Agilent 6400 Series Triple Quad LC/MS System

Installation Guide

Agilent Technologies

Notices

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Software Revision

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In This Guide...

This guide contains information to install the Agilent 6400 Series Triple Quad LC/MS System.

1 Instrument Installation

This chapter describes the steps that are needed to install the Triple Quad instrument.

2 Software Installation and Configuration

This chapter describes the steps that are needed to install the MassHunter software.

3 Installation Completion and System Verification

In this chapter, you finish the hardware setup.

4 Installation and Verification of Other Sources

This chapter contains the steps needed to install and verify the operation of the APCI and APPI sources.

5 Other Setup Tasks

This chapter contains tasks that are related to setting up your Triple Quad instrument.

6 Reference

This chapter contains parameters for verifying performance.

Content

1 Instrument Installation

This chapter describes the steps that are needed to install the Triple Quad instrument.

Figure 1 6490 Triple Quad LC/MS

Step 1. Unpack and set up the Triple Quad instrument

NOTE

Do not open the shipping containers until an Agilent representative is present to verify the contents of each carton. Warranty claims for missing items are honored only if an Agilent representative is on site to verify the contents of each shipping container as it is unpacked.

Checkout and tuning samples are shipped separately to comply with shipping regulations and to ensure prompt delivery. Make sure that these samples have been stored correctly, as indicated in the instructions in the box.

1. Carefully examine all containers for external signs of damage. If damage is discovered, immediately contact the carrier and the Agilent Service Management.

   Retain shipping containers and material until contents are checked for completeness and instrument performance is verified.
2. Check off each item on the packing list, and verify the serial numbers.
3. Record the serial numbers in the installation documentation.
4. Report any discrepancies to Agilent Technologies.
5. Check that site preparation is complete, which includes power and gas supplies and chemical supplies needed for installation and performance verification.
6. Check the line voltage.

   Verify the proper line voltage and outlet ratings according to the instructions given in the *Site Preparation Guide*.

Step 2. Prepare to install the system

Before you begin, check that you have these parts:

- Utility knife and needle nose pliers

1 Unpack and set up the Triple Quad instrument.

a Cut the tie wraps around the package. See Figure 2.

Figure 2 Triple Quad instrument shipping container

b Lift the boxes off the top of the instrument. See Figure 3.

Figure 3 Instrument unboxed on shipping pallet (6460 pictured)

c Remove the front, top, right, back-left, and lower front-left cosmetic covers so that you can remove the shipping brackets. You do not need to remove the upper front-left cover.

To remove the top cover, remove the two screws at the back side of the top cover that secure the back of the top cover to the instrument chassis (see Figure 4). These screws are only needed when shipping the instrument. You do not need to reinstall the screws when you reinstall the top cover.

Figure 4 Remove shipping screws from back of top panel. The screws to remove are circled in this picture.

d Remove the shipping brackets (a total of 7). You will need to use a 13-mm wrench.

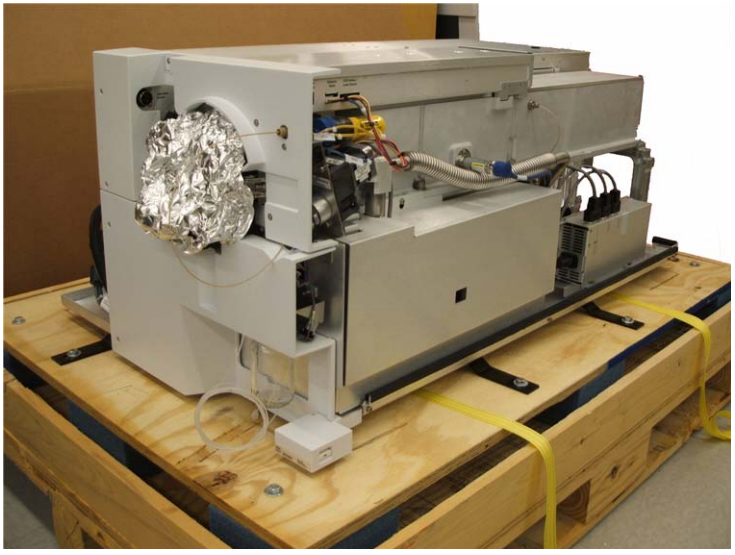


Figure 5 Triple Quad on shipping pallet with covers removed (6460 pictured)

e Take an inventory of the contents of the packages.

f Record serial numbers. The serial number label for the Triple Quad instrument is located on the lower left corner to the left of the instrument power push button.

2 Use four people, one at each corner of the instrument, to lift the Triple Quad instrument onto a stable bench or table.

WARNING **Do not lift the Triple Quad without enough assistance to do so safely. The instrument weighs up to 115 kg (255 lbs). Be sure to use correct lifting procedures to avoid possible injury. Lift with your knees, not with your back. Keep your back straight while you bend your knees.**

NOTE Do NOT lift from under the power switch bracket on the front left corner of the instrument. The bracket will bend. See Figure 6.

Figure 6 Do *not* lift from under power switch bracket

Step 3. Set up the rough pump

For 6490

You will install two rough pumps.

1 Unpack the exhaust hose and stainless steel pump oil drip pan from the shipping kit.

2 Remove the foreline pump from its shipping container.

3 Set the pump into the pump oil-drip pan.

The pan is used to catch any small amounts of oil that may seep out of the pump. The pan can also hold all of the oil in the pump in case there is a catastrophic seal failure.

4 Cut an appropriate length of the 3/4-inch OD Tygon exhaust tubing. Connect one end of the tubing to the adapter at the pump exhaust, and connect the other end of the tubing to the lab exhaust connection.

5 Add the SW60 pump oil to the foreline pump.

6 Attach the foreline pump power cord to the LC/MS, making sure the foreline pump power switch is in the ON position.

CAUTION The pump is shipped dry. Oil must be used for proper operation. Failure to add oil before you pump down the system will damage the rough pump.

For 6490 instruments, make sure you plug both rough pumps to the pump expander box. See Figure 7.

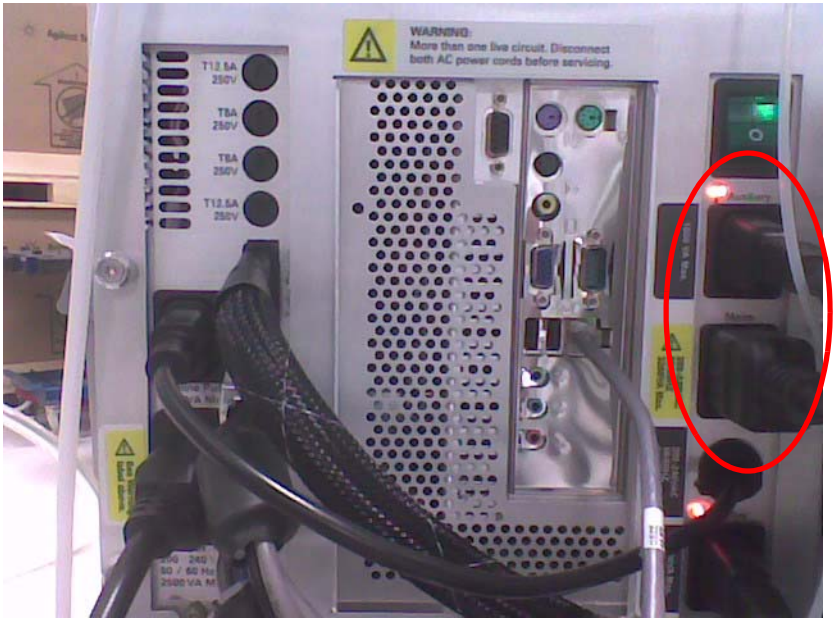


Figure 7 Back view of the 6490. Power Expansion Box AC outlets (circled) for the two rough pumps are towards the right.

For 6410, 6430 and 6460

1. Unpack the mist filter, exhaust hose, and pump oil drip pan from the shipping kit.
2. Remove the rough pump from its shipping container:
   - **a** Cut the straps that secure the cardboard box to the wooden pallet.
   - **b** Lift off the cardboard box.
   - **c** Set aside the two bottles of pump oil and funnel.
   - **d** Set aside the oil return kit. The felt plugs and screen inside the packaging are not needed and may be discarded.
   - **e** Use a pair of needle nose pliers to remove the metal clips that secure the pump to the wooden pallet.
3. Set the pump into the pump oil drip pan.

The pan is used to catch any small amounts of oil that may seep out of the pump. The pan can also contain all of the oil in the pump in case of a catastrophic seal failure.

4 Fill the rough pump with Inland 45 fluid.

The rough pump is drained of vacuum pump oil prior to shipment. Remove the oil fill cap and use the funnel to add Inland 45 pump fluid until the level in the site glass is approximately 2/3 full.

5 Install the oil mist filter.

Remove the plugged outlet connector and install the new KF-25 fitting that comes with the pump. Do not use the outlet nozzle as described in the Edwards manual and on the warning tag. Install the mist filter on the outlet port KF-25 fitting. See figure on the next page.

Make sure the mist filter drain plug is facing the rough pump ballast valve (toward the motor end of the pump).

6 Install the oil return line:

a Remove the drain plug from the mist filter and install the drain adapter.

b Install the brass restrictor into the black oil-return tubing.

c Connect the oil-return tubing between the rough pump ballast port fitting and the oil mist filter drain adapter. Make sure the end of the tubing with the brass restrictor is installed at the ballast port fitting.

d Use the plastic hose clips and a pair of needle nose pliers to secure the oil-return tubing.

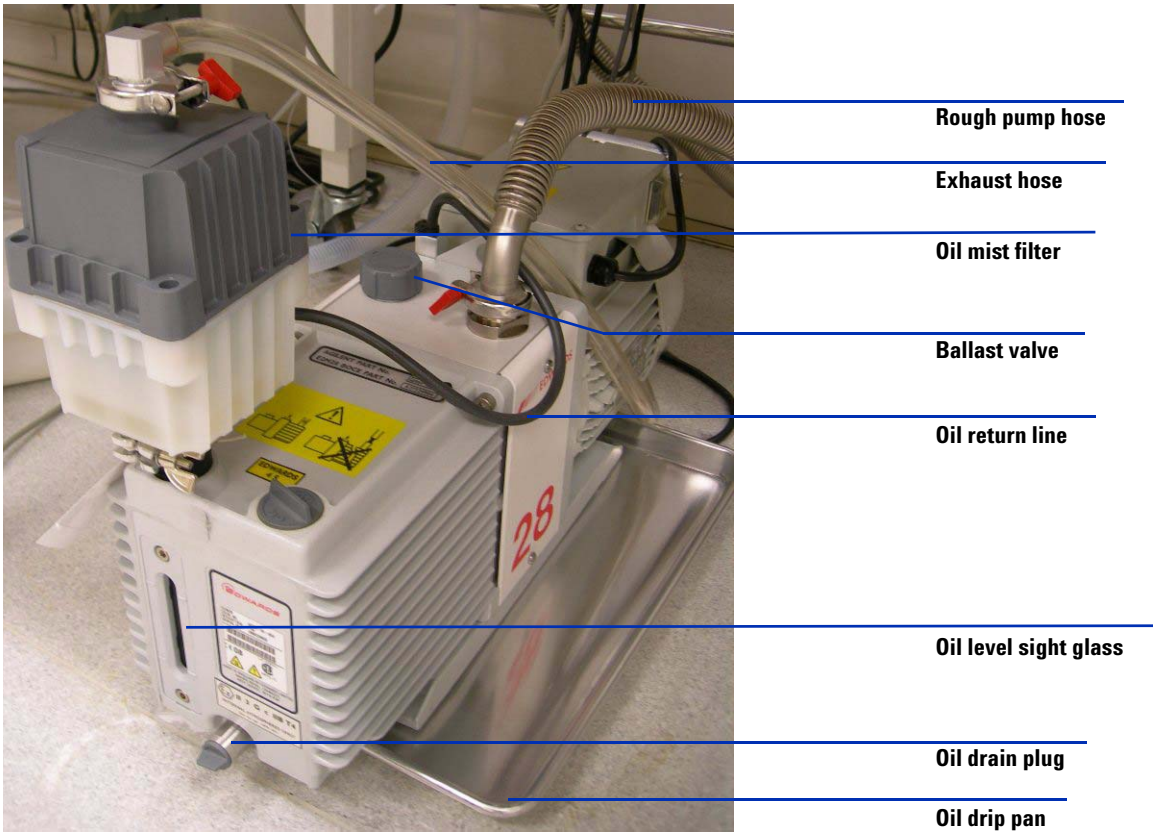


Figure 8 Example rough pump. Note that your pump may look slightly different.

7 Check that the ballast valve is closed.

Closing the ballast valve prevents additional air from being introduced to the pump. This allows the rough pump to reach a lower operating pressure.

CAUTION The ballast valve must be opened (fully counterclockwise) periodically to allow oil trapped in the mist filter to return to the pump. If the oil is not returned to the pump, the pump will eventually run dry and be damaged.

8 Find the Low Profile Exhaust Adaptor (p/n G1960-20003) and the O-ring (p/n 0905-1592). Put the O-ring on the exhaust adaptor.

9 Locate one KF-25 swing clamp (p/n 0100-1398), open the swing clamp, and use it to clamp the exhaust adaptor onto the oil mist filter. Connect the 1/2-inch I.D. Tygon exhaust tubing to the exhaust adaptor.

Step 4. Connect the Triple Quad instrument

1. Connect the rough hose (p/n G1969-20123) that is supplied in the ship kit to the KF-25 rough pump hose connection on the back left of the instrument.
2. Connect the rough pump hose to the KF-25 inlet adapter on the rough pump.
3. Use the supplied hook/loop fastener tape to secure the drain bottle into a secondary containment tub.
4. Connect the spray chamber drain tube to the 1-inch fitting on the drain bottle. The drain tube can also be routed out the front of the Triple Quad if needed.
5. Attach the calibrant delivery system (CDS)/inlet module waste tubing from the solvent selection valve on the Triple Quad to the ¼-inch fitting on the drain bottle.
6. Connect a length of the ½-inch Tygon tubing to the ½-inch fitting on the drain bottle, then connect the other end of the tubing to a vent connection that is separate from the vent used for the rough pump.

CAUTION **The drain bottle vent must be located away from the rough pump vent to prevent the rough pump exhaust from contaminating the Triple Quad spray chamber.**

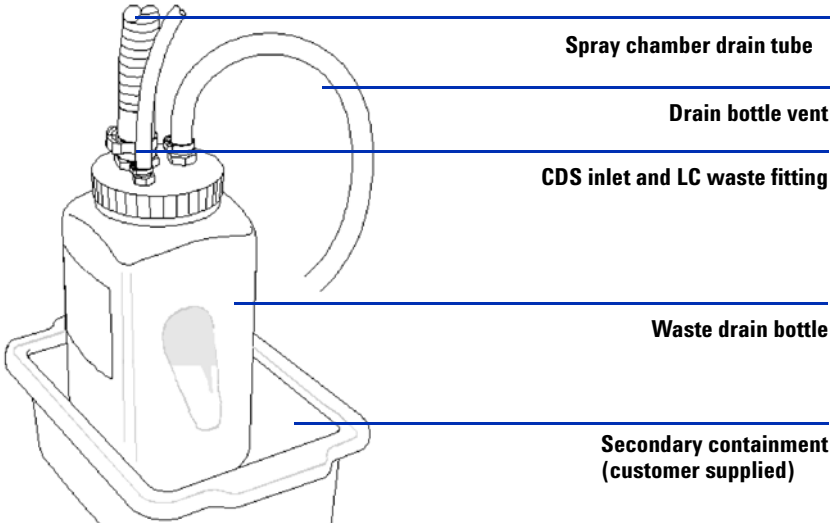


Figure 9 Drain bottle

7 Install the gas filter:

For 6410 and 6430:

a Cut an appropriate length of the 1/4-inch teflon gas tubing (G1946-80078) and connect to the nitrogen gas supply regulator or nitrogen generator.

b Use the supplied nut and ferrule kit (5183-0392) to attach the other length of tubing to the RMSN-4 gas filter. This configures the filter in series with the supply tubing. See Figure 10 as an example for assembling the gas trap in the correct configuration.

