: A warning indicates an operation that could cause personal injury if precautions are not followed.

WARNING! Electric Shock Hazard: This symbol indicates a warning of electrical shock hazard. Read the warning and follow all precautions before performing any operation described in the guide. Failure to do so can result in serious injury.

WARNING! Burn Hazard: This symbol indicates a warning of potential burns from hot surfaces. Read the warning and follow all precautions before performing any operation described in the guide. Failure to do so can result in serious injury.

WARNING! Biohazard: This symbol indicates a warning of biohazardous materials. Read the warning and follow all precautions before performing any operation described in the guide. Failure to do so can result in serious injury.

Caution: A caution indicates an operation that could cause damage to the instrument or loss of data if precautions are not followed.

Tip! Provides useful information that helps apply the techniques and procedures in the text for a specific need, and provides shortcuts, but is *not essential* to the completion of a procedure.

Note: A note emphasizes significant information in a procedure or description.

Qualified Personnel

After installing the system, the FSE (Field Service Employee) uses the *Customer Familiarization Checklist* to train the customer on system operation, cleaning, and basic maintenance. Only qualified AB SCIEX personnel shall install and service the equipment. Only personnel qualified by AB SCIEX shall operate and maintain the equipment. Contact an AB SCIEX FSE for more information.

Equipment Use and Modification

Use the system indoors in a laboratory that complies with the environmental conditions recommended in the system *Site Planning Guide*. If the system is used in an environment or in a manner not prescribed by AB SCIEX, the protection provided by the equipment can be impaired.

Unauthorized modification or operation of the system may cause personal injury and equipment damage, and may void the warranty. Erroneous data may be generated if operating the instrument while exceeding the recommended environmental conditions or operating with unauthorized modifications. Contact an AB SCIEX representative for more information on servicing the instrument.

Shipping Labels

Table 1-1 Labels on the Crate

External Labels	Definition	Action
 or 	TIP N TELL Blue beads in the arrow indicate that the container was tipped or mishandled.	Write on the Bill of Lading and check for damage. Any claims for tipping require a notation.

Regulatory Compliance

This system complies with the standards and regulations listed in this section. Applicable labels have been affixed to the system.

Australia and New Zealand

- **Electromagnetic Interference**—AS/NZ CISPR 11 (Class A)
- **Safety**—AS/NZ 61010-1

Canada

- **Electromagnetic Interference**—CAN/CSA CISPR11. This ISM device complies with Canadian ICES-001.

- **Safety**—CAN/CSA C22.2 No. 61010-1, and CAN/CSA C22.2 No. 61010-2-061

Europe

- **Electromagnetic Compatibility—**EN 55011 (Class A), EN 61326-1
- **Safety—**EN 61010-1, EN 61010-2-061
- **WEEE—**2002/96/EEC

United States

- **Electromagnetic Interference, FCC Part 15, Class A**—This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC (Federal Communications Commission Compliance) Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the operator's manual, can cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case you will be required to correct the interference, at your own expense. Changes or modifications not expressly approved by the manufacturer could void your authority to operate the equipment.
- **Safety**—UL 61010-1, IEC 61010-2-061

International

- **Electromagnetic Compatibility**—IEC 61326-1, IEC CISPR 11 Class A
- **Safety**—IEC 61010-1, IEC 61010-2-061

For more information, see the Declaration of Conformance included with the system.

Symbols and Labels on the Mass Spectrometer

Table 1-2 Labels on the Mass Spectrometer

External Labels	Definition
	**WARNING: NO USER SERVICEABLE PARTS INSIDE.** REFER SERVICING TO QUALIFIED PERSONNEL.
	EN61326—1:2006 CLASS A, GROUP 1, ISM EQUIPMENT

Table 1-2 Labels on the Mass Spectrometer (Continued)

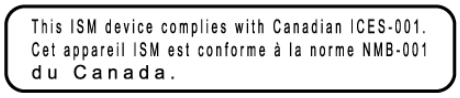
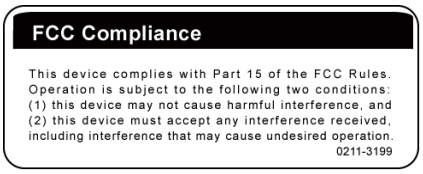
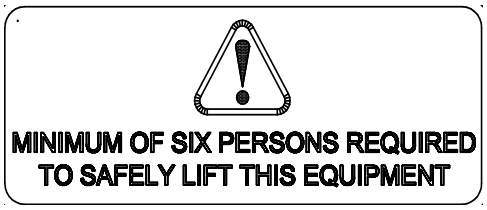
External Labels	Definition
	This ISM device complies with Canadian ICES-001. Cet appareil ISM est conforme à la norme NMB-001 du Canada.
	FCC Compliance. This device complies with Part 15 of the FCC Rules. Operation is subject to the following conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation. 0211-3199
	Do not dispose of equipment as unsorted municipal waste (WEEE).
FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.	This instrument is for research use only. It is not intended for use in diagnostic procedures.
	Caution, see accompanying documents. Minimum of 6 persons required to safely lift this equipment
	**WARNING**: Hot Surface Hazard.
	Operator Guide
	Follow operating instructions (mandatory)
	Alternating Current
A	Amperes (current)

Table 1-2 Labels on the Mass Spectrometer (Continued)

External Labels	Definition
	High voltage. Electrical Shock Hazard
	On (Mains supply)
	Off (Mains supply)
	Protective Earth (ground)
V	Volts (voltage)
V-A	Volts - Amperes (power)
W	Watts (power)

Occupational Health and Safety Symbols

This section describes some occupational health and safety symbols found in the laboratory environment.

Table 1-3 Chemical Hazard Symbols

Safety Symbol	Description
	Biohazard
	Corrosive or Caustic Chemical Hazard
	Explosion Hazard
	Oxidizing Chemical Hazard
	Poison Hazard
	Reactive Chemical Hazard

Table 1-3 Chemical Hazard Symbols (Continued)

Safety Symbol	Description
	Toxic Chemical Hazard

Table 1-4 Mechanical Hazard Symbols

Safety Symbol	Description
	Automated Machinery Hazard
	Crushing Hazard — From Above
	Crushing Hazard — From Side
	Fire Hazard
	Hot Surface Hazard
	Laser Radiation Hazard
	Lifting Hazard
	Magnetic Hazard
	Puncture Hazard

Table 1-5 Pressurized Gas Hazard Warning Symbols

Safety Symbol	Description
	Pressurized Gas Hazard

Mains Supply

WARNING! Electrical Shock Hazard: Use only qualified personnel for the installation of all electrical supplies and fixtures, and make sure that all installations adhere to local regulations.

The total combined system (instrument and roughing pump) power consumption is 2200 VA (50 or 60 Hz) at 230 VAC.

An external line transformer is not needed for the instrument, optional bench, or roughing pump.

Caution: Do not unpack or connect any components. The AB SCIEX FSE will unpack, connect, and configure the system for the proper operating voltage.

For information on system electrical specifications, refer to the Site Planning Guide.

Protective Conductor

The mains supply should include a correctly installed protective earth conductor that must be installed or checked by a qualified electrician before connecting the instrument.

Do not intentionally interrupt the protective conductor. Any interruption of the protective conductor is likely to make the installation dangerous.

Laboratory Ventilation

The venting of fumes and disposal of waste must be in accordance with all federal, state, provincial, and local health and safety regulations. The instrument shall be used indoors in a laboratory that complies with the environmental conditions recommended in the *Site Planning Guide* for the instrument. The source exhaust system must be vented either to an external fume hood or to an external exhaust system as recommended in the *Site Planning Guide* for the instrument.

Environmental Conditions

Use qualified personnel for the installation of electrical mains, heating, ventilation, and plumbing supplies and fixtures. Make sure that all installations follow local bylaws and biohazard regulations. For more information about the required environmental conditions for the system, refer to the *Site Planning Guide* for the instrument.

DANGER! Explosion Hazard: The instrument is not designed for operation in an explosive environment. Do not operate the instrument in an environment containing explosive gases.

WARNING! Asphyxiation Hazard: The use of instruments without adequate ventilation to outside air may constitute a health hazard. In addition, certain procedures required during the operation of the instrument may cause gases to be discharged into the exhaust stream; under these conditions, inadequate ventilation may result in serious injury. Take extreme care to vent exhaust gases properly.

WARNING! Toxic Chemical Hazard: Make sure the mass spectrometer system is connected to the local exhaust system and ducted to control hazardous emissions. The mass spectrometer system should only be used in a well-ventilated laboratory environment in compliance with local regulations and with appropriate air exchange for the work performed.

Note: In the USA, OSHA 29 CFR Part 1910-1450 requires 4 to12 air changes per hour in laboratories.

WARNING! Biohazard: This instrument or any part is not intended to act as a biological containment safety cabinet. For biohazardous material use, always apply local regulations for hazard assessment, control, and handling.

Instrument Disposal (Waste Electrical and Electronic Equipment)

Do not dispose of system components or subassemblies, including computer parts, as unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of WEEE (waste, electrical, and electronic equipment). To make sure that you safely dispose of this equipment, contact an FSE for instructions.

European Union customers: Contact a local AB SCIEX Customer Service office for complimentary equipment pick-up and recycling.

The 4500 series of instruments are intended for the qualitative and quantitative analysis of chemical species. The system includes a mass spectrometer, a Turbo V™ ion source, a computer, and the Analyst® software.

Theory of Operation

Mass spectrometry measures the mass-to-charge ratio of ions to identify unknown compounds, to quantify known compounds, and to provide information about the structural and chemical properties of molecules.

The 4500 series of instruments have a series of quadrupole filters that transmit ions according to their mass-to-charge (m/z) value. The first quadrupole in this series is the QJet Ion Guide located between the orifice plate and Q0. The QJet Ion Guide does not filter ions, but focuses them before they enter Q0. By prefocusing the larger ion flux created by the wider orifice, the QJet Ion Guide increases instrument sensitivity and improves the signal-to-noise ratio. In Q0 the ions are again focused before passing into Q1.

Q1 is a filtering quadrupole that sorts the ions before they enter Q2. Q2 is a collision cell in which an ions internal energy is increased though collisions with gas molecules to the point that molecular bonds break creating product ions. This technique allows users to design experiments that measure the m/z of product ions to determine the composition of the parent ions.

After passing through Q2 the ions enter Q3 for additional filtering, and then enter the detector. In the detector, the ions create a current that is converted into a voltage pulse. The voltage pulses leaving the detector are directly proportional to the quantity of ions entering the detector. The instrument monitors these voltage pulses and converts the information into a signal. The signal represents the ion intensity for a particular m/z value and the instrument displays this formation as a mass spectrum.

Data Handling

The Analyst software requires a computer running the Windows operating system. The computer with the associated system software works with the system controller and associated firmware to control the instrument and data acquisition. When operating the instrument, the acquired data is sent to the Analyst software where it can be displayed as either full mass spectra, intensity of single or multiple ions versus time, or total ion current versus time.

System Overview

Figure 2-1 *Front View* shows a system with a syringe pump, Turbo V ion source, a diverter valve, and the optional NanoSpray® ion source monitors.

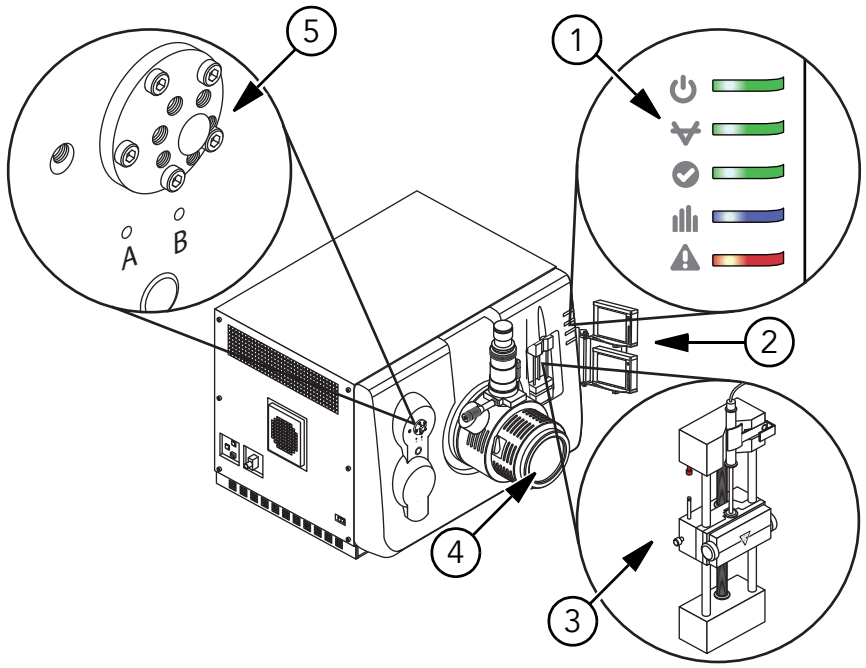


Figure 2-1 Front View

Item	Description	For more information refer to...
1	Instrument panel symbols	Table 2-1 on page 20.
2	Nanospray ion source monitors (optional)	*NanoSpray® Ion Source Operator Guide* located on the DVD that comes with the ion source.
3	Syringe pump	Adjust the Integrated Syringe Pump Position on page 22.
4	Turbo V ion source	Turbo V™ Ion Source User Reference
5	Diverter valve	If the optional DuoSpray™ ion source is installed, then the diverter valve is replaced with the DuoSpray valve. The diverter valve is installed below the DuoSpray valve. Refer to Configure the Integrated Syringe Pump on page 25. For information about the DuoSpray ion source, refer to the *DuoSpray™ Ion Source Operator Guide* located on the DVD that comes with the ion source.

Figure 2-2 *Rear and Side Views* on page 19 shows the location of the instrument connections, including the locations of the reset and vent buttons and the instrument switch.

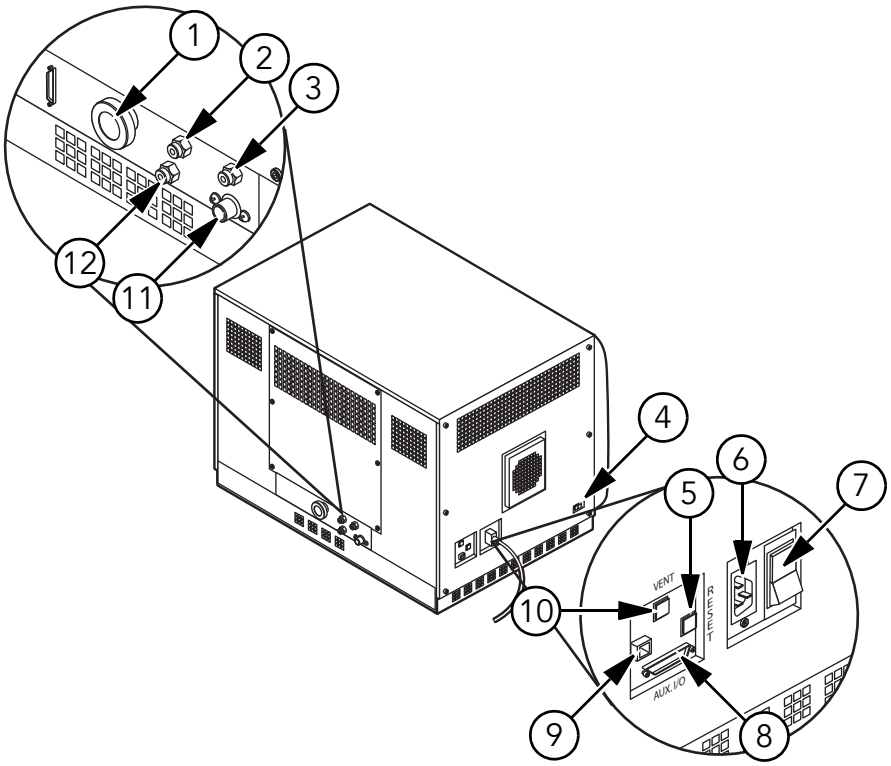


Figure 2-2 Rear and Side Views

Item	Description	For more information...
1	Roughing pump vacuum connection	Contact an AB SCIEX FSE.
2	Nitrogen gas supply (Curtain Gas™ supply, CAD gas)	Contact an AB SCIEX FSE.
3	Source exhaust supply	Contact an AB SCIEX FSE.
4	Source communication connection	Contact an AB SCIEX FSE.
5	Instrument reset button	Refer to Reset the System on page 21.
6	Mains supply connection	Refer to Turn on the System on page 20 or Shut Down the System on page 21.
7	Instrument switch (Up = On; Down = Off)	Refer to Turn on the System on page 20 or Shut Down the System on page 21.
8	Aux I/O connection	Refer to the *Peripheral Devices Setup Guide*.
9	Ethernet connection (connects the instrument and the computer)	Contact an AB SCIEX FSE.
10	Instrument vent (shutdown) button	Refer to Turn on the System on page 20 or Shut Down the System on page 21.
11	Source exhaust waste (to waste bottle)	Contact an AB SCIEX FSE.

Figure 2-2 Rear and Side Views (Continued)

Item	Description	For more information...
12	Air supply (Gas1/Gas2)	Contact an AB SCIEX FSE.

Instrument Panel Symbols

Table 2-1 shows the instrument status LEDs.

Table 2-1 Instrument Panel Symbols

Instrument LEDs	Color	Name	Description
	Green	Power	Lit when the instrument is powered up.
	Green	Vacuum	Lit when the correct vacuum has been achieved; and flashing if the vacuum is not at the correct vacuum (during pumpdown and venting.)
	Green	Ready	Lit when the instrument is in the Ready state. Instrument must be in the Ready state to operate.
	Blue	Scanning	Flashing when the instrument is acquiring data.
	Red	Fault	Lit when the instrument encounters a system fault.

After the instrument is turned on, all five LEDs illuminate. The power LED remains lit. The other four LEDs flash for two seconds and then turn off. The vacuum LED starts flashing. After the correct vacuum has been achieved this LED remains lit.

Turn on the System

WARNING! Personal Injury Hazard. Risk of personal injury or instrument damage. If the system must be moved, contact a Field Service Employee.

Note: Before operating the instrument, read the safety information in the Foreword.

Before the system is turned on, make sure the site requirements specified in the *Site Planning Guide* are met. This includes information on the mains supply and connections, source exhaust, compressed air, nitrogen, roughing pump, ventilation, exhaust, and site clearance.

1. Make sure there is clear access to the power cord. The power cord must be accessible in order to disconnect the instrument from the AC Mains.
2. Make sure the 4 L drain bottle is connected to the Exhaust Waste connection on the rear of the instrument and to the laboratory ventilation system.

3. Make sure that the mains supply cable is plugged in to the instrument.
4. Make sure the instrument and roughing pump mains supply cable are plugged into the 200 to 240 V electrical mains supply.
5. Make sure that the Ethernet cable is connected to both the instrument and the computer.
6. Turn on the roughing pump. The mains switch is located beside the mains supply input connection on the roughing pump.
7. Wait 5 minutes, then turn on the instrument switch.

Note: If the switch is already on, turn it off and then on.

8. Turn on the computer, if it was turned off.
9. Start the software.

Reset the System

- Press and hold the **Reset** button for 5 seconds.

  An audible click is heard when the relay activates. After approximately three minutes, the instrument should reach operating pressure.

Shut Down the System

1. Complete or stop any ongoing scans. For more information, refer to Stop Sample Acquisition on page 61.
2. Shut off the sample flow to the instrument.

   Caution: Potential Instrument Damage: Shut off the sample flow before the instrument is shut down.

3. In the Analyst software, deactivate the hardware profile, if active.
4. Close the software.
5. Press and hold the **Vent** button for three seconds.

   The turbo pump will spin down gradually.
6. Wait 15 minutes.
7. Turn off the roughing pump.

   The mains switch is located beside the mains supply input connection on the roughing pump.
8. Wait 15 minutes, then turn off the instrument switch.
9. Unplug the mains supply cable from the bulkhead on the left side of the instrument.
10. Unplug the roughing pump mains supply cable from the electrical mains supply.

Edit the Hardware Profile for the Integrated Syringe Pump

Make sure the syringe pump is seated properly to avoid damaging the syringe. For more information about creating and editing hardware profiles, refer to Create a Hardware Profile on page 31.

1. In the Navigation bar, under **Configure**, double-click **Hardware Configuration**.
2. Create or edit the hardware profile containing the instrument.
3. In the **Configuration** tab (Figure 2-3 *Configuration tab*), select **Use integrated syringe pump**.
4. Click **OK**.

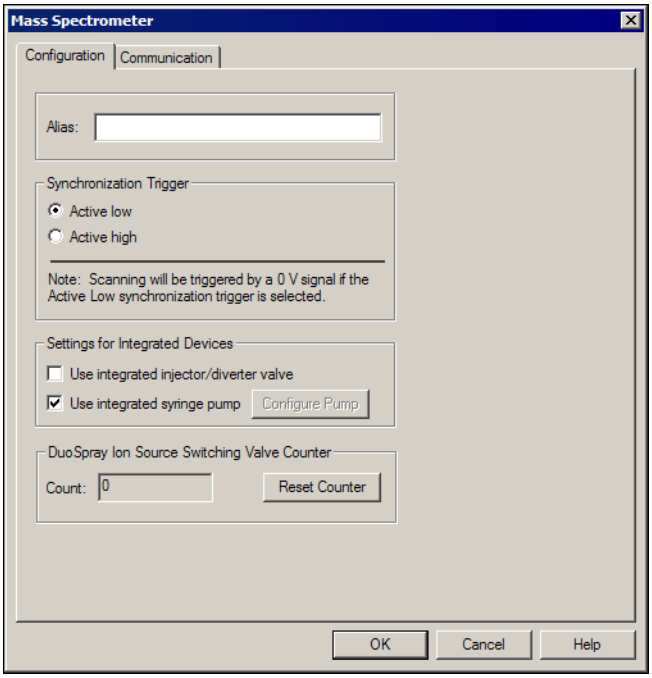


Figure 2-3 Configuration tab

5. Activate the hardware profile.

Adjust the Integrated Syringe Pump Position

1. Press the button on the right side of the syringe pump to lower the base and then insert the syringe as shown in Figure 2-4 *Lowering the syringe*.

   Make sure that the end of the syringe is flush against the base and that the shaft of the syringe rests in the cutout.

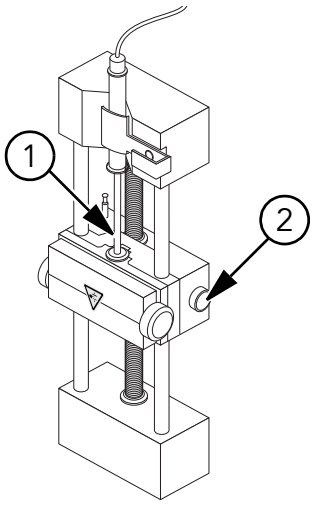


Figure 2-4 Lowering the syringe

Item	Description
1	Syringe plunger.
2	Release button. Press to raise or lower the base.

2. Adjust the post (Figure 2-5 *Safety stop*) so that it triggers the automatic syringe stop before the syringe plunger hits the bottom of the glass syringe.

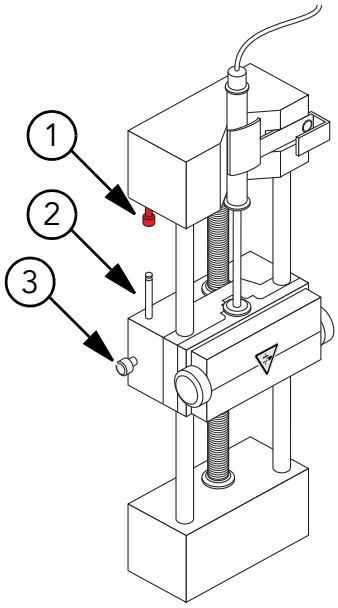


Figure 2-5 Safety stop

Item	Description
1	Automatic syringe stop. After the post hits the automatic syringe stop, the syringe pump stops.

Figure 2-5 Safety stop (Continued)

Item	Description
2	Post. Adjust the height to prevent the syringe plunger from hitting the syringe during sample infusion.
3	Post lock screw. Tighten the screw after the height of the post is adjusted.

3. Turn the side screws as shown in Figure 2-6 *Syringe pump* to secure the syringe.

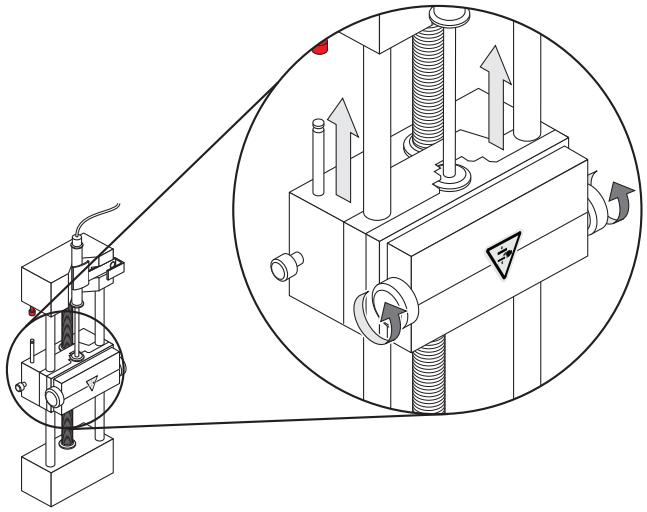


Figure 2-6 Syringe pump

4. On the instrument, press the button on the right side of the syringe pump (Figure 2-7 *Syringe pump LED*) to start the flow.

   The light next to the button illuminates when the syringe pump is in use.

Tip! The syringe pump can also be started using the Analyst software in Manual Tuning mode.

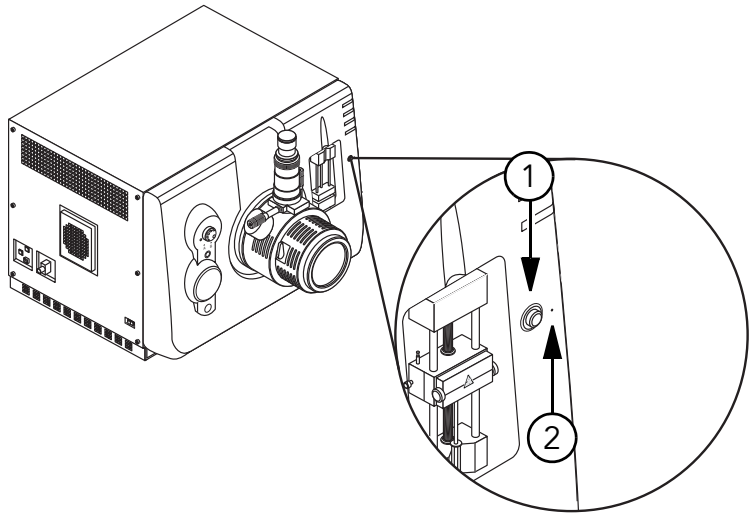


Figure 2-7 Syringe pump LED

Item	Description
1	Syringe pump on/off button
2	Syringe pump status LED

Configure the Integrated Syringe Pump

1. In the Navigation bar, under **Acquire**, double-click **Build Acquisition Methods**.
2. In the **Acquisition method** pane, click the **Syringe Pump** icon.

   The Syringe Pump method properties tab opens in the Acquisition Method Editor pane.
3. In the **Syringe Diameter (mm)** field, type the syringe diameter.
4. In the **Flow Rate** field, type the flow rate.
5. In the **Unit** list, select the units of flow.
6. Save the file.

Configure the Diverter Valve

The diverter valve is a two position, 6 port valve. If the valve is in Position A (Figure 2-9 *Diverter valve—injector mode position A*), the sample flows through the external loop. When the valve is switched to Position B (Figure 2-10 *Diverter valve—injector mode position B*), the sample is injected. For more information about creating and editing hardware profiles, refer to Create a Hardware Profile on page 31.

1. In the Navigation bar, under **Configure**, double-click **Hardware Configuration**.
2. Create or edit the hardware profile containing the instrument.
3. In the **Configuration** tab (Figure 2-8 *Configuration tab*), select **Use integrated injector/diverter valve**.

4. Click **OK**.

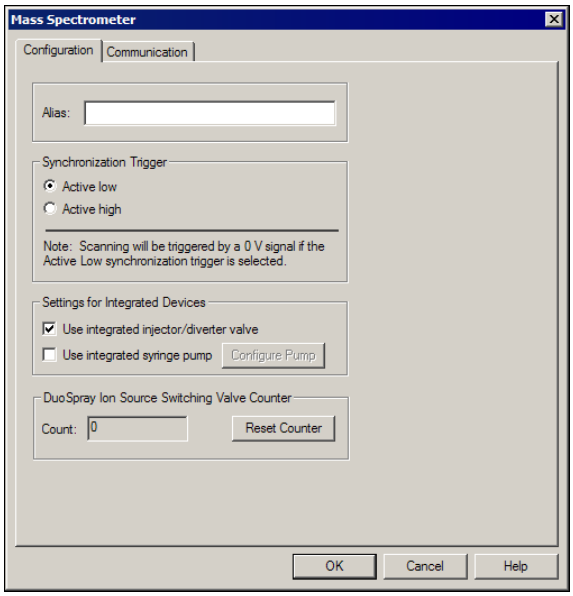


Figure 2-8 Configuration tab

5. Activate the hardware profile.

Plumb the Diverter Valve

The diverter valve should be plumbed for both injector and diverter modes.

