3200 QTRAP® LC/MS/MS System

Hardware Guide

AB SCIEX

Contents

System Information

Before you operate any instrument, you should read, understand, and follow all safety precautions to prevent personal injury or damage to the instrument. Before you operate this instrument, make sure you have read the *Safety Practices* guide.

Warnings and labels on the instrument are shown with international symbols. These safety practices are intended to supplement your federal, state or provincial, and local health and safety regulations and laws. The information provided covers instrument-related safety with regard to the operation of the instrument. It does not cover every safety procedure that should be practised. Ultimately, you and your organization are responsible for compliance with federal, state, provincial, and local EHS legal requirements and for maintaining a safe laboratory environment. For additional information, refer to the *Chemical Rubber Company Handbook of Laboratory Safety* and *Prudent Practices for Handling Chemicals in Laboratories*.

Symbols and Conventions

The following conventions are used throughout the guide:

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

WARNING!   A warning indicates an operation that could cause personal injury or damage to property if precautions are not followed.

Caution!   A caution indicates an operation that could cause damage to the instrument if precautions are not followed.

Note: A note provides additional information that is highlighted for the reader.

Principles of the System

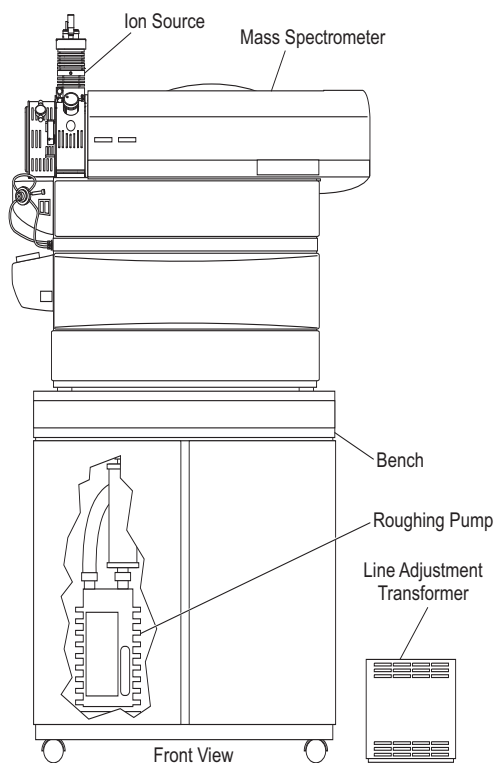The 3200 QTRAP® LC/MS/MS system includes a hybrid triple quadrupole/LIT (linear ion trap) mass spectrometer, a Turbo V™ ion source, a computer, and the Analyst® software.

> ⚠️ **WARNING! If you need to move the system, contact an FSE to assist you. Risk of personal injury or instrument damage.**

This section describes features and applications of the 3200 QTRAP system.

Figure 1-1   3200 QTRAP instrument and bench

Applications for the System

The 3200 QTRAP system is best suited to three specific applications:

- Quantitative analysis of small molecules
- Qualitative analysis of small molecules
- Qualitative analysis of proteins and peptides

Quantitative Analysis of Small Molecules

This application involves measurement of specific molecular weight compounds, such as a drug or metabolite in a liquid sample, and their resulting fragment ions for determining the exact quantity of the compound in the sample of interest. Quantitation is performed using a standard curve of mass spectrometer signal intensity for various known concentrations of the compound. The signal in the test sample is then compared to the standard curve to determine the concentration.

The typical scan type used for this application is MRM (Multiple Reaction Monitoring).

Qualitative Analysis of Small Molecules

This application involves MS analysis of low molecular weight (typically less than 1000 Da) compounds. This is for the purpose of identification and structural characterization of the chemical compounds in a liquid sample. In addition, this general application is used when the researcher wants to confirm what species are in the sample.

The typical scan type used for this application in the 3200 QTRAP system is an EMS (Enhanced MS) scan for intact molecular weight analysis. For more detailed structural analysis using tandem mass spectrometry, EPI (Enhanced Product Ion) and MS3 (MS/MS/MS) scans are performed.

Additionally, the 3200 QTRAP system allows you to combine triple quadrupole and LIT scan modes to achieve both qualitative and quantitative analyses in a single LC run.

Qualitative Analysis of Proteins and Peptides

This application involves determination of molecular weights and sequences of protein and peptide samples. This is for the purposes of identification of the compounds or for structural characterization. This general application is used when the researcher wants to know what molecular weight species and what sequences of peptides or proteins are in the sample.