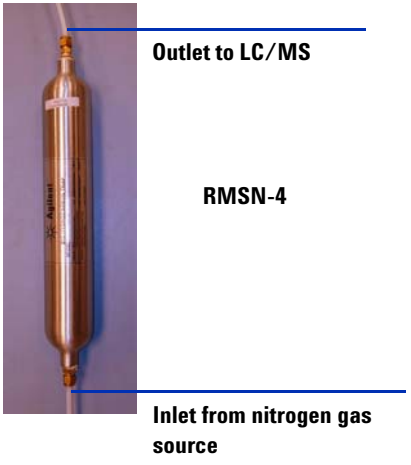


Figure 10 Gas filter connected in series

For 6460 and 6490:

a Cut an appropriate length of the 1/4-inch teflon gas tubing (p/n G1946-80078) and connect to the nitrogen gas supply regulator or nitrogen generator.

b Locate the 2 1/4-inch brass T-unions (0100-0088) and loosen the brass nuts on the T-unions.

c Connect the other end of the supply tubing to the T-union.

d Cut four 4-inch lengths of teflon tubing from the supplied 10 meter length (G1946-80078).

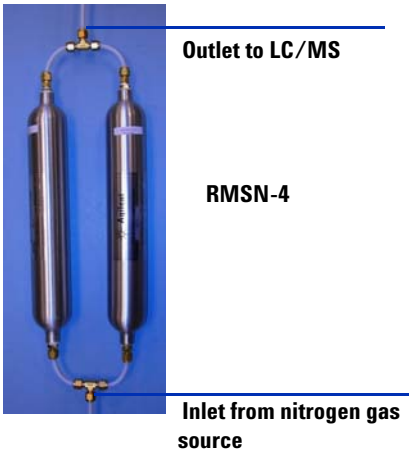


Figure 11 Gas filter connected in parallel

e Connect two of the 4 inch lengths to each side of the T-unions. Use the supplied nut and ferrule kit (5183-0392) to attach the other side 4 inch the tubing to the two RMSN-4 gas filters. This configures the filters in parallel. See Figure 11 as an example for assembling the gas traps in the correct configuration.

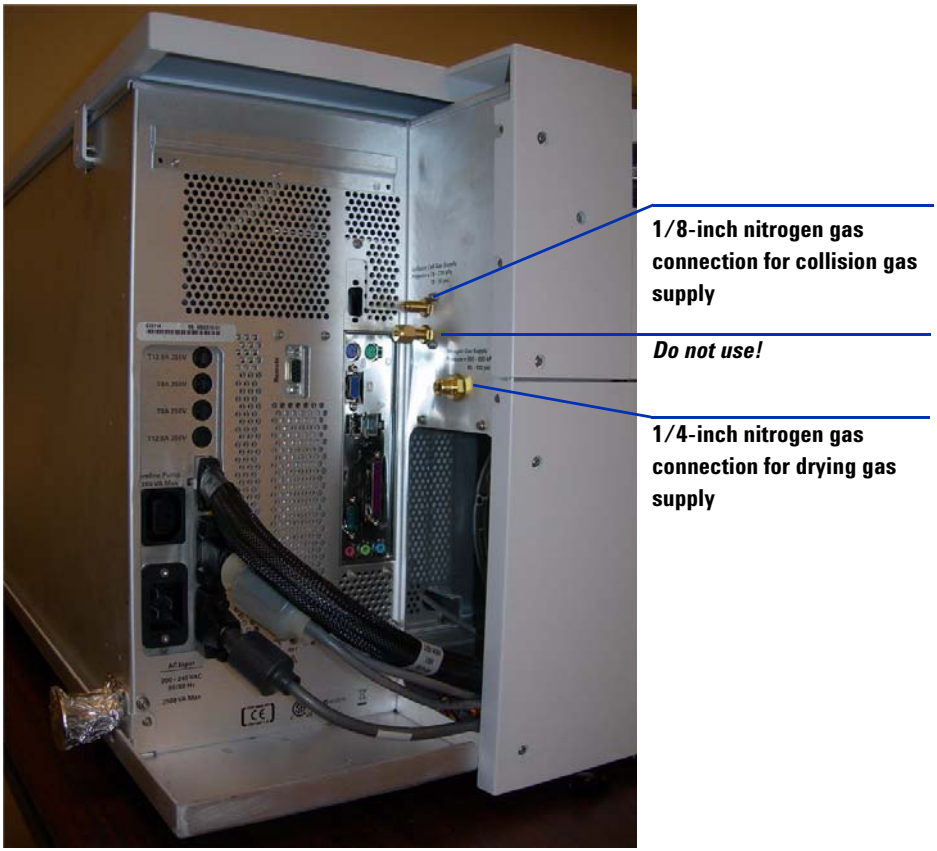


Figure 12 Power and gas connections on back left of Triple Quad

8 Set the pressure on the nitrogen supply regulator at 80 to 100 psi (550 to 690 kPa). Turn on the nitrogen gas for a few minutes to purge the tubing and gas conditioner before you connect the nitrogen gas supply to the Triple Quad. Regulator pressures above 110 psi (760 kPa) cause nitrogen waste due to release from the bleed valve on the flow-control module.

9 Use the tubing cutters (p/n 8710-1709) supplied in the ship kit to cut an appropriate length of the 1/8-inch copper supply tubing (p/n 5180-4196) to connect from the outlet fitting on the collision cell nitrogen tank regulator to the nitrogen gas conditioner (p/n RMSN-2) for the collision cell gas, then cut a second length of the 1/8 -inch copper supply tubing to connect from the nitrogen gas conditioner to the collision cell gas inlet fitting on the left

side of the Triple Quad (see Figure 12). Secure the gas filters in a vertical position with tie wraps.

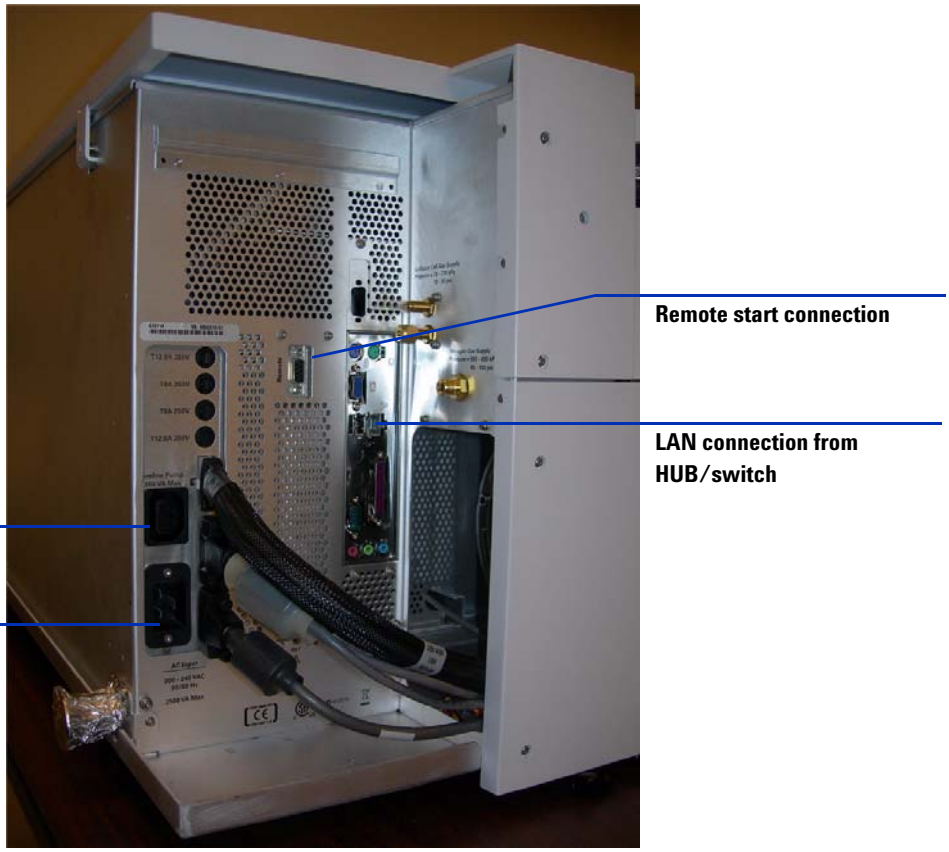


Figure 13 Power and electrical connection at back left side of Triple Quad

10 Set the pressure on the Collision Cell supply regulator at 10 to 30 psi (70 to 210 kPa).

CAUTION Do not attach the Collision Cell gas fitting to the nitrogen supply regulator at 80 to 100 psi (550 to 690 kPa). Pressure to the Collision Cell Gas Flow Module greater than 30 psi (210 kPa) can cause fewer collisions to be available for secondary collision induced dissociation (CID) and poor peak shapes, as well as poor high vacuum. The Collision Cell can use the same nitrogen supply as the regular supply (i.e. for drying gas, nebulizer, CDS pressure) if it is the required purity (99.999%) but it must be down-regulated to 30 psi (210 kPa).

11 Connect the remote start cable to its connector on the back left side of the Triple Quad. See Figure 13.

12 Connect the rough pump power cord into the rough pump connector on the back left side of the Triple Quad (see Figure 13).

13 Verify the front power switch is in the OFF position.

14 Plug the Triple Quad power cord into the Triple Quad power connector on the Triple Quad and the other end of the Triple Quad power cord into the wall outlet. See Figure 13.

15 Plug the Power Expansion box power cord into the wall outlet.

Step 5. Install the source

You can use an electrospray, the multimode source or the HPLC-Chip for 6410 check out.

ESI with Agilent Jet Stream Technology (6460 only)

Before you begin, check that you have these parts:

- Electrospray with Agilent Jet Stream Technology
- ESI-L Low Concentration Tuning Mix (p/n G1969-85000)

1 Remove the top cover by loosening the T10 lock screw and lifting off the instrument chassis.

2 Remove the electrospray interface with Agilent Jet Stream Technology from the packaging inside the instrument chassis next to the e-Module. See Figure 14.

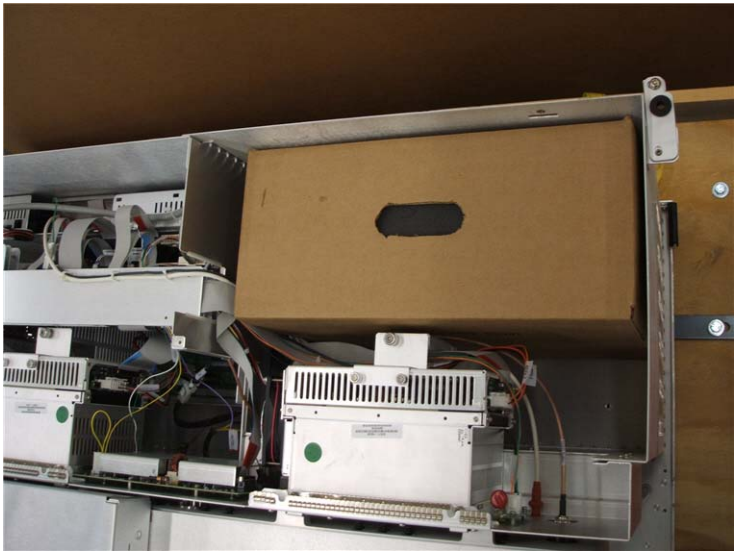


Figure 14 Packaging for ESI with Agilent Jet Stream Technology

3 Remove the foil that covers the spray chamber mount, and remove the shipping cover from the Electrospray spray chamber.

4 Put the nebulizer in the nebulizer adjustment fixture supplied in the shipping kit and check that the nebulizer needle is properly adjusted. The nebulizer needle should be even with the end of the nebulizer nozzle.

5 Install the nebulizer in the spray chamber.

6 Install the spray chamber on the spray chamber mount, close the spray chamber, and fasten the latch. You may need to adjust the latch to ensure that the O-ring seals completely. Use a ¼-inch x 5/16-inch wrench to loosen the lock nut, then adjust the latch to the proper fit, then tighten the lock nut so that the latch maintains its adjustment.

7 Connect the high voltage and vaporizer cables to the connectors on the left side panel of the instrument.

8 Connect the 1/8-inch nebulizing gas tubing from the Triple Quad mainframe to the nebulizer gas fitting.

9 Connect the 1/8-inch sheath gas tubing from the Triple Quad mainframe to the sheath gas fitting on the ESI with Agilent Jet Stream Technology.

10 Connect the PEEK tubing from the selection valve (inside the front cover) to the top of the nebulizer.

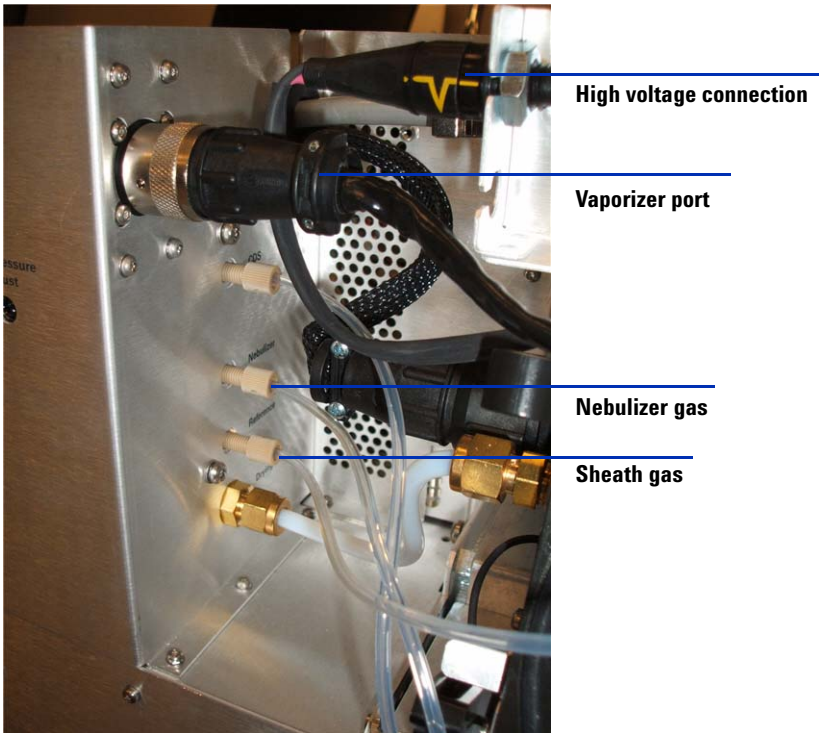


Figure 15 Connections for ESI with Agilent Jet Stream Technology

11 Rinse the calibrant bottle three times with hot (60°C) water and then three times with methanol. Then rinse it once with acetonitrile.

12 Add the ESI-L Low Concentration Tuning Mix to the calibrant bottle.

CAUTION Never use aliphatic, aromatic or halogenated hydrocarbons in the CDS. These solvents are not compatible with the O-ring in the CDS.

13 Turn on the Triple Quad front power switch to start the pump-down of the Triple Quad.

The rough pump will become quieter within a few seconds.