Plumb the Diverter Valve in Injector Mode

The diverter valve is a two position, 6 port valve. If you put the valve in Position A (Figure 2-9), the sample flows through the external loop. When you switch the valve to Position B (Figure 2-10), the sample is injected.

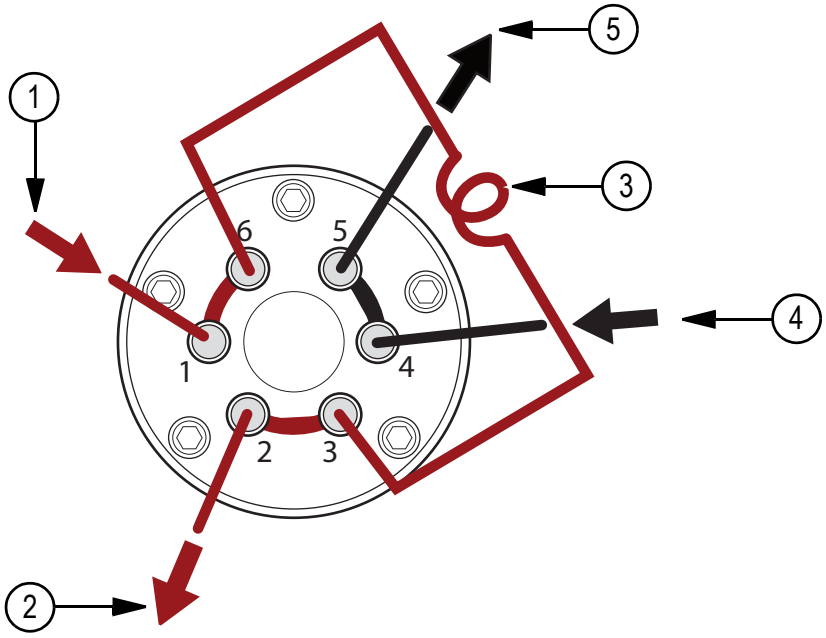


Figure 2-9 Diverter valve—injector mode position A

Item	Description
1	Sample in
2	Waste out
3	Sample loop
4	Mobile phase in
5	To column

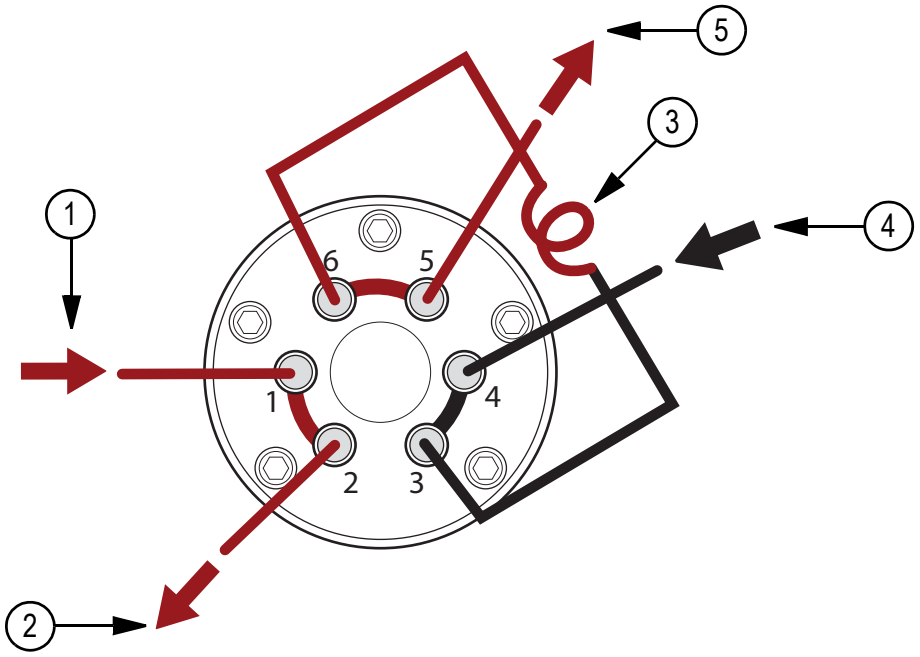


Figure 2-10 Diverter valve—injector mode position B

Item	Sample in
1	Sample in
2	Waste out
3	Sample loop
4	Mobile phase in
5	To column

Plumb the Diverter Valve in Diverter Mode

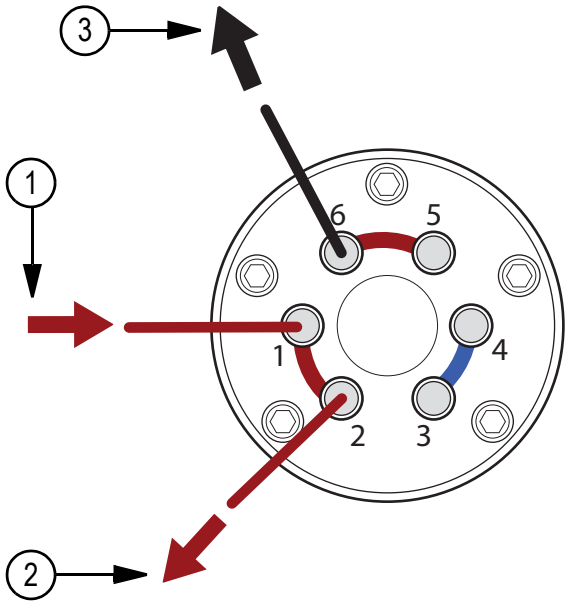


Figure 2-11 Diverter valve—diverter mode position A

Item	Description
1	Sample in
2	Waste out
3	To column

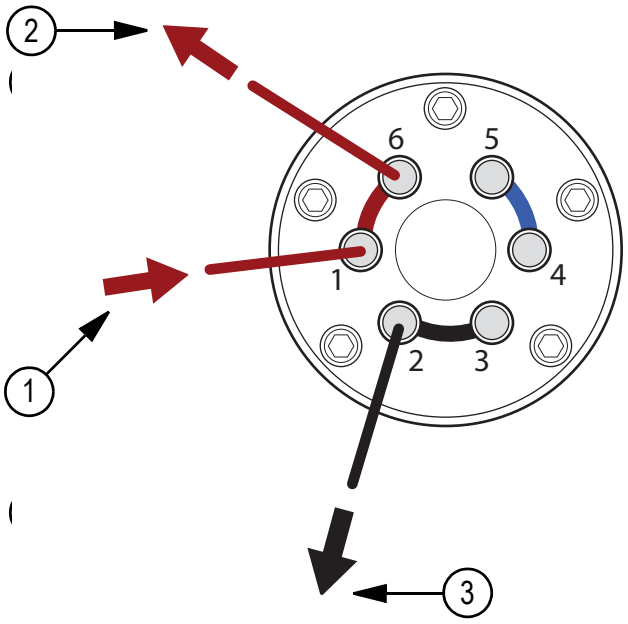


Figure 2-12 Diverter valve—diverter mode position B

Item	Sample in
1	Sample in
2	To column
3	Waste out

Instrument Safe Fluids

The following fluids can safely be used with the instrument:

- Methanol (0 to 100%)
- Acetonitrile (0 to 100%)
- Water
- Formic acid (0 to 1%)
- Ammonium acetate (0 to 1%)

Note: This list is not complete. Do not use any other fluid until confirmation is received from AB SCIEX that it will not present a hazard.

Hardware Profiles

A hardware profile tells the software what instrument and devices to use, and how the instrument and the devices are configured and connected to the computer.

Each hardware profile must include a mass spectrometer. Before creating an acquisition method, make sure that all devices used in the method are included in the hardware profile. In the configuration options for the mass spectrometer, ensure that the syring pump is enabled if it will be used during acquisition.

The devices configured in the active hardware profile and selected in the Add/Remove Device Method dialog appear as icons in the Acquisition Method Browser pane. Only devices included in the active hardware profile can be used to create acquisition methods.

For information about configuring devices, refer to the *Peripheral Devices Setup Guide*. For a list of the supported devices, refer to the *Software Installation Guide* for the Analyst® software.

Create a Hardware Profile

The user can create multiple hardware profiles, but only one profile can be active at any time.

1. In the Navigation Bar, under **Configure**, double-click **Hardware Configuration**.

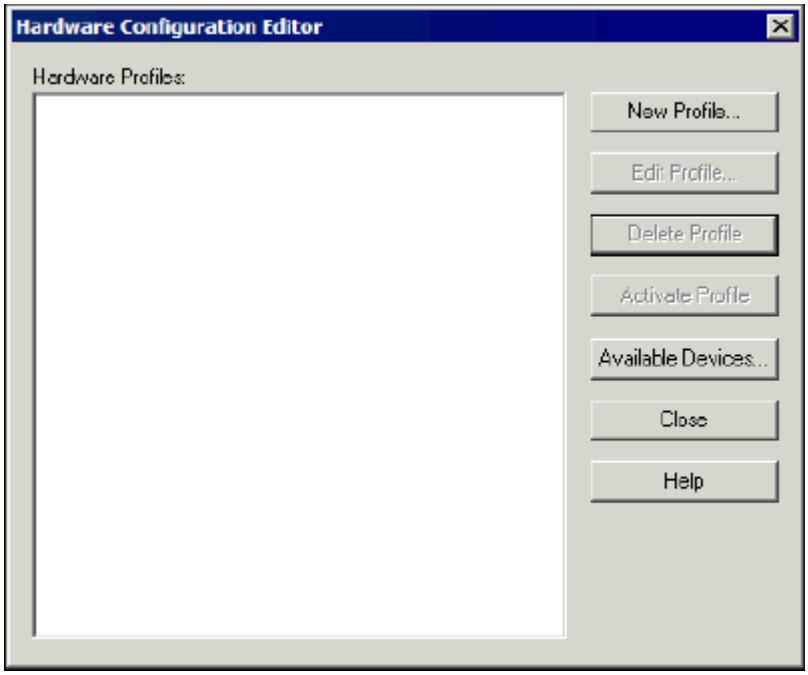


Figure 3-1 Hardware Configuration Editor dialog

2. In the **Hardware Configuration Editor** dialog, click **New Profile**.

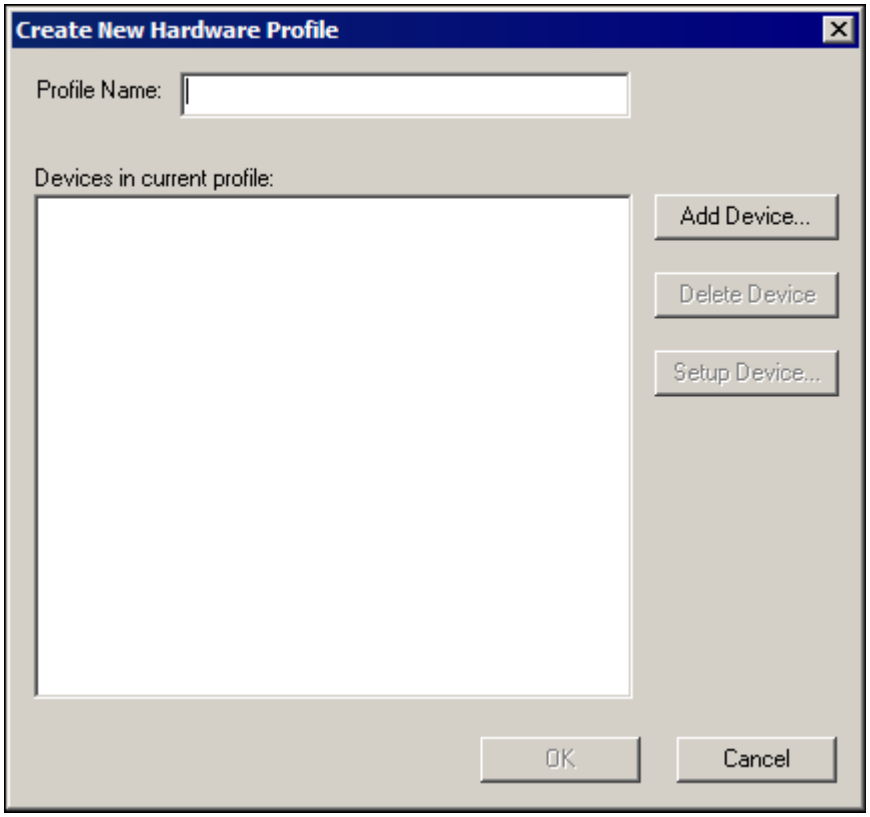


Figure 3-2 Create New Hardware Profile

3. In the **Profile Name** field, type a name.
4. Click **Add Device**.

   In the Available Devices dialog, in the Device Type field, Mass Spectrometer is the preset value.

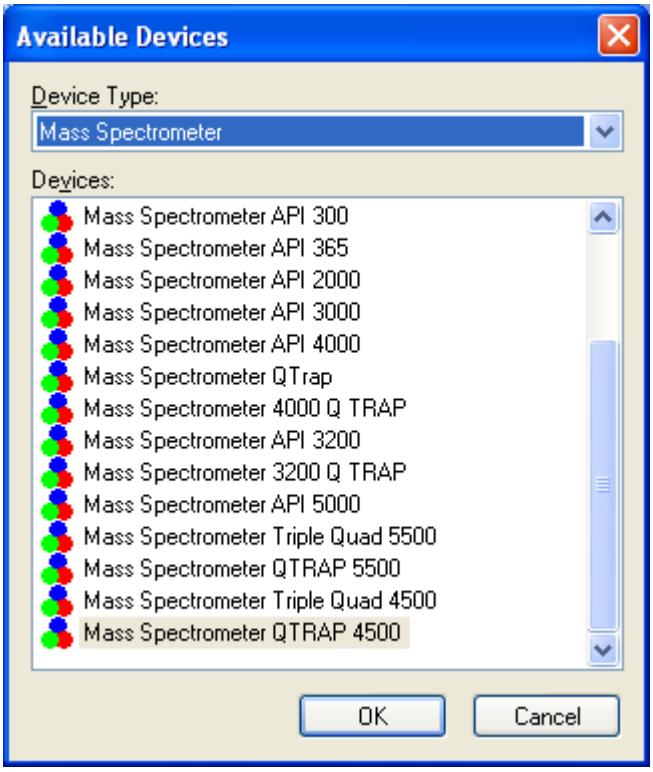


Figure 3-3 Available Devices dialog

5. In the **Devices** list, select the instrument.
6. Click **OK**.
7. In the **Devices in current profile** list, select the instrument.
8. Click **Setup Device**.
9. Select the features in the **Configuration** tab and **Communication** tab as required.
10. Click **OK** to return to the **Create New Hardware Profile** dialog.
11. Click **Add Device**
12. Add and configure each device that is used with the instrument. Refer to Add Devices to a Hardware Profile on page 34.
13. In the **Create New Hardware Profile** dialog, click **OK**.
14. In the **Hardware Configuration Editor**, click the hardware profile.
15. Click **Activate Profile**.

    The check mark turns green. If a red x is shown, then there is an issue with the hardware profile activation. For more information, refer to Troubleshooting Hardware Profile Activation.

Tip! A hardware profile does not have to be deactivated before activating another. Click a hardware profile and then click **Activate Profile**. The other profile is deactivated automatically.

16. Click **Close**.

Add Devices to a Hardware Profile

Devices must be configured to enable the software to communicate with them.

When the software is installed, the driver required for each device is also installed. After the devices are physically connected to the computer, configure the device.

1. Open the Hardware Configuration Editor.
2. In the **Hardware Profiles** list, deactivate the hardware profile.
3. Click **Edit Profile**.
4. Click **Add Device**.
5. In the **Available Devices** dialog, in the **Device Type** list, select the device.
6. Click **OK**.

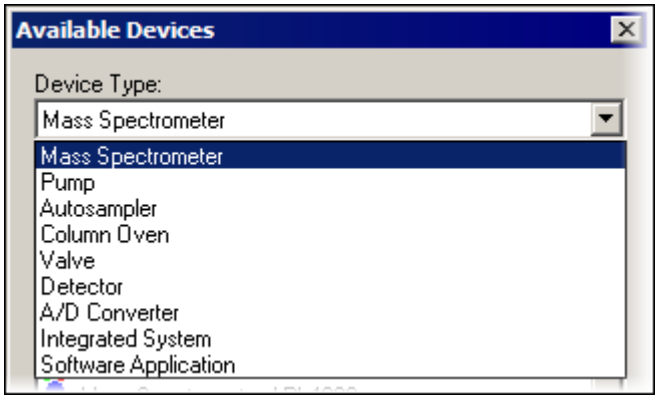


Figure 3-4 Available Devices dialog

7. In the **Devices in current profile** list, select the device.
8. Click **Setup Device**.

   A dialog containing configuration values for the device opens.
9. In the **Communication** tab, in the **Alias** field, type a name or other identifier. (Optional)

Note: For devices using serial communication, make sure that the serial port selected matches the serial port to which the device is physically connected. If a serial expansion cable is used, then the number selected in the profile is the number on the cable plus two.

Note: The Alias field may also be referred to as the Name box and may be found on another tab under Alias.

- If the device uses Serial Port as a communication interface, in the COM Port Number list, select the COM port that the device is connected to.
- If the device uses Ethernet as a communication interface, type the IP address assigned to the device by the administrator or use the corresponding host name for the address.

- If the device uses a GPIB board as a communication interface, do not change the settings for the GPIB board.

The rest of the preset values for the device are likely appropriate; do not change them. For information about the Configuration and Communication tabs, refer to the Help.

10. To restore the device preset values, in the **Communication** tab, click **Set Defaults**.
11. To save the configuration, click **OK**.
12. Repeat steps 4 to 9 for each device.
13. In the **Create New Hardware Profile** dialog, click **OK**.
14. To activate the hardware profile, in the **Hardware Configuration Editor**, click the hardware profile.
15. Click **Activate Profile**.

    The check mark turns green. If a red x is shown, then there is an issue with the hardware profile activation. For more information, refer to Troubleshooting Hardware Profile Activation.

Tip! A hardware profile does not have to be deactivated before activating another hardware profile. Click a hardware profile and then click **Activate Profile**. The other profile is deactivated automatically.

16. Click **Close**.

Troubleshooting Hardware Profile Activation

If a hardware profile fails to become active, a dialog appears indicating which device in the profile failed. A failed profile may be due to communications errors.

1. Read the error message generated. Depending on the message, there may be an issue with a device or how the communication is set up.
2. Verify that the device has power and is turned on.
3. Verify that the COM port assigned to the device is correct.

Tip! On computers with two built-in serial ports, the first port on the serial port expansion card is usually COM3, even though the cable indicates P1.

4. Verify that the communication settings with the device (for example, dip switch settings) are set correctly and match the settings in the **Communication** tab.
5. Turn off the device.
6. Wait 10 seconds
7. Turn on the device.

   Wait until all device power-up activities are complete before trying to activate the hardware profile again. Some devices may require 30 seconds or more to complete the power-up activities.
8. Activate the hardware profile.

9. If the issue persists, delete the failing profile and then create a new one.
10. If the issue persists, contact technical support.

Create Projects and Subprojects

To use a subproject structure within a project, create the subproject structure when the project is created.

1. Click **Tools > Project > Create Project**.
2. In the **Project name** field, type a project name.
3. (Optional) To use subprojects, select the required folders and then use the arrow buttons to move them to the **Subproject folders** list.

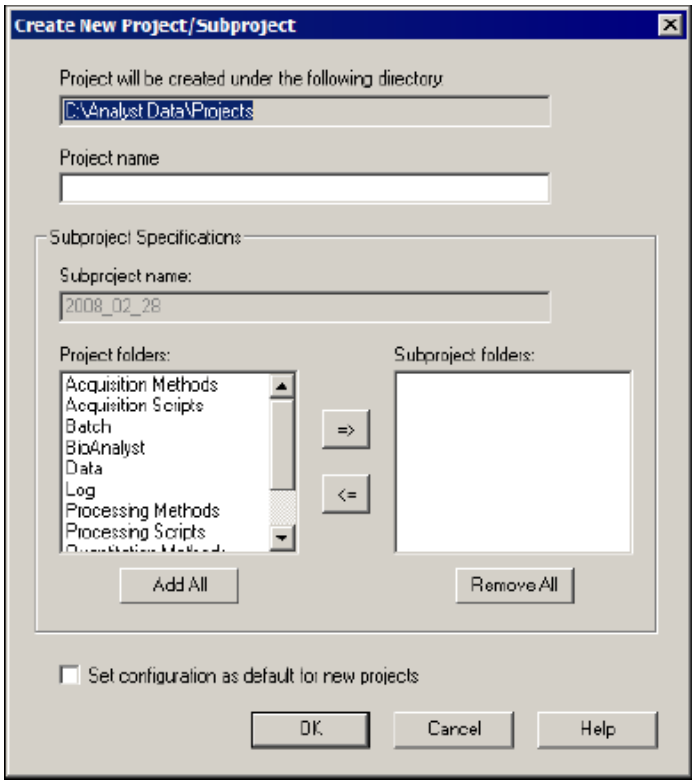


Figure 3-5 Create new Project/Subproject dialog

4. (If subprojects are used.) In the **Subproject name** field, type a name for the first subproject or use the existing date.
5. To use this project and subproject folder organization for all new projects, select the **Set configuration as default for new projects** check box.

   All new projects are created with this folder configuration.
6. Click **OK**.

Create a Subproject

Subprojects can only be created in a project that has an existing subproject structure.

1. On the Project toolbar, in the **Project** list, select the project.
2. Click **Tools > Project > Create Subproject**.
3. In the **Subproject name box**, type a name for the subproject or use the existing date.
4. Click **OK**.

Copy a Subproject

The user can copy a subproject from another project that has existing subprojects. If the copied subprojects contain folders that also exist in the project folder, then the software uses the project level folders

1. Click **Tools > Project > Copy Subproject**.
2. In the **Copy Subproject** dialog, click **Browse** to navigate to the subproject source.
3. Click **OK**.
4. In the **Source Subproject** list, select the subproject.
5. Click **Browse** to navigate to the subproject destination.
6. In the **Target Subproject** field, type the name.
7. Click **OK**.
8. Do one of the following:
   - To copy all folders and files from the **Subproject Source** into the **Subproject Destination**, select the **Copy Contents** check box.
   - To copy only the folders in the same structure into the **Subproject Destination**, make sure that the **Copy Contents** check box is cleared.
9. Click **Copy**.

Installed Project Folders

Three project folders are installed with the software: API Instrument, Default, and Example.

API Instrument Folder

The API Instrument folder is unique and very important to the correct functioning of the instrument. The API Instrument folder contains the information required for tuning and calibrating the instrument. This information includes parameter settings files, reference files, instrument data files that contain calibration and resolution information, and the acquisition methods used during automatic tuning. The API Instrument folder also contains data files for manual tuning runs that were performed using the Start button rather than the Acquire button. These data files are saved automatically in the API Instrument folder in the Tuning Cache folder and named with the date and time they were created. The Tuning Cache is automatically cleared periodically.

Default Folder

The Default folder contains folders that are present in new projects and serves as a template for new projects.

Example Folder

The Example folder contains sample methods and data files. Users can practice working with the Explore or Quantitate modes using the example data files. The example files are sorted into subfolders by instrument type and application area.

Back up the API Instrument Folder

Back up the API Instrument folder regularly and after routine maintenance has been performed.

1. Copy the API Instrument folder, paste it to a different location, preferably to another computer, and then rename the folder. Use the date and an instrument reference if there is more than one instrument when folder is named; for example, API Instrument__4000QTRAP3_010107
2. To recover the folder, rename the current API Instrument folder, and then copy the backup into the Projects folder and then change its name back to API Instrument.

Table 3-1 Icons on the Toolbar

Icon	Name	Function
	New Subproject	Creates a subproject. Subprojects can only be created later in the process if the project was originally created with subprojects.
	Copy Subproject	Copies a Subproject folder. Subprojects can be copied only from another project that has existing subprojects. If the same folders exist at both the project and subproject levels, the software uses the project level folders.

Run the Verify instrument performance option weekly or after the instrument is cleaned to confirm that the system is working properly. In general, the calibration and resolution for quadrupole are fine for 3 to 6 months unless the system loses vacuum. For LIT (linear ion trap) systems, the resolution should also be fine for 3 to 6 months but the calibration should be done approximately monthly. If the system loses vacuum, then check the calibration and resolution before using the system. For more information about tuning and calibration, refer to the *Advanced User* Guide and the *Manual Tuning Tutorial*.

Required material

- Tuning solutions that are supplied in the Standards Chemical Kit shipped with the system. If needed, a new Kit can be ordered from AB SCIEX.
- 5 ml, 1 ml, and 250 µl serial gas-tight syringes.
- PEEK (red) sample tubing.

Prerequisites

- Make sure that a printer is configured.
- Make sure that the spray is stable and that the correct tuning solution is being used.

Optimize the Instrument

The following procedure describes how to verify the performance of the instrument. For more information about using the other instrument performance options, refer to the Help.

1. In the Navigation Bar, under **Tune and Calibrate**, double-click **Manual Tuning**.
2. Run a calibration method and confirm that there is a stable TIC and that the peaks of interest are present in the spectrum.
3. In the Navigation Bar, under **Tune and Calibrate**, double-click **Instrument Optimization**.
4. Click **Verify instrument performance**.
5. Click **Next**.
6. Click **Approved Tuning**.
7. Click **Next.**
8. Select a **Tuning Solution**.

   Depending on the solution selected, different modes are available.

   i. Click a polarity.
   ii. If available, click **Q1** and **Q3** in the **Quad** section. If available, click the required scan speeds.
   iii. If available, click the scan speeds in the **LIT** section.

iv. If available, click MS/MS/MS options and then click **Next**.

9. If the **Select a mode** page opens, select **Automatic** and then click **Next.**
10. Click **GO**.

    The Verifying or Adjusting Performance screen opens. After the process has completed, the Results Summary opens. For more information, refer to the Help.
11. Depending on the fields selected, the user will be prompted to change solutions for the various scan types and polarities.

Verify or Adjust Performance

The top left corner shows the part of the instrument that is being tuned.

Current Spectrum: This graph shows the spectrum of the current scan, the optimal scan selected by the software, or the scan at the current parameter value when the software results are viewed in interactive mode.

The Instrument Optimization Decision Plots, in the top right graph, dynamically show the intensity versus voltage curves of the parameters that are currently being optimized.

Results Summary

The Results Summary, shown in Figure 4-1, is a record of any instrument settings changes that were made by the Instrument Optimization software. This includes the location of data files and instrument settings backups, as well as step-by-step changes and results during optimization. In addition, the Results Summary shows a verification report. This report contains a snapshot of the mass spectrum for each relevant mass for the scan modes being verified. The spectrum is labelled with the target mass, where the mass was found, mass shift, peak width, and peak intensity. The spectrum can be used as a visual record of peak shape or scan mode performance. A summary table of results follows the spectra.

The Results Summary is saved as a document in the folder indicated at the top of the report. Users can print the Results Summary or open a previously saved Results Summary.

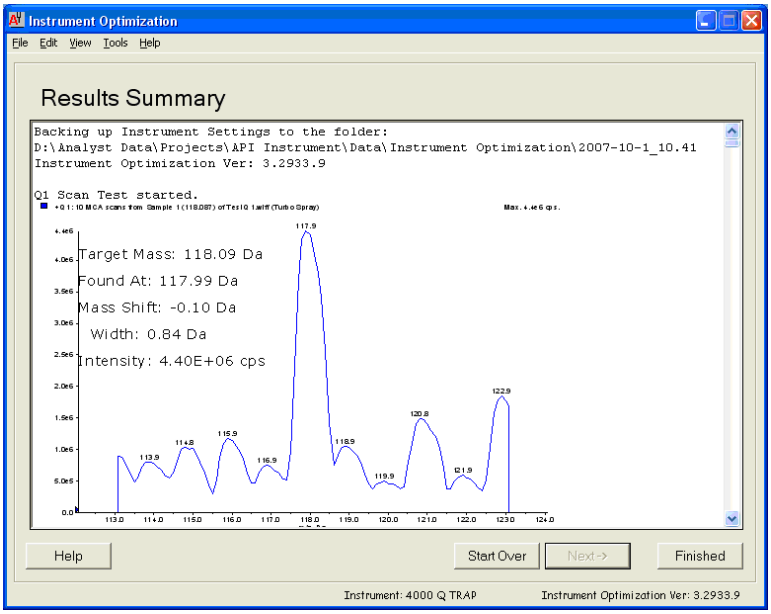


Figure 4-1 Results Summary

Basic Acquisition Methods 5

An acquisition method consists of experiments and periods. Use the Acquisition Method Editor to create a sequence of periods and experiments for the instrument and devices.