The typical scan types used for this application in the 3200 QTRAP system are EMS (Enhanced MS) and EMC (Enhanced Multi-Charge) scans for intact molecular weight analysis. ER (Enhanced Resolution) scans are used to obtain higher resolution spectra to determine the molecular weight and charge state of a multiply charged ion. This allows for more accurate identification of the sample using protein database searching. For more detailed structural analysis using tandem mass spectrometry (MS/MS), EPI (Enhanced Product Ion) scans are performed.

Features of the Instrument

The 3200 QTRAP system has the following features:

- The standard Turbo V™ ion source provides the plug-and-play TurboIonSpray® probe and APCI probe, a durable ceramic interface, and compatibility with high flow rates.

- Additional compatible ion sources, including the DuoSpray™ ion source, PhotoSpray® ion source, and NanoSpray® III ion source components, provide further flexibility.

- Scanning in a mass range of m/z 5 to 2000 in quadrupole mode and m/z 50 to 1700 in LIT mode.

- LINAC® collision cell eliminates cross-talk and allows for multi-component analysis during an LC run.

- Integrated switching valve and integrated syringe pump provide convenient sample introduction flexibility.

  - IDA (Information Dependent Acquisition) allows for the combination of triple quadrupole and LIT scan modes, maximizing the amount of information that can be acquired in an LC scan.

- Comprehensive, fully integrated set of software applications. See the AB SCIEX Web site for information on software products (www.absciex.com).

- Compatible with metabolite ID software, which automates metabolite identification, providing rapid, high-confidence results from your experiments.

How the Instrument Works

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. For an overview of the movement of ions through the system, see Figure 1-2.
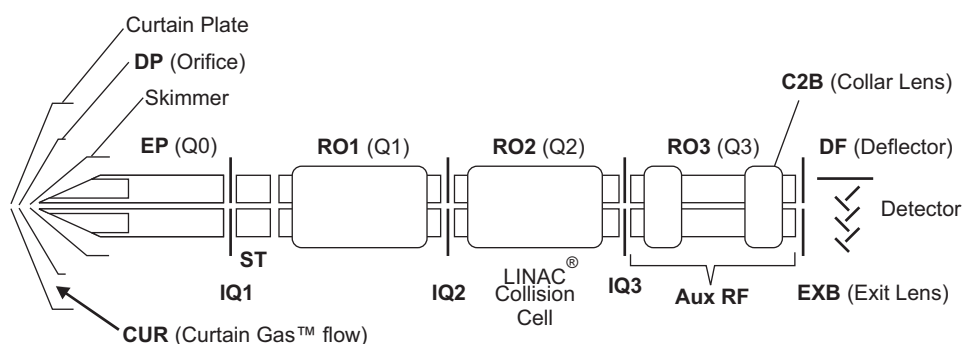
Figure 1-2 Ion optics path

The 3200 QTRAP system can use any of several ion source and probe combinations to produce ions from liquid samples. The standard Turbo V ion source, which ships with the instrument, can use either the TurboIonSpray probe or the APCI (atmospheric pressure chemical ionization) probe. The instrument is configured to perform complex MS/MS analysis, but for less rigorous analytical requirements it can perform single MS scans.

About Quadrupole Mode

The 3200 QTRAP system has a series of quadrupole filters that transmit ions according to their m/z (mass-to-charge ratios). Ions entering the ion path are first focused by Q0 before passing into Q1.

Q1 is a filtering quadrupole that sorts the ions before they enter Q2, a LINAC collision cell in which ions can be broken into fragments by collisions with gas molecules. This technique allows for the design of 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 and the instrument displays this formation as a mass spectrum.

About LIT Mode

The 3200 QTRAP system LIT functionality provides a number of enhanced modes of operation. A common factor of the enhanced modes is that ions are trapped in the Q3 quadrupole region and then scanned out to produce full spectrum data. Many spectra are rapidly collected in a short period of time and are significantly more intense than spectra collected in a comparable standard quadrupole mode of operation.

During the collection phase, ions pass through the Q2 collision cell where the CAD gas focuses the ions into the Q3 region. The Q3 quadrupole is operated with only the main RF voltage applied. Ions are prevented from passing through the Q3 quadrupole rod set and are reflected back by an exit lens to which a DC barrier voltage is applied. After the fill time elapses (a time defined by the user, or determined by the Dynamic Fill Time feature), a DC barrier voltage is applied to a Q3 entrance lens (IQ3). This confines the collected ions in Q3 and stops further ions from entering. The entrance and exit lens DC voltage barriers and the RF voltage applied to the quadrupole rods confine the ions within Q3.