Agilent G1948B Electrospray

Before you begin, check that you have these parts:

- Agilent G1948B Electrospray interface (the G1948A Electrospray interface is not supported)
- Nebulizer spacer kit (p/n G1946-60254)
- ESI-L Low Concentration Tuning Mix (p/n G1969-85000)

1. Remove the foil that covers the spray chamber mount, and remove the shipping cover from the Electrospray spray chamber.
2. Install the nebulizer spacer as follows:
   a. Use a flat-bladed screwdriver to remove the two nebulizer shoulder screws from the top of the spray chamber.
   b. Use the two m3x8 Torx T10 screws to install the nebulizer spacer onto the top of the spray chamber.
   c. Install the two nebulizer shoulder screws into the top of the nebulizer spacer.
3. Put the nebulizer in the nebulizer adjustment fixture supplied in the shipping kit and check that the nebulizer needle is properly adjusted. The nebulizer needle should be even with the end of the nebulizer nozzle.
4. Install the nebulizer in the spray chamber.
5. Install the spray chamber on the spray chamber mount, close the spray chamber, and fasten the latch. You may need to adjust the latch to ensure that the O-ring seals completely. Use a ¼-inch x 5/16-inch wrench to loosen the lock nut, then adjust the latch to the proper fit, then tighten the lock nut so that the latch maintains its adjustment.
6. Connect the 1/8-inch nebulizing gas tubing from the Triple Quad mainframe to the nebulizer gas fitting.
7. Connect the PEEK tubing from the selection valve (inside the front cover) to the top of the nebulizer.
8. Rinse the calibrant bottle three times with hot (60°C) water and then three times with methanol. Then rinse it once with acetonitrile.
9. Add the Electrospray calibrant to the calibrant bottle.

CAUTION Never use aliphatic, aromatic or halogenated hydrocarbons in the CDS. These solvents are not compatible with the O-ring in the CDS.

10 Turn on the Triple Quad front power switch to start the pump-down of the Triple Quad.

The rough pump will become quieter within a few seconds.

G1978B Multimode Source

1 Install these parts from the Multimode and APCI enablement kits that ship with the G1978B Multimode source into the Aux module on the Triple Quad LC/MS:

- APCI High Voltage Power Supply (p/n G1946-80058)
- Multimode HV board (p/n G1960-61015)
- Valve board—APCI HV PS cable (p/n G1960-60802)
- Valve board—APCI Needle Interlock cable (p/n G1960-60856)
- Multimode HV board cable (p/n G1960-60858)
- Multimode Power Data cable (p/n G1960-60878)
- MMI-L Low Concentration Tuning Mix (p/n G1969-85020)

2 Connect the multimode and corona needle cables to the respective connectors on the left side panel of the Triple Quad LC/MS.

3 Put the nebulizer in the nebulizer adjustment fixture supplied in the shipping kit and check that the nebulizer needle is properly adjusted.

The nebulizer needle should be even with the end of the nebulizer nozzle.

4 Install the nebulizer in the spray chamber.

5 Install the spray chamber on the spray chamber mount, close the spray chamber, and fasten the latch. You may need to adjust the latch to ensure that the O-ring seals completely. Use a ¼-inch x 5/16-inch wrench to loosen the lock nut, then adjust the latch to the proper fit, then tighten the lock nut so that the latch maintains its adjustment.

6 Connect the 1/8-inch nebulizing gas tubing from the Triple Quad mainframe to the nebulizer gas fitting.

7 Connect the PEEK tubing from the selection valve (inside the front cover) to the top of the nebulizer.

8 Rinse the calibrant bottle three times with hot (60°C) water and then three times with methanol. Then rinse it once with acetonitrile.

9 Add the Multimode calibrant to the calibrant bottle.

CAUTION Never use aliphatic, aromatic or halogenated hydrocarbons in the CDS. These solvents are not compatible with the O-ring in the CDS.

10 Turn on the Triple Quad front power switch to start the pump-down of the Triple Quad.

The rough pump will become quieter within a few seconds.

Step 6. Install the Agilent 1100 or 1200 series LC system

Before you begin, check that you have these parts:

- HPLC grade methanol, or better
- HPLC grade water, or better

1 Install the Agilent 1100 or 1200 LC system. Follow its installation documents. Other LCs are not supported.

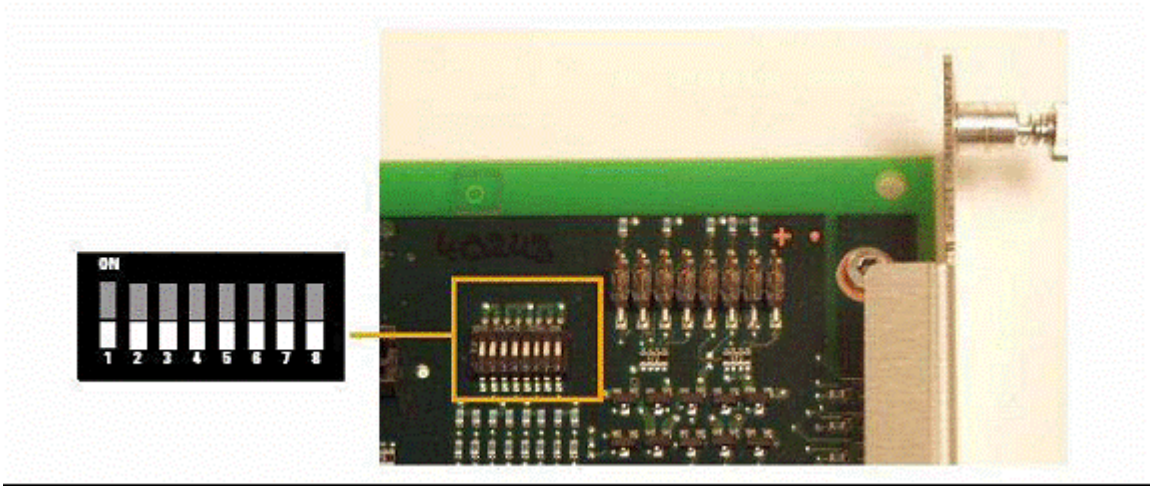
2 Connect the remote start cable from the Triple Quad to a Remote Start connector on the LC sampler, pump or other detector module only.

3 Install an Agilent G1369A LAN Interface card into the LC detector module. If a detector module is not present, install the Agilent G1369A LAN Interface card into the pump module. Before you install the LAN Interface card, set switches 5 and 6 to the ON position. This will force the Agilent LAN Interface Card to use its default IP address and subnet mask.

NOTE

Note: For a G1315C/D DAD SL, the LAN interface is built into the module main board. To set the G1315C/D to the proper IP address, set switch positions 7 and 8 to the ON or UP position. By default, switch positions 7 and 8 are in the down position. Refer to the *Agilent 1200 Series Diode Array and Multiple Wavelength Detector User Manual* (part number G1315-90012) for more information.

The default IP address is 192.168.254.11 and the default subnet mask is 255.255.255.0.



CAUTION **Do not use any other LAN interface card besides the G1369A (e.g. the older Jet Direct card J4100A).**

2
Software Installation and Configuration

If you purchased a bundled Triple Quad instrument system, the MassHunter Workstation software has been installed for you. In this case, skip to "Step 7. Install and configure Microsoft Excel 2007" and complete the steps that remain.

Step 1. Set up the computer system

1. Set up the PC:
   a. Verify that the line voltage is correct.
   b. Unpack the PC and put it on a suitable benchtop or table.
   c. Connect the keyboard, mouse, monitor and printer to the computer.
2. Verify that two network cards are available in the PC.

   One of these is usually a “built-in” card, and the second is located in an accessory slot. Follow the installation documentation supplied with the PC to install and verify components as needed.
3. Install the network hub:
   a. Put the hub on the table next to the PC.
   b. Plug the power supply into the hub.
   c. Connect the power supply to a power outlet.
4. Connect one end of a Category 5, shielded twisted pair (STP) cable to any port (1 to 7) on the network hub. Connect the other end of the cable to the 10/100 Base-T network card in slot 1 of the PC. The card should be labeled **LC/MS**.

   Do not connect to port 8 on the hub/switch.
5. Connect a second Category 5 STP LAN cable from one of the open ports 1 to 7 on the hub to the Agilent LAN Card in the 1100 or 1200 pump or detector.
6. Connect a third Category 5 STP LAN cable from one of the open ports 1 to 8 on the hub to the LAN interface connection on the LAN/MS control card in the Triple Quad instrument.
7. Install the printer. See the installation documentation supplied with the printer.
8. Turn on the printer, hub, monitor, and PC, in that order.

The computer comes loaded with software and the appropriate drivers for the installed accessories. The initial Administrator password is provided on a sticker on the PC.

Step 2. Check PC network card configuration

1 Click **Start > Control Panel > Network Connections**.

2 From the list of network connections, right-click the network connection that corresponds to the instrument LAN connection, then click **Properties**.

3 Scroll down the list to Internet IP Protocol, click it, then click **Properties**.

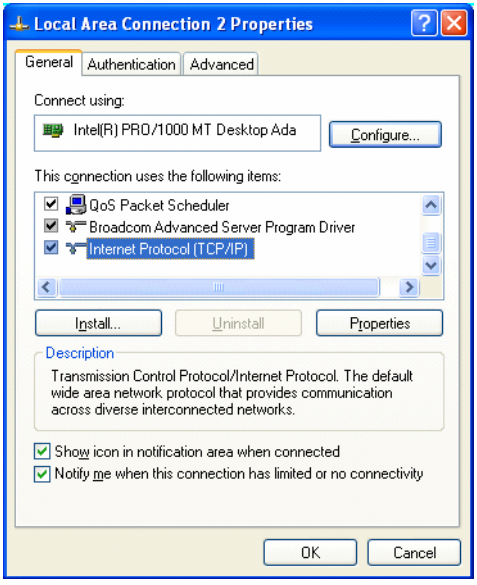


Figure 16 Local Area Connection Properties dialog box - General tab

4 Make sure the Network Adapter is set to these settings:

IP address: 192.168.254.1

Subnet mask: 255.255.255.0

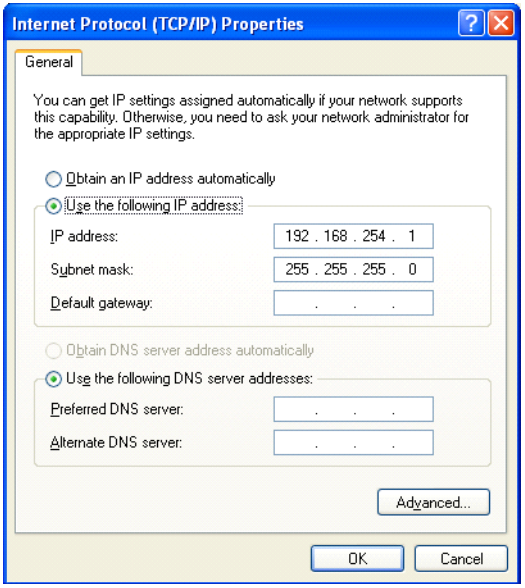


Figure 17 Internet Protocol (TCP/IP) Properties dialog box

Step 3. Prepare for installation

1 If this installation is an upgrade, use the **Add or Remove Programs** command in Control Panel to remove programs that you will be upgrading, if they are installed on your system. *Do not remove programs for which you need only to install a service pack.*

- Excel 2003
- Agilent MassHunter Workstation Qualitative Analysis
- Agilent MassHunter Workstation Quantitative Analysis
- Agilent MassHunter Workstation Quantitative Analysis Reporting

NOTE Remove only the programs that you plan to upgrade at this time. Do not remove the Agilent MassHunter Workstation Data Acquisition or the MassHunter Optimizer program at this time.

2 *Optional.* Back up the data, method and worklist files in the **D:\MassHunter** folder. Use the back-up method of your choice.

The uninstallation program keeps your data, method and worklist files. It also backs up your tuning and hardware setting files, which are restored during installation. Do not move, remove or rename the D:\MassHunter folder, or your tuning and hardware settings will not be saved.

3 Run Windows Update to make sure you have the latest critical updates and security fixes.

Make sure Windows Update is completed before you continue.

CAUTION If you do not run Windows Update, your system may be vulnerable to security problems. Confirm that the LAN power management has not been reactivated by a Windows Update program.

Step 4. Install the Data Acquisition program

The Data Acquisition program is pre-installed on the shipping PC when ordered together with the instrument as a bundle. If the program is already installed, continue at "Step 7. Install and configure Microsoft Excel 2007" on page 49.

If this is an upgrade from the same revision, such as from B.03.01 to B.03.01 SP1, continue at "Step 5. Install the Qualitative Analysis program" on page 43.

1 Check that no other program is running on your system, including Windows Update.

2 Put the Data Acquisition installation disk into the disk drive.

The welcome screen appears. See Figure 18.

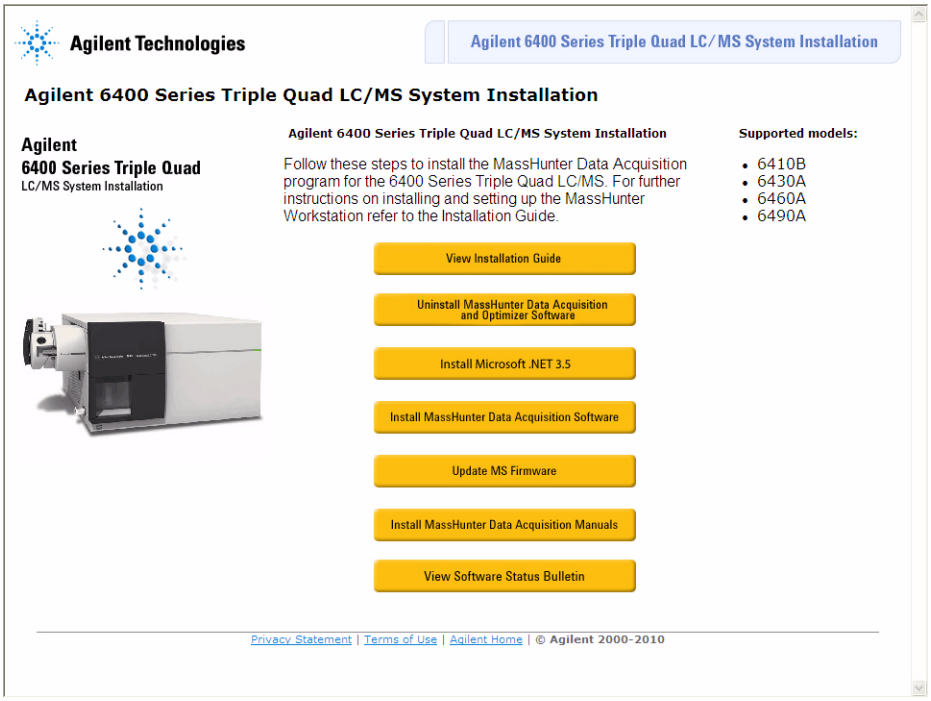


Figure 18 Installation Welcome Screen

3 If this is an upgrade installation, click **Uninstall MassHunter Data Acquisition and Optimizer Software**, then follow the instructions on the screen to remove the current program.

4 Click **Install MassHunter Data Acquisition Software**, then follow the instructions on the screen to install the software.

- In the License Agreement screen, click **I accept the terms of the license agreement**, then click **Next**.
- In the Choose Destination Location screen, click **Next** to accept the default destination location.

  The default is **C:\Program Files\Agilent\MassHunter\Workstation\Acq**.
- In the Choose location for customer data files screen, check that the default of `D:\MassHunter` is displayed in the text box, then click **Next**. See Figure 19.