Create Acquisition Methods

Only devices configured in the active hardware profile appear in the Acquisition Method Browser pane. Any devices added to the hardware profile must also be added to existing acquisition methods. For more information about devices, refer to the *Peripheral Devices Setup Guide.*

1. In the Navigation Bar, under **Acquire**, double-click **Build Acquisition Method**.
2. In the **Acquisition Method Properties** tab, select a **Synchronization Mode**.
3. Select the **Auto-Equilibration** check box. (Optional)
4. Enter the desired equilibration time in minutes, if Auto-Equilibration is selected.
5. In the **Acquisition method** pane, click the Mass Spec icon.
6. In the **MS** tab, select a scan type.
7. Type values in the fields as required. For more information refer to Parameters on page 46.
8. In the **Advanced MS** tab, type values in the fields as required. For more information refer to Parameters on page 46.
9. Click a device icon.
10. Select the parameters for the devices as required.
11. Add any additional periods and experiments.
12. Click **File** > **Save**.

Add an Experiment

1. Right-click the period and then click **Add experiment**.

   An experiment is added below the last experiment in the period.
2. In the **Acquisition Method Editor** pane, select the appropriate device or instrument parameters.

Add a Period

- In the **Acquisition method** pane, right-click the **Mass Spec** icon, and then click **Add period**.

  A period is added below the last period created.

Copy an Experiment into a Period

Prerequisite: Multi-period method

- In the **Acquisition method** pane, press CTRL, and then drag the experiment to the period.

  The experiment is copied below the last experiment in the period.

Copy an Experiment within a Period

Use this procedure to add the same or similar experiments to a period if most or all of the parameters are the same.

- Right-click the experiment and then click **Copy this experiment**.

Scan Techniques

MS: In MS scans, also referred to as single MS scans, ions are separated according to their mass-to-charge ratio. A single MS scan may be used to determine the molecular weight of a compound. Single MS scans can also be referred to as survey scans. MS scans do not provide any information as to the chemical make-up of the ions other than the mass-to-charge ratio. Perform MS/MS or MS/MS/MS scan types to obtain more information about the ions.

MS/MS: MS/MS scans are used to help identify or confirm a molecular species. In MS/MS, a precursor ion can be fragmented in one of two locations.

- For triple quadrupole instruments, fragmentation occurs in the collision cell.
- For LIT instruments, fragmentation can occur in the collision cell or the linear ion trap.

If enough energy is used, the precursor ion fragments to produce characteristic product ions.

MS/MS/MS: The LIT instrument MS/MS/MS scans go one step further than MS/MS scans. A fragment that is produced in the collision cell is fragmented further in the trap to give more structural information about the molecular ion.

Quadrupole-Mode Scan Types

Triple quadrupole instruments have high-sensitivity MRM (Multiple Reaction Monitoring) capabilities required for quantitation experiments. In addition, they have highly specific scan types such as precursor ion and neutral loss scans that allow a more advanced search to be performed on the components of the samples.

Q1 MS (Q1): A full scan using the first quadrupole (Q1). The ion intensity is returned for every mass in the scan range.

Q1 Multiple Ions (Q1 MI): A zero width scan type using the first quadrupole (Q1). The ion intensity is returned for the specified masses only.

Q3 MS (Q3): A full scan using the third quadrupole (Q3). The ion intensity is returned for every mass in the scan range.

Q3 Multiple Ions (Q3 MI): A zero width scan type using the third quadrupole (Q3). The ion intensity is returned for the specified masses only.

MRM (MRM): An MS/MS scan in which a user-selected ion is passed through the first quadrupole (Q1) and fragmented in the second quadrupole (Q2). The third quadrupole (Q3) is then used to specify which fragment ion enters the detector. This scan mode is used primarily for quantitation.

Product Ion (MS2): MS/MS full scan where the first quadrupole (Q1) is fixed to transmit a specific precursor ion and the third quadrupole (Q3) scans a defined mass range. Used to identify all of the products of a particular precursor ion.

Precursor Ion (Prec): MS/MS scan where the third quadrupole (Q3) is fixed at a specified mass-to-charge ratio to transmit a specific product ion and the first quadrupole (Q1) scans a mass range. Used to confirm the presence of a precursor ion or more commonly used to identify compounds sharing a common product ion.

Neutral Loss (NL): MS/MS scan where both the first quadrupole (Q1) and the third quadrupole (Q3) scan a mass range, a fixed mass apart. A response is observed if the ion chosen by the first analyzer fragments by losing the neutral loss (the fixed mass) specified. Used to confirm the presence of a precursor ion or more commonly used to identify compounds sharing a common neutral loss.

LIT-Mode Scan Types

The LIT-mode scans use the third quadrupole, Q3, as a linear ion trap. Ions are trapped and stored in the trap before being scanned out, giving increased sensitivity. In addition, MS/MS/MS can be performed in the linear ion trap, providing more information about the sample.

Enhanced MS (EMS): Ions are scanned in Q1 to the linear ion trap where they are collected. These ions are scanned out of Q3 to produce single MS type spectra.

Enhanced Multi-Charge (EMC): This scan is similar to the EMS scan except that before scanning the ions out of the linear ion trap, there is a delay period in which low-charge state ions (primarily singly-charged ions) are allowed to preferentially escape from the linear ion trap. When the retained ions are scanned out, the multiply-charged ion population dominates the resulting spectrum.

Enhanced Product Ion (EPI): This scan type is used to obtain a high quality MS/MS spectrum on a specific ion. The fragmentation is done in the collision cell and thus provides the information-rich MS/MS spectrum typical of collisionally activated dissociation fragmentation. In this scan mode, the precursor ion to be fragmented is first selected in Q1 with a mass window of 1 to 4 Da wide, filtering out all other ions. The precursor ion is fragmented by collisionally-activated dissociation (CAD) gas in the Q2 collision cell. The fragment ions generated are captured in the linear ion trap and then scanned out at one of three scan speeds, depending on the required fragment ion resolution.

For IDA experiments, the Product Of field is set to 30 Da by default, and this value should not be changed.

Enhanced Resolution (ER): This scan is similar to the EMS scan except that a small 30 Da mass around the precursor mass is scanned out of the linear ion trap at the slowest scan rate to produce a narrow window of the best-resolved spectra.

MS/MS/MS (MS3): In this scan mode, a precursor ion is selected by Q1 and fragmented with collisionally activated dissociation in the Q2 collision cell. The resulting product ions are all transmitted to the linear ion trap, where a single product ion is then isolated. The isolated ion is further fragmented in the linear ion trap, and the resulting product ions are scanned out of the ion trap at one of three scan speeds. As with any in-trap CID technique, there is a low mass cut-off for the second MS/MS step due to the condition that the lowest mass fragment and precursor

must be simultaneously stable in the trap. For the QTRAP® instruments, this means that we lose ions lower than 28 percent of the mass of the precursor ion when performing MS3 experiments. This phenomenon is often referred to as the 1/3rd cut-off rule.

Time Delayed Frag (TDF): Product ions are generated and collected in the linear ion trap. During the first part of the collection period, the lower mass ions are not collected in the linear ion trap. During the second part of the collection period, all masses over the mass range of interest are collected. The resulting enhanced product ion spectra are simplified compared to EPI scan type spectra. The nature of the spectra aids in the interpretation of the structure and fragmentation pathways of the molecule of interest. This scan type is not applicable to the AB SCIEX 4500 QTRAP and AB SCIEX 5500 QTRAP systems.

About Spectral Data Acquisition

Spectral data can be acquired in one of three modes, as shown in the following table.

Table 5-1 Spectral Data Acquisition

Mode	Description
Profile	The preset value is 0.1 Da. Profile data is the data generated by the instrument and corresponds to the intensity recorded at a series of evenly spaced discrete mass values. For example, for a mass range 100 Da to 200 Da and step size 0.1, the instrument scans 99.95 to 100.05 (records as value 100), 100.05 to 101.15 (records as value 101)…199.95 to 200.05 (records as value 200).
Peak Hopping	The preset value is 1.0 Da. Peak Hopping is a mode of operating a mass spectrometer in which large steps (approximately 1 Da) are made. It has the advantage of speed (less data steps are made) but with the loss of peak shape information.
Centroid	The instrument scans as in Profile mode, but creates a centroid of the data, replacing found peaks with the intensity-weighted center of gravity for each peak. Centroid data has the advantage of significantly reducing file size. The disadvantage is that peak shape information is lost, and if data has been collected as a centroid it cannot be altered. It is recommended to use Profile mode and centroid the data post-acquisition.

Parameters

The working parameters are the set of instrument parameters currently being used.

- Source and gas parameters (these parameters can change depending on the ion source used)
- Compound parameters
- Resolution parameters
- Detector parameters

For more information about instrument parameter values and ranges, refer to Appendix B: 4500 Series Instrument Requirements and Parameters.

The following figure shows the location of the parameters on the ion optics path.

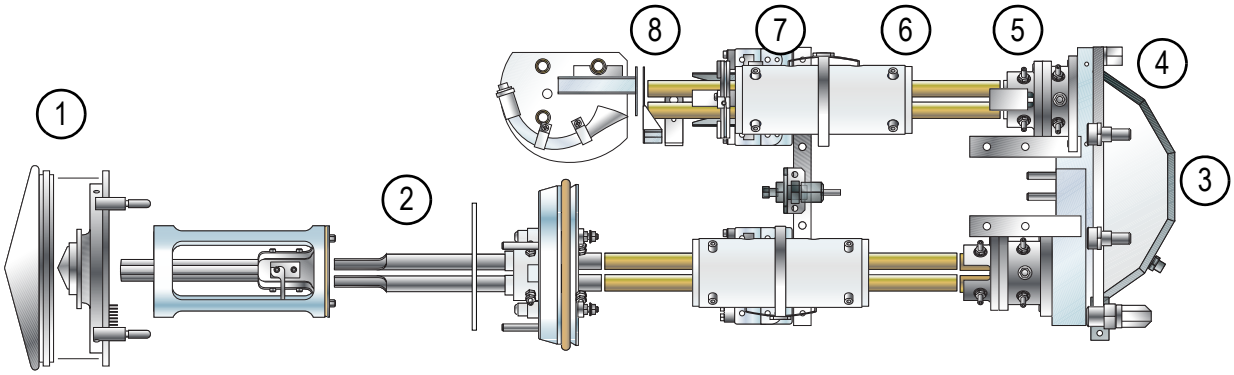


Figure 5-1 Ion optics path and parameters

Item	Parameter	Parameter Type	Use	Scan Type
1	IS (Ion Transfer Voltage)	Source and gas	For the PhotoSpray® source, the IS parameter controls the voltage that transfers the ions from the primary ionization region towards the curtain plate orifice.	All
1	NC (Needle Current)	Source and gas	The NC parameter controls the current applied to the corona discharge needle in the APCI probe, used in the Turbo V™ source. The discharge ionizes solvent molecules, which in turn ionize the sample molecules.	All
1	ihe (Interface Heater)	Source and gas	The ihe parameter switches the interface heater on and off. Heating the interface helps maximize the ion signal and prevents contamination of the ion optics. This should always stay on. The button controlling the interface heater reads ON when the interface heater is on.	All
1	IHT (Interface Heater Temperature)	Source and gas	The IHT parameter controls the temperature of the NanoSpray® interface heater and is only available if the NanoSpray ion source and interface are installed.	All
1	sdp	Source and gas	The sdp parameter controls the selection of the DuoSpray™ ion source probes: TurboIonSpray probe or APCI probe	

Figure 5-1 Ion optics path and parameters (Continued)

Item	Parameter	Parameter Type	Use	Scan Type
1	DP (Declustering Potential)	Compound	The DP parameter controls the voltage on the orifice, which controls the ability to decluster ions between the orifice and the skimmer (or for systems with a QJet® Ion Guide, between the orifice and QJet Ion Guide). It is used to minimize the solvent clusters that may remain on the sample ions after they enter the vacuum chamber, and, if required, to fragment ions. The higher the voltage, the higher the energy imparted to the ions. If the DP parameter is too high, unwanted fragmentation may occur. Use the preset value and optimize for the compound.	All
1	CUR (Curtain Gas)	Source and gas	The CUR parameter controls the gas flow of the Curtain Gas™ interface. The Curtain Gas interface is located between the curtain plate and the orifice. It assists in solvent evaporation and prevents solvent droplets from entering and contaminating the ion optics. The gas flow should be maintained as high as possible without losing sensitivity.	All
1	IS (IonSpray Voltage)	Source and gas	The IS parameter controls the voltage applied to the electrode that ionizes the sample in the ion source. It depends on the polarity and it affects the spray stability and the sensitivity. This parameter can be compound-dependent and should be optimized for each compound.	All
1	GS1 (Gas 1)	Source and gas	The GS1 parameter controls the nebulizer gas. The nebulizer gas helps generate small droplets of sample flow and affects spray stability and sensitivity.	All

Figure 5-1 Ion optics path and parameters (Continued)

Item	Parameter	Parameter Type	Use	Scan Type
1	GS2 (Gas 2)	Source and gas	The GS2 parameter controls the auxiliary, or turbo, gas. It is used to help evaporate the solvent to produce gas phase sample ions.	All
1	TEM (Temperature)	Source and gas	The TEM parameter controls the temperature of the turbo gas in the TurboIonSpray® probe or the temperature of the probe in the heated nebulizer (or APCI) probe.	All
2	EP (Entrance Potential)	Compound	The EP parameter controls the potential difference between the voltage on Q0 and ground. The entrance potential guides and focuses the ions through the high-pressure Q0 region. Use the preset value.	All
2	Q0 Trapping	Compound	The Q0 trapping parameter controls the storage of ions in the Q0 region. It is used to increase sensitivity and duty cycle by trapping ions in the Q0 region while ions are being mass-selectively ejected from the LIT. Use fixed fill time with this parameter. Either select or clear the feature based on the experiment. The recommended fixed fill time to use with Q0 trapping is 20 ms or greater.	EMS, EMC, EPI, ER, and MS/MS/MS,

Figure 5-1 Ion optics path and parameters (Continued)

Item	Parameter	Parameter Type	Use	Scan Type
3	CAD Gas	Source and gas	The CAD parameter controls the pressure of collision gas in the collision cell during Q3, MS/MS-type, and LIT scans. For Q3 scans, the collision gas helps to focus the ions as they pass through the collision cell; the preset for the CAD parameter is in fixed mode. For MS/MS-type scans, the collision gas aids in fragmenting the precursor ions. When the precursor ions collide with the collision gas, they can dissociate to form product ions. For LIT scans, the collision gas helps to focus and trap ions in the LIT. Use the preset value and optimize for the compound.	Q3 MI, Q3 MS, MRM, Prec, NL, EMS, ER, EPI, MS/MS/MS, EMC, and TDF
3	CE (Collision Energy)	Compound	The CE parameter controls the potential difference between Q0 and Q2 (collision cell). It is used only in MS/MS-type scans. This is the amount of energy that the precursor ions receive as they are accelerated into the collision cell, where they collide with gas molecules and fragment. Use the preset value and optimize for the compound.	MRM, MS2, Prec, NL, and LIT
3	CES (Collision Energy Spread)	Compound	The CES parameter, in conjunction with the Collision Energy (CE), determines which three discreet collision energies are applied to the precursor mass in an Enhanced Product Ion (EPI) or MS/MS/MS (MS3) experiment when CES is used. By typing a collision energy spread value, CES is automatically turned on. Use the preset value and optimize for the compound.	EPI and MS/MS/MS

Figure 5-1 Ion optics path and parameters (Continued)

Item	Parameter	Parameter Type	Use	Scan Type
4	CXP (Collision Cell Exit Potential)	Compound	The CXP parameter is only used in Q3 and MS/MS-type scans, where it transmits the ions into Q3. Use the preset value and optimize for the compound.	Q3, MRM, MS2, Prec, and NL
4	TDF CE (Time Delayed Fragmentation Collision Energy)	Compound	This is the amount of energy that the precursor ions receive as they are accelerated into Q3, where they collide with gas molecules and fragment. Use the preset value.	TDF
5	Q3 Entry Barrier	Compound	The Q3 Entry Barrier parameter is used to transfer the ions from Q2 into the LIT. Use the preset value.	EMS, EMC, EPI, ER, and MS/MS/MS
5	Q3 Empty Time	Compound	The Q3 Empty Time parameter controls the amount of time that singly-charged ions are removed from the LIT. Use the preset value.	EMC
	MS/MS/MS Fragmentation Time	Compound	The MS/MS/MS Fragmentation Time parameter controls the amount of time that the excitation energy is applied. It is used in combination with the excitation energy to fragment the isolated second precursor ion. Use the preset value.	MS/MS/MS
6	MCS (Multi-Charge Separation) Barrier	Compound	The MCS Barrier parameter controls the voltage used when eliminating the singly-charged ions from the LIT. Use the preset value.	EMC
6	Q3 Cool Time	Compound	The Q3 Cool Time parameter controls the amount of time that the precursor ions are allowed to cool prior to collection of their product ions. Use the preset value.	TDF

Figure 5-1 Ion optics path and parameters (Continued)

Item	Parameter	Parameter Type	Use	Scan Type
7	Fixed LIT Fill Time	Compound	The Fixed LIT Fill Time parameter controls the amount of time that the LIT fills with ions. Use the preset value.	EMS, EPI, MRM, and MS/MS/MS
7	DFT (Dynamic Fill Time)	Compound	DFT will dynamically calculate the length of time that ions are collected in the LIT based on the incoming ion signal. When DFT is turned on the signal is optimized to either increase sensitivity or minimize space-charging. Either select or clear the feature based on the experiment. In the **Tools** > **Settings** > **Method Options** dialog, the Dynamic Fill Time settings are optimized for the 10000 Da/s scan speed. These settings are also suitable for other LIT scan speeds.	EMS, EPI, ER, and MS/MS/MS
8	CEM (CEM)	Detector	The CEM parameter controls the voltage applied to the detector. The voltage controls the detector response.	All

Acquisition Method Editor Icons

Table 5-2 Acquisition Method Editor Icons

Icon	Name	Function
	Mass Spec	Shows the MS tab in the Acquisition Method Editor.
	Period	Right-click to add an experiment, add an IDA Criteria Level, or delete the period.
	Autosampler	Opens the Autosampler Properties tab.
	Syringe Pump	Opens the Syringe Pump Properties tab.
	Column Oven	Opens the Column Oven Properties tab.
	Valve	Opens the Valve Properties tab.
	DAD	Opens the DAD Method Editor. For more information about the DAD, refer to Show DAD Data on page 78.
	ADC	Opens the ADC Properties tab. For more information about the ADC, refer to Show ADC Data on page 72.

A batch is a collection of information about the samples. Batches tell the software the order in which to analyze the samples. For more information about importing batches, refer to the *Advanced User Guide*.

Set Queue Options

The queue goes one by one through the list, running each sample with the selected acquisition method. After all the samples have been acquired, the queue stops and the instrument goes into Standby mode. In Standby mode, the LC pumps are turned off and some instrument voltages are turned off.

The user can change the length of time the queue runs after the last acquisition has finished, before it puts the instrument into Standby mode. For more information about the other fields in the Queue Options dialog, refer to the Help.

1. In the Navigation Bar, click **Configure**.
2. Click **Tools > Settings > Queue Options**.

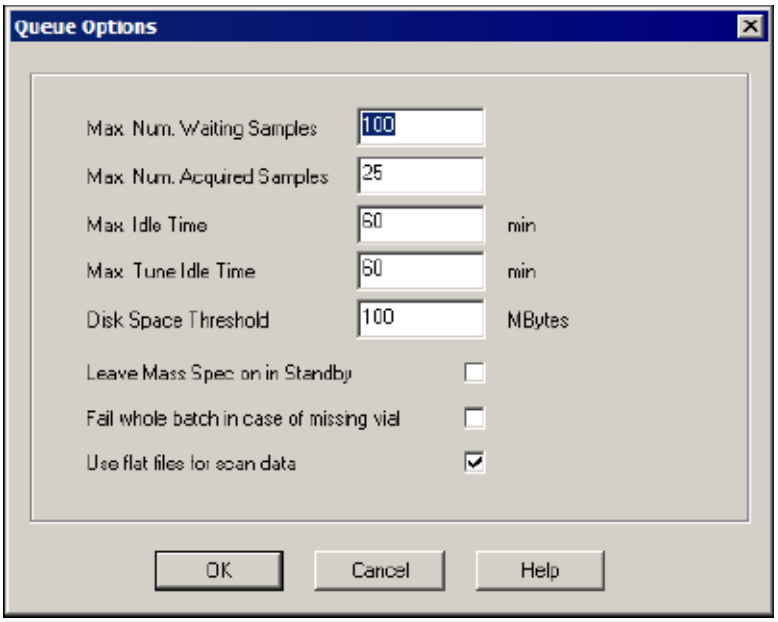


Figure 6-1 Queue Options dialog

3. In the **Max Idle Time** field, type the length of time the queue will wait after acquisition is completed before going into Standby mode. The preset value is 60 minutes.
4. If gas cylinders are used, adjust this time to make sure that the gas in the cylinders is not depleted.
5. If an LC method is used, then before the run is started, make sure that there is enough solvent in the reservoirs for the primary flow rate for all of the sample runs and the maximum idle time.

Create and Submit a Batch

For more information about adding quantitation information to a batch, refer to the *Advanced User Guide*.

1. In the Navigation Bar, under **Acquire**, double-click **Build Acquisition Batch**.

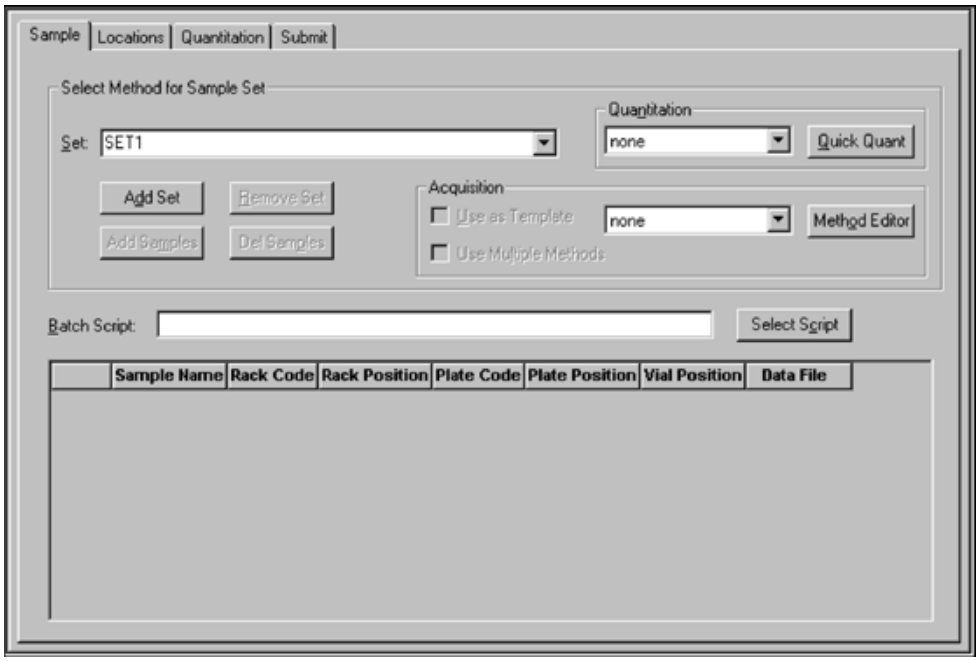


Figure 6-2 Batch Editor

2. In the **Sample** tab, in the **Set** list, type a name.
3. Click **Add Set**.
4. Click **Add Samples**.

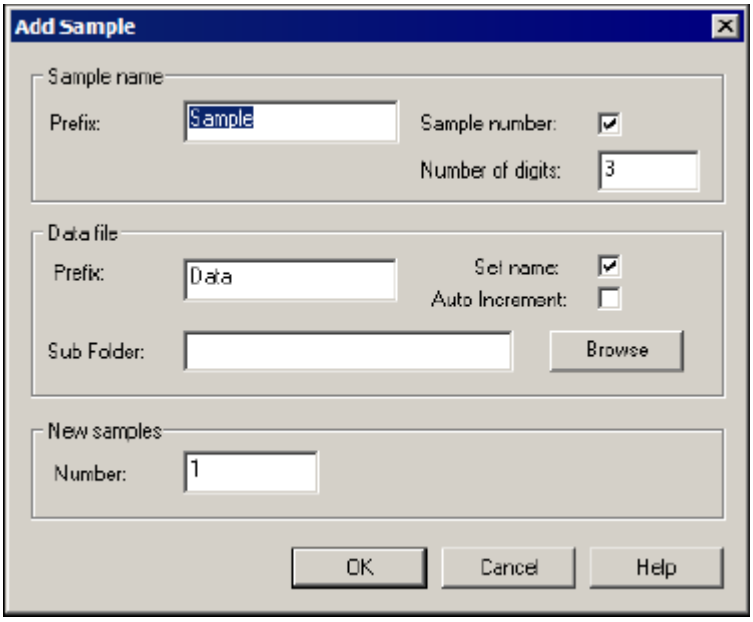


Figure 6-3 Add Sample dialog

5. In the **Sample name** section, in the **Prefix** field, type a name for the samples.
6. To add incremental numbering to the end of the sample name, select the **Sample number** check box.
7. If the **Sample number** check box is selected, in the **Number of digits** field, type the number of digits to include in the sample name.

   For example, if 3 is typed, the sample names would be samplename001, samplename002, samplename003.
8. In the **Data file** section, in the **Prefix** field, type a name for the data file that will store the sample information.
9. Select the **Set name** check box to use the set name as part of the data file name.
10. Select the **Auto Increment** check box to increment the data file names automatically.

Note: The data for each sample can be stored in the same or separate data file. The names of the data file will have numerical suffixes starting from 1.

11. In the **Sub Folder** field, type a name.

    The folder is stored in the Data folder for the current project. If the Sub folder field is left blank, the data file is stored in the Data folder and a subfolder is not created.
12. In the **New samples** section, in the **Number** field, type the number of new samples.
13. Click **OK**.

    The sample table fills with the sample names and data file names.

Tip! Fill Down and Auto Increment options are available in the right-click menu after a single column heading or several rows in a column are selected.

14. In the **Sample** tab, in the **Acquisition** section, select a method from the list.

    Depending on how the system is set up, specific information for the autosampler must be entered. Even if the injection volume is set in the method, the injection volume can be changed for one or more samples by changing the value in the injection volume column.

Tip! To use different methods for some of the samples in this set, select the Use Multiple Methods check box. The Acquisition Method column is shown in the Sample table. Select the acquisition method for each sample in this column.

15. To change the injection volumes from the volumes listed in the method, in the **Inj. Volume (µL)** column, type the injection volume for each sample.
16. To set sample locations, do one of the following:
    - Set Sample Locations in the Batch Editor on page 59
    - Select Vial Positions using the Locations Tab on page 59
17. Click the **Submit** tab.
18. If the **Submit Status** section contains a message about the status of the batch, do one of the following:
    - If the message indicates that the batch is ready for submission, proceed to step 19.
    - If the message indicates that the batch is not ready for submission, make the changes as indicated by the message.
19. Click **Submit**.

Change Sample Order

The order of the samples can be edited before they are submitted to the Queue.

- In the Submit tab, double-click any of the numbers on the far left of the table (a very faint square box is visible), and then drag them to the new location

Acquire Data

The system should not be in Tune mode when sample acquisition is started. Also, if the system has been previously run that day and has not yet been set to Standby, sample acquisition will start automatically.

1. In the Navigation Bar, click **Acquire**.
2. Click **View > Sample Queue**.

The Queue Manager opens with all submitted samples.

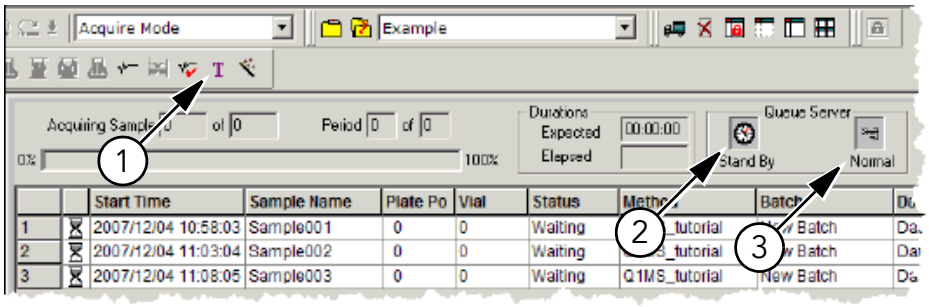


Figure 6-4 Queue Manager

Item	Description
1	The Tune icon should not be pressed in.
2	Queue status.
3	Queue Server should be in Normal mode. For more information, refer to Queue States on page 63.