During the scan out phase, the voltage on the exit lens and the auxiliary RF voltage are ramped simultaneously with the main RF voltage for increased resolution and sensitivity as compared to quadrupole scan modes. An auxiliary AC frequency is applied to the Q3 quadrupole. The main RF voltage amplitude is ramped from low to high values, which sequentially brings masses into resonance with the auxiliary AC frequency. When ions are brought into resonance with the AC frequency, they acquire enough axial velocity to overcome the exit lens barrier and are axially ejected towards the mass spectrometer ion detector. Full spectra data can be acquired from the ions collected in Q3 by rapidly scanning the main RF voltage.

Parts of the Instrument

This section contains diagrams of the instrument and general descriptions of the main components.
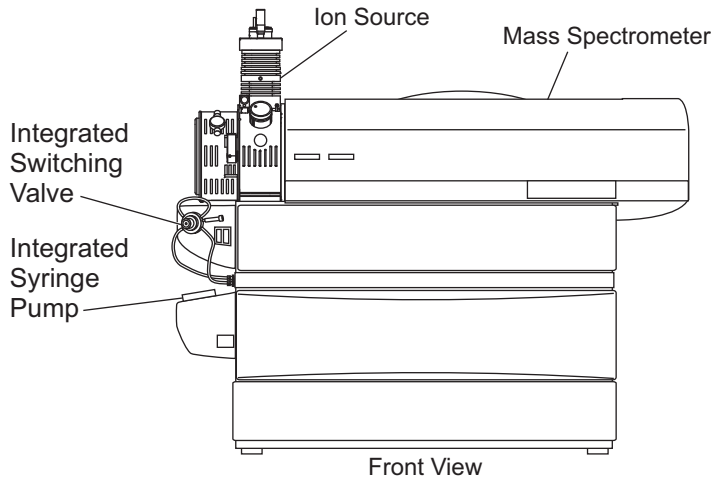
Figure 1-3 Front view
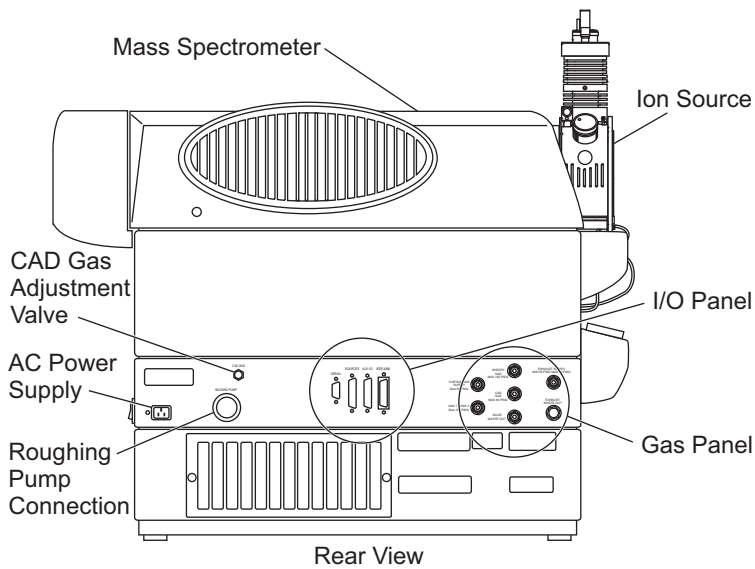
Figure 1-4 Back view

Mains Controls

The 3200 QTRAP system controls are located near the right rear corner of the instrument. This area contains the mains switch and the mains supply inlet.
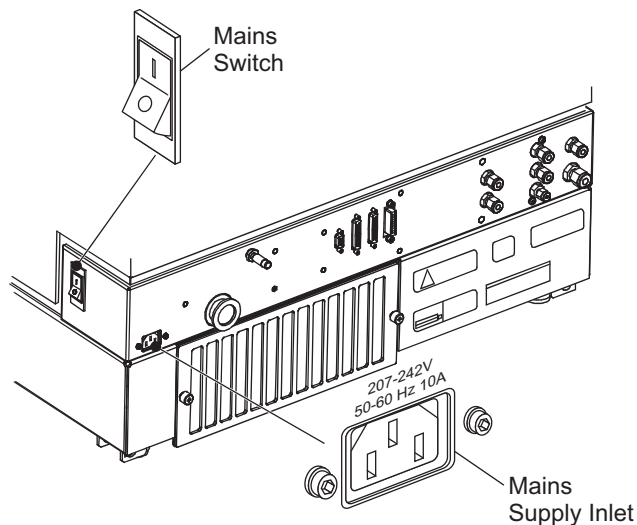
Figure 1-5   Mains switch and supply connection

Sample Introduction Features and Status Indicators

This section describes the features and controls housed on the front left area of the instrument, adjacent to the ion source housing.

The Instrument Status lights indicate the status of the instrument vacuum. When the operational vacuum conditions are satisfied and the instrument is in analysis mode, the Ready light (green) is illuminated and the Fault light (red) is extinguished. The Fault light flashes when a vacuum fault is detected. In Pump-Down mode, the Ready light flashes for the duration of the sequence.