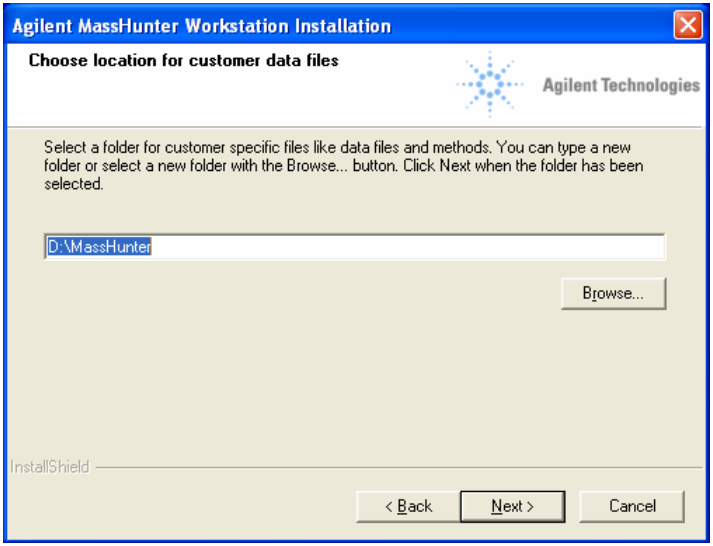


Figure 19 Choose location for customer data files screen

- In the Start Copying Files screen, review the settings then click **Next**.

  The Setup Status screen now appears.
- If you get a message that indicates that the screen resolution is *not* set to the required 1280 x 1024 resolution, click **OK**.

The best resolution for MassHunter is either 1600 x 1200 (for 4 x 3 aspect ratio) or 1920 x 1080 (for 16 x 9).

- If you see a Notepad window that prompts you to install other options, close the Notepad window.
- When the InstallShield Wizard Complete message is displayed, click **Finish**.

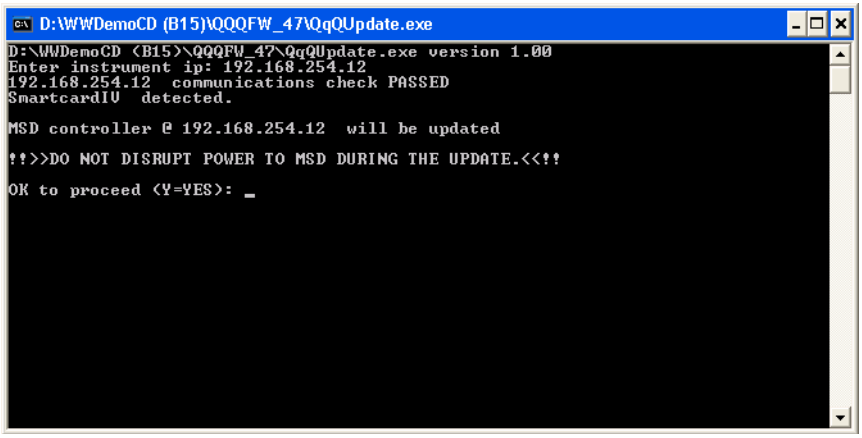
5 Click Install Microsoft .NET 3.5 and follow the instructions, if any, to install Microsoft .NET Framework 3.5.

6 If this installation is a software-only upgrade:

a Click **Upgrade MS Firmware**.

b Press **Enter** to accept the default IP address for the Triple Quad instrument (`192.168.254.12`).

c When prompted, type **Y** for yes to proceed with update.

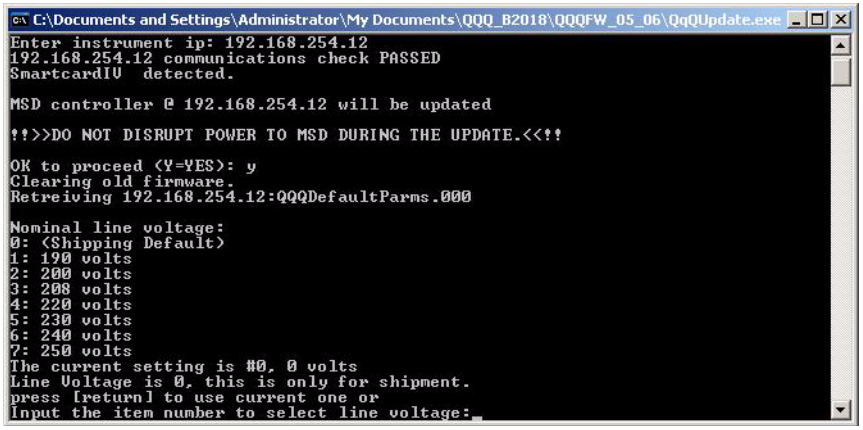


7 If the instrument is using an ESI with Agilent Jet Stream Technology and this is a new installation:

a In Windows Explorer, find the folder **\Support\QQQFirmware\QQQFW_0*x_xx***, where *x_xx* represents the version of the shipping firmware shipping, then run **QqQUpdate.exe**.

b Type the IP address of the Triple Quad instrument (192.168.254.12) and press **Enter**.

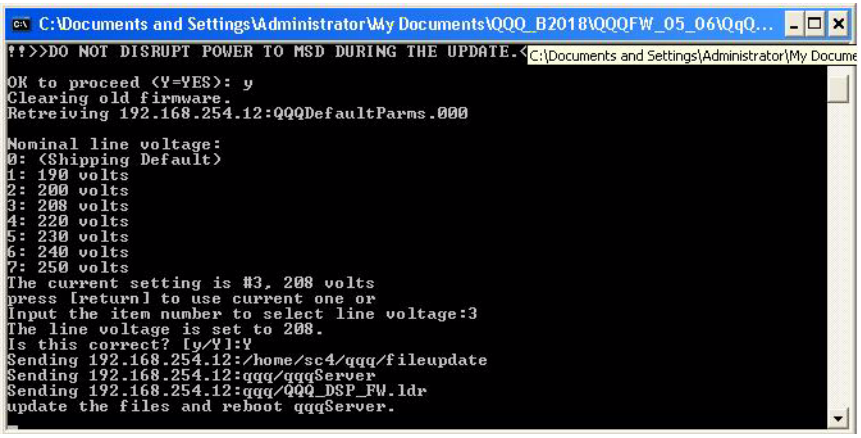
c When prompted, enter the country-specific nominal input voltage. Verify with a Digital Multi-Meter (DM) using the AC Voltage measuring function.



For example, in the United States, the closest nominal voltage for the Triple Quad instrument is 208V so you would select option 3.

CAUTION The ESI with Agilent Jet Stream Technology uses 95% input voltage duty cycle to heat properly. Improper settings can severely hamper its performance.

d When prompted, confirm input voltage set in the previous step.



e Reboot the LC/MS interface card, or turn on and off the instrument, when prompted.

Once the firmware update has completed, you will be prompted to close the DOS window.

8 *Optional.* Click **Install MassHunter Data Acquisition Manuals** to copy the user guides to the location of your choice.

The installation program automatically copies the MassHunter user guides onto your computer so that they can be opened from online Help. The user guides are also available on the installation disk in the Manuals folder. Click this button only if you want an additional set of user guides copied to the location of your choice.

Step 5. Install the Qualitative Analysis program

The Qualitative Analysis and Quantitative Analysis programs are pre-installed on the shipping PC when ordered together with the instrument as a bundle. If the programs are already installed, continue with "Step 7. Install and configure Microsoft Excel 2007" on page 49.

1 Check that no other program is running on your system, including Windows Update.

   Installation of the Qualitative Analysis program will fail if Windows Update is running, even in the background.

2 Install the Qualitative Analysis program:

   a Put the Qualitative Analysis installation DVD into the DVD drive.

   b If you are installing only the service pack for the Qualitative Analysis program, continue at step 4.

   c From the installation disk, double-click **Setup.exe**.

   d In the License Agreement screen, click **I accept the terms of the license agreement**, then click **Next**.

   e In the Choose Destination Location screen, click **Next** to accept the default destination location.

      The default is **C:\Program Files\Agilent\MassHunter\Workstation\Qual**.

   f In the Choose location for customer data files screen, check that the default of `D:\MassHunter` is displayed in the text box, then click **Next**. See Figure 20.

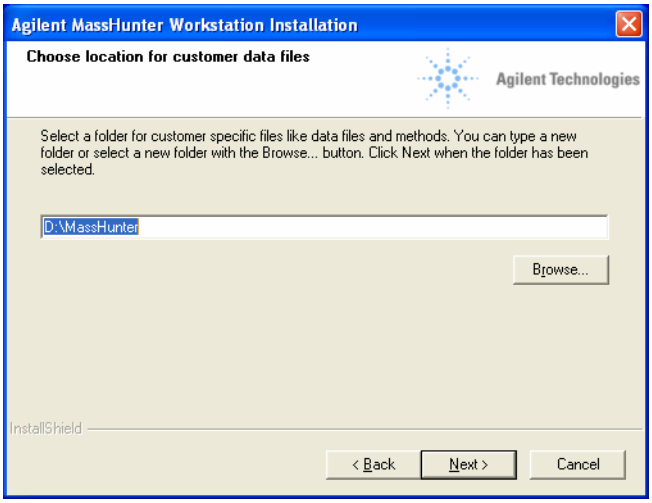


Figure 20 Location for data files

g In the Start Copying Files screen, review the settings then click **Next**.

h If you get a message that indicates that the screen resolution is not set to the required 1280 x 1024 resolution, click **OK**.

The best resolution for MassHunter is either 1600 x 1200 (for 4 x 3 aspect ratio) or 1920 x 1080 (for 16 x 9).

i If you see a Notepad window that prompts you to install other options, close the Notepad window.

3 When the InstallShield Wizard Complete message is displayed, click **Finish**.

4 From within a Windows file manager window, check the installation disk for any folder that includes the word **Service Packs**.

5 If one or more service pack files or folders exist on the installation disk, then for each service pack file:

a Double-click the name of the most recent service pack to run the installation program.

You may need to open folders inside the service pack folder to find the installation file. The name of service pack installation files typically end in **SP *x*.exe**.

b Follow the instructions to install the service pack.

Step 6. Install the Quantitative Analysis program

1 Check that no other program is running on your system.

Installation of the Quantitative Analysis program will fail if Windows Update is running, even in the background.

2 Install the Quantitative Analysis program:

a Put the Quantitative Analysis installation disk into the disk drive.

b If you are installing only the service pack for the Quantitative Analysis program, continue at step 3.

c From the root directory of the installation disk, double-click **setup_quant.exe**.

d When prompted, select the language for the installation.

e In the Welcome to the InstallShield Wizard window, click **Next** to continue with the installation.

f In the License Agreement window, click **I accept the terms in the license agreement**, then click **Next**.

g In the Destination Folder window, click **Next** to accept the default destination location.

The default is **C:\Program Files\Agilent\MassHunter\Workstation\Quant**.

h In the Customer Data Folder window, click the **Change** button and select the path used to store data files, click the **OK** button, then click **Next**.

This data file location is typically the path used by the MassHunter Data Acquisition Workstation to store acquired data.

i When the Choose Desktop Icons window appears, mark the check boxes for the instruments that apply. Clear the check boxes for the instruments that do not apply. Click **Next** (Figure 21).

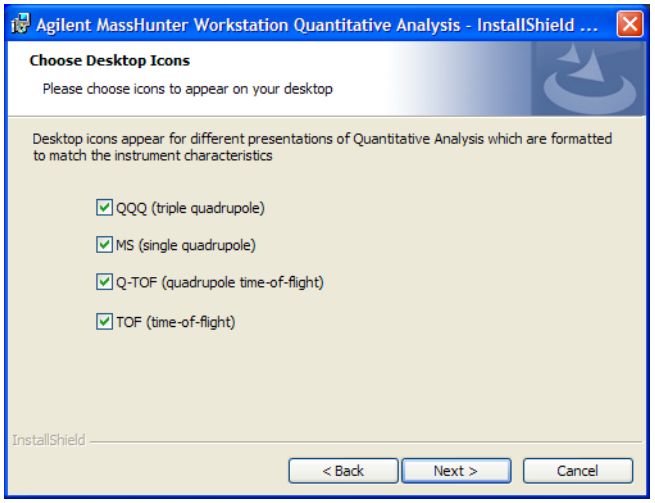


Figure 21 Choose desktop icons window

j When the Ready to Install the Program window appears, click **Install** to begin.

When the installation begins, you will see the Installing Agilent MassHunter Workstation Quantitative Analysis window.

k When the InstallShield Wizard Completed message is displayed, click **Finish.**

l Confirm that the icons you selected appear on your desktop (Figure 22).

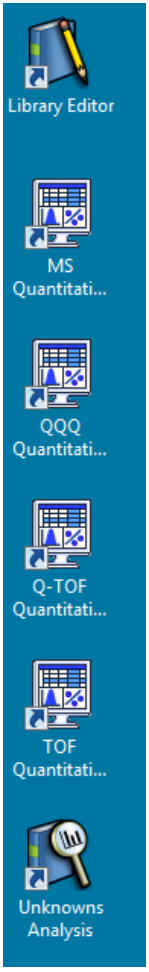


Figure 22 Desktop with Icons

3 From within a Windows file manager window, check the installation disk for any folder that includes the word **Service Packs**.

4 If one or more service pack files or folders exist on the installation disk, then for each service pack file:

   a Double-click the name of the most recent service pack to run the installation program.

You may need to open folders inside the service pack folder to find the installation file. The name of service pack installation files typically end in **SP *x*.exe**.

b Follow the instructions to install the service pack.

Step 7. Install and configure Microsoft Excel 2007

If you are doing an upgrade installation, continue at "Step 8. Install Service Packs for Data Acquisition" on page 53.

For new installations, do this step even if Excel is already installed on your system.

Agilent MassHunter Workstation software supports Excel 2007 or Office 2007.

1 Close all MassHunter Workstation programs.

2 Click **Start > All Programs > Agilent > MassHunter Workstation > Acq Tools > Remove MassHunter Processes** to make sure that all MassHunter processes are stopped.

3 Start the installation:

   a Follow the instructions that came with Excel 2007 or Office 2007 to start the installation.

   b If you are prompted to enter the Product Key, do so.

   If you do not enter the product key now, Excel 2007 will remind you later, which may interfere with report automation.

   c If you are installing Excel 2007, or if you are installing Office 2007 and you want to install all Office options, click **Install Now**. Continue at step 5.

   d If you are installing Office 2007 and you want to customize your installation, then click **Customize**.

4 If Excel 2007 is already installed, or you are doing a customized Office 2007 installation:

   - For Excel 2007, check that the disk icon next to **Microsoft Office** appears white. If not, right-click **Microsoft Office** and click **Run all from My Computer**. Then click **Continue** and continue the installation as instructed.
   - For Office 2007, check that the disk icon next to each of these programs appear white. If not, right-click the program name and click **Run all from My Computer**:

     Microsoft Office Excel
     Office Shared Features
     Office Tools

Click **Continue** and continue the installation as instructed.

Figure 23 Microsoft Office installation options. For Excel 2007, you will only see Microsoft Office Excel, Office Shared Features and Office Tools.

5 When you are prompted to get updates, click **Go to Office Online**. Follow the instructions to update Excel 2007 or Office 2007.

Make sure Service Pack 2 is installed for optimal performance of MassHunter reporting.

Figure 24 Click the **Go to Office Online** button to update Excel.

6 Check that Excel security for MassHunter Workstation add-ins is configured:

a Open Excel.

b Click the Microsoft Office Button  .

c Click **Excel Options**. See Figure 25.

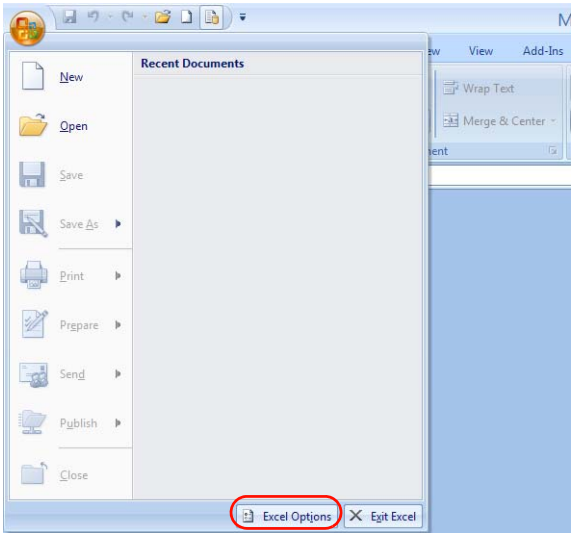


Figure 25 Excel Options button

d Click **Trust Center** in the navigation pane.

e Click **Trust Center Settings**. See Figure 26.