3. Click **Acquire > Start Sample**.

Set Sample Locations in the Batch Editor

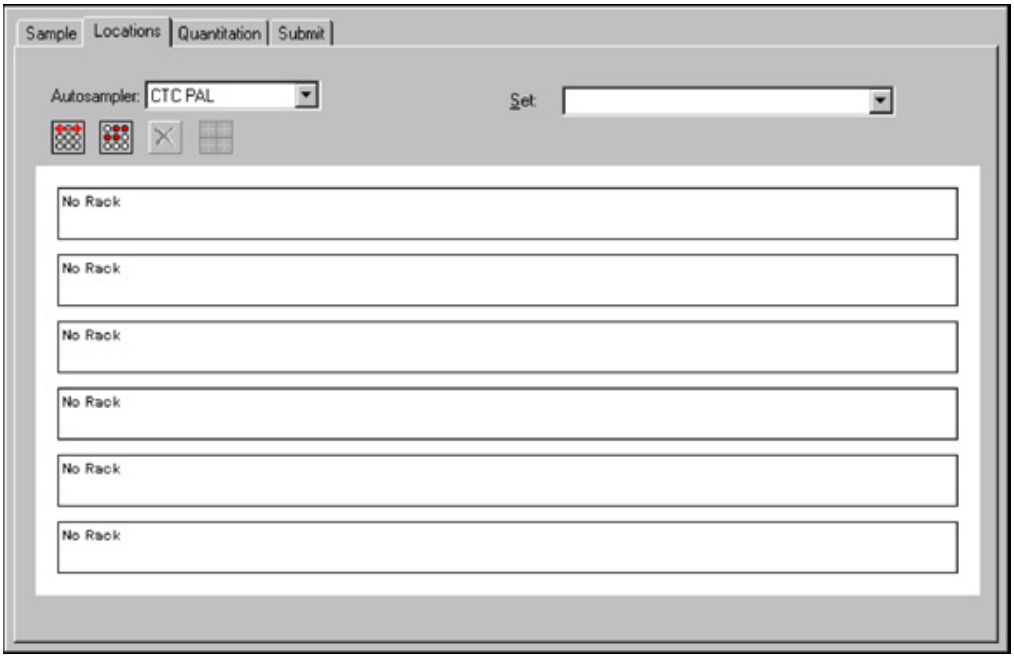
If an autosampler is used in the acquisition method, then the vial positions of the samples must be defined in the acquisition batch. Define the location in the Sample tab or in the Locations tab. For more information about creating batches, refer to Create and Submit a Batch on page 56.

Note: Depending on the autosampler being using, it may not be necessary to type details in additional columns.

1. In the **Sample** tab, in the **Set** list, select the set.
2. For each sample in the set, do the following if applicable:
   - In the **Rack Code** column, select the rack type.
   - In the **Rack Position** column, select the position of the rack in the autosampler.
   - In the **Plate Code** column, select the plate type.
   - In the **Plate Position** column, select the position of the plate on the rack.
   - In the **Vial Position** column, type the position of the vial in the plate or tray.
3. Save the file.

Select Vial Positions using the Locations Tab

1. In the **Batch Editor**, click the **Locations** tab.



2. In the **Set** list, select the set.
3. In the **Autosampler** list, select the autosampler.

   The appropriate number of rack spaces for the autosampler is shown in the graphic rack display.
4. In the space associated with the rack, right-click and then select the rack type.

   The plates or trays are shown in the rack.
5. Double-click one of the rectangles.

   The circles depicting the wells or vials for the plate or tray appear.
6. To select whether samples are marked by row or column, click **Row/Column Selection**.

   If the button shows a red horizontal line, the Batch Editor marks the samples by row. If the button shows a red vertical line, the Batch Editor marks the samples by column.
7. Click the sample wells or vials in the order to be analyzed. Click a selected well or vial again to clear it.
8. Save the file.

Tip! To auto fill in the samples, hold down the Shift key and then click the first and last vial within a set. To perform multiple injections from the same vial, hold down the Ctrl key and then click the vial location. The red circle changes to a green circle.

Stop Sample Acquisition

When a sample acquisition is stopped, the current scan finishes before the acquisition is stopped.

1. In the Queue Manager, click the sample in the queue after the point where acquisition should stop.
2. In the Navigation Bar, click **Acquire**.
3. Click **Acquire > Stop Sample**.

   The queue stops after the current scan in the selected sample is complete. The sample status in the Queue Manager (Local) window changes to Terminated, and all other samples following in the queue are waiting.
4. To continue processing the batch, click **Acquire > Start Sample**.

Batch and Acquisition Method Editor Tips

Table 6-1 Tips

To do this...	...do this
To change all the values in a column simultaneously	click a column heading and then right-click. From the menu, use the Auto Increment and Fill Down commands to change the values in the column. This also works for multiple cells in the same column.
To change an existing acquisition method	from the list, select the method and then click Method Editor. To create a new acquisition method, from the list, select None and then click Method Editor. Only experienced users should use this feature. Do not use this feature if the Use Multiple Methods option is used.
To apply a previously created quantitation method	select the method from the Quantitation menu.
To select more than one well or vial at a time	hold down the Shift key and then click the first and last well or vial in the range.

Batch Editor Right-Click Menu

Right-click in the Batch Editor table to access the following options.

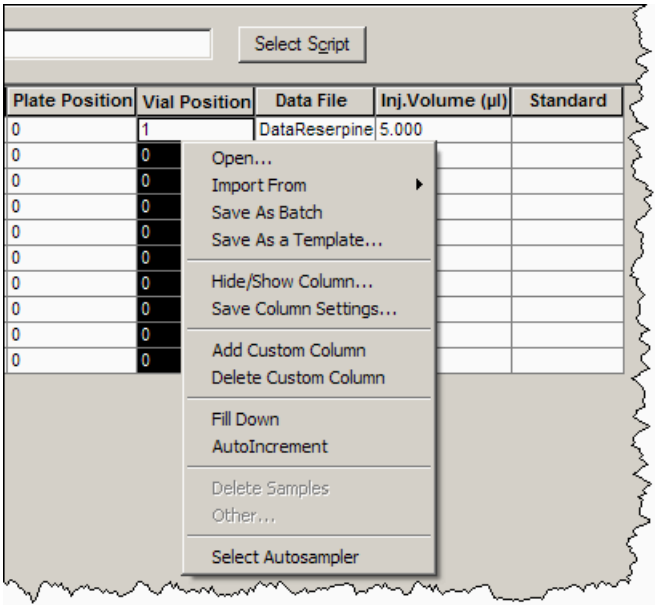


Figure 6-5 Batch Right-Click menu

Menu	Function
Open	Opens a batch file.
Import From	Imports a file.
Save As Batch	Saves the batch.
Save As a Template	Saves the batch as a template. Used with the Express View feature.
Hide/Show Column	Hides or shows a column.
Save Column Settings	Saves the batch column settings.
Add Custom Column	Adds a custom column.
Delete Custom Column	Deletes a custom column.
Fill Down	Fills the same data into the selected cells.
Auto Increment	Automatically increments data into the selected cells.
Delete Samples	Deletes the selected row.
Select Autosampler	Selects an autosampler.

Queue States and Device Status

The Queue Manager shows queue, batch, and sample status. Detailed information about a particular sample in the queue can also be viewed.

Queue States

The current state of the queue is indicated in the Queue Server.

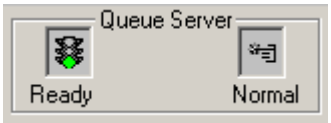


Figure 6-6 Queue Server indicator showing Normal mode

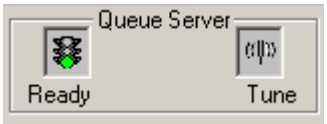


Figure 6-7 Queue Server indicator showing Tune mode

The first icon in Figure 6-6 shows the queue state. The second icon indicates whether the queue is in Tune mode (for tuning) or Normal mode (for running samples). Table 6-2 shows the various queue states.

Table 6-2 Queue States

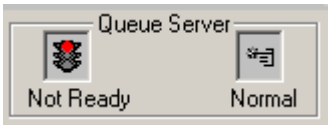
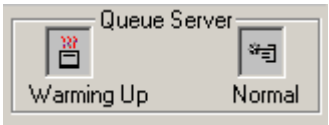
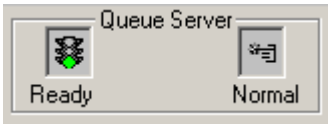
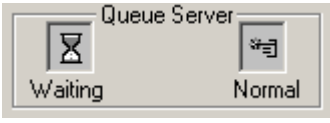
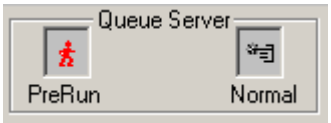
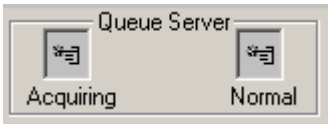
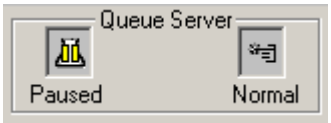
Icons	State	Definition
	Not Ready	In the Not Ready state, the hardware profile is deactivated and the queue is not accepting any sample submissions.
	Stand By	In the Stand By state, the hardware profile has been activated, but all devices are idle. Pumps are not running and gases are turned off.
	Warming Up	In the Warming Up state, the instrument and devices are equilibrating, columns are being conditioned, the autosampler needle is being washed, and column ovens are reaching temperature. The period of equilibration is selected by the operator. From this state, the system can go to the Ready state.
	Ready	In the Ready state, the system is ready to start running samples and the devices have been equilibrated and are ready to run. In this state, the queue can receive samples and will run after samples are submitted.
	Waiting	In the Waiting state, the system will automatically begin acquisition when the next sample is submitted.

Table 6-2 Queue States (Continued)

Icons	State	Definition
	Prerun	In the Prerun state, the method is being downloaded to each device and device equilibration is occurring. This state occurs before the acquisition of each sample in a batch.
	Acquiring	In the Acquiring state, the method is run and data acquisition occurs.
	Paused	In the Paused state, the instrument has been paused during acquisition.

View Instrument and Device Status Icons

Icons representing the instrument and each device in the active hardware configuration appear on the status bar in the bottom right corner of the window. The user can view the detailed status of an LC pump to check if the LC pump pressure is appropriate, or view the detailed status of the instrument to check the temperature of the ion source.

- On the status bar, double-click the icon for the device or instrument.

  The Instrument Status dialog opens.

Table 6-3 Instrument and Device Status (showing the instrument icon)

Status	Icon	Background Color	Description
Idle		Green or yellow	The device is not running. If the background color is yellow, the device should be equilibrated before it is ready to run. If the background color is green, the device is ready to run.
Equilibrating		Green or yellow	The device is equilibrating.
Waiting		Green	The device is waiting for a command from the software, from another device, or for some action by the operator.
Running		Green	The device is running.

Table 6-3 Instrument and Device Status (showing the instrument icon) (Continued)

Status	Icon	Background Color	Description
Aborting		Green	The device is aborting a run.
Downloading		Green	A method is being transferred to the device.
Ready		Green	The device is not running, but is ready to run.
Error		Red	The device has encountered an error that should be investigated.

Note: For each status the background color can also be red. This situation means that the device encountered an error while in that status.

Queue Right-Click Menu

Right-click in the Queue table to access the following options.

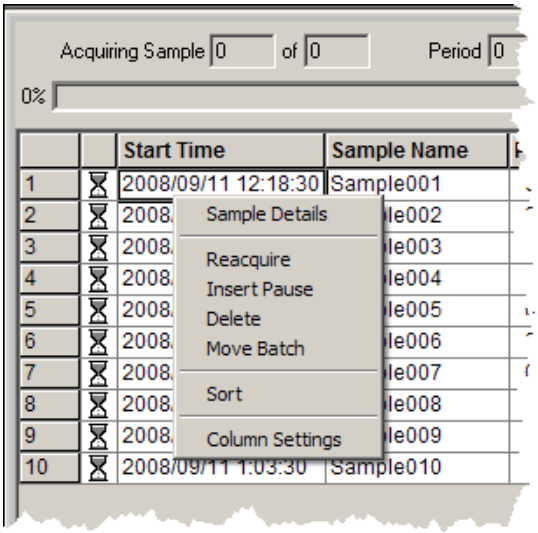


Figure 6-8 Queue Manager Right-Click Menu

Menu	Function
Sample Details	Opens the Sample Details dialog.
Reacquire	Acquires a sample again.
Insert Pause	Inserts a pause, in seconds, between two samples.
Delete	Deletes either the batch or the selected samples.

Figure 6-8 Queue Manager Right-Click Menu (Continued)

Menu	Function
Move Batch	Moves the batch within the queue.
Sort	Sorts by the preselect column.
Column Settings	Changes the column settings.

Icon Quick Reference: Acquire Mode

Table 6-4 Acquire Mode Icons

Icon	Name	Function
	View Queue	View the sample queue.
	Instrument Queue	View a remote instrument station.
	Status for Remote Instrument	View the status of a remote instrument.
	Start Sample	Starts the sample in the queue.
	Stop Sample	Stops the sample in the queue.
	Abort Sample	Aborts a sample acquisition in the middle of the processing of that sample.
	Stop Queue	Stops the queue before it has completed processing all the samples.
	Pause Sample Now	Inserts a pause in the queue.
	Insert Pause before Selected Sample	Inserts a pause before a specific sample.
	Continue Sample	Continues acquiring the sample.
	Next Period	Starts a new period.
	Extend Period	Extends the current period.
	Next Sample	Stops acquiring the current sample and to start acquiring the next sample.

Table 6-4 Acquire Mode Icons (Continued)

Icon	Name	Function
	Equilibrate	Click to select a method to use to equilibrate the devices. This method should be the same as the method used with the first sample in the queue.
	Standby	Puts the instrument in Standby mode.
	Ready	Puts the instrument in Ready mode.
	Reserve Instrument for Tuning	Reserves the instrument for tuning and calibrating.
	IDA Method Wizard	Starts the IDA Method Wizard.

Analyzing and Processing Data 7

Use the sample files installed in the Example folder to learn how to view and analyze data using the most common analysis and processing tools. For more information about the following topics, refer to the *Advanced User Guide*.

- Labelling graphs
- Overlaying and summing spectra or chromatograms
- Performing background subtractions
- Smoothing algorithms
- Working with smoothed data
- Working with centroid data
- Working with contour plots
- Working with the fragment interpretation tool
- Working with library databases and library records

Open Data Files

Prerequisite: The Example project is selected.

1. In the Navigation Bar, under **Explore**, double-click **Open Data File**.

   The Select Sample dialog opens.
2. In the **Data Files** list, double-click **Triple Quad**.
3. Select **QuanData.wiff**.
4. In the **Samples** list, select sample AP13-020.
5. Click **OK**.

   The data acquired from the sample is shown. If data is still being acquired, the mass spectrum, DAD/UV trace, and TIC continue to update automatically.

Tip! To turn off the automatic update on the mass spectrum, right-click the mass spectrum and then click **Show Last Scan**. If there is a check mark beside **Show Last Scan**, then the spectrum will update in real time.

Navigate Between Samples in a Data File

Prerequisite: The Example project is selected.

Table 7-9 shows the navigation icons used in this procedure. If samples were saved in separate data files, then open each file individually.

1. In the Navigation Bar, under **Explore**, double-click **Open Data File**.
2. In the **Data Files** list, double-click **Triple Quad**.
3. Select **QuanData.wiff**.

4. To skip to the next sample in the data file, click the icon with the arrow pointing to the right.
5. To skip to a non-sequential sample, click the icon with the arrow curving to the right.
6. In the **Select Sample** dialog, in the **Sample** list, select the sample.
7. To go to the previous sample in the data file, click the icon with the arrow pointing to the left.

Show Experimental Conditions

The experimental conditions used to collect data are stored in the data file with the results. The information contains the details of the acquisition method used: the MS acquisition method (that is, the number of periods, experiments and cycles) including instrument parameters, and HPLC device method (LC pump flow rate). In addition, it also contains the MS resolution and mass calibration tables used for the sample acquisition. Table 7-1 shows the software functionality available when the user views the file information.

Note: If data is acquired from more than one sample into the same .wiff file, the file information pane will not refresh automatically as the user scrolls through the samples. Close the file information pane and then reopen it to view the details for the next sample in the .wiff file.

- Click **Explore** > **Show** > **Show File Information**.

  The File Information pane opens below the graph.

Tip! To create an acquisition method from the file information pane, right-click the file information pane and then click **Save Acquisition Method.**

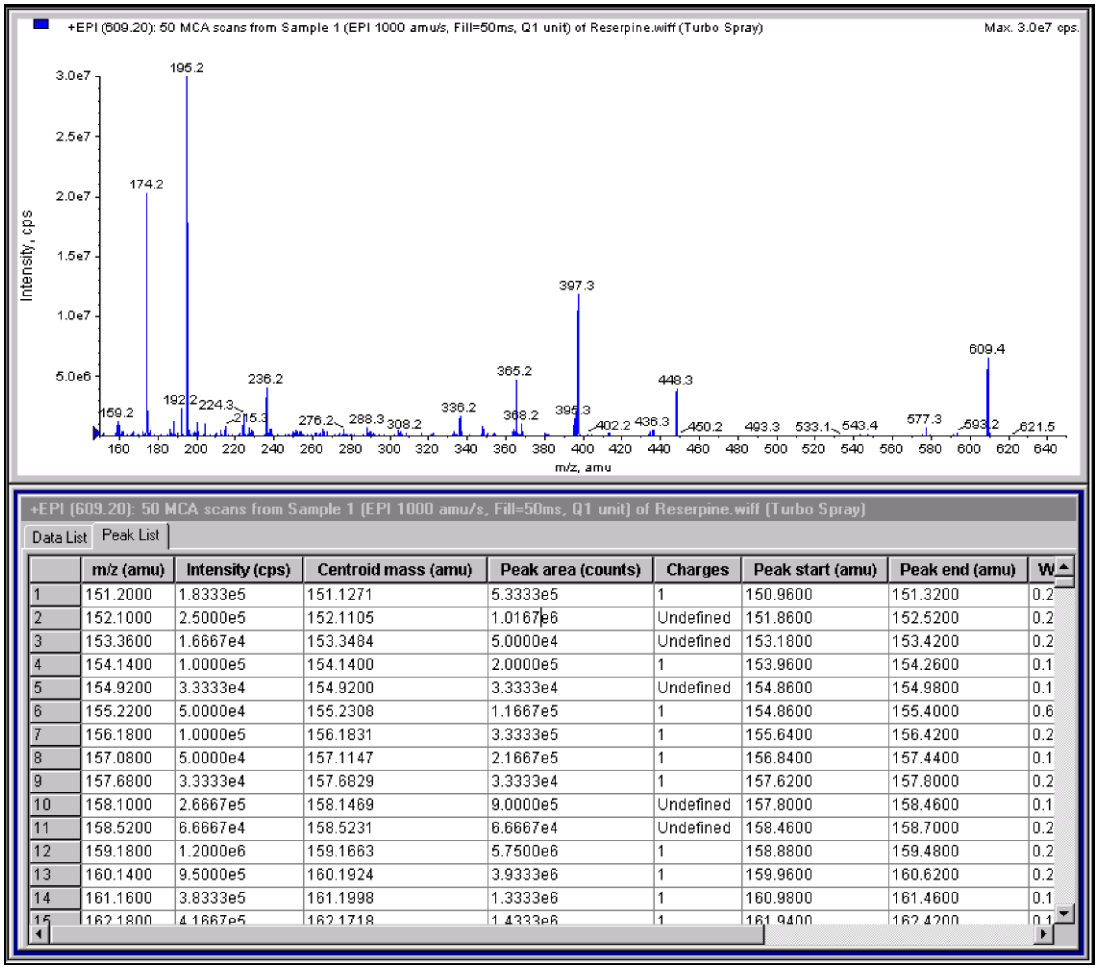
Table 7-1 Right-Click Menu for Show File Information Pane

Menu	Function
Copy	Copies the selected data.
Paste	Pastes data.
Select All	Selects all the data in the pane.
Save To File	Saves data in an .rtf file.
Font	Changes the font.
Save Acquisition Method	Saves the acquisition method as .dam file.
Save Acquisition Method to CompoundDB	Opens the Specify Compound Information dialog. Select the IDs and molecular weights to be saved in the compound database.
Delete Pane	Deletes the pane.

Show Data in Tables

- With a data file open, click **Explore** > **Show** > **Show List Data**.

  The data is shown in a pane below the graph.



	m/z (amu)	Intensity (cps)	Centroid mass (amu)	Peak area (counts)	Charges	Peak start (amu)	Peak end (amu)	W
1	151.2000	1.8333e5	151.1271	5.3333e5	1	150.9600	151.3200	0.2
2	152.1000	2.5000e5	152.1105	1.0167e6	Undefined	151.8600	152.5200	0.2
3	153.3600	1.6667e4	153.3484	5.0000e4	Undefined	153.1800	153.4200	0.2
4	154.1400	1.0000e5	154.1400	2.0000e5	1	153.9600	154.2600	0.1
5	154.9200	3.3333e4	154.9200	3.3333e4	Undefined	154.8600	154.9800	0.1
6	155.2200	5.0000e4	155.2308	1.1667e5	1	154.8600	155.4000	0.6
7	156.1800	1.0000e5	156.1831	3.3333e5	1	155.6400	156.4200	0.2
8	157.0800	5.0000e4	157.1147	2.1667e5	1	156.8400	157.4400	0.1
9	157.6800	3.3333e4	157.6829	3.3333e4	1	157.6200	157.8000	0.2
10	158.1000	2.6667e5	158.1469	9.0000e5	Undefined	157.8000	158.4600	0.1
11	158.5200	6.6667e4	158.5231	6.6667e4	Undefined	158.4600	158.7000	0.2
12	159.1800	1.2000e6	159.1663	5.7500e6	1	158.8800	159.4800	0.2
13	160.1400	9.5000e5	160.1924	3.9333e6	1	159.9600	160.6200	0.2
14	161.1600	3.8333e5	161.1998	1.3333e6	1	160.9800	161.4600	0.1
15	162.1800	4.1667e5	162.1718	1.4333e6	1	161.9400	162.4200	0.1

Table 7-2 RIght-Click Menu for the Spectral Peak List Tab

Menu	Function
Column Options	Opens the Select Columns for Peak List dialog.
Save As Text	Saves the data as text file.
Delete Pane	Deletes the pane.

Table 7-3 Right-Click Menu for the Chromatographic Peak List Tab

Menu	Function
Analyst Classic Parameters	Opens the Analyst Classic dialog.
IntelliQuan Parameters	Opens the Intelliquan dialog.
Save As Text	Saves the data as text file.
Delete Pane	Deletes the pane.

Show ADC Data

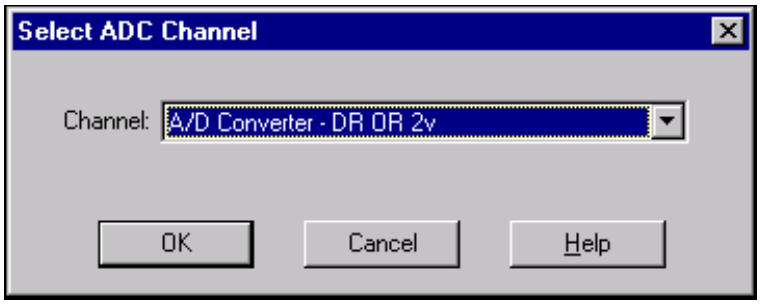
ADC (analog-to-digital converter) data is acquired from a secondary detector (for example from a UV detector through an ADC card), and is useful for comparison with mass spectrometer data. To have ADC data available, acquire the data and the mass spectrometer data simultaneously and save it in the same file.

Prerequisite: The Example project is selected.

1. In the Navigation Bar, under **Explore**, double-click **Open Data File**.

   The Select Sample dialog opens.
2. In the **Data Files** list, double-click **Devices**, and then click **Adc16chan.wiff**.
3. In the **Samples** list, select a sample.
4. Click **OK**.
5. Click **Explore > Show > Show ADC Data**.

   The Select ADC Channel dialog opens.



6. In the **Channel** list, select a channel.
7. Click **OK**.

   The ADC data opens in a new pane below the active pane.

Show Basic Quantitative Data

1. In the Peak List tab, right-click and select **Show Peaks in Graph.**

   Peaks appear in two colors.
2. To change the peak finding algorithm settings right-click and then select either **Analyst Classic Parameters** or **Intelliquan Parameters**, which ever is active.
3. To remove the colored peaks, right-click in the **Peak List** tab and then clear **Show Peaks in Graph**.

Chromatograms

Table 7-4 Chromatograms

Types of chromatograms	Purpose
TIC (Total Ion Chromatogram)	A chromatographic display generated by plotting the intensity of all ions in a scan against time or scan number. When a data file is opened, it is preset to appear as a TIC. If the experiment contains only one scan, it is shown as a spectrum. For more information about using the available icons, refer to Table 7-8 *Graph Options* on page 81. If the MCA check box is selected during acquisition of the data file, then the data file opens to the mass spectrum. If the MCA check box is not selected, then the data file opens with the TIC
XIC (Extracted Ion Chromatogram)	An ion chromatogram created by taking intensity values at a single, discrete mass value, or a mass range, from a series of mass spectral scans. It indicates the behavior of a given mass, or mass range, as a function of time.
BPC (Base Peak Chromatogram)	Chromatographic plot that shows the intensity of the most intense ion within a scan versus time or scan number.
TWC (Total Wavelength Chromatogram)	A chromatographic display created by summing all of the absorbance values in the acquired wavelength range and then plotting the values against time. It consists of the summed absorbances of all ions in a scan plotted against time in a chromatographic pane.
XWC (Extracted Wavelength Chromatogram)	A subset of TWC. An XWC shows the absorbance for a single wavelength or the sum of the absorbance for a range of wavelengths.
DAD (Diode Array Detector)	A UV detector that monitors the absorption spectrum of eluting compounds at one or more wavelengths.

Show TICs from a Spectrum

Prerequisite: The Example project is selected.

1. In the Navigation Bar, under **Explore**, double-click **Open Data File**.

   The Select Sample dialog opens.
2. In the **Data Files** list, double-click **LIT**, and then click **Reserpine.wiff**.
3. Click **OK**.
4. Click **Explore > Show > Show TIC**.

   The TIC opens in a new pane.

Tip! Right-click inside a pane containing a spectrum and then click **Show TIC**.

Show a Spectrum from a TIC

1. In a pane containing a TIC, select a range.
2. Click **Explore > Show > Show Spectrum**.

   The spectrum opens in a new pane.

Tip! Double-click in the TIC pane at a particular time to show the spectrum.

Generate XICs

The user can generate XICs only from single period, single experiment chromatograms or spectra. To obtain an XIC from multi-period or multi-experiment data, split the data into separate panes by clicking the triangle that is under the x-axis. For more information about using the available icons, refer to Table 7-8 *Graph Options* on page 81.

There are several methods for extracting ions to generate an XIC, depending on whether chromatographic or spectral data is used. Table 7-5 contains a summary of methods that can be used with chromatograms and spectra.

Table 7-5 Summary of XIC Generation Methods

Method	Use with chromatogram	Use with spectrum	Extraction
Selected range	No	Yes	The selected range method extracts ions from a selected area in a spectrum.
Maximum	No	Yes	The maximum method extracts ions from a selected area in a spectrum using the most intense peak in the selected area. This creates an XIC using the maximum mass from the selected spectral range.
Base peak masses	Yes	No	The base peak masses method can be used only with BPCs (Base Peak Chromatograms.) Use the Use Base Peak Masses command to extract ions results in an XIC with a different colored trace for each mass. If the selection includes multiple peaks, the resulting XIC will have an equal number of colored traces representing each mass.
Specified masses	Yes	Yes	The specified masses method extracts ions from any type of spectrum or chromatogram. Select up to ten start and stop masses for which to generate XICs.

To generate an XIC using a selected range

1. In the Navigation Bar, under **Explore**, double-click **Open Data File**.

   The Select Sample dialog opens.
2. In the **Data Files** list, double-click **LIT**, and then click **Reserpine.wiff**.
3. Click **OK**.
4. To select a range inside the pane, click and hold the left mouse button at the start of the range and then drag the cursor to the stop point and release.

   The selection is highlighted in blue.
5. Click **Explore > Extract Ions > Use Range**.

   An XIC of the specified selection opens in a pane below the spectrum pane. The experiment information at the top of the pane contains the mass range and the maximum intensity in counts per second.

To generate an XIC using the maximum peak

1. In the Navigation Bar, under **Explore**, double-click **Open Data File**.

   The Select Sample dialog opens.
2. In the **Data Files** list, double-click **LIT**, and then click **Reserpine.wiff**.
3. Click **OK**.
4. Select a range.