Directly below the ion source housing is a set of conveniently integrated sample introduction features. An integrated switching valve with associated Load and Inject buttons (marked A and B, respectively) can be used as a manual injector when optimizing for an analyte using FIA, or for diverting LC flow when any precipitate or salts initially come off the LC column. Below that, an integrated syringe pump is available for infusing standards when calibrating the instrument or optimizing for an analyte.
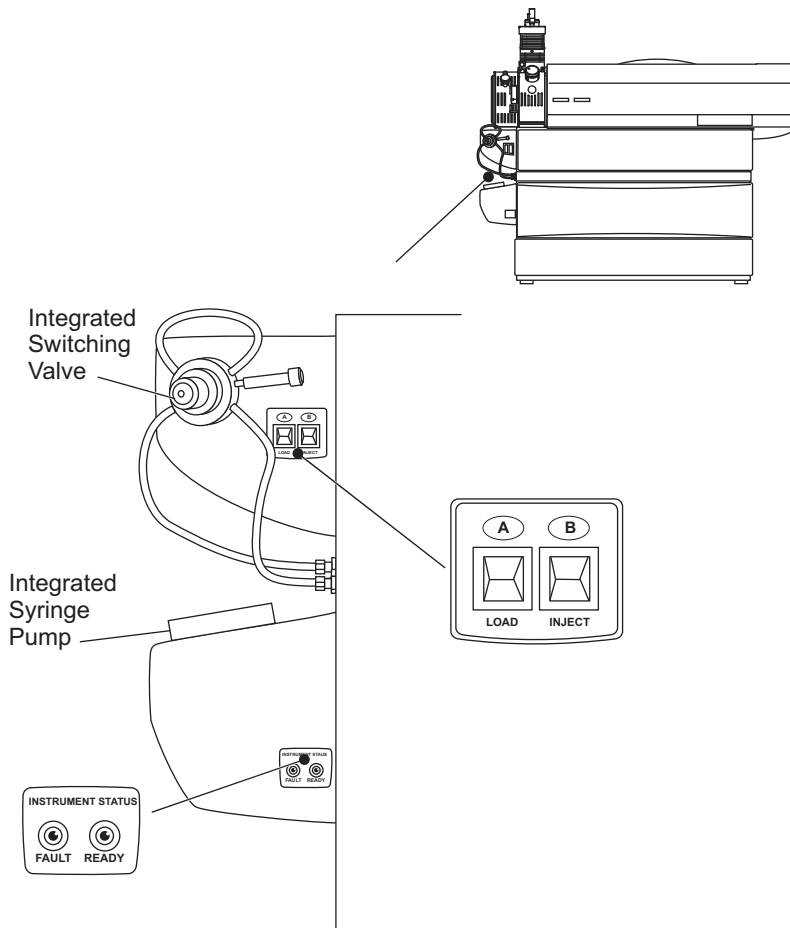
Figure 1-6 Sample introduction features and status indicators

I/O Panel

The input/output panel is located at the center back of the chassis. This panel contains several connection ports for the system:

- The IEEE-488 (GPIB) port provides a connection to the system's computer.

- The ion sources port facilitates communication to optional ion sources, such as the control valve for the DuoSpray ion source, or provision of 24 VDC for the PhotoSpray ion source.

- The AUX I/O outlet allows for communication with necessary peripherals, such as autosamplers, UV detectors, and so forth.

- A serial port provides a connection point for additional device communications.
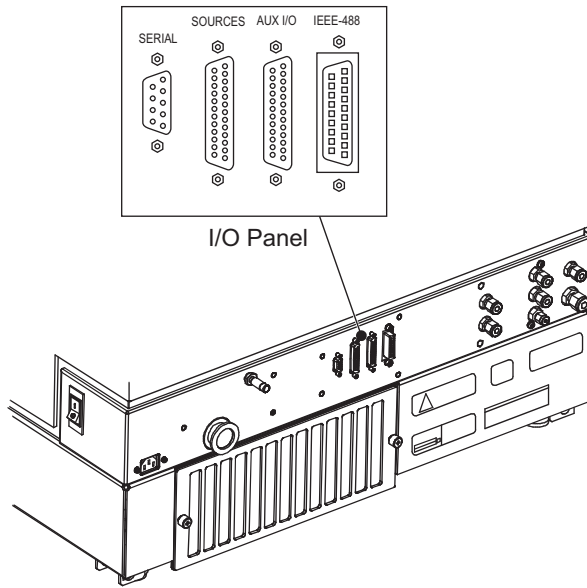
Figure 1-7 I/O panel