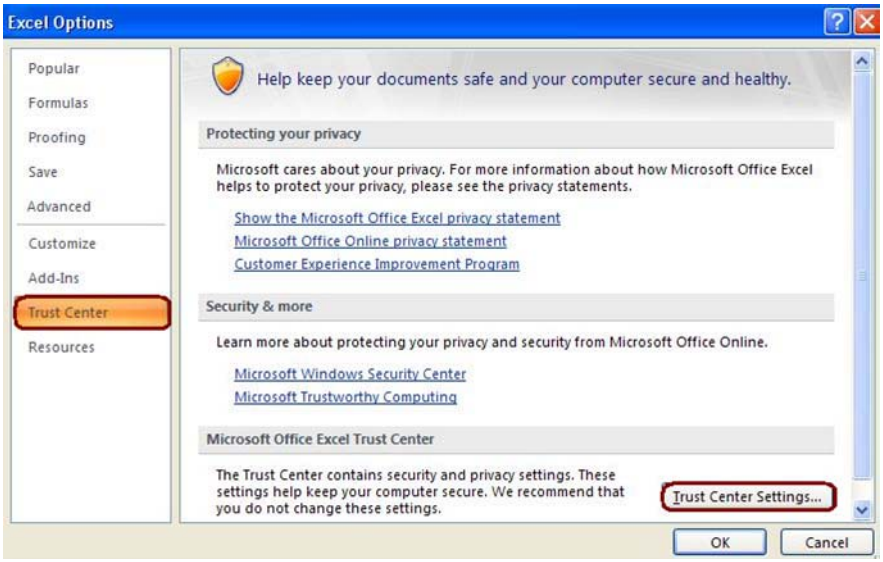


Figure 26 Excel Options window

f Make sure that the **Trust access to the VBA project object model** check box is marked.

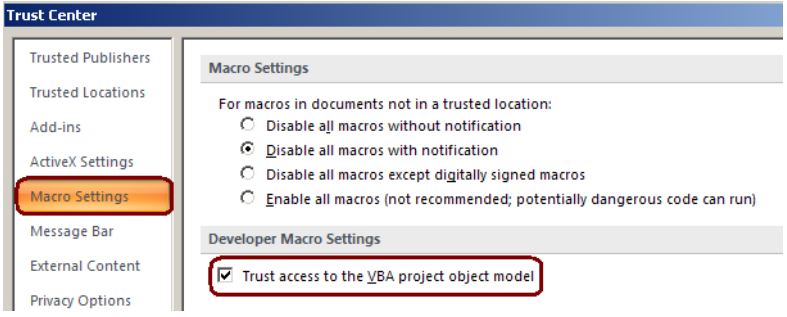


Figure 27 Mark the **Trust access to the VBA project object model** check box.

g Click **OK** in the Trust Center window.

h Click **OK** in the Excel Options window.

Step 8. Install Service Packs for Data Acquisition

1 Put the Data Acquisition installation disk into the disk drive.

2 From within a Windows file manager window, check the installation disk for any folder that includes the word **Service Packs**.

3 If one or more service pack files or folders exist on the installation disk, then for each service pack file:

   a Double-click the name of the most recent service pack to run the installation program.

   You may need to open folders inside the service pack folder to find the installation file. The name of service pack installation files typically end in **SP *x*.exe**.

   b Follow the instructions to install the service pack.

Step 9. Install Compliance programs (optional)

Do these steps if you need to enable compliance. You must install the MassHunter Data Acquisition program before you install the MassHunter Quantitative Analysis Compliance program.

1 Install the Quantitative Compliance program:

   a Open the folder **SetupCompliance** on the Quantitative Analysis installation disk.

   b Double-click **setup.exe**.

2 Enable Compliance for the Data Acquisition program.

   Refer to the *MassHunter Data Acquisition Compliance Software Quick Start Guide* for details.

The compliance programs are installed and enabled.

Step 10. Install Quantitative Analysis Reporting

Do this step only if you need to upgrade Quantitive Analysis Reporting.

1 Check that no other program is running on your system.

Installation of the Quantitative Analysis Reporting program will fail if Windows Update is running, even in the background.

2 Install the Quantitative Analysis Reporting program:

a Put the Quantitative Analysis installation disk into the disk drive.

b If you need only to install the service pack for Quantitative Reports, continue at step 3.

c From the root directory of the installation disk, double-click **setup_quantreport.exe**.

d In the Welcome to the InstallShield Wizard window, click **Next** to continue with the installation.

e In the License Agreement window, click **I accept the terms of the license agreement**, then click **Next**.

f If needed, in the Destination Folder window, click **Change** to change the default destination location for the report templates.

The default is **D:\MassHunter\Report Templates\Quant**.

g Click **Next** to continue.

h When the Ready to Install the Program window appears, click **Install** to begin.

When the installation begins you will see the Installing Agilent MassHunter Workstation Quantitative Analysis Reporting window.

i When the InstallShield Wizard Completed message is displayed, click **Finish.**

3 From within a Windows file manager window, check the installation disk for any folder that includes the word **Service Packs**.

4 If one or more service pack files or folders exist on the installation disk, then for each service pack file:

a Double-click the name of the most recent service pack to run the installation program.

You may need to open folders inside the service pack folder to find the installation file. The name of service pack installation files typically end in **SP *x*.exe**.

b Follow the instructions to install the service pack.

5 If you plan to use a PCL6 printer, download the print driver patch from http://support.microsoft.com/kb/935843.

This patch is needed to correctly print reports in A4 format to HP PCL6 printers.

6 Set the Microsoft Image Writer as the default printer:

a Click **Start > Control Panel**.

b Double-click **Printers and Faxes**.

c Right-click **Microsoft Office Document Image Printer** and click **Set as Default Printer.**

The Microsoft Image Writer formats the Excel workbooks three to four times faster for networked printers because the formatting page set-up information is stored on the local computer. The report is processed by the Microsoft Image Writer, but it is actually printed on the printer that is selected in the MassHunter program.

CAUTION If you do not set up a default printer, such as the Microsoft Office Document Image Printer, reporting can fail.

7 Install the Save as PDF add-in.

a On the Qualitative Analysis or Quantitative Analysis installation disk, open the folder **Supplemental\tools\Excel2007**.

b Double-click **SaveAsPDFandXPS.exe**.

c Follow the instructions to install the add-in.

This plug-in lets you save and send reports in PDF format.

NOTE If you remove a MassHunter program and then install it again, you may also need to re-install the PDF plug-in.

Step 11. Check Excel Add-Ins for MassHunter

1 Close all MassHunter Workstation programs.

2 Click **Start > All Programs > Agilent > MassHunter Workstation > Acq Tools > Remove MassHunter Processes** to make sure that all MassHunter processes are stopped.

3 Start Excel 2007.

If you are warned that Excel is unable to find a MassHunter Add-In, click **OK**.

4 Open the Add-Ins dialog box:

a Click the Microsoft Office button, then click **Excel Options**.

b From the navigation pane, click **Add-Ins**.

c Click the **Go** button next to the **Manage** drop-down list.

5 Verify that check boxes are marked for all add-ins that begin with **Masshunter**, then click **OK**. See Figure 28.

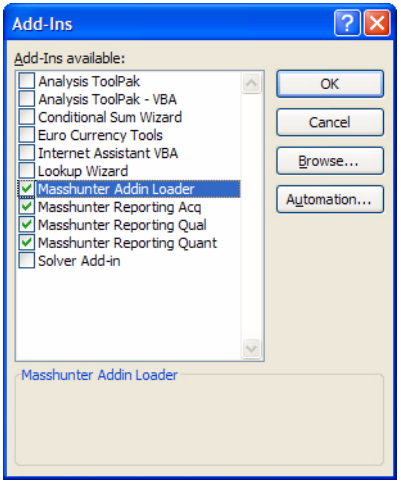


Figure 28 All MassHunter add-in check boxes marked

Step 12. Configure the instrument

In this step, you configure the Triple Quad instrument and the 1100/1200 LC.

1 Open the **Agilent MassHunter** folder on the desktop, then double-click **Acq Tools**.

2 Double-click the **Instrument Configuration** tool.

3 In the Instrument Configuration dialog box:

   a If you want to change the name of the instrument, type a new name for **Instrument Name**.

   b Click **Agilent 6400 Series Triple Quadrupole**.

   c Mark the **Agilent 1100/1200/1290 System Access** check box.

   d Click **Configure**.

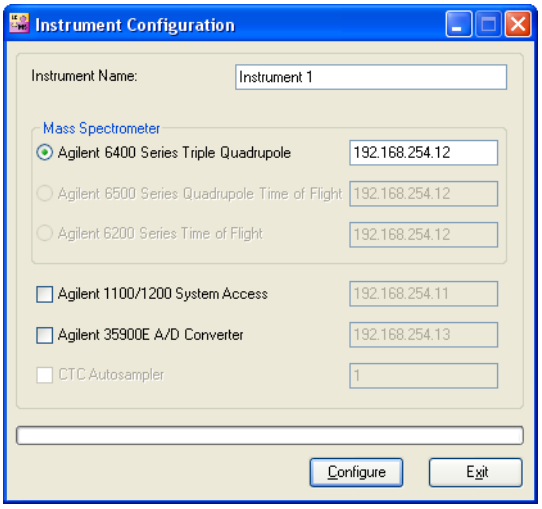


Figure 29 Instrument Configuration Dialog Box

4 When configuration is completed, click **OK**.

Step 13. Copy the support folder to the computer

In this step, you copy the **\Support** folder from the Data Acquisition installation disk to the **D:\MassHunter** folder.

1 In a Windows Explorer window, open the installation disk drive.

2 Right-click the **Support** folder and click **Copy**.

3 Right-click the **D:\MassHunter** folder and click **Paste**.

4 In Windows Explorer, right-click the **D:\MassHunter\Support** folder and click **Properties**.

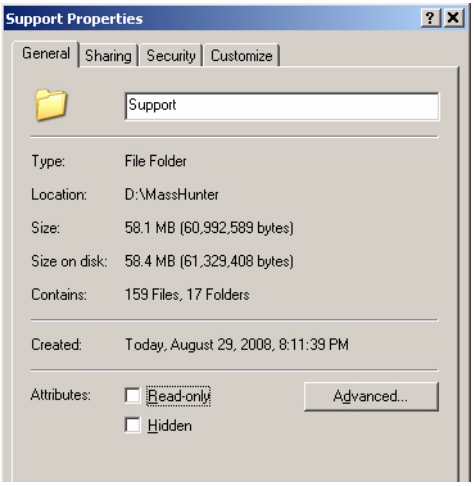


Figure 30 Support Properties dialog box

5 Clear the **Read-only** check box, and then click **Apply**.

The Confirm Attribute Changes dialog box will be displayed.

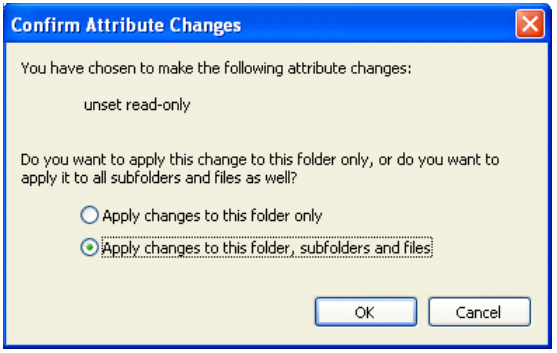


Figure 31 Confirm Attribute Changes dialog box

6 Click **Apply changes to this folder, subfolders and files**, then click **OK**.

Step 14. Confirm the LC firmware revision

1. Start the Data Acquisition program.
2. Click **File > Print > Instrument Configuration**.
3. Go to http://www.chem.agilent.com/en-US/Support/Downloads/firmware/Pages/LC.aspx and check that you have the most recent firmware for your instrument.
4. If you need to update your LC firmware, follow the instructions on the web site. Make sure you use the latest firmware update tool, as instructed on the web site.
5. Check that the firmware for the G1369A Agilent LAN Interface card is the latest version available:
   a. Start a Command Prompt in Windows.
   b. Type `telnet 192.168.254.11` in the Command Prompt window and press **Enter**.
   c. Type `/` in the Telnet window.

   The LAN Interface card displays its current configuration information.

   The LAN Interface card requires 1.10 firmware or higher. If an upgrade is needed, please .

Step 15. Finish the installation

1. Create a system recovery disk of your installed system.

   Follow the instructions for the Backup Solution Bundle (G1030-64002) included in the Agilent 6400 Series Triple Quad LC/MS System bundle.

2. Install or enable any other program that you will use with your MassHunter workstation. Refer the installation guide or quick start guide for these programs.

3. Make sure that the trust settings in Excel are set for all MassHunter users, including the guest account that is set up for compliance. See step 6 of "Step 7. Install and configure Microsoft Excel 2007".

4. Install the programs that are part of the Agilent Software Backup Solution and do a system back-up of the computer.

   Refer to the *H1172A Backup Solution Installation and User Guide* and the *Agilent Software Backup Solution Hardware and Software Installation Checklist* for details.

3
Installation Completion and System Verification

This chapter contains the steps needed to complete the hardware setup and verify the performance specifications of the installed system.

Step 1. Passivate the 1100/1200 LC

If you have a 6490, this step is required as part of MS-specific cleaning. This procedure comes from Service Note 0110-086. (For an alternate procedure, see Service Note 1200-022.) For other models, do this step if you want to clean the LC more efficiently.

If passivation is not sufficient for your purposes, repeat the flushing procedure.

CAUTION Do not include the UV-VIS detector in the passivation procedure because you can damage the quartz cell window.

Required Parts

- 500 mL HPLC grade Water
- 100 mL concentrated nitric acid (65%) (Fluka, 84385, 250ml)
- 100 mL 25% (v/v) aqueous ammonia solution (Fluka, 509857, 100ml)
- 1 mL formic acid 90% (Fluka, 94318, 50ml)
- pH paper sticks with pH range from 4.5 - 10 (Roth, C731)
- 1 x 0100-1847 adapter AIV (for G1311A quaternary pumps only
- 2 x 0100-1847adapter SSV (for G1312A binary pumps only)
- 3 x 1 liter solvent bottles
- 1 x 500 mL beaker
- 1 x 1000 mL flask for waste
- Personal protective equipment as needed

WARNING **Follow appropriate laboratory procedures and safety precautions for the solvents in use, including lab coat, goggles, gloves, and splash shield. This flushing procedure uses concentrated (65%) nitric acid and half concentration aqueous ammonia solution.**

1 Prepare solvents:
   - 15% ammonia solution: Dilute 60 mL ammonia solution (25%) with 40 mL water.
   - 0.1% (v/v) formic acid solution: Add 100 µL of concentrated formic acid to 100 mL of water.

2 For G1312A binary pumps only, disconnect tubings and install bottle head assay:
   - **a** Remove solvent inline filters from tubing.
   - **b** Remove the bottle head assay from the degasser.
   - **c** Remove the solvent line from the degasser to the pump.
   - **d** Remove the line from the MCGV to the active inlet valve (AVI).
   - **e** Install the bottle head assay directly into the active inlet valve (AIV) using the adapter (0100-1847) to fit for both A and B channels.
   - **f** Set %B to 50% for the remainder of the tests. If optional solvent select valve (SSV) is installed bypass this as well.