   The selection is highlighted in blue.
5. Click **Explore > Extract Ions** > **Use Maximum**.

   An XIC of the maximum peak specified selection opens below the spectrum pane. The experiment information at the top of the pane contains the mass range and the maximum intensity in counts per second.

To generate an XIC using base peak masses

1. In a BPC, select the peak from which to extract ions.

   The selection is highlighted in blue.
2. Click **Explore > Extract Ions** > **Use Base Peak Masses**.

   An XIC of the specified selection opens below the spectrum pane. The experiment information at the top of the pane shows the mass range and the maximum intensity in counts per second.

To extract ions by selecting masses

1. Open a spectrum or chromatogram.
2. Click **Explore > Extract Ions** > **Use Dialog**.

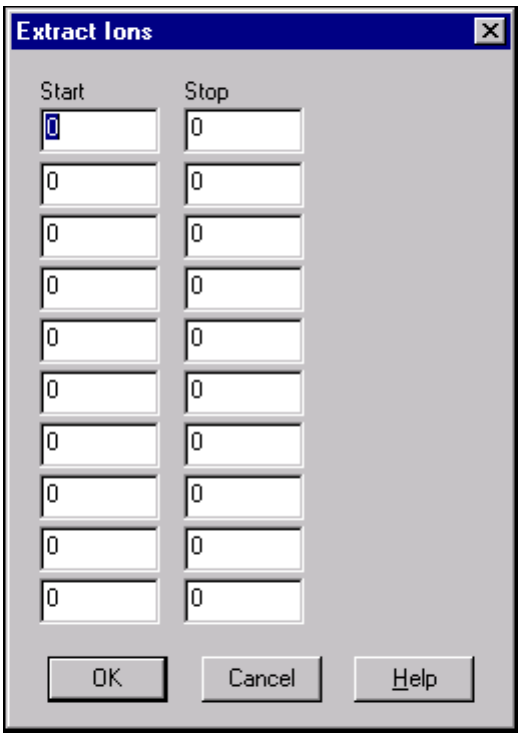


Figure 7-1 Extract Ions dialog

3. Type the values for each XIC to be created. If a stop value is not typed, then the range is defined by the start value.
   - In the **Start** field, type the start value (lower value) for the mass range.
   - In the **Stop** field, type the stop value (higher value) for the mass range.
4. Click **OK**.

   An XIC of the selection opens below the chromatogram pane. The experiment information at the top of the pane includes the masses and the maximum intensity in counts per second.

Generate BPCs

BPCs can be generated only from single period, single experiment data. For more information about using the available icons, refer to Table 7-8 *Graph Options* on page 81.

1. Select an area within a TIC.

   The selection is highlighted in blue.
2. Click **Explore > Show > Show Base Peak Chromatogram**.

   The selections are shown in the Start Time and End Time fields.

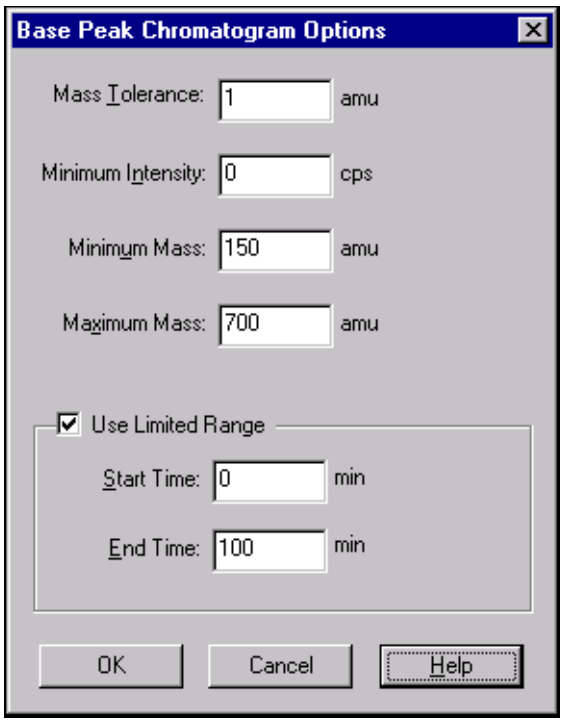


Figure 7-2 Base Peak Chromatogram Options dialog

3. In the **Mass Tolerance** field, type the value to dictate the mass range used to find a peak. The software finds the peak using a value twice the typed range (± the mass value).
4. In the **Minimum Intensity** field, type the intensity below which peaks are ignored by the algorithm.
5. In the **Minimum Mass** field, type the mass that determines the beginning of the scan range.
6. In the **Maximum Mass** field, type the mass that determines the end of the scan range.
7. To set the start and end times, select the **Use Limited Range** check box and do the following:
   - In the **Start Time** field, type the time that determines the start of the experiment.
   - In the **End Time** field, type the time that determines the end of the experiment.
8. Click **OK**.

   The BPC is generated in a new pane.

Generate XWCs

The user can extract up to three ranges from a DAD spectrum to generate the XWC. For more information about using the available icons, refer to Table 7-8 *Graph Options* on page 81.

1. Open a data file that contains a DAD spectrum.
2. Anywhere in the pane, right-click and then click **Extract Wavelengths**.

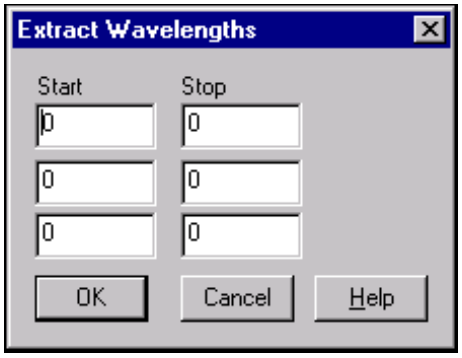


Figure 7-3 Extract Wavelengths dialog

3. Type start and stop values.
4. Click **OK**.

   The XWC opens in a pane below the DAD spectrum.

Show DAD Data

The user can view DAD data in chromatogram or spectrum form, the same as mass spectrometer data.

1. Open a data file containing data acquired with a DAD.
2. The TWC, which is analogous to a TIC, opens in a pane below the TIC.
3. In the TWC pane, click a point to select a single point in time, or highlight an area of the spectrum to select a range of time.
4. Click **Explore > Show > Show DAD Spectrum**.

   The DAD spectrum opens in a pane below the TWC. The y-axis shows absorbance and the x-axis shows wavelength.

Tip! If the pane with the TWC is closed, click a point anywhere in the TWC to open it again. Click **Explore** > **Show** > **Show DAD TWC**.

Generate TWCs

A TWC shows total absorbance (mAU) on the y-axis plotted against time on the x-axis. For more information about using the available icons, refer to Table 7-8 *Graph Options* on page 81.

1. Open a data file that contains a DAD spectrum.
2. Click **Explore > Show > Show DAD TWC**.

   The TWC opens in a pane below the DAD spectrum.

Tip! Right-click inside the pane containing the DAD spectrum and then click Show DAD TWC.

Adjust the Threshold

The threshold is an invisible line drawn parallel to the x-axis of a graph that sets a limit below which the software will not include peaks in a spectrum. The line has a handle, represented by a blue triangle to the left of the y-axis. Click the blue triangle to view a dotted line that represents the threshold. The threshold can be raised or lowered, but changing the threshold value does not change the data. The software does not label any peaks in the region that lies below the threshold.

1. Open a data file.
2. Adjust the threshold using one of the following steps:
   - To raise the threshold, drag the blue triangle up the y-axis. To lower the threshold, drag the blue triangle down.
   - Click **Explore > Set Threshold**. In the **Threshold Options** dialog that opens, type the threshold value.
   - Click **Explore > Threshold**.

   The graph updates to show the new threshold. Peak labeling and the peak list are also updated.

Tip! To view the current threshold value, move the pointer over the threshold handle.

Table 7-6 Right-Click Menu for Chromatogram Panes

Menu	Function
List Data	Lists the data points and integrates chromatograms.
Show Spectrum	Generates a new pane.
Show Contour Plot	Shows a color-coded plot of a data set, where the color represents the intensity of the data at that point. Only certain MS modes are supported.
Extract Ions	Extracts a specific ion or set of ions from a selected pane and then generates a new pane containing a chromatograph for the specific ions.
Show Base Peak Chromatogram	Generates a new pane containing a base peak chromatogram.
Show ADC Data	Generates a new pane containing the UV data trace, if acquired.
Spectral Arithmetic Wizard	Opens the Spectral Arithmetic Wizard.
Save to Text File	Generates a text file of the pane, which can be opened in Excel or other programs.
Save Explore History	The Explore History File records changes to processing parameters, also called Processing Options, when a .wiff file is processed in Explore mode. The processing history is stored in a file with an .EPH (Explore Processing History) extension.
Add Caption	Adds a caption at the cursor point in the pane.
Add User Text	Adds a text box at the position of the mouse cursor.

Table 7-6 Right-Click Menu for Chromatogram Panes (Continued)

Menu	Function
Set Subtract Range	Sets the subtract range in the pane.
Clear Subtract Range	Clears the subtract range in the pane.
Subtract Range Locked	Locks or unlocks the subtract ranges. If the subtract ranges are not locked then each subtract range can be moved independently. The subtract ranges are preset to locked.
Delete Pane	Deletes the selected pane.

Table 7-7 Right-Click Menu for Spectra Panes

Menu	Function
List Data	Lists the data points and integrates chromatograms.
Show TIC	Generates a new pane containing the TIC.
Extract Ions	Extracts a specific ion or set of ions from a selected pane and then generates a new pane containing a chromatograph for the specific ions.
Save to Text File	Generates a text file of the pane, which can be opened in Excel or other programs.
Save Explore History	The Explore History File records changes to processing parameters, also called Processing Options, when a .wiff file is processed in Explore mode. The processing history is stored in a file with an .EPH (Explore Processing History) extension.
Add Caption	Adds a caption at the cursor point in the pane.
Add User Text	Adds a text box at the position of the mouse cursor.
Show Last Scan	Shows the scan prior to the selection.
Select Peaks For Label	In this dialog, select the parameters to reduce peak labeling.
Delete Pane	Deletes the selected pane.
Add a Record	Add records and compound-related data including spectra to the library. An active spectrum is required to perform this task.
Search Library	Searches the library without constraints or with previously saved constraints.
Search With Constraints	Searches using the Search Constraints dialog.

Data Processing

Graphical data can be processed many ways. This section provides information and procedures for using some of the most commonly used tools.

The user can zoom in on part of a graph to view a particular peak or an area in greater detail in both spectra and chromatograms. The user can also zoom in repeatedly to view smaller peaks.

Graphs

The same data can be examined in different ways. Data can also be kept for comparison purposes before performing processing operations such as smoothing or subtraction.

A window contains one or more panes arranged in such a way that all the panes are fully visible and they do not overlap.

Panes may be of variable or fixed size. Panes are automatically tiled within the window and are arranged into column and row format. If the size of a window is changed, the panes within the window change in size to accommodate the new size. A window cannot be sized to the point where any of the panes would become smaller than its minimum size.

Two or more windows or panes containing similar data can be linked, for example, spectra with similar mass ranges. As one pane or window is zoomed in, the other pane zooms in simultaneously.

For example, the user can link an XIC to the BPC from which the XIC was extracted. Zooming in the BPC also zooms the XIC, so that both chromatograms show the same magnification.

Table 7-8 Graph Options

To do this...	use this menu option...	...or click this icon
Copy a graph to a new window	• Select the graph to copy. Click **Explore > Duplicate Data** > **In New Window**.	
Rescale graph to its original size	• Select the graph. Click **Explore > Home Graph**.	

Move a pane	• Select the graph. Click **Window > Move Pane**.
	• Select the pane or window and then drag it to the new position. This position can be within the same window or within another window.
	A four-headed arrow is shown when the cursor is on the boundary of the active window or pane.
	  • If the pane is at the top or bottom of the target pane, the pane moves above or below that pane, respectively.
	  • If the pane is at the left or right of the target pane, the pane moves to the left or right of that pane, respectively.
	  • If the pane is at any other position, the pane moves to the target row. The drop shadow of the pane as the pane is moved around indicates its new position.
		

Link panes	1. With the two graphs open, click one to make that pane active. 2. Click **Explore > Link** and then click the other pane.	

Table 7-8 Graph Options (Continued)

To do this...	use this menu option...	...or click this icon
Remove linking	• Close one of the panes. Click **Explore > Remove Link**	
Delete a pane	• Select the graph. Click **Window > Delete Pane**.	
Lock a pane	• Select the graph. Click **Window > Lock Panes**.	
Hide a pane	• Select the graph. Click **Window > Hide Pane**.	
Maximize a pane	• Select the graph. Click **Window > Maximize Pane**.	
Tile panes	• Select the graph. Click **Window > Tile all Panes**.	

Zoom in on the y-axis

1. Position the pointer to the left of the y-axis and then drag vertically away from the starting point.

   A box is drawn along the y-axis representing the new scale.

Note: Take care when zooming in on the baseline. Zoom in too low and the zoom-in box disappears.

2. Release the mouse button to draw the graph to the new scale.

Zoom in on the x-axis

1. Position the pointer under the x-axis to either side of the area to expands and then drag away from the starting point in a horizontal direction to expand the area of interest.
2. Release the mouse button to redraw the graph to the new scale.

Tip! To return the graph to the original scale, double-click on either axis. To restore the entire graph to original scale, click **Explore** > **Home Graph**.

Table 7-9 Explore Quick Reference: Chromatograms and Spectrum

Icon	Name	Function
	Open File	Opens files.
	Show Next Sample	Goes to the next sample.
	Show Previous Sample	Goes to the previous sample.
	GoTo Sample	Opens the Select Sample dialog.
	List Data	Views the data in tables.
	Show TIC	Generates a TIC from a spectrum.
	Extract Using Dialog	Extracts ions by selecting masses.
	Show Base Peak Chromatogram	Generates a BPC.
	Show Spectrum	Generates a spectrum from a TIC.
	Copy Graph to new Window	Copies the active graph to a new window.
	Baseline Subtract	Opens the Baseline Subtract dialog.
	Threshold	Adjusts the threshold.
	Noise Filter	Click to use the Noise Filter Options dialog to define the minimum width of a peak. Signals below this minimum width are regarded as noise.
	Show ADC	View ADC data.
	Show File Info	Shows the experimental conditions used to collect the data.
	Add arrows	Add arrows to the x-axis of the active graph.

Table 7-9 Explore Quick Reference: Chromatograms and Spectrum (Continued)

Icon	Name	Function
	Remove all arrows	Remove arrows from the x-axis of the active graph.
	Offset Graph	Click to compensate for slight differences in the time during which the ADC data and the mass spectrometer data were recorded. This is useful when overlaying graphs for comparison.
	Force Peak Labels	Labels all the peaks.
	Expand Selection By	Sets the expansion factor for a portion of a graph to be viewed in greater detail.
	Clear ranges	Return the expanded selection to normal view.
	Set Selection	Click to type start and stop points for a selection. This provides more accurate selection than is possible by highlighting the region using the cursor.
	Normalize to Max	Click to scale a graph to maximum, so that the most intense peak is scaled is to full scale, whether or not it is visible.
	Show History	View a summary of data processing operations performed on a particular file, such as smoothing, subtraction, calibration, and noise filtering.
	Open Compound Database	Opens the compound database.
	Set Threshold	Adjusts the threshold.
	Show Contour Plot	Shows selected data as either a spectrum graph or an XIC. Additionally, for data acquired by a DAD, a contour plot can show selected data as either a DAD spectrum or an XWC.
	Show DAD TWC	Generates a TWC of the DAD.
	Show DAD	Generates a DAD.
	Extract Wavelength	Extracts up to three wavelength ranges from a DAD spectrum to view the XWC.

Analyzing and Processing Quantitative Data 8

Use the sample files found in the Example folder to learn how to select samples for quantitation, how to select preset queries and create table-specific queries, and how to analyze the acquired data. For more information about the following topics, refer to the *Advanced User Guide*.

- Metric Plots
- Layout of a Results Table

Quantitative Analysis

Quantitative analysis is used to find the concentration of a particular substance in a sample. By analyzing an unknown sample and comparing it to other samples containing the same substance with known concentrations (standards), the software can calculate the concentration of the unknown sample. The process involves creating a calibration curve using the standards and then calculating the concentration for the unknown sample. The calculated concentrations of each sample are then available in a Results Table.

Quantitation Methods

A quantitation method is a set of parameters used to generate peaks in a sample. The quantitation method can include parameters used to locate and integrate peaks, generate standard curves, and calculate unknown concentrations. A previously saved quantitation method can be selected from the Quantitation menu in the batch. For information about creating a batch, refer to Create and Submit a Batch on page 56.

The user can create a quantitation method before data acquisition and then apply the method to the quantitative data automatically after the batch is complete. Alternatively, a quantitation method can be created and applied post-acquisition.

Three tools can be used to create a quantitation method: the Quantitation Wizard, the Build Quantitation Method, and Quick Quant.

Build Quantitation Method

The Build Quantitation Method does not generate a Quantitation Results Table although the method can subsequently be used in the Quantitation Wizard to create a Results Table. The Build Quantitation Method can also be used to change existing quantitation methods. This is the most flexible way of creating a quantitation method.

Quantitation Wizard

With the Quantitation Wizard, a Results Table is generated at the same time as the quantitation method. Alternatively, an existing quantitation method can be used to quantitate different sets of data. This is the most common way of creating a quantitation method.

Quick Quant

Quick Quant is part of the Batch Editor. Use Quick Quant to add compound concentrations prior to data acquisition. Because a sample has not been acquired, a representative sample cannot be be selected or any peaks reviewed. With this process, only the method components are defined.

About Results Tables

Results Tables summarize the calculated concentration of an analyte in each unknown sample based on the calibration curve. Results Tables also include the calibration curves as well as statistics for the results. The user can customize the Results Table and view the Results Tables in layouts.

The data from a Results Table can be exported to a .txt file for use in other applications, such as Microsoft Excel. The user can also export data in the table or just the data in the visible columns.

Quantitation Methods and Results Tables

For the following procedures, use the sample data that is installed with the software. PK Data contains the batches Mix_Batch1 and Mix_Batch2. These sample batches are used to demonstrate the usefulness of metric plots to isolate problematic samples. The ions scanned were reserpine (609.4/195.0), minoxidil (210.2/164.2), tolbutamide (271.3/91.1) and rescinnamine (635.4/221.2), which is the internal standard. Batch 1 contains no errors in terms of sample preparation, whereas Batch 2 contains a QC sample where the internal standard was added twice (sample QC2).

Create a Method using the Quantitation Method Editor

Prerequisites:

- The Example project is selected.
- The Analyst Classic algorithm is used.

1. In the Navigation Bar, under **Quantitate**, double-click **Build Quantitation Method**.

   The Select Sample dialog opens.
2. In the **Data Files** list, double-click the **Triple Quad** folder.
3. Select **Mix_Batch_2. wiff**.

   The samples in the selected data file appear in the Samples list.
4. Click **OK**.
5. In the **Internal Standards** table, do the following:
   i. In the **Name** column, select rescinnamine.
   ii. In the **Q1/Q3** column, select 635.400/221.185 for each standard.

Note: If the Compound ID field was populated for the samples and internal standards in the acquisition method, then in the Internal Standards table, when a value in the Q1/Q3 field is selected, the Name field is automatically populated.

6. In the **Analytes** table, do the following:

i. In the **Name** column, select reserpine.
ii. In the **Internal Standard** column, from the list, select the internal standard to be associated with each analyte.
iii. In the **Q1/Q3 column,** select 609.400/195.039.
iv. If required, add one or more of the other compounds for a more complex analysis.

Note: If the Compound ID field was populated for the samples and internal standards in the acquisition method, then in the Analytes table, the Name field and Q1/Q3 field are populated.

7. Click the **Integration** tab.
8. In general, the preset integration parameters are suitable for most peaks. If the integration is not suitable, then change the algorithm.
9. Click the **Show or Hide Parameters** icon to show the additional integration algorithms.
10. Click the **Calibration** tab. The preset parameters are suitable for these samples.
11. Save the quantitation method.

    The new method can be used when a batch is created in the Batch Editor or when the Quantitation Wizard is used to generate a Results Table.

Note: The quantitation method can only be used in the current project unless it is copied to another project. To do this, click **Tools > Project > Copy Data**. A new project must be created and selected to be available for use.

Create a Results Table using the Quantitation Wizard

Prerequisites

- The Example project is selected.
- The Analyst Classic algorithm is used.

1. In the Navigation Bar, under **Quantitate**, double-click **Quantitation Wizard**.

   The Create Quantitation Set - Select Samples page opens.
2. In the **Available Data Files** list, double-click the **Triple Quad** folder.
3. Select **Mix_Batch_2.wiff**.
4. Click **Add All Files**.
5. Click **Next**.

   The Create Quantitation Set - Select Settings & Query page opens.
6. In the **Default Query** section, click **Select Existing: Query**.
7. In the **Query** list, select **Accuracy 15%**.
8. Click **Next**.

Note: To create a query at the same time, refer to Create a Standard Query on page 88.

The Create Quantitation Set - Select Method page opens.

9. Click **Choose Existing Method**.
10. In the Method list, select **PK Data_Mix.qmf**.
11. Click **Finish**.

    The Results Table opens.

Tip! To add or remove samples in the Results Table, click **Tools > Results Table > Add/Remove Samples**.

12. Save the Results Table.

Create a Standard Query

A query and a standard query can be created numerous ways. The following is one example. For more information about creating queries, refer to the Help.

1. In the Navigation Bar, under **Quantitate**, double-click **Quantitation Wizard**.
2. In the **Create Quantitation Set - Select Samples** page, select samples.
3. Click **Next**.
4. In the **Select Settings & Query** page, in the **Default Query** section, select **Create New Standard Query**.
5. Type a query name.

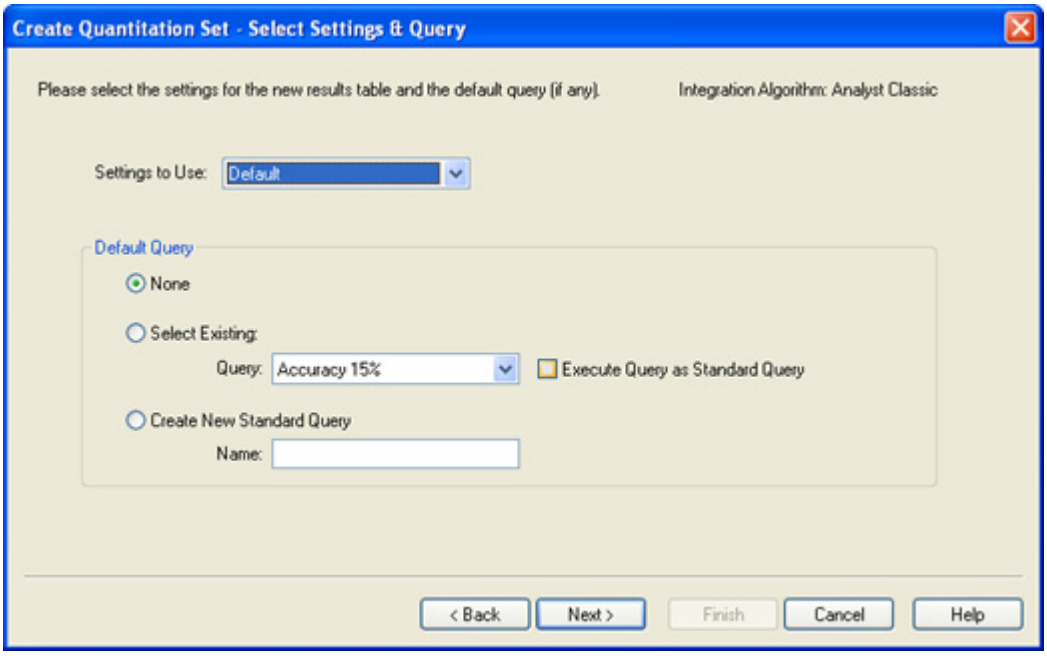


Figure 8-1 Create Quantitation Set - Select Settings & Query page

6. Click **Next**.

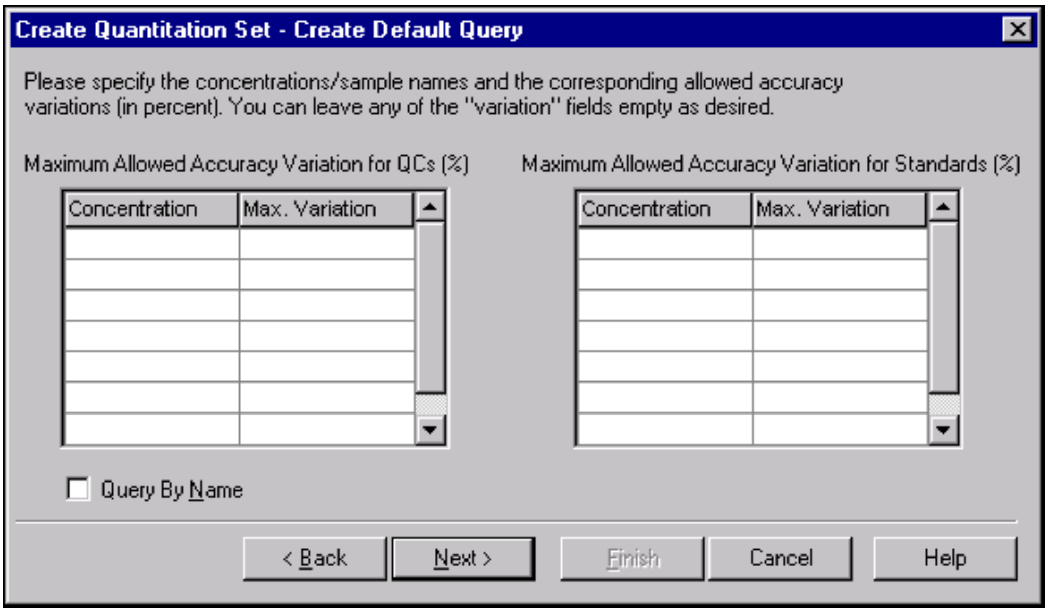


Figure 8-2 Create Default Query page

7. In the **Maximum Allowed Accuracy Variation for QCs (%)** table in the **Max. Variation** column, type the maximum allowable percent of variation for each QC (for example, 5 is ±5%) in the same row as the corresponding concentration. If the concentrations were not specified during acquisition, they do not appear here. In that case, type them in the **Concentration** column.
8. In the **Maximum Allowed Accuracy Variation for Standards (%)** table, in the **Max. Variation** column, type the maximum allowable percent of variation for each

standard (for example, 10 is ±10%) in the same row as the corresponding concentration. If the concentrations were not specified during acquisition, they do not appear here. In that case, type them in the **Concentration** column.

9. Click **Next**.

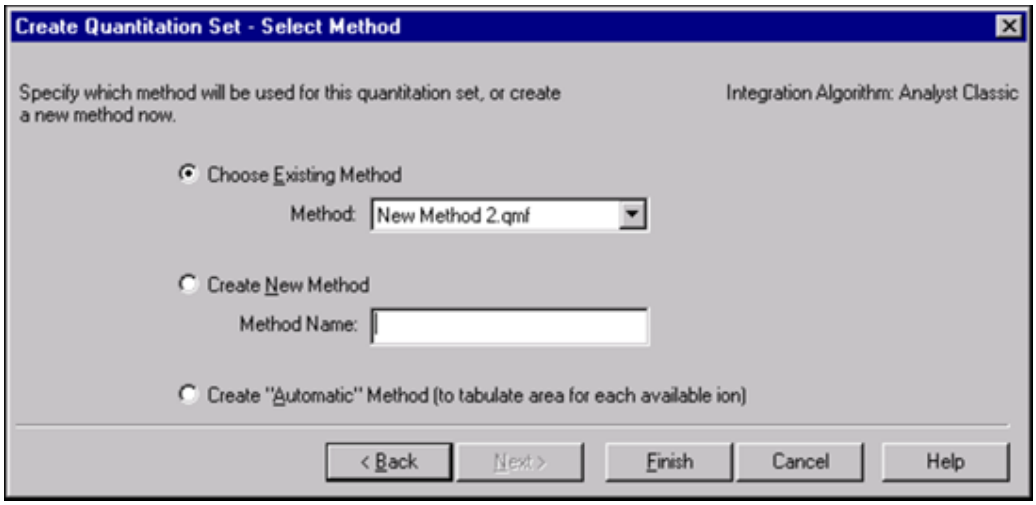


Figure 8-3 Create Quantitation Set - Select Method page

10. Select or create a method.
11. Click **Finish**.

    The query is applied as a standard query. The query results appear as a Pass or Fail entry in the Standard Query Status column of the Results Table.

Tip! To return to the full view, right-click and then click **Full**.