Gas Panel

The gas panel houses the gas supply connections and the external connections for the ion source exhaust system.
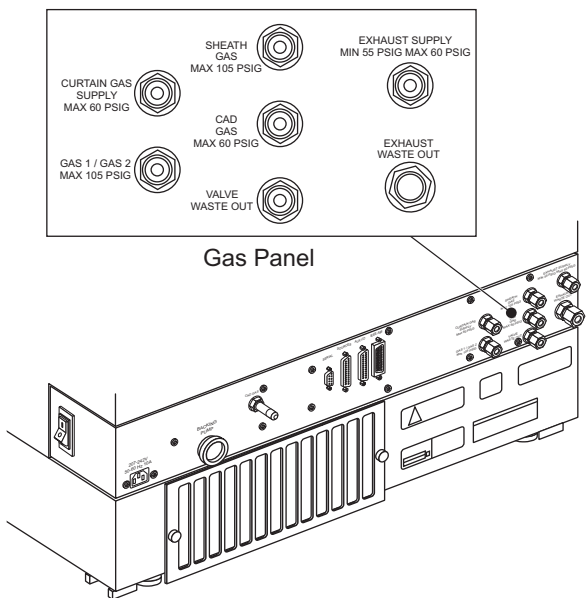
Figure 1-8 Gas panel

Ion Source Exhaust System

The ion source exhaust system is a safety feature that isolates the sample vapors and exhaust products from the laboratory environment. The ion source requires that this exhaust system be properly connected and functioning. The exhaust pump draws the solvent vapors from the enclosed ion source housing and delivers them to the exhaust-waste-out fitting, and then to a waste bottle outside the instrument. It is recommended that these vapors be passed through this waste bottle and then vented to a fume hood or outside port.

> **WARNING!   Hazardous Materials: Take all necessary precautions to ensure the safe disposal of the ion source exhaust. Follow all applicable local regulations.**
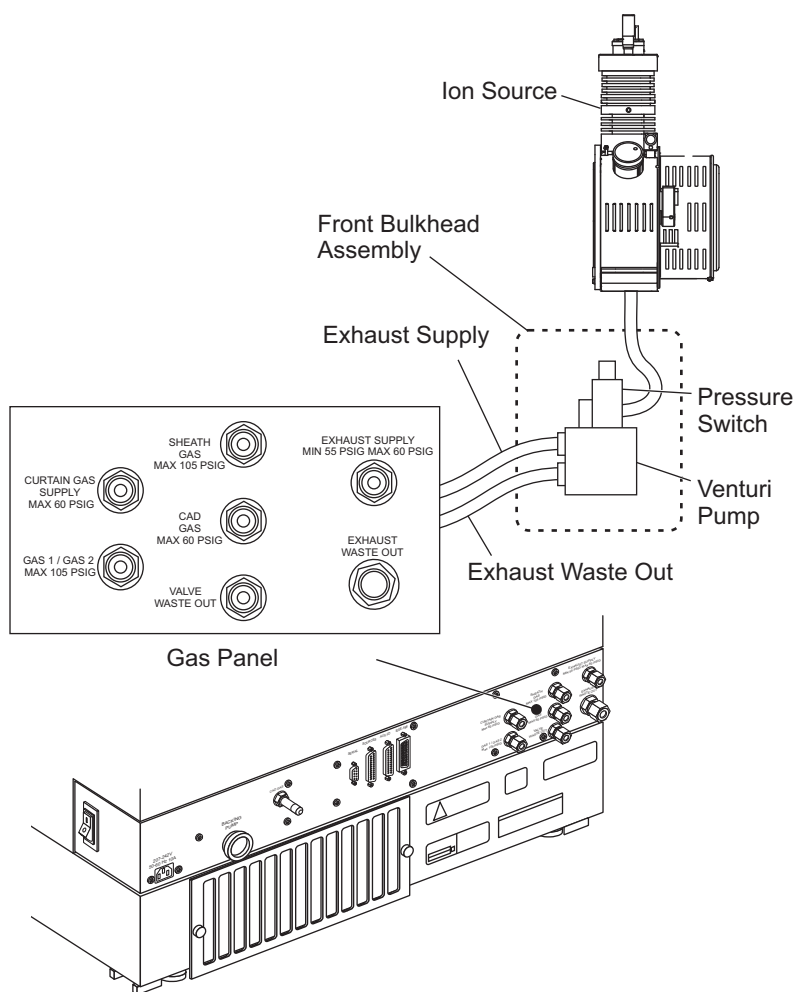
Figure 1-9 Ion source exhaust system

A filtered nitrogen or zero air gas supply (oil free) is delivered to the source exhaust pump at pressures indicated on the gas panel at the back of the instrument. The preceding figure shows the exhaust supply connection points for the 3200 QTRAP system.