3 For G1311A quaternary pumps only, disconnect tubings and install bottle head assay:
   - **a** Remove solvent inline filters from tubing. Remove the bottle head assay “A” from the degasser.
   - **b** Remove the solvent line “A” from the degasser to the pump.
   - **c** Remove the line from the MCGV to the active inlet valve (AVI).
   - **d** Install the AIV adapter (0100-1847) into the active inlet valve inlet.
   - **e** Set channels B/C and D to OFF.

CAUTION Solvent can drip from line coming from Multi Channel Gradient Valve (MCGV) if the above steps are not followed. You can bypass MCGV and SSV because they do not contain stainless steel. If not bypassed during the tests, all channels (even if not connected) must be flushed thoroughly with water.

4 Connect the capillaries:
   a Check that the purge valve outlet is connected to the waste container.
   b Connect a capillary from the outlet of the pump to the ALS.
   c Connect a capillary from the ALS to TCC inlet of heater (any position).
   d Connect 2 to 3 capillaries to the outlet of the heater so that you have spare SST capillaries that are passivated for your application work.

CAUTION If buffer was in the channel(s), flush with water overnight before you start the passivation procedure.

5 Place the bottle head assemblies into a beaker with HPLC water and flush at 5 mL/minute for 5 minutes with the purge valve open. Close the purge valve and pump for an additional 5 minutes.

6 Open the purge valve, change the solvent to concentrated nitric acid and flush with 5 (2.5) mL/minute for approximately 5 minutes.

7 Reduce the flow to 0.5 (0.25) mL/minute.

8 Close the purge valve and pump nitric acid for approximately 30 minutes.

9 Open purge valve, replace the nitric acid with water and flush twice (each time fresh) with water at 5 (2.5) mL/minute for 5 minutes.

10 Open the purge valve, replace the water with the 15% ammonia solution and flush with 5 (2.5) mL/minutes. Close the purge valve and flush another 3 minutes.

11 Flush the threads of the fittings:
   a Loosen the 1/16-inch fittings which are accessible from the front of the instrument (active inlet valve, outlet ball valve and purge valve), one after the other.
   b Put a piece of tissue below the fitting.
   c Let approximately 100 µL of ammonia flow over the threads of the fitting.

12 Open the purge valve, change the solvent to distilled water and flush with 5 (2.5) mL/minute for 5 minutes. Close the purge valve and flush a further 3 minutes.

13 Open the purge valve, replace the water by 0.1% formic acid in water and flush with 5 (2.5) mL/minutes for 5 minutes. Close the purge valve and flush for a further 3 minutes.

14 Open 1/16-inch fittings as described in and repeat step 11 to flush the threads.

15 Open the purge valve, change the solvent to distilled water and flush with 5 (2.5) mL/minute for 5 minutes. Close the purge valve and flush a further 3 minutes.

16 Use pH paper sticks to check that the water that flows out of the waste line is back to neutral pH.

17 Reinstall the solvent inlet filters, reconnect the SSV if it been bypassed, reinstall the degasser. Operate as normal.

Step 2. Condition the 1100 / 1200 LC

Required Parts

- High-purity HPLC grade isopropanol (supplied by customer)
- Premixed Flushing Solvent (p/n G1969-85026)
- High Purity Water (p/n 8500-2236)
- High Purity Methanol (p/n 8500-1867)
- High Purity Acetonitrile (p/n G2453-85050)
- 5 M formic acid (p/n G2453-85060)

1. Start up the Data Acquisition program.
2. Condition the LC as specified in its installation document.
3. Disconnect any column(s) that may be installed.
4. Rinse the LC solvent bottles three times with hot (60°C) water, then rinse them three times with methanol.
5. Add the 500 mL of flushing solvent (p/n G1969-85026) to a clean solvent bottle and connect it to channel A.
6. Set up the pump module to deliver the flushing solvent for 15 minutes at 3 mL/minute. Set up the method for LC only acquisition. Do 5 injections with the flushing solvent to clean the injector path and sample loop.
7. Flush out system overnight with the flushing solvent.
   a. Set up the pump module for 0.5 mL/minute flow with the flushing solvent.
   b. Set the drying gas flow to: 10 L/minute.
   c. Set the nebulizer pressure to 40 psi.
   d. Set the drying gas temperature to 350°C.
   e. For 6460/6490: Set sheath gas temperature to 350°C.
   f. For 6460/6490: Set sheath gas flow to 10 L/minute.
   g. With the flushing solvent, bypass the Triple Quad spray chamber by right-clicking on the **MS QQQ** icon and clicking **LC > Waste** in the Data Acquisition program.

      If the MS is dirty, click **LC > MS** instead to also flush the MS.
   h. Flush the system overnight with the flushing solvent.

8 After flushing overnight with the flushing solvent, put 100% isopropyl alcohol in a clean solvent bottle and connect it to channel A. Prime the channel, and then flush for 30 minutes at 1 mL/minute with the 100% isopropyl alcohol to remove the flushing solvent.

9 Put 250 mL of 100% methanol in a clean solvent bottle, connect it to channel A, and prime the channel.

10 Install the 2.1 x 50 mm x 1.8 micron SB-C18 Rapid Resolution column supplied with the instrument

Flush the column out with 100% methanol for 1 hour at 1 mL/minute/minute.

11 Use the high purity solvents that are shipped with the system: Acetonitrile (p/n G2453-85050); Water (p/n 8500-2236); 5 M formic acid (p/n G2453-85060). Add 1 mL of the 5 M formic acid solution to 1 liter of water for a final concentration of 5 mM.

12 Pour 1 liter of acetonitrile in another clean solvent bottle.

13 Connect the 1 liter of water with 5 M formic acid to channel A and 1 liter of acetonitrile to channel B with 30:70 ratio, and flush the system for 1 hour at 1 mL/minute. Make sure to flush through the Rapid Resolution checkout column. Make sure that the solvent stream is going into the Triple Quad (MS spray chamber).

Step 3. Prepare the performance evaluation samples

In this step, you dilute the supplied performance evaluation samples to the concentrations needed for the Triple Quad system checkout. Use the diluted samples within a day of dilution. Refrigerate the intermediate (first) dilution in the supplied bottle.

- Always rinse the graduated pipettes and volumetric flasks thoroughly with deionized water before and between each use.
- Use polypropylene labware for preparing performance evaluation samples, since glass vessels introduce unacceptable levels of sodium.
- Always rinse the autosampler vials and caps with the solvent mix used for sample dilution before filling them with the performance verification samples. This minimizes any background that can be contributed by the vials and caps. The vials may be run uncapped if the septa are found to be a source of background contamination.

Required Parts

- 1 mL graduated pipette (p/n 9301-1423)
- 2 x 100 mL volumetric flasks (p/n 9301-1344)
- Positive-mode performance evaluation sample, Agilent G2423A
- Plastic bottle for storing first dilution (p/n 9301-1433)

1. Transfer 1 mL of 5 ng/µL reserpine (p/n G2423A) to a 100 mL volumetric flask. Use a clean graduated pipette.
2. Dilute to the 100 mL mark with 30:70 water:acetonitrile. Transfer 1 mL of the first dilution to a 100 mL volumetric flask. Use a clean graduated pipette.
3. Dilute to the 100 mL mark with 30:70 water:acetonitrile.

   This provides 500 fg/µL at 2 µL injection volume, which equals the final 1 pg/µL reserpine concentration that is needed for performance verification.

Table 1 6490 Performance Verification Summary, MRM Mode

	6490 ESI with Agilent Jet Stream Technology Positive MRM Mode
Sample	Reserpine, 5 ng/µL
Concentration after dilution	500 fg/µL
Injection volume	2 µL
Total sample amount injected	1 pg
Sample order number (p/n)	G2423A
Solvent	30:70 water:acetonitrile
Method	6490 ESI_AJT Pos MRM Checkout.m
Worklist	6490 ESI_AJT Pos MRM Checkout.wkl
Performance specifications	10,000:1 (1x RMS)

Table 2 6430 and 6460 Performance Verification Summary, MRM Mode

	6460 ESI with Agilent Jet Stream Technology Positive MRM Mode	**6430, 6460 ES G1948B Positive MRM Mode**
Sample	Reserpine, 5 ng/µL	Reserpine, 5 ng/µL
Concentration after dilution	500 fg/µL	500 fg/µL
Injection volume	2 µL	2 µL
Total sample amount injected	1 pg	1 pg
Sample order number (p/n)	G2423A	G2423A
Solvent	30:70 water:acetonitrile	30:70 water:acetonitrile
Method	6460 ESI_AJT Pos MRM Checkout.m	6400 ESI Pos MRM Checkout.m

Table 2 6430 and 6460 Performance Verification Summary, MRM Mode (continued)

	6460 ESI with Agilent Jet Stream Technology Positive MRM Mode	**6430, 6460 ES G1948B Positive MRM Mode**
Worklist	6460 ESI_AJT Pos MRM Checkout.wkl	6400 ESI Pos MRM Checkout.wkl
Performance specifications	1000:1 (1x RMS)	300:1 (1 x RMS)

Table 3 6410 Performance Verification Summary, MRM Mode

	ES G1948B Positive MRM Mode	**ES G1978B Positive MRM Mode**
Sample	Reserpine, 5 ng/µL	Reserpine, 5 ng/µL
Concentration after dilution	500 fg/µL	500 fg/µL
Injection volume	2 µL	2 µL
Total sample amount injected	1 pg	1 pg
Sample order number (p/n)	G2423A	G2423A
Solvent	30:70 water:acetonitrile	30:70 water:acetonitrile
Method	6400 ESI Pos MRM Checkout.m	MMI-ES Pos MRM Reserpine Checkout.m
Worklist	6400 ESI Pos MRM Checkout.wkl	MMI-ES Pos Reserpine Checkout.wkl
Performance specifications	150:1 (1 x RMS)	150:1 (1 x RMS)

Step 4. Review methods and run worklist

Before you begin, copy the methods and worklists according to your instrument model:

Table 4 Methods and worklists to copy

If you have...	Copy...	From...	To...
6490 ESI with Agilent Jet Stream Technology	6490 ESI_AJT Pos MS2 Scan.m 6490 ESI_AJT Pos MRM Checkout.m	D:\MassHunter\Support\Checkout\6490 Methods and Worklist\Pos	D:\MassHunter\Methods
	6490 ESI_AJT Pos MRM Checkout.wkl	D:\MassHunter\Support\Checkout\6490 Methods and Worklist\Pos	D:\MassHunter\Worklists
6460 ESI with Agilent Jet Stream Technology	6460 ESI_AJT Pos MS2 Scan.m 6460 ESI_AJT Pos MRM Checkout.m	D:\MassHunter\Support\Checkout\6460 Methods and Worklist\Pos	D:\MassHunter\Methods
	6460 ESI_AJT Pos MRM Checkout.wkl	D:\MassHunter\Support\Checkout\6460 Methods and Worklist\Pos	D:\MassHunter\Worklists
6410, 6430, 6460 ESI	6400 ESI Pos MRM Reserpine Checkout.m 6400 ESI Pos MS2 Scan.m	D:\MassHunter\Support\Checkout\6400 Methods and Worklist\Pos\ESI Pos MRM Checkout	D:\MassHunter\Methods
	ESI Pos Reserpine Checkout.wkl	D:\MassHunter\Support\Checkout\6400 Methods and Worklist\Pos\ESI Pos MRM Checkout	D:\MassHunter\Worklists

Depending on your instrument model, go to:

- "For 6490 - ESI with Agilent Jet Stream Technology, Positive MRM Mode" on page 75
- "For 6460 - ESI with Agilent Jet Stream Technology, Positive MRM Mode" on page 77
- "For 6410/6430/6460 - G1948B Electrospray Interface, Positive MRM Mode" on page 79
- "For 6410 - G1978B Multimode Interface, Positive MRM Mode" on page 81.

For 6490 - ESI with Agilent Jet Stream Technology, Positive MRM Mode

1 Verify that the Collision Cell gas flow rate produces a high vacuum gauge reading in the range of 5.0 to 5.8 x $10e^{-5}$ torr.

To view the high vacuum gauge reading, click the **Cell** tab in the Tune Context and check that the high vacuum gauge reading is within range. If not, see "To reset the Collision Cell gas flow rate" on page 87.

2 Start the Data Acquisition program, change the **Context** to **Tune**, and start an **Autotune**.

After the autotune has completed, you may need to wait up to 30 minutes to allow for the calibrant solution to be pumped out of the Triple Quad. This minimizes any background signal attributable to the calibrant. In addition, you can sonicate the nebulizer in a small graduated cylinder filled with acetonitrile for 10 minutes.

NOTE Parameters in the method can be modified by your Agilent representative for optimal response.

3 Change the Context to **Acquisition**, click **File > Load** and then load the method **6490 ESI_AJT Pos MRM Checkout.m**. The checkout method includes the following acquisition parameters. Edit if needed.

- 2 µL injection
- 4°C autosampler temperature
- 10 seconds needle wash (flushport 25:75 water:methanol, 0.1% formic acid)
- water, 0.1% formic channel A: acetonitrile, 0.1% formic channel B
- gradient

Time	%B
0.2	10
0.8	100
1.0	100
1.1	10

- 0.4 mL/minute
- 2 minutes run time

- 200°C Drying gas temperature (can be optimized, typically between 130 to 200°C)
- 12 L/minute Drying gas flow (can be optimized, typically between 12 to 15 L/minute)
- 20 psi nebulizer pressure (can be optimized, typically between 20 to 25 psi)
- 400°C Sheath gas temperature
- 12 L/minute Sheath gas flow
- 3500 V capillary voltage
- 500 V nozzle voltage (can be optimized, typically between 0 to 500 V)
- 41 V collision cell energy (can be optimized, typically between 37 to 42 V)
- fragmentor voltage is fixed and determined by iFunnel and autotune
- 3 V cell accelerator voltage (can be optimized, typically between 1 to 3 V)
- 200 V Delta EMV (can be optimized, typical between 200 to 350 V)
- MS1 Resolution set to Wide and MS2 Resolution set to Unit

4 Make sure that for channels A and B, 90:10 water with 5 mM formic acid:acetonitrle are selected as the LC solvent.

5 Click **File > Load** and then load the method **6490 ESI_AJT Pos MS2 Scan.m**.

6 Put the vials into the LC autosampler.

- Position #1: An empty, uncapped vial
- Position #2: A vial containing the solvent used for dilution (this is the solvent blank)
- Position #3: A vial containing the reserpine sample (500 fg/µL)

7 From the **File** menu click **Load** and then load the worklist: **6490 ESI_AJT Pos MRM Checkout.wkl**.

The worklist is set up to do one injection of the solvent blank using the **6490 ESI_AJT Pos MS2 Scan.m** method in order to collect background ion data, then using the **6490 ESI_AJT Pos MRM Checkout.m** method for the remaining runs, one injection of the empty vial, five injections of the solvent blank, and five injections of the reserpine sample. After that, it runs the **6490 ESI_AJT Pos MRM Checkout.m** method to run five more injections of the reserpine sample with 0.1 µL injection volume.

For 6460 - ESI with Agilent Jet Stream Technology, Positive MRM Mode

1 Verify that the Collision Cell gas flow rate produces a high vacuum gauge reading in the range of 1.9 to 2.3 x $10e^{-5}$ torr.

To view the high vacuum gauge reading, click the **Cell** tab in the Tune Context and check that the high vacuum gauge reading is within range. If not, see "To reset the Collision Cell gas flow rate" on page 87.

2 Start the Data Acquisition program, change the **Context** to **Tune**, and start an **Autotune**.

After the autotune has completed, you may need to wait up to 30 minutes to allow for the calibrant solution to be pumped out of the Triple Quad. This minimizes any background signal attributable to the calibrant. In addition, you can sonicate the nebulizer in a small graduated cylinder filled with acetonitrile for 10 minutes.