Results Table Right-Click Menu

Right-click in the Results Table table to access the following options.

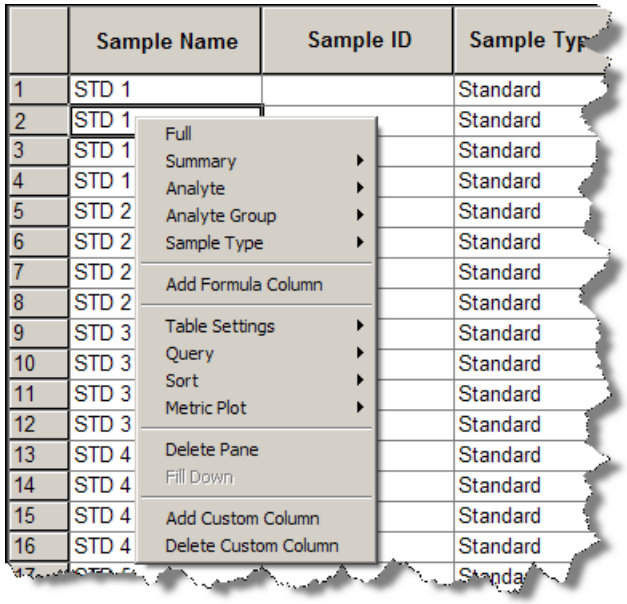


Figure 8-4 Results Table Right-Click Menu

Menu	Function
Full	Shows all the columns.
Summary	Shows specific columns.
Analyte	Shows a specific analyte.
Analyte Group	Create an analyte group.
Sample Type	Shows samples of a specific type or all samples.
Add Formula Column	Adds a formula column.
Table Settings	Edits or selects a table setting.
Query	Creates or selects a query.
Sort	Click to create a sort or to sort by index.
Metric Plot	Creates a metric plot.
Delete Pane	Deletes the active pane.
Fill Down	Fills the same data into the selected cells.
Add Custom Column	Adds a custom column.
Delete Custom Column	Deletes the selected custom column.

Peak Review and Manual Integration of Peaks

Use peak review to survey the peaks that the software has identified, and then redefine the peak or the start and end points where necessary.

After identifying the analytes and internal standards that the software must find, the software searches for the peaks in the samples. When the software identifies a peak, it shows the

chromatograms for each analyte and internal standard in the Create Quantitation Method: Define Integration page of the Standard Wizard or on the Integration tab of the Full Method Editor. The user can confirm the peaks that are found or change the quantitation method to better define the peaks.

Review Peaks

During peak review, the user may want to view a peak in its entirety—or may want to examine the baseline to find out how well the software found the start and end points of the peak. Use the automatic zooming feature to do either.

To help the software find a peak, define the exact start and end points of the peak and background manually. These changes will apply only to that individual peak unless the global method is updated.

Tip! To review an individual peak, right-click on a point on the curve and then click Show Peak. The software shows the Peak Review window with the selected peak.

1. Right-click in the **Results Table**, click **Analyte**.
2. Select a sample.
3. Click **Tools > Peak Review > Pane**.

   The peaks appear below the Results Table with only the peaks listed in the Results Table.
4. Right-click in the pane and then click **Options**.
5. In the **Peak Review Options** dialog, in the **Appearance** section, change **Num. rows** to **1** and **Num. columns** to **2**.
6. In the **Automatic Zooming** section, click **Zoom Y axis to**: **100% of largest peak** to show the entire peak.

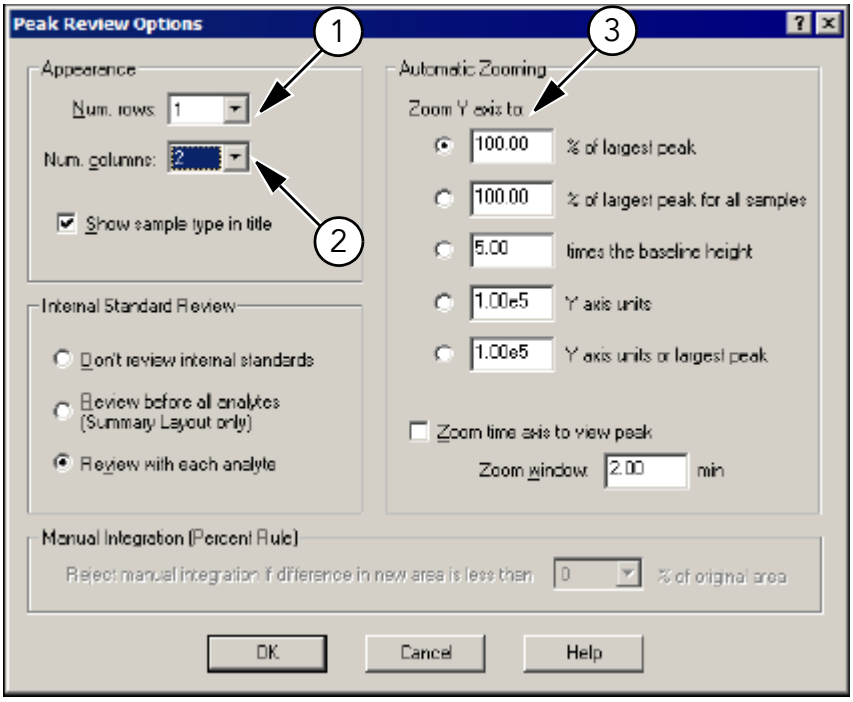


Figure 8-5 Peak Review Options dialog

Item	Definition
1	Num. rows
2	Num. columns
3	Zoom Y axis to 100% of largest peak

7. Click **OK**.
8. To move through the peaks, click the right-pointing arrow. For more information, refer to Figure 8-6 *Peak Review Pane* on page 94.S
9. Go to the second injection of standard 3.

   In this example, the peak can be integrated closer to the baseline by selecting the Specify Parameters option.

Tip! To move to a specific peak in the Peak Review pane, select the corresponding row in the Results Table.

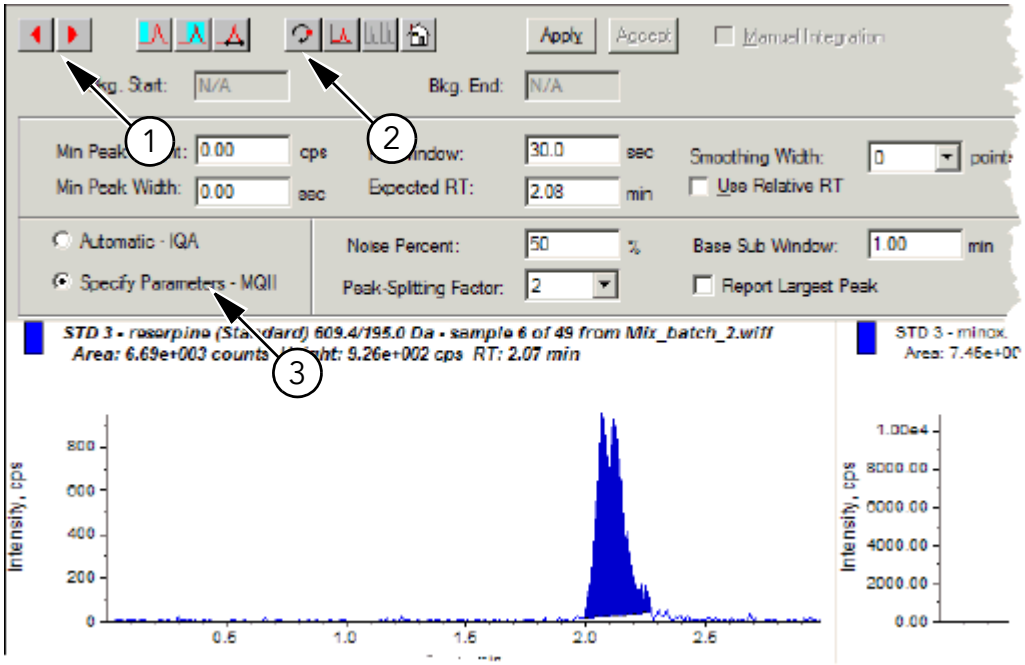


Figure 8-6 Peak Review Pane

Item	Description
1	Arrows: click to move through the peaks.
2	Show or Hide Parameters: click to show the integration parameters.
3	Integration parameters: click to change the parameters.

10. Click **Show or Hide Parameters** twice.
11. Click **Specify Parameters -MQ III**.
12. Change the **Noise Percent** value.
13. Click **Apply**.

    The peak is integrated closer to the baseline.
14. If the change does not improve the peak integration, then adjust the **Noise Percent** parameter until the optimal value is found.
15. To update the algorithm for all peaks, right-click in the pane and then click **Update Method**.

Note: The Update Method function only updates the algorithm values for that specific analyte (or internal standard) and not all analytes.

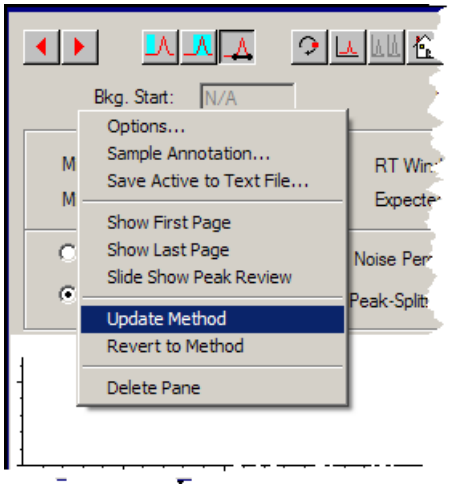


Figure 8-7 Update Method

Manually Integrate Peaks

Manually integrating peaks should be done last. Manually integrate peaks only if all the peaks have not been found after adjusting and updating the algorithm parameters. This is done to limit person-to-person variability.

Note: Peaks that are manually integrated, or where the algorithm was changed for only that peak, are identified as such in the Record Modified column of the Results Table, as are the peaks that have algorithm parameter changes for a sample but not updated to the entire analyte group.

1. In the **Peak Review** pane, click **Manual Integration Mode**.

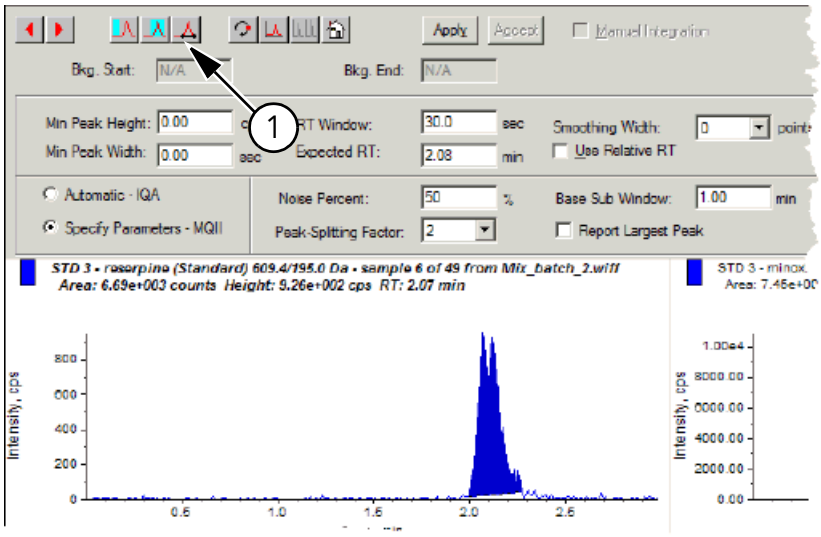


Figure 8-8 Peak Review pane: Manual integration

Item	Description
1	Manual Integration Mode

2. Zoom in on the lower 10% of the peak.

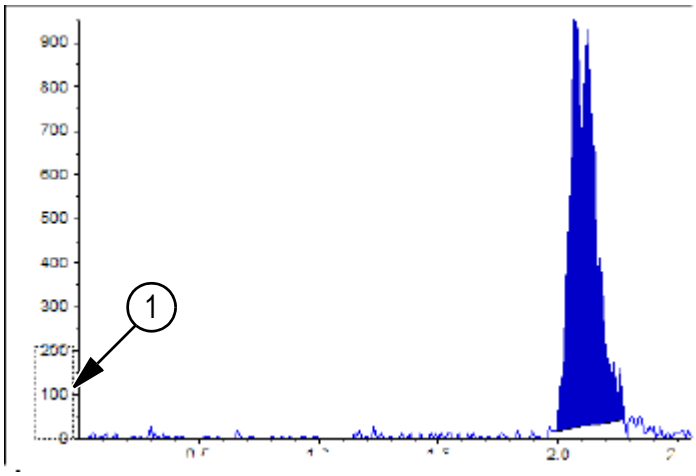


Figure 8-9 Peak Review pane: Zooming in on a peak

Item	Description
1	Lower 10% of the peak

3. Position the cross-hair where the start of the peak is to be defined and then drag the cross-hair to where the end of the peak is to be defined.

   The software shades the area bounded by the base and sides of the peak. Peak parameters are gray as they are no longer applicable because the peak was drawn manually.

4. Do one of the following:
   - To make this change permanent, click **Accept**.
   - To discard the changes, clear the **Manual Integration** check box.

Tip! If a peak was correct as originally selected, right-click the peak and then click **Revert to Method**.

Peak Review Right-Click Menu

Right-click in the Peak Review window or pane to access the following options.

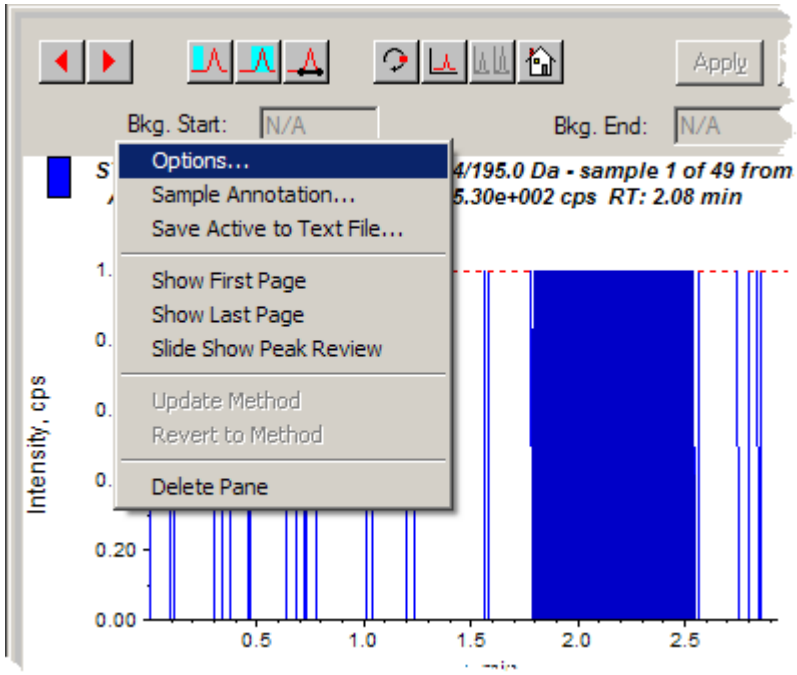


Figure 8-10 Peak Review Right-Click Menu

Menu	Function
Options	Opens the Peak Review Options dialog.
Sample Annotation	Opens the Sample Annotation dialog.
Save Active to Text File	Saves the selected peak as a text file.
Show First Page	Goes to the first sample.
Show Last Page	Goes to the last sample.
Slide Show Peak Review	Opens the slide show.
Update Method	Updates the algorithm for all peaks.
Revert to Method	Click to have a redefined peak reselected based on the current quantitation method.
Delete Pane	Deletes the active pane.

Calibration Curves

Use calibration curves to find the calculated concentration of samples, including QC (quality control) samples. QC samples are added to a batch to estimate the data quality and accuracy of standards in the batch. QC samples have known analyte concentrations but are treated as unknowns so that the measured concentrations can be compared to the actual value.

The calibration curve is generated by plotting the concentration of the standard versus its area or height. If an internal standard is used, the ratio of the standard concentration/internal standard versus the ratio of the standard peak height or area to the internal standard peak height or area is plotted. The area or height ratio of a sample is then applied to this curve to find the concentration

of the sample, as shown in the Results Table. A regression equation is generated by this calibration curve according to the regression that was specified. The regression equation is used to calculate the concentration of the unknown samples.

View Calibration Curves

The user can view the calibration curve and change the regression options in an open Results Table. If there are two or more Results Tables open, the calibration curves can be overlaid. To overlay curves, the method used to create the tables must be the same.

Plot a calibration curve to see the curve used for regression. The Calculated Concentration field in the Results Table reflects any changes resulting from the fit of the curve to the points of the standard.

Note: This option is available only when a Results Table is open in the workspace.

1. With a Results Table open, click **Tools > Calibration > Pane**.

   The Calibration Curve pane containing the calibration curve opens.

2. If there is more than one analyte, then use the following steps to view the calibration curve for another analyte:

   i. In the **Analyte** list, select an analyte.
   ii. If required, in the next list, select **Area** or **Height**.

3. To change the regression options for the calibration curve, do the following:

   i. Click **Regression**.

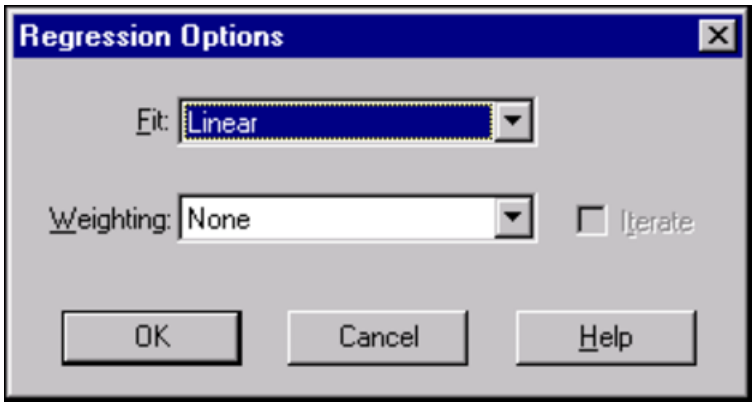


Figure 8-11 Regression Options dialog

   ii. In the **Fit** list, select **Linear**.
   iii. In the **Weight** list, select **1 / x**.
   iv. Click **OK**.

   The calibration curve opens. The user can review individual peaks on the curve or exclude points from the curve to produce a better curve.

4. If required, repeat these steps to create a more appropriate curve.
5. To save the changes, click **Accept**.

Overlay Calibration Curves

Note: To examine the curve for one table more closely, right-click on the curve and click Active Plot. Select the curve to be plotted on top.

1. With two or more open Results Tables, view a calibration curve for one of the tables.
2. Right-click the calibration curve and then click **Overlay**.

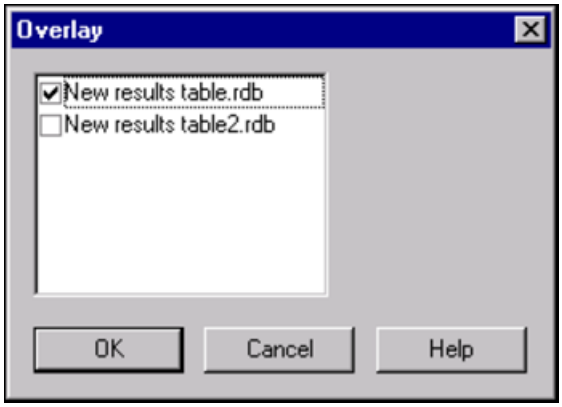


Figure 8-12 Overlay dialog

3. Select the tables to overlay with the current curve.
4. Click **OK**.

   The software plots the curves for all selected tables on the same graph.

Calibration Curve Right-Click Menu

Right-click in the Calibration window or pane table to access the following options.

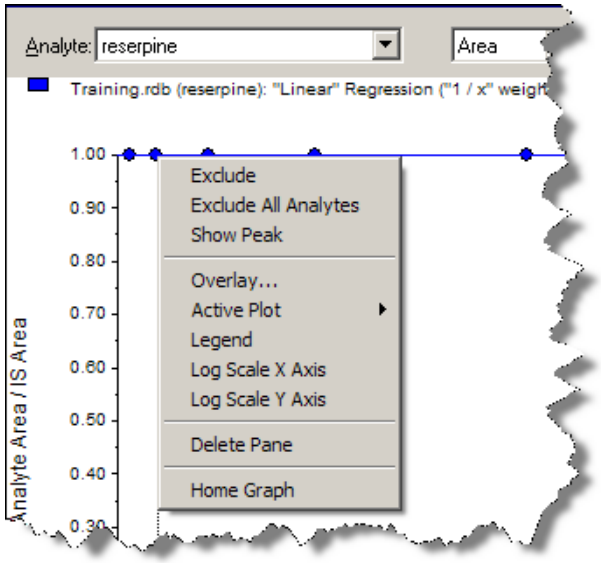


Figure 8-13 Calibration Curve Right-Click Menu

Menu	Function
Exclude (Include)	Right-click the point and then click **Exclude** to exclude the point from the curve. Right-click the point and then click **Include** to include the dropped point.
Exclude All Analytes (Include All Analytes)	Right-click a point and then click **Exclude All Analytes** to exclude all the analytes from the curve. Right-click a point and then click **Include All Analytes** to include the points.
Show Peak	Click to review an individual peak.
Overlay	Overlays two graphs.
Active Plot	Determines which plot is active.
Legend	Shows the graph legend.
Log Scale X Axis*	Click to use a log scale for the X axis.
Log Scale Y Axis*	Click to use a log scale for the Y axis.
Delete Pane	Deletes the active pane.
Home Graph	Scales the graph to its original size
*** A log scale arranges the data points in a more manageable view so that the effect of all points can be monitored simultaneously. For this view, select Log Scale Y Axis versus Log Scale X and not just the log of one axis.**	

Sample Statistics

Use the Statistics window to view the statistics samples, typically for standards and QCs (quality controls). The data from each available batch in the Results Table opens in tabular form in the grid and a row of data is shown for each standard or QC concentration.

View the Statistics for Standards and Qcs

When viewing more than one Results Table, statistical information about the standards and QCs for additional batches in the Statistics window can be obtained. This allows the user to compare results between batches and look for trends in the standards or QCs.

1. With a Results Table open, click **Tools > Statistics**.
2. In the **Statistics Metric** list, select **Concentration**.
3. In the **Analyte Name** field, select an analyte.
4. In the **Sample Type** field, select **Standard**.

   The results appear.
5. Look at the **%CV** and **Accuracy** columns.

   The %CV shows the coefficient of variance between the measurements of a single parameter, for example the area. Accuracy shows how close the plotted point is to the interperlated value.

6. If required, select the **Display Low/High values** check box and then examine the **Low**, **High**, and **Mean** for each row in the grid. Each row represents standards that have the same concentration levels.
7. Select another analyte.

   The results appear on a per-analyte basis.
8. To check for Quality Control variations at the same concentration levels, select **QC** in the **Sample Type** field.

Compare Results Between Batches

The number of analytes and the analyte names must be the same for the data to be combined in the Statistics pane.

1. Open the Results Tables.
2. Click **Tools > Statistics**.
3. Do one of the following:
   - To arrange the results by Results Table, in the **Conc. as Rows** list, select **Group By Batch**.
   - To arrange the results in order of concentration, in the **Conc. as Rows** list, select **Group By Concentration**.
   - To arrange the results in order of concentration, but without a row showing the statistics for each group or batch, in the **Conc. as Rows** list, select **Group By Concentration (no All)**.

   The software sorts the results. At the end of each batch or group, one or two additional rows appear: All (statistics for all results tables in that group) and Average (statistics on the statistics for that batch or group).

Table 8-1 Integration Tab and Quantitation Wizard Icons

Icon	Name	Function
	Set parameters from Background Region	Use the selected peak.
	Select Peak	Use the selected background.
	Manual Integration Mode	Click to manually integrate peaks.
	Show or Hide Parameters	Click to toggle the peak-finding parameters between shown and hidden.
	Show Active Graph	Shows the analyte chromatogram only.
	Show Both Analyte and IS	Shows the analyte and its associated chromatogram (available only when an associated internal standard exists).

Table 8-1 Integration Tab and Quantitation Wizard Icons (Continued)

Icon	Name	Function
	Use Default View for Graph	Returns to the preset (view all data) view (if, for example, the user has zoomed in on a chromatogram).

Table 8-2 Results Table Icons

Icon	Name	Function
	Sort Ascending by Selection	Click to sort the selected column by ascending values.
	Sort Descending by Selection	Sorts the selected column by descending values.
	Lock or Unlock Column	Locks or unlocks the selected column. A locked column cannot be moved.
	Metric Plot by Selection	Creates a metric plot from the selected column.
	Show all Samples	Shows all the samples in the Results Table.
	Delete Formula Column	Deletes formula columns.

Table 8-3 Icon Quick Reference: Quantitate Mode

Icon	Name	Function
	Add/Remove Samples	Adds or removes samples from the Results Table.
	Export as Text	Saves the Results Table as a text file.
	Modify Method	Click to open a .wiff file.
	Peak Review - Pane	Opens peaks in a pane.
	Peak Review - Window	Opens peaks in a window.
	Calibration - Pane	Opens the calibration curve in a pane.
	Calibration - Window	Opens the calibration curve in a window.

Table 8-3 Icon Quick Reference: Quantitate Mode (Continued)

Icon	Name	Function
	Show First Peak	Shows the first peak in the pane or window.
	Show Last Peak	Shows the last peak in the pane or window.
	Show Audit Trail	Shows the audit trail for the Results Table.
	Clear Audit Trail	Clears the audit trail for the Results Table.
	Statistics	Opens the Statistics window.
	Report Generator	Opens the Reporter software.

Use the ion source for electrospray ionization with the TurboIonSpray® probe, or for chemical ionization with the APCI (atmospheric pressure chemical ionization) probe. Applications for the ion source include qualitative method development and qualitative and quantitative analysis.

WARNING! Toxic Chemical Hazard: Use the ion source only if you have knowledge of and training in the proper use, containment, and evacuation of poisonous or injurious materials used with the ion source. Any poisonous or injurious materials introduced into the equipment will be present in the ion source and exhaust output.

Introduction to the Ion Source

Figure A-1 shows the parts of the ion source.

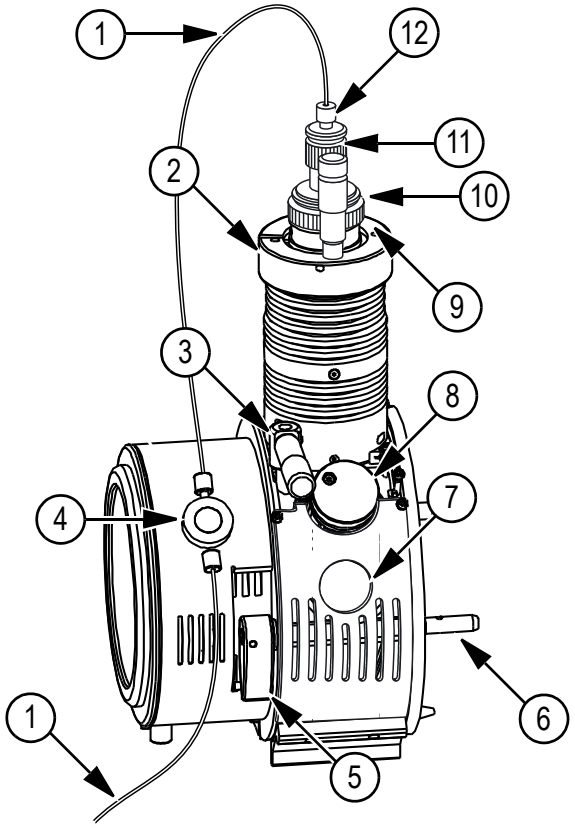


Figure A-1 Ion source components

Item	Description
1	Sample tubing

Figure A-1 Ion source components (Continued)

Item	Description
2	Probe tower
3	X-axis adjustment knob used to position the probe on the horizontal axis for ion source sensitivity adjustments
4	Grounding union
5	One of two source latches that secure the ion source to the mass spectrometer
6	Guide pin
7	Window port
8	Turbo heater
9	Bronze retaining ring
10	Y-axis adjustment knob used to position the probe on the vertical axis for ion source sensitivity adjustments
11	Electrode adjustment nut
12	Sample tubing nut

Probes

Choose the probe and method most suitable for the compound in the sample stream flow.

Table A-1 Specifications of the Ion Source

Parameter	TurboIonSpray® probe	APCI probe
Ion source temperature range	Probe temperature from ambient temperature to 750°C, depending on liquid flow	Probe temperature from 50°C to 750°C, depending on liquid flow
Liquid chromatography	Interfaces to any liquid chromatography system	
Nebulizer gas (Gas 1) Heater gas (Gas 2)	Refer to the *Site Planning Guide* for the mass spectrometer.	