Vacuum Chamber

The vacuum chamber houses the mass filter rail including the ion optics, the quadrupole rod sets, the collision cell, and the ion detector.

Shutting Down and Turning on the System

Use the following procedures if you need to shut down or turn on the system.

To shut down the system

1. In the Analyst software, complete or stop any ongoing scans.

2. Turn off the sample flow to the instrument.

> ⚠️ **Caution!   Potential Instrument Damage: Shut off the sample flow before you shut down the instrument.**

3. In the Analyst software, deactivate the hardware profile and then close the application software.

4. Stop the Analyst Service. (See the Analyst software Help system.)

> ⚠️ **Caution!   Leave the roughing pump running for a minimum of 15 minutes after turning off the instrument's main power switch. When the main power switch is turned off, the turbo pumps continue to rotate without power for a few minutes and continue to provide vacuum to the vacuum chamber. If, during this time, the roughing pump is turned off, the pressure in the vacuum line between the roughing pump and the turbo pumps increases. The increase in back pressure can create an incorrect load on the turbo pumps' bearings and can cause a catastrophic failure of the turbo pumps.**

> ⚠️ **Caution!   If the instrument is to be shut down for any length of time, we recommend that the vacuum chamber be vented to prevent exhaust from the roughing pump being sucked back into the vacuum chamber. To vent the vacuum chamber, follow steps 5 to 7.**

5. Turn off the main power to the instrument. As you face the instrument where the instrument name is visible and with the ion source to your left, the switch is located on the bulkhead at the back right corner of the chassis.

6. After fifteen minutes, turn off the roughing pump. The power switch is located beside the power supply input attachment on the roughing pump.

> 📝 **Note:** The roughing pump has its own power toggle switch and must be turned off manually. The roughing pump is not controlled remotely by the system controller.

7. Let the vacuum chamber vent naturally through the orifice for ten minutes to allow the instrument to reach atmospheric pressure.

8. Unplug the mains supply cord to the instrument from the rear bulkhead near the mains switch. See Figure 1-5.

To turn on the system

Certain conditions outside the direct control of the instrument firmware must be satisfied before the turbo pumps will be initiated. The Curtain Gas™ supply must be turned on at the ion source, and the roughing pump must be turned on manually. Interlocks (pressure switches) monitored by the firmware detect if the Curtain Gas supply and the roughing pump are switched on. If the interlocks are not satisfied, the turbo pumps are not initiated.

> **Caution!** **If the ion source is removed, the system electronics will be disabled, interrupting any data acquisition tasks. The turbo pump and the vacuum system will not be affected.**

1. Turn on the roughing pump, if it was turned off. The power switch is located beside the power supply input attachment on the roughing pump.

    > **Note:** The roughing pump has its own power toggle switch and must be turned on manually. The roughing pump is not controlled remotely by the system controller.

2. Make sure that all gas supplies are flowing correctly to the instrument.

3. Plug the mains supply cord into the bulkhead.

4. Turn on the mains switch.

5. Make sure that the GPIB (general purpose interface bus) cable is connected to both the instrument and the computer.

6. Turn on the computer, if it was turned off, and then start the Analyst software.

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. If you are uncertain about a specific fluid, do not use the fluid until confirmation is received from AB SCIEX that it will not present a hazard.

Ion Source/Gas Parameters

The parameters in Table 1-1 are optimized for different LC conditions involving flow rate. For more information about the parameters, see the Analyst software Help system.

Note: AB SCIEX recommends that you run the instrument with the Curtain Gas flow set to at least 20 to maintain good instrument performance.

Table 1-1 Ion Source/Gas Parameters

Parameter	Value
Curtain Gas (CUR)	20
IonSpray Voltage (IS)	5000
Temperature (TEM)	700
Ion Source Gas (GS1)	60
Ion Source Gas 2 (GS2)	60

Turbo V Ion Source Settings

Table 1-2 shows the recommended starting values for the X- and Y-axis parameters. For more information, see the *Turbo V™ Ion Source Operator's Guide.*

Table 1-2 Vertical and Horizontal Parameters

Parameter	Value
X-axis	5
Y-axis	5

Compound Parameters

In general, you can use the preset values for most of the parameters in Table 1-3. For more information about the parameters, see the Analyst software Help system.

Table 1-3 Compound Parameters

Parameter	Value
CAD Gas	Use the preset value and optimize for your compound.
DP (Declustering Potential)	Use the preset value and optimize for your compound.
EP (Entrance Potential)	Use the preset value.
CXP (Collision Cell Exit Potential)	Use the preset value and optimize for your compound.
CE (Collision Energy)	Use the preset value and optimize for your compound.
CES (Collision Energy Spread)	Use the preset value and optimize for your compound.
Fixed LIT Fill Time	Use the preset value.