NOTE Parameters in the method can be modified by your Agilent representative for optimal response.

3 Change the Context to **Acquisition**, click **File > Load** and then load the method **6460 ESI_AJT Pos MRM Checkout.m**. The checkout method includes the following acquisition parameters:

- 2 µL injection
- isocratic from channel A and B 30:70 at 0.4 mL/minute
- 1 minute run time
- 325°C Drying gas temperature
- 10 L/minute Drying gas flow
- 20 psi nebulizer pressure
- 400°C Sheath gas temperature
- 12 L/minute Sheath gas flow
- 4000 V capillary voltage
- 500 V nozzle voltage
- 41 V collision cell energy (can be optimized, typical between 37 to 41 V)
- 150 V fragmentor voltage (can be optimized, typical between 140 to 250 V)
- 200 V Delta EMV (can be optimized, typical between 0 to 200 V)

- MS1 Resolution set to Wide and MS2 Resolution set to Unit

4 Click **File > Load** and then load the method **6460 ESI_AJT Pos MS2 Scan.m**.

5 Put the vials into the LC autosampler.

- Position #1: An empty, uncapped vial
- Position #2: A vial containing the solvent used for dilution (this is the solvent blank)
- Position #3: A vial containing the reserpine sample (500 fg/µL)

6 From the **File** menu click **Load** and then load the worklist: **6460 ESI_AJT Pos MRM Checkout.wkl**.

The worklist is set up to do one injection of the solvent blank using the **6460 ESI_AJT Pos MS2 Scan.m** method in order to collect background ion data, then using the **6460 ESI_AJT Pos MRM Checkout.m** method for the remaining runs, one injection of the empty vial, five injections of the solvent blank, and five injections of the reserpine sample.

For 6410/6430/6460 - G1948B Electrospray Interface, Positive MRM Mode

1 Verify that the Collision Cell gas flow rate produces a high vacuum gauge reading in the range of 1.9 to 2.3 x $10e^{-5}$ torr.

To view the high vacuum gauge reading, click the **Cell** tab in the Tune Context and check that the high vacuum gauge reading is within range. If not, see "To reset the Collision Cell gas flow rate" on page 87.

2 Start the Data Acquisition program, change the **Context** to **Tune**, and start an **Autotune**.

After the autotune has completed, you may need to wait up to 30 minutes to allow for the calibrant solution to be pumped out of the Triple Quad. This minimizes any background signal attributable to the calibrant. In addition, you can sonicate the nebulizer in a small graduated cylinder filled with acetonitrile for 10 minutes.

NOTE Parameters in the method can be modified by your Agilent representative for optimal response.

3 Change the **Context** to **Acquisition**, click **File > Load** and then load the method **6400 ESI Pos MRM Checkout.m**. The checkout method includes the following acquisition parameters:

- 2 µL injection
- isocratic from channel A and B 30:70 at 0.4 mL/minute
- 1 minutes run time
- 325°C Drying gas temperature
- 10 L/minute Drying gas flow (can be optimized, typical 10 to 13 L/minute)
- 35 psi nebulizer pressure (can be optimized, typical 20 to 35 psi)
- 4000 V capillary voltage
- 37 V collision cell energy (can be optimized, typical between 37 to 41 V)
- 240 V fragmentor voltage (can be optimized, typical between 140 to 250 V)
- 200 V Delta EMV (can be optimized, typical between 200 to 400 V)
- MS1 Resolution set to Wide and MS2 Resolution set to Unit

4 Edit the method to ensure that for channels A and B, 30:70 water:acetonitrile with 5mM formic acid is selected as the LC solvent.

5 Click **File > Load** and then load the method **6400 ESI Pos MS2 Scan.m**.

6 Put the vials into the LC autosampler.

- Position #1: An empty, uncapped vial
- Position #2: A vial containing the solvent used for dilution (this is the solvent blank)
- Position #3: A vial containing the reserpine sample (500 fg/µL)

7 From the **File** menu click **Load** and then load the worklist: **6400 ESI Pos MRM Checkout.wkl**.

The worklist is set up to do one injection of the solvent blank using the **6400 ESI Pos MS2 Scan.m** method in order to collect background ion data, then using the **6400 ESI Pos MRM Checkout.m** method for the remaining runs, one injection of the empty vial, five injections of the solvent blank, and five injections of the reserpine sample.

For 6410 - G1978B Multimode Interface, Positive MRM Mode

1 Verify that the Collision Cell gas flow rate produces a high vacuum gauge reading in the range of 2.7 to 3.3 x 10e-5 torr.

To view the high vacuum gauge reading, click the **Cell** tab in the Tune Context and check that the high vacuum gauge reading is within range. If not, see "To reset the Collision Cell gas flow rate" on page 87.

2 Start the Data Acquisition program, change the **Context** to **Tune**, and start an **Autotune**.

After the autotune has completed, you may need to wait up to 30 minutes to allow for the calibrant solution to be pumped out of the Triple Quad. This minimizes any background signal attributable to the calibrant. In addition, you can sonicate the nebulizer in a small graduated cylinder filled with acetonitrile for 10 minutes.

3 Change the Context to **Acquisition**, click **File > Load** and then load the method **MMI-ES Pos MRM Reserpine Checkout.m**. The checkout method includes the following acquisition parameters:

- 2 µl injection
- isocratic from channel A at 0.4 mL/min.
- 1 minute run time
- 250°C Drying gas temperature
- 5 L/min. Drying gas flow (can be optimized, typical 10 to 13 L/minutes)
- 60 psi nebulizer pressure (can be optimized, typical 35 to 40 psi)
- 2000 V capillary voltage
- 250°C Vaporizer temperature
- 2000 V charge voltage
- 37 V collision cell energy (can be optimized, typical between 37 to 41 V)
- 240 V fragmentor voltage (can be optimized, typical between 140 to 250 V)
- 400 V Delta EMV (can be optimized, typical between 200 to 400 V) starting with Acquisition program version B.02.01
- MS1 Resolution set to Wide and MS2 Resolution set to Unit

4 Edit the method to ensure that for channel A, the 75:25 methanol:water solution with 5mM ammonium formate is selected as the LC solvent.

5 Click **File > Load** and then load the method **MMI-ES Pos MS2 Scan.m**.

6 Edit the method to ensure that for channel A, 75:25 methanol:water solution with 5mM ammonium formate is selected as the LC solvent.

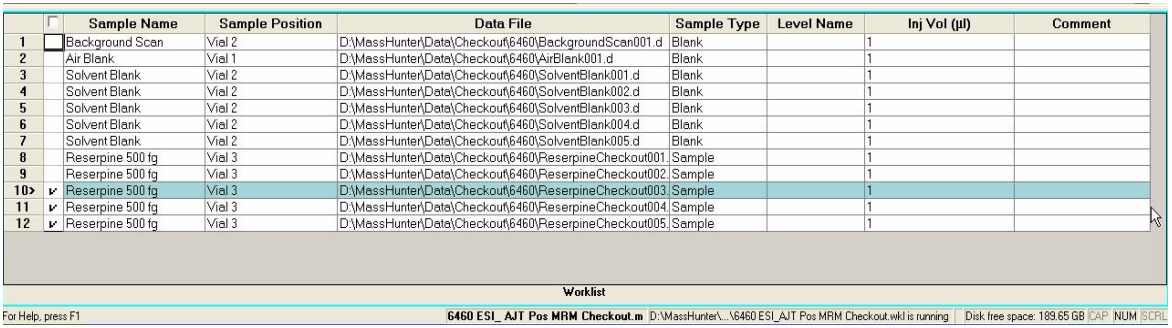
7 Put the vials into the LC autosampler.

- Position #1: An empty, uncapped vial
- Position #2: A vial containing the solvent used for dilution (this is the solvent blank)
- Position #3: A vial containing the reserpine sample (500 fg/µL)

8 From the **File** menu click **Load** and then load the worklist: **MMI-ES Pos Reserpine Checkout.wkl**.

The worklist is set up to do one injection of the solvent blank using the **ESI Pos MS2 Scan.m** method in order to collect background ion data, then using the **ESI Pos MRM Reserpine Checkout.m** method for the remaining runs, one injection of the empty vial, five injections of the solvent blank, and five injections of the reserpine sample.

Step 5. Verify the Triple Quad sensitivity



		Sample Name	Sample Position	Data File	Sample Type	Level Name	Inj Vol (µl)	Comment
1		Background Scan	Vial 2	D:\MassHunter\Data\Checkout\6460\BackgroundScan001.d	Blank		1	
2		Air Blank	Vial 1	D:\MassHunter\Data\Checkout\6460\AirBlank001.d	Blank		1	
3		Solvent Blank	Vial 2	D:\MassHunter\Data\Checkout\6460\SolventBlank001.d	Blank		1	
4		Solvent Blank	Vial 2	D:\MassHunter\Data\Checkout\6460\SolventBlank002.d	Blank		1	
5		Solvent Blank	Vial 2	D:\MassHunter\Data\Checkout\6460\SolventBlank003.d	Blank		1	
6		Solvent Blank	Vial 2	D:\MassHunter\Data\Checkout\6460\SolventBlank004.d	Blank		1	
7		Solvent Blank	Vial 2	D:\MassHunter\Data\Checkout\6460\SolventBlank005.d	Blank		1	
8		Reserpine 500 fg	Vial 3	D:\MassHunter\Data\Checkout\6460\ReserpineCheckout001.d	Sample		1	
9		Reserpine 500 fg	Vial 3	D:\MassHunter\Data\Checkout\6460\ReserpineCheckout002.d	Sample		1	
10>	✓	Reserpine 500 fg	Vial 3	D:\MassHunter\Data\Checkout\6460\ReserpineCheckout003.d	Sample		1	
11	✓	Reserpine 500 fg	Vial 3	D:\MassHunter\Data\Checkout\6460\ReserpineCheckout004.d	Sample		1	
12	✓	Reserpine 500 fg	Vial 3	D:\MassHunter\Data\Checkout\6460\ReserpineCheckout005.d	Sample		1	

Figure 32 Reserpine checkout worklist

1. Review the worklist to be sure that the method and data paths are correct, and that the data file names given in the worklist are unique and have not already been acquired.
2. Run the worklist.
3. When the worklist is finished, calculate signal-to-noise for each injection:
   a. Load each solvent blank and reserpine sample data file into the Qualitative Analysis program.
   b. Generate Extracted Ion Chromatograms of the 195.1 ion.

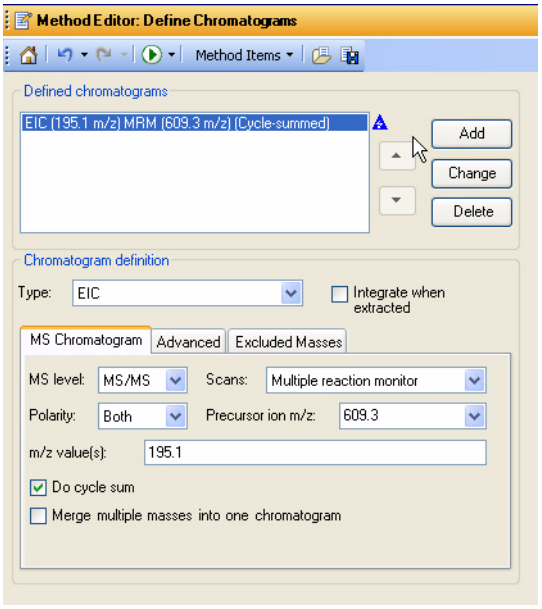
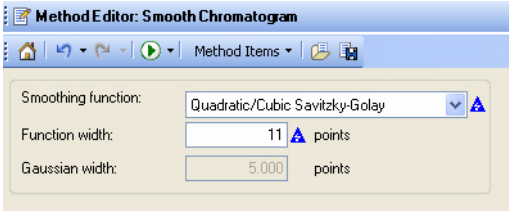


Figure 33 Extract Chromatogram dialog box

c Smooth the extracted chromatogram by setting **Function** to **Quadratic/Cubic Savitzky-Golay**, and **Function width** to **11** points.



d Integrate each reserpine peak, and click **Calculate Signal to Noise**.

e Calculate the signal-to-noise using **Height**.

f Under Noise Measurement, for **Noise definition**, select **RMS** and select **X 1**.

g For **Noise regions**, type `0.100 - 0.350`. (Make sure that the noise region does not include the reserpine peak.)

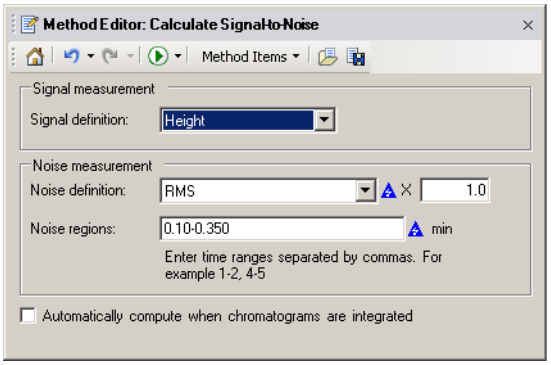


Figure 34 Calculate Signal to Noise dialog box

4 Generate a printout of each signal-to-noise calculation report for each solvent blank and reserpine injection. Include the chromatogram in the printout.

5 Open the Excel spreadsheet **D:\MassHunter\Support\Checkout\Sensitivity Checkout Report.xls**. Fill in the values to calculate the average signal to noise and save the spreadsheet.

The average signal-to-noise must be greater than that listed in Table 5.

Table 5 Minimum average signal-to-noise

Instrument Model	Source	Average Signal-to-Noise (1 x RMS)
6460	ESI with Agilent Jet Stream Technology	1000:1
6430	ESI	300:1
6410	ESI	150:1
6410	Multimode	150:1

6 Generate a printout of the Excel signal-to-noise report.

	A	B	C	D	E	F
1	**6460 Triple Quad LC/MS Sensitivity Checkout Report**					
2						
3						
4	**Instrument Serial Number:**	US83310889				
5						
6	**Date:**	28-Aug-2008				
7						
8						
9						
10		Signal-to-Noise				
11	Blank #1	0.27				
12	Blank #2	0.38				
13	Blank #3	0.42				
14	Blank #4	0.3				
15	Blank #5	0.41				
16	Blank Ave. S/N =	0				
17						
18			Area	Height		
19	Reserpine #1:	1207.2	924.00	404.00		
20	Reserpine #2:	1368.5	973.00	419.00		
21	Reserpine #3:	1583.7	959.00	412.00		
22	Reserpine #4:	1132.6	1044.00	448.00		
23	Reserpine #5:	2086	1091.00	471.00		
24	Reserpine Ave. S/N =	1476	998.20	430.80	AVE	
25			67.80	27.94	STD	
26			**6.79%**	**6.49%**	RSD	
27						
28	**Signal-to-Noise =**	**1475:1**				
29	(Reserpine Ave. - Blank Ave.)	PASS				
30						
31						

If verification in negative mode is required, verify the instrument with the parameters listed in Table 6. Use the Agilent ES Negative Ion Performance Standard (p/n G1946-85005) that is included with your instrument.

Table 6 Electrospray Negative Mode

Sample	Acid Red 4 10 ng/µl in 50:50 water / IPA
Concentration after dilution	1 pg/µl
Injection volume	1 µl
Total sample amount injected	1 pg
Sample order number	G1946-85005
Solvents	50:50 isocratic 100% Water (no modifiers) 100% Acetonitrile (no modifiers)
Method	ESI Neg MRM Acid Red 4 Checkout.m
Worklist	ESI Neg MRM Acid Red 4 Checkout.wkl

To reset the Collision Cell gas flow rate

Most parameters like the Collision Cell gas flow rate should work with values set at the factory. In case the default flow rate does not give a high vacuum gauge reading in the range of 1.9 to 2.3 x $10e^{-5}$ for a 6460/6430 and 2.7 to 3.3 x 10^{-5} for 6410, you will need to do two steps:

- Determine the optimal flow rate (DAC steps) to achieve the correct high vacuum gauge reading.
- Enter this value into a Data Acquisition configuration file.