The TurboIonSpray probe produces ions through ion evaporation. The APCI probe vaporizes the sample before inducing ionization through atmospheric pressure chemical ionization. This process is induced by a corona discharge needle as the ions pass through the ion source housing to the interface region.

All of the data acquired using the ion source is identified with an abbreviation representing the probe used to acquire the data (TIS for the TurboIonSpray probe, HN for the APCI probe).

TurboIonSpray® Probe

The TurboIonSpray probe is suited for LC/MS/MS analyses. The sensitivity that is achieved with this technique is dependent on both flow rate and analyte. At higher flow rates, ionization efficiency increases, resulting in improved sensitivity. Compounds with extremely high polarity and low surface activity usually show the greatest sensitivity increases. The TurboIonSpray

technique is mild enough to be used with labile compounds, such as peptides, proteins, and thermally labile pharmaceuticals.

When the heater is turned off, the TurboIonSpray probe functions as a conventional IonSpray™ ion source. It also functions with flow rates from 5 µL/min to 3000 µL/min and it vaporizes 100% aqueous to 100% organic solvents.

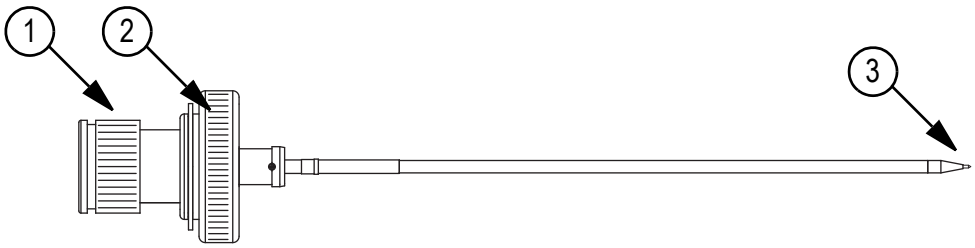


Figure A-2 Parts of the TurboIonSpray® probe

Item	Description
1	Electrode adjustment nut (black collar) that adjusts the extension of the electrode tip
2	Bronze retaining ring that fastens the probe to the probe tower on the ion source housing
3	Electrode tip through which samples are sprayed into the sample inlet area of the ion source

APCI Probe

The APCI probe is suitable for:

- Ionization of compounds that do not readily form ions in solution. These are usually non-polar compounds.
- Creation of simple APCI spectra for MS/MS experiments.
- High-throughput analyses of complex and dirty samples. It is less sensitive to ion suppression effects.
- Rapid sample introduction by flow injection with or without an LC column

The APCI probe can accept the entire effluent, without splitting, at flow rates from 50 µL/min to 3000 µL/min (through a wide bore column). It can vaporize volatile and labile compounds with minimal thermal decomposition. The rapid desolvation and vaporization of the droplets and entrained analyte minimizes thermal decomposition and preserves molecular identity for ionization by the corona discharge needle. Buffers are readily tolerated by the ion source without significant contamination and the flash vaporization of the sprayed effluent allows up to 100% water to be used without difficulty.

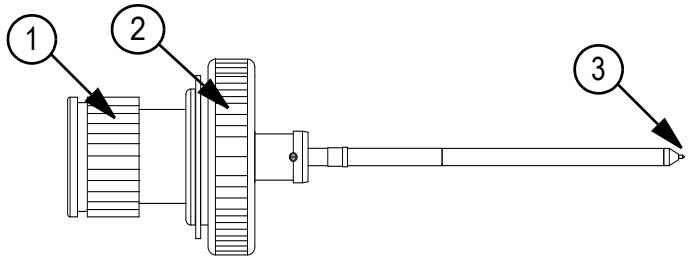


Figure A-3 Parts of the APCI probe

Item	Description
1	Electrode adjustment nut (black collar) that adjusts the extension of electrode tip
2	Bronze retaining ring that fastens the probe to the probe tower on the ion source housing
3	Electrode tip through which samples are sprayed into the sample inlet area of the ion source

Gas and Electrical Connections

Gas and high-voltage electrical connections enter through the front plate of the interface and connect internally through the ion source housing. When the ion source is installed on the mass spectrometer, all of the electrical and gas connections are made.

Ion Source Latch

A latch disables the high-voltage power supply for the mass spectrometer and the source exhaust system if:

- The ion source housing is not installed or is improperly installed.
- A probe is not installed.
- The mass spectrometer senses a gas fault.

Source Exhaust System

WARNING! Toxic Chemical Hazard: Be sure to use the source exhaust system to safely remove sample vapor exhaust from the laboratory environment. For requirements for the source exhaust system, refer to the *Site Planning Guide* for the mass spectrometer.

A passive pressure exhaust system removes ion source gases through a drain port without introducing chemical noise. The drain port connects through a drain chamber and a source exhaust pump to a drain bottle, and from there to a customer-supplied exhaust ventilation system. For more information on the requirements for the source exhaust system, refer to the *Site Planning Guide* for the mass spectrometer.

WARNING! Toxic Chemical Hazard: Vent the source exhaust system to an external fume hood or an external vent to prevent hazardous vapors from being released into the laboratory environment.

A pressure switch mounted on the source exhaust pump measures the pressure in the source exhaust line. If the pressure in the line rises above the set point while the probes are installed, the high-voltage power supply is turned off.

Installation

The interior of the ion source is visible through the tempered glass windows on the side and end of the ion source housing. The ion source housing is connected to the vacuum interface housing and is held in position by two source latches.

WARNING! Electrical Shock Hazard: Install the ion source on the mass spectrometer as the last step in this procedure. High voltage is present when the ion source is installed on the equipment.

Required Parts (supplied)
• Ion source housing assembly • Probe • Ion source hardware kit (Do not discard the empty package. Use it to store the ion source when not in use.) • Adjust the black electrode adjustment nut on the probe to move the electrode tip inside the electrode tube. For optimum stability and performance, the electrode tip should extend between 0.5 mm and 1.0 mm from the end of the probe.

Install the Ion Source

Install the probe in the ion source housing before installing the ion source. Always remove the ion source from the mass spectrometer before exchanging probes. Refer to Remove the Ion Source on page 117.

When the ion source is installed, the mass spectrometer recognizes the ion source and displays the ion source identification in the software.

Install the Probe in the Ion Source Housing

If the probe is not properly installed in the ion source housing, then high-voltage power for the mass spectrometer and source exhaust system is turned off.

WARNING! Electrical Shock Hazard: Make sure that the ion source housing is completely disconnected from the mass spectrometer before proceeding.

WARNING! Electrical Shock Hazard: When installing the ion source, install the probe before installing the ion source on the mass spectrometer.

Caution: Potential Equipment Damage: Do not let the protruding electrode tip touch any part of the ion source housing, to avoid damaging the probe.

Caution: Potential Equipment Damage: Make sure that the corona discharge needle tip is turned away from the orifice when using the TurboIonSpray probe.

1. Insert the probe into the tower.
2. Align the hole on the probe with the alignment pin at the top of the ion source housing. Refer to Figure A-1 on page 105.
3. Gently push down on the probe so that the contacts engage with those in the tower.
4. Turn the bronze retaining ring over the probe and push it down to engage its threads with the threads on the tower.
5. Tighten the ring until it is finger-tight.

   When using the APCI probe, make sure that the corona discharge needle tip is pointed toward the orifice.

Connect the Source Tubing

1. Insert a 30 cm piece of red PEEK tubing into the sample tubing nut.
2. Install the sample tubing nut on the fitting at the top of the probe.
3. Tighten the sample tubing nut until it is finger-tight.

Install the Ion Source

1. Make sure that the source latches on the side of the ion source are pointing up in the 12:00 position.
2. Align the ion source with the vacuum interface, making sure that the latches on the ion source are aligned with the sockets in the vacuum interface.
3. Push the ion source gently against the vacuum interface and then rotate the ion source latches, shown in Figure A-1 on page 105, fully downwards to lock the ion source into place.

Connect the Sample Tubing and Cables

WARNING! Toxic Chemical Hazard: Make sure that the sample tubing nut is tightened properly before operating this equipment. If the sample tubing nut is not tight, the sample may leak, and you may be exposed to dangerous chemicals.

WARNING! Electrical Shock Hazard: Do not bypass the grounding union connection. The grounding union provides safety grounding between the mass spectrometer and the sample introduction device.

1. Connect red PEEK tubing from the sample supply device to the grounding union on the ion source.
2. Connect the other end of the red PEEK tubing to the grounding union.

Tip! Refer to Figure A-1 on page 105 for a picture on the parts referenced in this procedure.

Optimization

Optimize the ion source whenever the analyte, flow rate, or mobile phase composition changes.

Use appropriate analytical procedures and practices to minimize external dead volumes. Prefilter samples so that the capillary tubing in the sample inlets is not blocked by particles, precipitated samples, or salts.

Optimize the TurboIonSpray® Probe

Optimize performance while injecting a known compound and monitor the signal of the known ion. Adjust the parameters to maximize the signal-to-noise ratio and signal stability.

Caution: Potential Equipment Damage: If the LC system connected to the mass spectrometer is not controlled by the Analyst software, then do not leave the mass spectrometer unattended while in operation. The LC system can flood the ion source housing when the mass spectrometer goes into Standby mode.

Note: If the IonSpray voltage is too high, a corona discharge can occur. It is visible as a blue glow at the tip of the TurboIonSpray probe. This will result in decreased sensitivity and stability of the ion signal.

Table A-2 Typical Values for Optimizing the TurboIonSpray Probe

Parameters	LC flow rate			Operational range
	5 µL/min to 50 µL/min	200 µL/min	1000 µL/ min	5 µL/min to 3000 µL/min
Probe X-axis position	3 mm to 8 mm			0 mm to 10 mm
Probe Y-axis position	5 mm to 10 mm	0 mm to 5mm		0 mm to 13 mm
The optimal X-axis position is within 0 mm to 3 mm on either side of the orifice. The optimal Y-axis position is within 3.0 mm to 7.0 mm of the orifice.				

Adjust the TurboIonSpray Probe Position

At low flow rates, the probe can be adjusted to its lowest Y-axis position. For high flow rates, position the probe higher than the orifice. The curtain plate orifice should remain clear of solvent or solvent droplets at all times.

For multiply-charged proteins and peptides introduced at a few microliters per minute, position the sprayer nozzle higher than the curtain plate orifice.

1. Before starting the sample flow, let the ion source warm up for 30 minutes or until the ion source housing is warm to the touch.

   The 30-minute warm-up stage prevents solvent vapors from condensing in the cold probe.
2. In the Analyst® software, in **Tune and Calibrate** mode, double-click **Manual Tune.**
3. Open the method to optimize the ion source.
4. Look through the window of the ion source housing to view the position of the probe.
5. Set the X-axis adjustment knob to 5 and the Y-axis adjustment knob to 5.
6. Infuse or inject the sample.
7. Monitor the signal in the software.
8. Use the X-axis adjustment knob to adjust the probe position in small increments until the best signal or signal-to-noise ratio is achieved.

   The probe may optimize slightly to either side of the orifice.

Tip! It is easier to optimize signal and signal-to-noise with FIA or on-column injections.

9. Use the Y-axis adjustment knob to adjust the probe position in small increments until the best signal or signal-to-noise ratio is achieved.

Note: The vertical position of the probe depends on flow rate. At low flow rates, the probe should be closer to the orifice. At higher flow rates, the probe should be farther away.

WARNING! Toxic Chemical Hazard: Make sure that the electrode tip extends past the end of the probe, to prevent the escape of hazardous vapor from the ion source.

10. Adjust the black electrode adjustment nut on the probe to move the electrode tip relative to the sprayer tube.

    Typically, the optimum extension of the electrode from the sprayer tube is 0.5 mm to 1.0 mm. After the probe is optimized, it needs only minor adjustment.

Optimize Gas 1, Gas 2, Curtain Gas, and IonSpray Voltage

Optimize nebulizer gas (Gas 1) for best signal stability and sensitivity. The heater gas (Gas 2) aids in the evaporation of solvent, which helps to increase the ionization of the sample. However, too high a temperature can cause premature vaporization of the solvent at the TurboIonSpray probe tip, especially if the probe is too low, which will result in signal instability and a high chemical background noise. Similarly, a high heater gas flow could produce a noisy or unstable signal.

1. On the **Source/Gas** tab in the **Tune Method Editor**, type a starting value for **Ion Source Gas 1 (GS1)**.

   Typical values are between 40 psi and 60 psi for GS1.
2. Type a starting value for **Ion Source Gas 2 (GS2)**.

Typical values are between 30 psi and 50 psi for GS2.

Note: Gas 2 is used with higher flow rates typical with an LC system and in conjunction with increased temperature.

3. Type a starting value for **IonSpray Voltage (IS)** or **IonSpray Voltage Floating (ISVF)**.
4. Adjust GS1 and GS2 in increments of 5 psi until the best signal or signal-to-noise ratio is achieved.
5. In the **Curtain Gas (CUR)** field, type **20**.
6. Increase CUR to the highest possible gas flow rate without a noticeable signal drop.
7. Adjust IS or ISVF in increments of 100 V to maximize signal to noise.

Optimize the Turbo Heater Temperature

The quantity and type of sample affects the optimal TurboIonSpray probe temperature. At higher flow rates the optimal temperature increases. Normal optimization is usually performed in increments of 50°C.

WARNING! Toxic Chemical Hazard: Vent the source exhaust system to an external fume hood or an external vent to prevent hazardous vapors from being released into the laboratory environment.

1. On the **Source/Gas** tab in the **Tune Method Editor**, type a starting value for **Temperature (TEM)**.
2. Wait for the signal to stabilize, to make sure that the ion source is at the correct temperature.
3. Adjust the temperature parameter as required until the best signal or signal-to-noise ratio is achieved.

Optimization Tips

- Use the highest temperature possible when optimizing compounds. A temperature of 700°C is common for many compounds. High temperatures help keep the ion source clean and reduce background noise.
- Use the highest Curtain Gas flow rate (CUR) possible without decreasing the signal. This helps to:
  - Prevent penetration of the Curtain Gas flow, which can produce a noisy signal
  - Prevent contamination of the orifice
  - Increase the overall signal-to-noise ratio
- Direct the liquid spray from the TurboIonSpray probe away from the orifice in order to:
  - Prevent contamination of the orifice
  - Prevent piercing of the Curtain Gas flow, which can create an unstable signal
  - Prevent electrical shorting due to the presence of the liquid

- Use the lowest IonSpray voltage possible without losing signal. Focus on signal-to-noise and not just signal.

Optimize the APCI Probe

Optimize performance by injecting a known compound and monitoring the signal of the known ion. Adjust the parameters to maximize the signal-to-noise ratio.

Table A-3 Typical Values for Optimizing the APCI Probe

Parameter	Typical value	Operational range
Probe X-axis position	5 mm	Scale 0 mm to 10 mm
Probe Y-axis position	5 mm	Scale 0 mm to 13 mm
The optimal X-axis position is within 0 mm to 2 mm on either side of the orifice. The optimal probe Y-axis position is within 3.0 mm to 7.0 mm of the orifice.		

Warm up the APCI Probe

Caution: Potential Equipment Damage: If the LC system connected to the mass spectrometer is not controlled by the Analyst software, then do not leave the mass spectrometer unattended while in operation. The LC system can flood the ion source housing when the mass spectrometer goes into Standby mode.

1. On the **Source/Gas** tab in the **Tune Method Editor**, in the **Curtain Gas (CUR)** field, type **20**.

   Operate the APCI probe with the highest Curtain Gas flow rate possible without a noticeable signal drop.
2. In the **Ion Source Gas 1 (GS1)** field, type **20**.
3. In the **Temperature (TEM)** field, type **400**.
4. Before starting the sample flow, let the ion source warm up for 30 minutes or until the ion source housing is warm to the touch.

   The 30-minute warm-up stage prevents solvent vapors from condensing in a cold probe.
5. Turn on the pump.
6. Wait until the APCI probe reaches a temperature at which the solvent mist is cleared from the ion source housing (about 10 minutes).

Adjust the Corona Discharge Needle

When using the APCI probe, make sure that the corona discharge needle is pointing toward the orifice.

WARNING! Electrical Shock Hazard: Follow this procedure to avoid contact with the high voltages applied to the corona discharge needle and the curtain plate.

1. Use the slotted screwdriver to rotate the plastic screw on the top of the needle.

2. Look through the glass window to make sure that the needle is aligned with the tip facing the orifice.

Adjust the APCI Probe Position

Make sure that the curtain plate orifice remains clear of solvent or solvent drops at all times. The position of the probe relative to the curtain plate orifice affects sensitivity and signal stability. The probe vertical (Y-axis) position is dependent on flow rate. For low flow rates, move the probe to its lowest position. For higher flow rates, move the probe above the curtain plate orifice.

1. In **Tune and Calibrate** mode, double-click **Manual Tune**.
2. Run the method to optimize the ion source.
3. Look through the window of the ion source housing to view the position of the probe.
4. Set the X-axis adjustment knob to 5 and the Y-axis adjustment knob to 5.
5. Use FIA to inject the sample or use a Tee connection with a syringe to infuse the sample at a high flow rate.
6. Monitor the signal in the Analyst software.
7. Use the X-axis adjustment knob to adjust the probe in small increments until the best signal or signal-to-noise ratio is achieved.

Tip! It is easier to optimize signal and signal-to-noise with FIA or on-column injections.

8. Use the Y-axis adjustment knob to adjust the probe in small increments until the best signal or signal-to-noise ratio is achieved.

   The APCI probe optimizes toward the orifice plate. After the probe is optimized, it needs only minor adjustment.

Optimize the Nebulizer Current

The ion source is controlled by current and not by voltage. Select the appropriate current for the acquisition method, regardless of ion source selection position. Use the PEEK adjustment knob to position the corona discharge needle so that the tip of the needle points right at the center of the probe.

- Start with a **Nebulizer Current (NC)** value of 1 and increase until the best signal or signal-to-noise ratio is achieved.

  The NC (Nebulizer Current) applied to the corona discharge needle usually optimizes between 1 µA and 5 µA in positive mode. If there are no changes in signal when increasing the current, then leave the current at the lowest value that provides the best signal or signal-to-noise ratio.

Optimize the APCI Probe Temperature

The quantity and type of solvent affects the optimal APCI probe temperature. At higher flow rates, the optimal temperature increases.

Optimization is usually performed in increments of 50°C.

WARNING! Toxic Chemical Hazard: Vent the source exhaust system to an external fume hood or an external vent to prevent hazardous vapors from being released into the laboratory environment.

1. On the **Source/Gas** tab in the **Tune Method Editor**, type a starting value for the **Temperature (TEM)**.
2. Adjust the temperature parameter as required until the best signal or signal-to-noise ratio is achieved.

Maintenance

To determine how often to clean the ion source or perform preventive maintenance, consider the following:

- Compounds tested
- Cleanliness of the preparation methods
- Amount of time an idle probe contains a sample
- Overall system run time

These factors can cause changes in mass spectrometer performance, indicating that maintenance is required.

Perform periodic gas leakage tests and general maintenance inspections to be sure of safe operation of the system. Clean the ion source regularly to keep it in good working condition.

Caution: Potential Instrument Damage: Use only the recommended cleaning method to avoid damaging the equipment.

Required Tools
• 1/4 inch open-ended wrench
• 9/64 inch Allen key (supplied)
• 5 mm Allen key
• 2.5 mm Allen key
• Phillips screwdriver
• Slotted screwdriver

Clean the Probes

Flush the ion source periodically, regardless of the type of compounds sampled. Do this by setting up a method in the Analyst software specifically for performing a flushing operation.

1. Switch to a mobile phase that is 50:50 water:acetonitrile or 50:50 water:methanol.

2. In the Analyst software, set **TEM** between 500 and 600, **GS1** and **GS2** to at least 40, and Curtain Gas™ flow to the highest setting possible.
3. Wait until the TEM setpoint is reached.
4. Infuse or inject mobile phase through the sample tubing and probe at 1 mL/min for about 10 to 15 minutes.
5. Make sure that the probe and sample tubing are flushed thoroughly.

Remove the Ion Source

Always remove the ion source from the mass spectrometer before you perform any maintenance on the ion source or exchange probes.

WARNING! Hot Surface Hazard: Surfaces of the ion source become hot during operation. Let the ion source cool for at least 10 minutes before starting any maintenance procedures.

1. Stop any ongoing scans.
2. Shut down the sample stream.
3. Using the Analyst software, put the mass spectrometer in Standby mode. Refer to the Analyst software Help.
4. Let the ion source cool for at least 10 minutes.
5. Disconnect the sample tubing from the grounding union.
6. Turn the two source latches upward to release the ion source.
7. Pull the ion source gently away from the vacuum interface.
8. Put the ion source on a clean, secure surface.

Remove the Probe

Always remove the ion source from the mass spectrometer before you perform any maintenance on the probe or change probes.

WARNING! Electrical Shock Hazard: Disconnect the source from the mass spectrometer before starting any maintenance procedures.

1. Remove the ion source from the mass spectrometer. Refer to Remove the Ion Source.
2. Loosen the 1/8-in. sample tubing nut and remove the sample tubing from the probe.
3. Loosen the bronze retaining ring that fastens the probe to the ion source housing.
4. Gently pull the probe straight up out of the tower. Do not let the tip of the probe touch anything during removal or storage.
5. Put the probe on a secure, clean surface.

Clean the Electrode Tube

Clean the electrode tube periodically, or when performance decreases.

This procedure applies to both the TurboIonSpray® and APCI probes. Use this procedure to remove the electrode tube for cleaning. If the electrode tube cannot be cleaned, then use this procedure to replace it with a new part.

WARNING! Electrical Shock Hazard: Remove the ion source from the mass spectrometer before starting any maintenance procedures.

1. Remove the ion source from the mass spectrometer.
2. Remove the probe from the ion source. Refer to Remove the Ion Source on page 117 and Remove the Probe on page 117.
3. Remove the electrode adjustment nut. Hold the probe with the tip pointing downwards so the spring remains inside the probe as the electrode tube is withdrawn. Refer to Figure A-4.

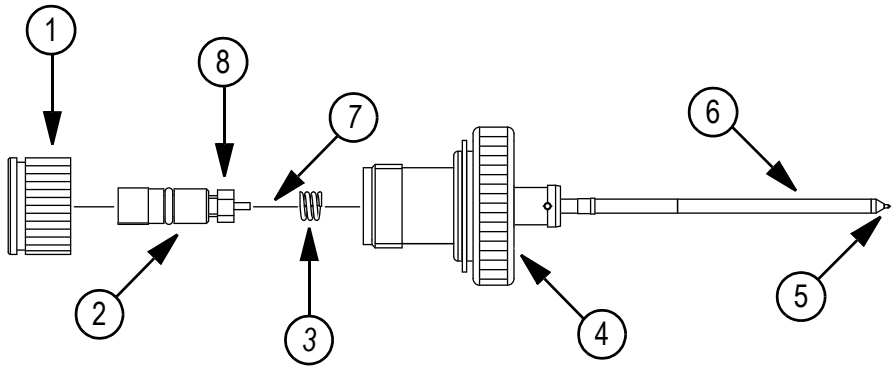


Figure A-4 Probe - expanded view

Item	Description
1	Electrode adjustment nut
2	PEEK union
3	Spring
4	Bronze retaining ring
5	Electrode tip
6	Sprayer tube
7	Electrode tube
8	1/4-inch retaining nut

4. Pull the PEEK union and the attached electrode tube from the probe. Refer to Figure A-4.
5. Use the 1/4 inch open-ended wrench to remove the retaining nut that holds the electrode tube in the PEEK union.

6. Remove the electrode tube from the retaining nut.
7. Clean the electrode tube with a 50:50 methanol:water solution, by running the solution through the electrode tube or by soaking the tube in an ultrasonic bath.

Replace the Electrode

1. Insert the electrode tube into the retaining nut and then into the PEEK union fitting.

   Make sure that the electrode tube is inserted as far into the PEEK union fitting as it will go. If there is a gap between the electrode tube and its seat inside the union fitting, a dead sample volume may occur.
2. Align the electrode tube with the narrow opening in the sprayer tube, and then insert the PEEK union fitting and attached electrode tube into the probe. Be careful not to bend the electrode tube.
3. Make sure that the spring is still inside the probe and then tighten the electrode adjustment nut.
4. Insert the probe into the tower, taking care not to allow the tip of the probe to touch any part of the ion source housing.
5. Push down the bronze retaining ring to engage its thread with the thread on the ion source housing and then tighten the ring.
6. Insert the sample tubing into the sample tubing nut, insert the sample tubing nut into the fitting at the top of the probe, and then tighten the sample tubing nut until it is finger-tight.
7. Install the ion source on the mass spectrometer. Refer to Install the Ion Source on page 109.
8. Adjust the electrode tip to specification. Refer to Adjust the Electrode Tip Extension on page 119.

Adjust the Electrode Tip Extension

The electrode tip extension should be adjusted for best performance. The optimal setting is compound-dependent. The distance that the electrode tip extends affects the shape of the spray cone, and the cone shape affects mass spectrometer sensitivity.

WARNING! Toxic Chemical Hazard: Make sure that the electrode tip extends past the end of the probe, to prevent the escape of hazardous vapor from the ion source.

- Adjust the black electrode adjustment nut on the top of the probe to extend or retract the electrode tip. The electrode tip should extend between 0.5 mm and 1.0 mm from the end of the probe as shown in Figure A-5.

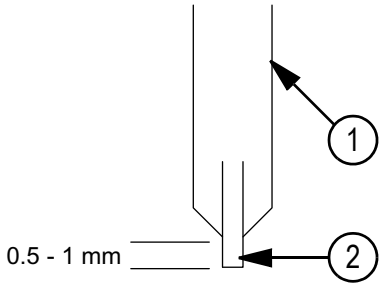


Figure A-5 Electrode tip extension adjustment

Item	Description
1	Probe
2	Electrode

Replace the Corona Discharge Needle

The corona discharge needle tip may become so corroded that it must be cut off from the corona discharge needle. If this occurs, replace the corona discharge needle.

WARNING! Electrical Shock Hazard: Remove the ion source from the mass spectrometer before starting any maintenance procedures.

WARNING! Piercing Hazard: The tip of the needle is extremely sharp. Take care to handle it safely.

1. Remove the ion source and probe from the mass spectrometer, refer to Remove the Ion Source on page 117 and Remove the Probe on page 117.

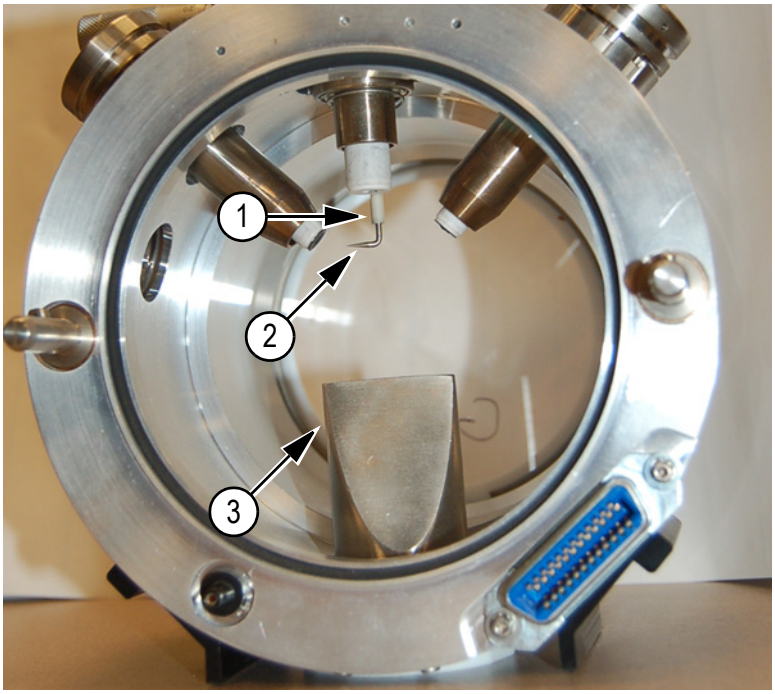


Figure A-6 Removing the corona discharge needle

Item	Description
1	Ceramic sleeve
2	Corona discharge needle tip
3	Exhaust chimney

2. Hold the corona discharge needle tip and, with the other hand, turn the corona discharge needle adjustment knob counter-clockwise to remove it.
3. Gently pull the corona discharge needle down through the exhaust chimney to remove it.
4. Insert the new needle through the exhaust chimney into the ceramic sleeve as far as it will go.
5. While holding the corona discharge needle tip, install and tighten the corona discharge needle adjustment knob.
6. Insert the probe, and then install the ion source housing on the mass spectrometer. Refer to Install the Ion Source on page 109.

Replace the Sample Tubing

Use the following procedure to replace the sample tubing if it has a blockage.

1. Stop the sample flow and make sure that any remaining gas has been removed through the source exhaust system.
2. Remove the ion source. Refer to Remove the Ion Source on page 117.
3. Disconnect the sample tubing from the probe and the union.