Table 1-3 Compound Parameters (cont'd)

Parameter	Value
DFT (Dynamic Fill Time)	Either select or deselect the feature based on your experiment.
	In the **Tools** > **Settings** > **Method Options** dialog box, the Dynamic Fill Time settings are optimized for the 10000 Da/s scan speed. These settings are also suitable for other LIT scan speeds.
Q0 Trapping	Either select or deselect the feature based on your experiment.
	The recommended fixed fill time to use with Q0 trapping is 20 ms or greater.
MCS (Multi-Charge Separation) Barrier	Use the preset value.
Q3 Entry Barrier	Use the preset value.
Q3 Empty Time	Use the preset value.
MS/MS/MS Fragmentation Time	Use the preset value.
Q3 Cool Time	Use the preset value.
TDF CE (Time Delayed Fragmentation Collision Energy)	Use the preset value.
IE1 (Ion Energy 1) — *For experienced instrument operators only*	Do not adjust.
IE3 (Ion Energy 3) — *For experienced instrument operators only*	Do not adjust.

Related Documentation

The guides and tutorials for the instrument and the Analyst software are installed automatically with the software and are available from the Start menu: All Programs > AB SCIEX. A complete list of the available documentation can be found in the online Help. To view the Analyst software Help, press F1.

Technical Support

AB SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the instrument or any technical issues that may arise. For more information, visit the Web site at http://www.absciex.com.

The following tables contain generic parameters for the 3200 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 2-1 3200 Series Instrument Parameters

Parameter ID	Access ID	Positive ion mode			Negative ion mode		
		Q1	Q3	MS/MS	Q1	Q3	MS/MS
CUR	CUR	10 10 to 50	10 10 to 50	10 10 to 50	10 10 to 50	10 10 to 50	10 10 to 50
CAD	CAD	0 n/a	2 0 to 12	3 0 to 12	0 n/a	2 0 to 12	3 0 to 12
IS[1]	IS	5000 0 to 5500	5000 0 to 5500	5000 0 to 5500	–4200 –4500 to 0	–4200 –4500 to 0	–4200 –4500 to 0
IS[2]	IS	1000 0 to 5500	1000 0 to 5500	1000 0 to 5500	–1000 –4500 to 0	–1000 –4500 to 0	–1000 –4500 to 0
IS[4]	IS	1500 0 to 2500	1500 0 to 2500	1500 0 to 2500	–1500 –2500 to 0	–1500 –2500 to 0	–1500 –2500 to 0
NC[3]	NC	1 0 to 5	1 0 to 5	1 0 to 5	–1 –5 to 0	–1 –5 to 0	–1 –5 to 0
NC[5]	NC	1 0 to 5	3 0 to 5	3 0 to 5	–3 –5 to 0	–3 –5 to 0	–3 –5 to 0
TEM[1,3,4,5]	TEM	0 0 to 750	0 0 to 750	0 0 to 750	0 0 to 750	0 0 to 750	0 0 to 750
OR	DP	20 0 to 400	20 0 to 400	20 0 to 400	–20 –400 to 0	–20 –400 to 0	–20 –400 to 0
Q0 (EP = –Q0)	EP	10 1 to 12	10 1 to 12	10 1 to 12	–10 –12 to –1	–10 –12 to –1	–10 –12 to –1
IQ1 (IQ1 = Q0 + offset)	IQ1	Q0 +(– 1) –2 to –1	Q0 +(– 1) –2 to –1	Q0 +(– 1) –2 to –1	Q0 + 1 1 to 2	Q0 + 1 1 to 2	Q0 + 1 1 to 2
[1] TurboIonSpray® ion source [2] NanoSpray® ion source [3] DuoSpray™ ion source (1 = TurboIonSpray ion source, 2 = Heated Nebulizer) [4] PhotoSpray® ion source [5] Heated Nebulizer [6] 1 = On, 0 = Off							

Table 2-1 3200 Series Instrument Parameters (cont'd)