4. Replace the sample tubing with the same length of tubing used previously.
5. Install the ion source. Refer to Install the Ion Source on page 109.
6. Resume the sample flow.

Troubleshooting

Table A-4 Troubleshooting

Symptom	Possible cause	Solution
The Analyst® software reports that the mass spectrometer is in Fault status.	The probe is not installed. The probe is not connected securely.	Install the probe. Refer to Install the Probe in the Ion Source Housing on page 109. Remove and replace the probe. Tighten the probe connection bronze ring securely. Refer to Remove the Probe on page 117 and Install the Probe in the Ion Source Housing on page 109.
The Analyst software indicates that the APCI probe is in use, but the TurboIonSpray® probe is installed.	F3 fuse is blown.	Contact an FSE.
The spray is not uniform.	The electrode is blocked.	Clean or replace the electrode. Refer to Clean the Electrode Tube on page 118.
Sensitivity is poor.	Solvent vapor or other unknown compounds are present in the analyzer region.	Optimize the Curtain Gas™ flow. Refer to Optimize the TurboIonSpray® Probe on page 111 or Optimize the APCI Probe on page 114.
During testing, the ion source fails to meet specifications.	The mass spectrometer has not passed the installation tests. The test solution was not prepared correctly.	Perform installation tests on the mass spectrometer with the default source. Confirm that the test solutions were prepared correctly. If the problem cannot be resolved, contact an FSE.
Background noise is high.	Temperature (TEM) is too high. Heater gas flow rate (GS2) is too high.	Optimize the temperature. Optimize heater gas flow.

Table A-4 Troubleshooting (Continued)

Symptom	Possible cause	Solution
Arcing or sparks occur.	The position of the corona discharge needle is incorrect.	Turn the corona discharge needle toward the curtain plate, and away from the stream of heater gas. Refer to Adjust the Corona Discharge Needle on page 114.

Consumables

The following tables list the orderable parts for the Turbo V™ ion source. The parts are available in the Consumables Kit for the mass spectrometer (PN 1026540).

Table A-5

Part #	Description	Quantity	Details
016316	TUBE*1 16 OD X .005 BORE	cm	Red PEEK tubing (0.005-inch bore)
016325	FITTING*PEEK 10 32 X 1 16 INCH	1	Brown PEEK fitting
016485	TUBE* 1 16 OD-0.0025 INCH ID PEEK	cm	Tan PEEK tubing (0.0025-inch bore)
019675	FITTING*TEE INSERT .25 BORE	1	TEE insert (0.25 mm bore)
025388	ELECTRODE*N	1	APCI electrode
025392	ELECTRODE*T	1	TurboIonSpray® electrode

Table A-6

Part #	Description	Quantity	Details
027947	FRU*KIT NEB NEEDLE	1	Corona discharge needle
027950	FRU*KIT ELECTRODE NEB	1	APCI electrode kit
1003263	FUSE*4A 250v 5X20 LONG DELAY	1	F3 fuse T4A 250 V, 5 mm × 20 mm time delay (Not used for AB SCIEX TripleTOF™ 5600 systems.)

Table A-7

Part #	Description	Quantity	Details
027460	OPT*ASSY NEB	1	APCI probe assembly
027461	OPT*ASSY TURBO	1	TurboIonSpray® probe assembly

4500 Series Instrument Requirements and Parameters

B

This appendix includes electrical and gas requirements and instrument parameters for the 4500 series instruments.

Electrical and Gas Requirements

The following table contains the values for nominal input voltage, input voltage fluctuation, frequency, maximum input current and maximum input power, where appropriate.

Table B-1 Electrical Requirements

Component	Values
Instrument	200 to 240 V, ±10% of nominal, 50/60 Hz, 10 A, 1000 VA
Roughing pump (Leybold)	200 to 240 V, ±10% of nominal, 50/60 Hz, 4.2 A (50 Hz), 4.7 A (60 Hz), 2420 VA (50 Hz), 2250 (60 Hz)
Roughing pump (Varian)	200 to 240 V, ±10% of nominal, 50/60 Hz, 1.76 A, 1200 VA
Instrument bench	100 to 240 V, ±10% of nominal, 50/60 Hz, 1 A, 240 VA
Computer	115/230 V, ±10% of nominal, 50/60 Hz, 6/3 A, 690 VA
Monitor	100 to 240 V, +6/–10% of nominal, 50/60 Hz, 1.5 A, 360 VA

The following table contains the gas requirements for the 4500 series of instruments.

Table B-2 Gas Requirements

Gas	Description	Requirements
Gas 1/Gas 2	Zero air	Zero grade air at 100 psi (max.); flow rate of 22 L/min.
Curtain Gas™ supply; CAD gas	UHP (ultra high purity) nitrogen (N2)	UHP N2 at 60 psi (max.); flow rate of 10 L/min.
Source exhaust	House air, zero air, or UHP N2	House air, zero air, or UHP N2 at 55 psi; flow rate of 8 L/min. Do not operate below 55 psi.

Instrument Parameters

The following table contains generic parameters for the 4500 series of instruments. The first number under each scan type is the preset value; the range of numbers is the accessible range for each parameter.

Table B-3 4500 Series Instrument Parameters

Access ID	Positive ion mode			Negative ion mode		
	Q1	Q3	MS/MS	Q1	Q3	MS/MS
CUR[6]	20 10 to 55	20 10 to 55	20 10 to 55	20 10 to 55	20 10 to 55	20 10 to 55
CUR[1][2][3][4][5]	10 10 to 55	10 10 to 55	10 10 to 55	10 10 to 55	10 10 to 55	10 10 to 55
CAD[a]	0 Fixed	6 Fixed	Medium Low, Medium, High	0 Fixed	6 Fixed	Medium Low, Medium, High
CAD[b]	0 Fixed	5 Fixed	9 0 to 12	0 Fixed	5 Fixed	9 0 to 12
IS[1][2]	5500 0 to 5500	5500 0 to 5500	5500 0 to 5500	–4500 –4500 to 0	–4500 –4500 to 0	–4500 –4500 to 0
IS[5] Ion Transfer Voltage	1500 0 to 2500	1500 0 to 2500	1500 0 to 2500	–1500 –2500 to 0	–1500 –2500 to 0	–1500 –2500 to 0
IS[6]	1000 0 to 4000	1000 0 to 4000	1000 0 to 4000	–1000 –4000 to 0	–1000 –4000 to 0	–1000 –4000 to 0
NC[3][4]	3 0 to 5	3 0 to 5	3 0 to 5	–3 –5 to 0	–3 –5 to 0	–3 –5 to 0
TEM[2][3][4][5]	0 0 to 750	0 0 to 750	0 0 to 750	0 0 to 750	0 0 to 750	0 0 to 750
DP[a]	200 0 to 300	100 0 to 300	100 0 to 300	–100 –300 to 0	–100 –300 to 0	–100 –300 to 0
DP[b]	130 0 to 300	130 0 to 300	120 0 to 300	–60 –300 to 0	–60 –300 to 0	–150 –300 to 0
EP	10 2 to 15	10 2 to 15	10 2 to 15	–10 –15 to –2	–10 –15 to –2	–10 –15 to –2
CEM[a]	1800 0 to 3300	1800 0 to 3300	1800 0 to 3300	1800 0 to 3300	1800 0 to 3300	1800 0 to 3300
CEM[b]	2000 0 to 3300	2000 0 to 3300	2000 0 to 3300	2000 0 to 3300	2000 0 to 3300	2000 0 to 3300
(1)IonSpray™ ion source (2)TurboIonSpray® ion source (3)Heated Nebulizer (4)DuoSpray™ ion source, 1=TIS, and 2=HN (5)PhotoSpray® ion source (6) NanoSpray® ion source (a) AB SCIEX QTRAP® 4500 system (b) AB SCIEX Triple Quad™ 4500 system						

Table B-3 4500 Series Instrument Parameters (Continued)

Access ID	Positive ion mode			Negative ion mode		
	Q1	Q3	MS/MS	Q1	Q3	MS/MS
GS1[a]	20 0 to 90	20 0 to 90	20 0 to 90	20 0 to 90	20 0 to 90	20 0 to 90
GS1[b]	15 0 to 90	15 0 to 90	15 0 to 90	15 0 to 90	15 0 to 90	15 0 to 90
GS2	0 0 to 90	0 0 to 90	0 0 to 90	0 0 to 90	0 0 to 90	0 0 to 90
IHT	150 0 to 250	150 0 to 250	150 0 to 250	150 0 to 250	150 0 to 250	150 0 to 250
sdp	1 1 or 2	1 1 or 2	1 1 or 2	1 1 or 2	1 1 or 2	1 1 or 2
IQ1 (IQ1 = Q0 + offset)	Q0 + (-0.5)	Q0 + (-0.5)	Q0 + (-0.5)	Q0 + 0.5	Q0 + 0.5	Q0 + 0.5
ST (ST = Q0 + offset)	Q0 + (-8) -12 to -5	Q0 + (-8) -12 to -5	Q0 + (-8) -12 to -5	Q0 + 8 12 to 5	Q0 + 8 12 to 5	Q0 + 8 12 to 5
IE1[a] (IE1 = Q0 - RO1)	1.6 0 to 3	n/a	2 0 to 3	-1 -3 to 0	n/a	-1 -5 to 0
IE1[b] (IE1 = Q0 - RO1)	0.9 0 to 3	n/a	0.9 0 to 3	-1 -3 to 0	n/a	-1 -5 to 0
IQ2[a] (IQ2 = Q0 + offset)	Q0 + (-10)	Q0 + (-11)	Q0 + (-10)	Q0 + 11	Q0 + 11	Q0 + 15
IQ2[b] (IQ2 = Q0 + offset)	Q0 + (-12)	Q0 + (-12)	Q0 + (-12)	Q0 + (-10)	Q0 + 10	Q0 + 10
RO2[a]	-20 Fixed	-20 Fixed	n/a	20 Fixed	20 Fixed	n/a
RO2[b]	-22 Fixed	-20 Fixed	n/a	18 Fixed	20 Fixed	n/a

(1)IonSpray™ ion source (2)TurbolonSpray® ion source (3)Heated Nebulizer (4)DuoSpray™ ion source, 1=TIS, and 2=HN (5)PhotoSpray® ion source (6) NanoSpray® ion source (a) AB SCIEX QTRAP® 4500 system (b) AB SCIEX Triple Quad™ 4500 system

Table B-3 4500 Series Instrument Parameters (Continued)

Access ID	Positive ion mode			Negative ion mode		
	Q1	Q3	MS/MS	Q1	Q3	MS/MS
CE[a] (CE = Q0 - RO2)	n/a	n/a	30 5 to 180	n/a	n/a	-30 -180 to -5
CE[b] (CE = Q0 - RO2)	n/a	n/a	53 5 to 180	n/a	n/a	-40 -180 to -5
ST3 (ST3 = RO2 + offset)	n/a	n/a	n/a	n/a	n/a	n/a
CXP[a] (CXP = RO2 - ST3)	n/a	15 0 to 55	15 0 to 55	n/a	-15 -55 to 0	-15 -55 to 0
CXP[b] (CXP = RO2 - ST3)	n/a	9 0 to 55	27 0 to 55	n/a	-17 -55 to 0	-12 -55 to 0
RO3	-50 Fixed	n/a	n/a	50 Fixed	n/a	n/a
IE3[a]	n/a	1.5 0 to 5	1.8 0 to 5	n/a	-1.100 -5 to 0	-1 -5 to 0
IE3[b]	n/a	2 0 to 5	1.5 0 to 5	n/a	-2.500 -5 to 0	-1.2 -5 to 0
DF[a]	-100 Fixed	-100 Fixed	-100 Fixed	100 Fixed	100 Fixed	100 Fixed
DF[b]	-250 Fixed	-250 Fixed	-250 Fixed	150 Fixed	150 Fixed	150 Fixed

(1)IonSpray™ ion source (2)TurboIonSpray® ion source (3)Heated Nebulizer (4)DuoSpray™ ion source, 1=TIS, and 2=HN (5)PhotoSpray® ion source (6) NanoSpray® ion source (a) AB SCIEX QTRAP® 4500 system (b) AB SCIEX Triple Quad™ 4500 system

Table B-4 QTRAP 4500 System Parameters for LIT Scan Types Only

Access ID	Positive Ion Mode	Negative Ion Mode
CUR[6]	20 10 to 55	20 10 to 55
CUR[1][2][3][4][5]	20 10 to 55	20 10 to 55

Table B-4 QTRAP 4500 System Parameters for LIT Scan Types Only (Continued)

Access ID	Positive Ion Mode	Negative Ion Mode
CAD	High Low; Medium; High	High Low; Medium; High
IS[(1)(2)]	5500 0 to 5500	-4500 -4500 to 0
IS[(5)]	1500 0 to 2500	-1500 -2500 to 0
IS[(6)]	1000 0 to 4000	-1000 -4000 to 0
NC[(3)(4)]	3 0 to 5	–3 –5 to 0
TEM[(2)(3)(4)(5)]	0 0 to 750	0 0 to 750
DP	100 0 to 300	–100 –300 to 0
EP	10 2 to 15	–10 –15 to –2
AF2	0.100 0 or 1	0.100 0 or 1
AF3	Mass-Speed Dependent 0 to 10	Mass-Speed Dependent 0 to 10
EXB	Mass-Speed Dependent –165 to 0	Mass-Speed Dependent 0 to 165
CEM	1800 0 to 3300	1800 0 to 3300
GS1	20 0 to 90	20 0 to 90
GS2	0 0 to 90	0 0 to 90
CES	0 0 to 50	0 0 to 50
CE (Q0 - ROS)	10 5 to 180	-30 -180 to -10

Table B-4 QTRAP 4500 System Parameters for LIT Scan Types Only (Continued)

Access ID	Positive Ion Mode	Negative Ion Mode
IHT	150 0 to 250	150 0 to 250
sdp	1 1 or 2	1 1 or 2

(1)IonSpray™ ion source (2)TurboIonSpray® ion source (3)Heated Nebulizer (4)DuoSpray™ ion source, 1=TIS, and 2=HN (5)PhotoSpray® ion source (6) NanoSpray® ion source

Table C-1 Tuning Frequency

	Calibration		Resolution Optimization	
Scan Type	**Frequency**	**Manual/ Automated**	**Frequency**	**Manual/Automated**
Q1 and Q3	3 to 6 months	Both	3 to 6 months	Both
LIT	Every 2 weeks; as required	Both	3 to 6 months	Automated only

Table C-2 Suggested Tuning Solutions for AB SCIEX Triple Quad™ 4500 System

System	Positive	Negative
AB SCIEX Triple Quad 4500 system	2×10^{-6} M PPG (1:50)	3×10^{-4} NEG PPG

Table C-3 Suggested Tuning Solutions for AB SCIEX QTRAP® 4500 System

	Q1 and Q3		LIT
Instrument	**Positive**	**Negative**	**Positive and Negative**
AB SCIEX QTRAP 4500 system	2×10^{-6} M PPG (1:50)	3×10^{-4} NEG PPG	1:100 Agilent mix

Table C-4 Q1 and Q3 PPG Positive Ion Scans

Instrument	Masses							
AB SCIEX Triple Quad 4500 system	59.0	175.1	500.3	616.5	906.7	1254.9	1545.1	1952.4
AB SCIEX QTRAP 4500 system	59.0	175.1	500.3	616.5	906.7	1254.9	1545.1	1952.4

Table C-5 Q1 and Q3 PPG Negative Ion Scans

Instrument	Masses							
AB SCIEX Triple Quad 4500 system	45.0	411.2	585.4	933.6	1223.8	1572.1	1863.3	1979.3
AB SCIEX QTRAP 4500 system	45.0	411.2	585.4	933.6	1223.8	1572.1	1863.3	1979.3

Table C-6 Masses and Polarity for the AB SCIEX QTRAP 4500 system (Agilent)

Instrument/ Polarity	Masses						
LIT Positive	118.087	322.049	622.030	922.010	1521.972	–	–
LIT Negative	112.985	431.982	601.978	1033.988	1633.949	–	–

Regularly clean and maintain the instrument for optimal performance. For information on tuning frequency, refer to Table C-1 *Tuning Frequency* on page 131.

The following table provides a recommended schedule for cleaning and maintaining the instrument. Contact your Qualified Maintenance Person to order consumable parts.

Table D-1 System Maintenance Tasks

Component	Frequency	Task	For more information, refer to...
Curtain plate	As needed	Clean	Clean the Curtain Plate
Orifice plate	As needed	Clean	Clean the Front of the Orifice Plate
QJet® ion guide	As needed	Clean	Contact an AB SCIEX FSE.
Q0 and IQ1 lens	As needed	Clean	Contact an AB SCIEX FSE.
Instrument air filter	Every 6 months	Inspect and clean or replace	Contact an AB SCIEX FSE.
Instrument surfaces	As needed	Clean	Surface Cleaning
Drain bottle	As needed	Empty	Empty the Drain Bottle
Roughing pump oil	Leybold Sogevac SV 28: Every 6 months Varian MS 30: Annually	Replace	Dependent on roughing pump. Contact an AB SCIEX FSE.
Electrode	As needed	Inspect and clean or replace	Clean the Electrode Tube
Corona discharge needle	As needed	Replace	Replace the Corona Discharge Needle

For “As needed” tasks, follow these guidelines:

- Clean the curtain plate, orifice plate, QJet ion guide, and Q0 region if system sensitivity degrades.
- Clean the instrument surfaces after a spill, or when they become dirty.
- Empty the drain bottle when it becomes full.

Contact an AB SCIEX representative for maintenance service and support.

Health and Safety Precautions

- Determine what chemicals may have been used in the instrument prior to service. Refer to Safety Data Sheets for the health and safety precautions that must be followed with chemicals.
- Work in a well-ventilated area.

- Always wear assigned personal protective equipment, including powder-free nitrile gloves, safety glasses, and a laboratory coat.
- Follow required electrical safe work practices.
- Avoid ignition sources when working with flammable materials, such as isopropanol, methanol, and other flammable solvents.
- Take care in the use and disposal of any chemicals. Potential risk of personal injury if proper handling and disposing of chemicals are not followed.
- Avoid skin contact with chemicals during cleaning, and wash hands after use.
- Comply with all local regulations for the handling of biohazard, toxic, or radioactive materials.

WARNING! Radiation Hazard, Biohazard, or Toxic Chemical Hazard: Determine whether instrument decontamination is required prior to cleaning. Instrument decontamination should be conducted prior to cleaning if radioactive materials, biological agents, or toxic chemicals have been used with an instrument.

Caution: Potential Instrument Damage: Rinse off any acid-containing cleaning solvents with water. Do not use chlorinated solvents because these may damage the QJet ion guide components

Surface Cleaning

Clean the external surfaces of the instrument after a spill, or when they become dirty.

- Using warm, soapy water and a soft cloth, wipe the external surfaces.

Empty the Drain Bottle

Empty the drain bottle when it becomes full.

WARNING! Biohazardous Material: Deposit biohazardous material in appropriately labelled containers. Potential risk of personal injury if proper handling and disposing of biohazardous materials are not followed

1. Shut Down the System.
2. Disconnect the tubes from the top of the drain bottle.

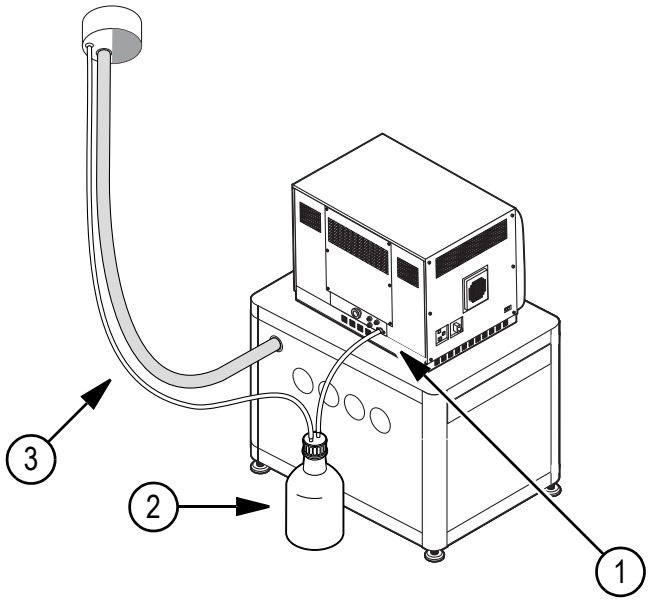


Figure D-1 Drain bottle

Item	Description
1	Connection to instrument.
2	Drain bottle (Drain bottle shown at the rear of the instrument to show connection points. The drain bottle is at the side of the instrument when installed.)
3	Connection to vent.

3. Unscrew the cap and dispose of the waste.
4. Replace the cap and connect the tubes.

Front-End Cleaning

Clean the instrument front-end using the routine cleaning method, to:

- Minimize unscheduled instrument downtime.
- Maintain optimum sensitivity.
- Avoid more extensive cleaning that requires a service visit.

Symptoms of contamination: Significant loss in sensitivity and increased background noise.

When contamination occurs, perform an initial routine cleaning. Clean up to and including the front of the orifice plate. If routine cleaning does not resolve issues with sensitivity, a full cleaning may be necessary.

This section provides instructions for performing routine cleaning without breaking vacuum and full cleaning under atmospheric pressure, after venting the instrument.

Note: Follow all applicable local regulations. For health and safety guidelines, refer to Health and Safety Precautions for more information.

Note: For consumables ordering information and inquiries, call 877-740-2129 (U.S. only), or visit www.absciex.com.

Required Tools and Materials

- Powder free gloves (nitrile recommended)
- Safety glasses
- Laboratory coat
- Fresh, high quality water (at least 18 Mohm de-ionized water [DI water] or ultra-pure HPLC-grade water). Old water can contain contaminants which can further contaminate the mass spectrometer.
- HPLC- or LCMS-grade methanol, isopropanol (2-propanol), or acetonitrile
- Cleaning solution. Use one of:
  - 100% methanol
  - 100% isopropanol
  - 50:50 acetonitrile:water solution (freshly prepared)
  - 50:50 acetonitrile:water with 0.1% acetic acid solution (freshly prepared)

Caution: Do not use chlorinated solvents.

Table D-2 Tools and Supplies Available from AB SCIEX

Description	P/N
Small polyester swab (thermally bonded)	1017396
Small lint-free wipe (11 cm x 21 cm); available in Consumables kits	WC018027

Best Practices

WARNING! Radiation Hazard, Biohazard, or Toxic Chemical Hazard: Determine whether instrument decontamination is required prior to cleaning. Instrument decontamination should be conducted prior to cleaning if radioactive materials, biological agents, or toxic chemicals have been used with an instrument.

- Always wear clean, powder-free gloves for the cleaning procedures.
- After cleaning the instrument components and before reassembling them, put on a clean pair of gloves.
- Do not use cleaning supplies other than those specified in this procedure.
- If possible, prepare cleaning solutions just before beginning.

- Prepare and store all organic solutions and organic-containing solutions in very clean glassware only. Never use plastic squirt bottles. Contaminants can leach from these bottles and further contaminate the mass spectrometer.
- Allow only the center area of the wipe to contact the instrument surface. Cut edges can leave fibers behind.

Tip! Wrap the wipe around a thermally-bonded polyester swab.

Figure D-2 Example: Folding the wipe

- Allow the wipe or swab to contact the surface once, and then discard it, to avoid cross-contamination.
- Larger parts of the vacuum interface, such as the curtain plate, may require several cleanings, using multiple wipes.
- To avoid contaminating the solution, pour the solution on the wipe or swab.
- Only moisten the wipe or swab slightly when applying water or cleaning solution. Water, more so than organic solvents, may cause the wipe to deteriorate, leaving residue on the instrument.

Prepare for Routine Cleaning

In routine cleaning, clean the curtain plate and the front of the orifice plate. Routine cleaning can be performed while the instrument remains under vacuum.

Note: Instruments with a NanoSpray® ion source may require a full cleaning for best results. Contact an AB SCIEX FSE.

1. Deactivate the hardware profile.

WARNING! Hot Surface Hazard: Surfaces of the ion source become hot during operation. Let the ion source cool for at least 10 minutes before starting any cleaning procedures.

2. Remove the ion source. Be sure to place the ion source in a safe location.
3. Wait at least 20 minutes for the curtain plate and orifice plate to cool.
4. Cover the source drain with the exhaust cover plate (if available), or a similar cover.

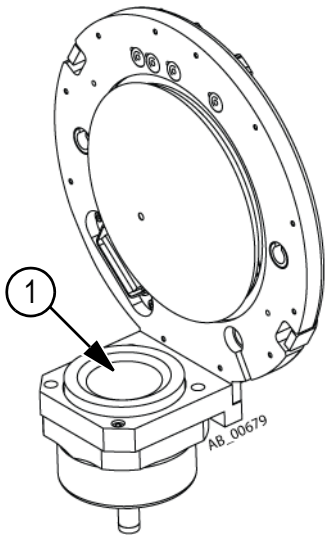


Figure D-3 Source drain on the vacuum interface

Item	Description
1	Source drain

Clean the Curtain Plate

1. Remove the curtain plate and then place it on a clean, stable surface.

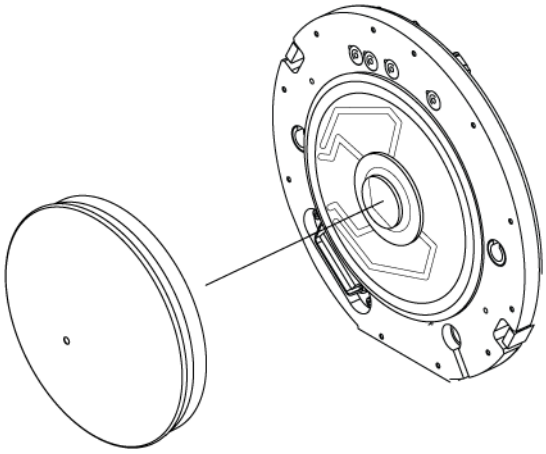


Figure D-4 Interface with curtain plate removed

2. Using wipes and water, clean both sides of the curtain plate.
3. Repeat step 2 using the cleaning solution.
4. Using a dampened wipe or small poly swab, clean the aperture.
5. Wait until the curtain plate is dry.
6. Inspect the curtain plate for solvent stains or lint, removing any residue with a clean, slightly damp lint-free wipe.

Note: Persistent spotting or filming is an indicator of contaminated solvent.

Clean the Front of the Orifice Plate

Note: If the standard orifice plate has a removable interface heater, do not remove the heater during cleaning.

1. When cleaning a NanoSpray orifice plate, remove the interface heater and clean it:
   i. Wipe the heater with a lint-free wipe dampened with water.
   ii. Wipe the heater with a lint-free wipe dampened with cleaning solution.
2. Moisten the lint-free wipe with water and then wipe the front of the orifice plate.

Caution: Potential Instrument Damage: Do not insert a wire or metal brush into the orifice, to avoid damaging the aperture.

3. Repeat step 2 using the cleaning solution.
4. Wait until the orifice plate is dry.
5. Inspect the orifice plate for solvent stains or lint, removing any residue with a clean, slightly damp lint-free wipe.

Note: Persistent spotting or filming is an indicator of contaminated solvent.

Put the Instrument Back into Service

1. Install the curtain plate on the front end of the instrument.
2. Remove the protection from the ion source drain.
3. Install the ion source on the mass spectrometer.
4. Tighten the ion source by turning the ion source release latches down into the locking position. (Refer to the ion source Operator's Guide.)
5. Activate the hardware profile.

Basic System Troubleshooting

E

This appendix contains basic information for troubleshooting basic system issues. Certain activities may be carried out by the AB SCIEX trained Qualified Maintenance Person in the laboratory. For advanced troubleshooting, contact an AB SCIEX Field Service Employee (FSE).

Table E-1 System Issues

Problem	Possible cause	Corrective action
Sensitivity loss	Instrument or ion source requires tuning and optimizing	For more information, refer to: • Instrument Tuning and Calibrating • Turbo V™ Ion Source User Reference appendix • Analyst® software Help system
	Dirty curtain plate	Refer to Clean the Curtain Plate for more information.
	Dirty orifice plate	Refer to Clean the Front of the Orifice Plate for more information.
	Dirty QJet® ion guide, Q0 or IQ0	Contact an AB SCIEX FSE or your local AB SCIEX trained Qualified Maintenance Person.
Frequent or extreme contamination of the QJet ion guide	Curtain Gas™ flow rate is too low.	Verify, and if applicable, increase the Curtain Gas flow rate.
Low vacuum pressure	Low roughing pump oil level.	Check the roughing pump oil level, and add oil if necessary. Contact an AB SCIEX FSE or your local AB SCIEX trained Qualified Maintenance Person.

For sales, technical assistance or service, contact an AB SCIEX FSE or visit the AB SCIEX Web site at **www.absciex.com** for contact information.