Parameter ID	Access ID	Positive ion mode			Negative ion mode		
		Q1	Q3	MS/MS	Q1	Q3	MS/MS
ST (ST = Q0 + offset)	ST	Q0 +(−5) −8 to −2	Q0 +(−5) −8 to −2	Q0 +(−5) −8 to −2	Q0 + 5 2 to 8	Q0 + 5 2 to 8	Q0 + 5 2 to 8
RO1 (IE1 = Q0 − RO1)	IE1	1 0.5 to 2	n/a	1 0.5 to 2	−1 −2 to −0.5	n/a	−1 −2 to −0.5
RO1 (RO1 = Q0 + offset)	RO1	n/a	Q0 + (−2) −2 to −0.5	n/a	n/a	Q0 + 2 0.5 to 2	n/a
IQ2 (CEP = Q0 − IQ2)	CEP	Mass Dependent 0 to 188	n/a	Mass Dependent 0 to 188	Mass Dependent −188 to 0	n/a	Mass Dependent −188 to 0
IQ2 (IQ2 = RO2 + offset)	IQ2	n/a	RO2 + 0 0 to 2	n/a	n/a	RO2 + 0 −2 to 0	n/a
RO2 (CE = Q0 − RO2)	CE	n/a	n/a	30 5 to 130	n/a	n/a	−30 −130 to −5
RO2	RO2	−100 −150 to −20	−20 −130 to −5	n/a	100 20 to 150	20 5 to 130	n/a
IQ3 (CXP = RO2 − IQ3)	CXP	n/a	Mass Dependent 0 to 58	5 0 to 58	n/a	Mass Dependent −58 to 0	−5 −58 to 0
IQ3	IQ3	−125 −200 to −100	n/a	n/a	125 100 to 200	n/a	n/a
RO3 (IE3 = RO2 − RO3)	IE3	n/a	4 0.5 to 8	4 0.5 to 8	n/a	−4 −8 to −0.5	−4 −8 to −0.5
RO3	RO3	−150 −200 to −150	n/a	n/a	150 150 to 200	n/a	n/a
EX	EX	−200 n/a	−200 n/a	−200 n/a	200 n/a	200 n/a	200 n/a

[1] TurboIonSpray® ion source [2] NanoSpray® ion source [3] DuoSpray™ ion source (1 = TurboIonSpray ion source, 2 = Heated Nebulizer) [4] PhotoSpray® ion source [5] Heated Nebulizer [6] 1 = On, 0 = Off

Table 2-1 3200 Series Instrument Parameters (cont'd)

Parameter ID	Access ID	Positive ion mode			Negative ion mode		
		Q1	Q3	MS/MS	Q1	Q3	MS/MS
DF	DF	−100	−100	−100	100	100	100
		−400 to 0	−400 to 0	−400 to 0	0 to 400	0 to 400	0 to 400
CEM	CEM	1800	1800	1800	1800	1800	1800
		500 to 3297	500 to 3297	500 to 3297	500 to 3297	500 to 3297	500 to 3297
GS1	GS1	20	20	20	20	20	20
		0 to 90	0 to 90	0 to 90	0 to 90	0 to 90	0 to 90
GS2	GS2	0	0	0	0	0	0
		0 to 90	0 to 90	0 to 90	0 to 90	0 to 90	0 to 90
ihe[6]	ihe	1	1	1	1	1	1
		0 to 1	0 to 1	0 to 1	0 to 1	0 to 1	0 to 1
C2	C2	0	0	0	0	0	0
		n/a	n/a	n/a	n/a	n/a	n/a
XA3	XA3	0	0	0	0	0	0
		n/a	n/a	n/a	n/a	n/a	n/a
XA2	XA2	0	0	0	0	0	0
		n/a	n/a	n/a	n/a	n/a	n/a
IHT[2]	IHT	40	40	40	40	40	40
		0 to 250	0 to 250	0 to 250	0 to 250	0 to 250	0 to 250
svp[3]	svp	1	1	1	1	1	1
		1 to 2	1 to 2	1 to 2	1 to 2	1 to 2	1 to 2
[1] TurboIonSpray® ion source [2] NanoSpray® ion source [3] DuoSpray™ ion source (1 = TurboIonSpray ion source, 2 = Heated Nebulizer) [4] PhotoSpray® ion source [5] Heated Nebulizer [6] 1 = On, 0 = Off							

Table 2-2 3200 QTRAP® System Parameters For LIT Scan Types Only

Parameter ID	Access ID	Positive ion mode	Negative ion mode
CAD	CAD	High Low–Medium–High	High Low–Medium–High
FI2	CEP	Mass Dependent 0 to 188	Mass Dependent −188 to 0
ROS (Q0 – RO2)	CE	30 5 to 130	−30 −5 to −130
AF2*	AF2	100 0 to 200	100 0 to 200
AF3	AF3	Mass-Speed Dependent 0 to 5	Mass-Speed Dependent 0 to 5
EXB	EXB	Mass-Speed Dependent −200 to 0	Mass-Speed Dependent 0 to 200
DF	DF	−400 n/a	400 n/a
C2B	C2B	Mass-Speed Dependent −500 to 500	Mass-Speed Dependent −500 to 500
CES	CES	0 −50 to 50	0 −50 to 50
* MS/MS/MS only ** Resolution Parameter			