1 Determine the optimal Flow Rate:

a Open a DOS window (click **Start > Run**, type `cmd` and click **OK**).

b Type `telnet 192.168.254.12 5123` to open a “back door” into the Triple Quad's MS Interface Card firmware.

c Press **Enter**.

d Type `msepeek 1605` at the **>** prompt to view the current value (in DAC Steps) for the Flow Rate.

You should get a response like this:

```
[61577.0]:   backdoor() command:msepeek 1605(12)
[61577.0]:   MSE_READ: address = 1605, value = 520.
```

520 is the factory default value.

e Find the high vacuum gauge reading in the **Cell** tab of the Manual Tune program.

f Use the syntax below to adjust the value for register 1605 until the high vacuum gauge reading in the Manual Tune program is in the range of 1.9 to 2.3 x $10e^{-5}$:

```
>msepoke 1605, value
```

For example, to set the 1605 register value to 550, type the following and press **Enter**.

```
>msepoke 1605, 550
```

You may need to try numbers that are above and below the default value.

g Write this number down.

2 Edit the acquisition parameter file so that the newly determined value will be applied whenever Data Acquisition starts up and will be used for tuning and data acquisition:

a Open the **D:\MassHunter\instr\default** folder, then open the appropriate .xml file with Notepad:

6460	G6460AHWParams.xml
6430	G6430AHWParams.xml
6410	G6410BHWParams.xml

b Locate the set point for the Collision Cell gas flow between the <flowrate> and </flowrate> tags. In this example, the set point for the flow rate is 520:

```
<cellFlow>
 <flowA>1</flowA>
 <flowB>0</flowB>
 <flowrate>520</flowrate>
</cellFlow>
```

c Change the flow rate set point to the value that you wrote down as the optimal flow rate. In this example, the value is changed to 550:

```
<cellFlow>
 <flowA>1</flowA>
 <flowB>0</flowB>
 <flowrate>550</flowrate>
</cellFlow>
```

d Click **File > Save** to save the file.

e Click **File > Exit** to close the file.

3 Restart the Data Acquisition program:

a Close the Data Acquisition program.

b Close all MassHunter engines from the MassHunter Acquisition Engine launcher. (Click the **Acq System Launcher** icon.)

c Restart the MassHunter engines.

d Start the Data Acquisition program.

You should now read the high vacuum value which you targeted for and can proceed with the installation as described.

4 Verify the Collision Cell gas flow:

a In the DOS window, type the following and press **Enter**:

```
>msepeek 1605
```

b Close the DOS window:

```
>bye
```

4 Installation and Verification of Other Sources

This chapter contains the steps needed to install and verify the operation of the APCI, multimode and APPI sources.

If the APCI, multimode APPI or HPLC-Chip/MS source was purchased with the Triple Quad instrument, you will need to install the source and verify its operation.

You can complete only a check tune with the different source types: G1948B and G1978B. Make sure you use the correct tune calibrant.

G1947B APCI Source

To install the G1947B APCI source

1. Install these parts from the APCI enablement kits that ship with the G1947B APCI source into the Aux module on the Triple Quad instrument:
   - APCI High Voltage Power Supply (p/n G1946-80058)
2. Pour the Electrospray calibrant back into its original bottle or another suitable container, rinse the calibrant bottle with acetonitrile, pour the APCI calibrant into the calibrant bottle, and attach the calibrant bottle back onto the CDS.
3. Remove the electrospray source and install the APCI source onto the spray chamber mounts.
4. Connect the vaporizer heater and APCI corona needle cables to the respective connectors on the left side panel of the Triple Quad instrument.

To verify the operation of the G1947B APCI source

There is no sensitivity checkout with the G1947B APCI source on the Triple Quad instrument. To verify its proper operation, you need to run a check tune.

1. Check the tuning of the APCI source:
   a. In the **Context** list, click **Tune** in the Data Acquisition program.
   b. Load the autotune file that was generated with the electrospray or multimode source.
   c. In the **Manual Tune** tab, mark the **Calibrant** check box, then acquire the data.
   d. Verify that the masses in the **MS1** and **MS2** tabs are correct for the loaded source before you manually tune the peaks.
   e. Verify that you have sufficient abundance for the tune peaks, that the tune peaks have peak widths of 0.7 ±0.05 amu, and that mass assignments are within ±0.15 amu of the target mass assignments.
2. Save the tune file.

G1978B Multimode Source

For more information on installing the multimode source, please refer to the multimode user guide and set-up guide.

To install the G1978B multimode source

If the multimode source is not already installed on your Triple Quad instrument, do these steps.

1 Install these parts from the Multimode and APCI enablement kits that ship with the G1978B Multimode source into the Aux module on the Triple Quad instrument:

For all models:

- APCI High Voltage Power Supply (p/n G1946-80058)
- Multimode HV board cable (p/n G1960-60858)

CAUTION

Do not install the G1960-65015 Multimode HV board on the 6460 or you may damage the ESI with Agilent Jet Stream Technology. The Multimode HV board is already installed on the 6460 and is backward-compatible with the G1978B multimode source.

The Multimode Power Data cable is already installed on the 6460.

2 Pour the electrospray tuning mix back into its original bottle or another suitable container, rinse the calibrant bottle with acetonitrile, pour the MMI-L tuning mix into the calibrant bottle, and attach the calibrant bottle back onto the CDS.

3 Remove the electrospray source and install the multimode source onto the spray chamber mounts.

4 Connect the multimode and corona needle cables to the respective connectors on the left side panel of the Triple Quad instrument.

To verify the operation of the G1978B multimode source

1 Check the tuning of the Multimode source:

   a In the **Context** list, click **Tune** in the Data Acquisition program.

   b Load the autotune file that was generated with the electrospray or multimode source.

   c

   d In the **Manual Tune** tab, mark the **Calibrant** check box, then acquire the data.

   e Verify that the masses in the **MS1** and **MS2** tabs are correct for the loaded source before you manually tune the peaks.

   f Verify that you have sufficient abundance for the tune peaks, that the tune peaks have peak widths of 0.7 ±0.05 amu, and that mass assignments are within ±0.15 amu of the target mass assignments.

2 Save the tune file.

G1971B APPI Source

To install the G1971B APPI source

1. Install the APPI USB to Serial Converter Cable (p/n 8121-1013) to the one of the available USB ports on the SmartCard on the left side of the Triple Quad instrument.
2. Pour the Electrospray calibrant back into its original bottle or another suitable container, rinse the calibrant bottle with acetonitrile, pour the APCI/APPI calibrant into the calibrant bottle, and attach the calibrant bottle back onto the CDS.
3. Remove the currently installed source and install the APPI source onto the spray chamber mounts.
4. Connect the APPI vaporizer heater cable to its connector on the left side of the Triple Quad instrument. Then connect the APPI serial cable to the USB/Serial Converter cable that was installed in step 1 of this topic.
5. Connect the APPI power supply cable to the APPI DB9 power connector and screw it in. This connector contains both serial and power interfaces.
6. Connect the other end of the serial cable to the Serial connector on the Smart card interface, which is located on the left side of the instrument chassis.
7. Plug the APPI power supply into an AC outlet using the power cord supplied with the APPI interface kit.

To verify the operation of the G1971B APPI source

There is no sensitivity checkout with the G1971B APPI source on the Triple Quad instrument. To verify its proper operation, you need to run a check tune to verify performance.

1. Check the tuning of the APPI source:
   a. In the **Context** list, click **Tune** in the Data Acquisition program.
   b. Load the autotune file that was generated with the electrospray or multimode source.
   c. In the **Manual Tune** tab, mark the **Calibrant** check box, then acquire the data.
   d. Verify that the masses in the **MS1** and **MS2** tabs are correct for the loaded source before you manually tune the peak.
   e. Verify that you have sufficient abundance for the tune peaks, that the tune peaks have peak widths of 0.7 ±0.05 amu, and that mass assignments are within ±0.15 amu of the target mass assignments.
2. Save the tune file.

HPLC-Chip/MS Cube Interface

Follow the steps in this section to do verification with the HPLC-Chip/MS Cube Interface.

Step 1. Prepare performance verification sample

1 Prepare the HSA peptides standard (p.n. G2455-85001) by adding 500 µL of 15:85 acetonitrile:water with 0.1% formic acid to the HSA peptide standard (500 pmol/vial).

2 Mix well on a vortex mixer to completely dissolve the standard.

The resulting stock solution is 1 pmol/µL and contains 7 peptides. Only 1 peptide will be used for the quantitation checkout.

3 Create each of the dilutions listed in Table 7:

a In a conical bottom polypropylene autosampler vial, put the volume of standard in Table 7.

b Add the volume of 15:85 acetonitrile:water with 0.1% formic acid solvent in Table 7.

The conical bottom polypropylene autosampler vials (p/n 5188-2788) and top with the appropriate caps (p/n 5182-0541) are included in the shipment.

Step E is optional and is used for collecting information.

Table 7 Dilutions for HSA peptides standard

Sample	Volume of Standard	Volume of Solvent	Final Concentration
A	10 µL of 1 pmol/µL solution	90 µL	100 fmol/µL
B	10 µL of A	90 µL	10 fmol/µL
C	10 µL of B	90 µL	1 fmol/µL
D	10 µL of C	90 µL	100 amol/µL
E	10 µL of D	90 µL	10 amol/uL

4 Create a blank vial of 15:85 acetonitrile:water with 0.1% formic acid.

Use the same conical vials and caps used for the HSA samples.

5 Prepare a vial containing 60:40 acetonitrile:water with 1% TFA to clean the injector before doing low level samples.

This solution works well for solubilizing hydrophobic peptides. If a clean blank cannot be achieved after running several injections of this solution, remove the needle seat (with seat capillary) and place it seat-side down in a beaker with this solution, then sonicate for 5 to 10 minutes.

Step 2. Prepare the HPLC-Chip/MS

- Copy the files from the Support folder to the Methods and Worklists folders according to Table 8.

Table 8 Methods and worklists to copy

Copy...	From...	To...
6400 HPLC-Chip HSA Checkout.m	D:\MassHunter\Support\Checkout\6400 Methods and Worklist\Pos\HPLC-Chip HSA Checkout	D:\MassHunter\Methods
6400 HPLC-CHip HSA Checkout.wkl	D:\MassHunter\Support\Checkout\6460 Methods and Worklist\Pos\HPLC-Chip HSA Checkout	D:\MassHunter\Worklists

If you want to create your own method and worklist, do these steps:

1 Create a worklist in MassHunter Acquisition program to run the prepared samples. See Figure 35.

If you are time-limited, you can do a single injection of each, but triplicate injections are preferred. The 10 amol HSA level is optional.

Remember to condition a new chip with 500 fmol of HSA 3 times. Then clean up with blanks and the solubilizing solution.

	Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Inj Vol (µl)	Comment
1	blank	P1-D1	43mm-9min-hsa-mrm-575-unit.m	blank-r001.d	Blank		1	
2	blank	P1-D1	43mm-9min-hsa-mrm-575-unit.m	blank-r002.d	Blank		1	
3	blank	P1-D1	43mm-9min-hsa-mrm-575-unit.m	blank-r003.d	Blank		1	
4	100 amol/ul HSA	P1-F5	43mm-9min-hsa-mrm-575-unit.m	100amol-r001.d	Calibration	1	1	
5	100 amol/ul HSA	P1-F5	43mm-9min-hsa-mrm-575-unit.m	100amol-r002.d	Calibration	1	1	
6	100 amol/ul HSA	P1-F5	43mm-9min-hsa-mrm-575-unit.m	100amol-r003.d	Calibration	1	1	
7	1 fmol/ul HSA	P1-F6	43mm-9min-hsa-mrm-575-unit.m	1fmol-r001.d	Calibration	2	1	
8	1 fmol/ul HSA	P1-F6	43mm-9min-hsa-mrm-575-unit.m	1fmol-r002.d	Calibration	2	1	
9	1 fmol/ul HSA	P1-F6	43mm-9min-hsa-mrm-575-unit.m	1fmol-r003.d	Calibration	2	1	
10	10 fmol/ul HSA	P1-F7	43mm-9min-hsa-mrm-575-unit.m	10fmol-r001.d	Calibration	3	1	
11	10 fmol/ul HSA	P1-F7	43mm-9min-hsa-mrm-575-unit.m	10fmol-r002.d	Calibration	3	1	
12	10 fmol/ul HSA	P1-F7	43mm-9min-hsa-mrm-575-unit.m	10fmol-r003.d	Calibration	3	1	
13	100 fmol/ul HSA	P1-F8	43mm-9min-hsa-mrm-575-unit.m	100fmol-r001.d	Calibration	4	1	
14	100 fmol/ul HSA	P1-F8	43mm-9min-hsa-mrm-575-unit.m	100fmol-r002.d	Calibration	4	1	
15	100 fmol/ul HSA	P1-F8	43mm-9min-hsa-mrm-575-unit.m	100fmol-r003.d	Calibration	4	1	

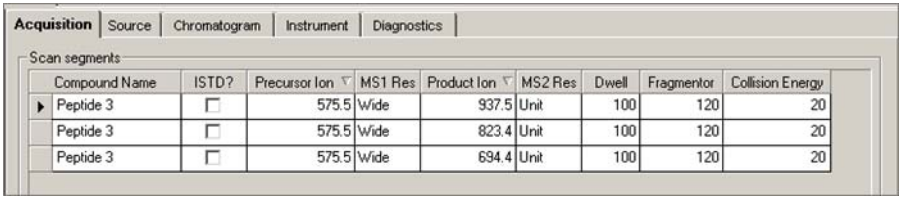
Figure 35 HPLC-Chip/MS Worklist

2 Create the QQQ acquisition method as shown below:

Nanoflow Pump		
Solvents	A1: 3:97 acetonitrile:water with 0.1% formic acid B1: 90:10 acetonitrile:water with 0.1% formic acid	
Flow	600 nL/min. with fast gradient solvent consumption	
Max pressure	175 bar	
Stop time	9 minutes	
Post time	0 minutes	
Gradient	Time	%B
	0	3.0
	7	70.0
	7.1	3
Capillary Pump		
Solvent	A1: 97:3 water:acetonitrile with 0.1% formic acid	
Flow	4.00 µL/min. (primary flow 200 µL/min.)	
Max pressure	175 bar	
Micro wellplate sampler		
Injection volume	1 µL	
Needle wash	enabled for 5 sec in flushport	
Needle flush solvent	20:80 methanol:water with 0.1% formic acid	
Bottom sensing	On	
Vial offset	0	
ALS Therm	On, 40°C	
HPLC-Chip MS Interface		
Injection flush volume	4 µL	
Pumps	Intelligent Sample Loading is activated.	
Chip Cube Timetable	Valve to enrichment at 7.5 min.	
QQQ MRM Method		
Ionization mode	positive HPLC-Chip	
Time filter	on	
Time Filter Width	0.07	
Dry gas flow	5 L/min.	

Dry gas temp	325°C
Capillary Voltage	1700-1800 V is typical
Time segments	1
Delta EMV	0 V (can go up to 200 V if more noise is desired)

MRM scan segments:



Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy
Peptide 3	☐	575.5	Wide	937.5	Unit	100	120	20
Peptide 3	☐	575.5	Wide	823.4	Unit	100	120	20
Peptide 3	☐	575.5	Wide	694.4	Unit	100	120	20

Step 3. Analyze the results

1 In the Quantitative Analysis program, create a batch and add the samples.

2 Create a calibration curve and use the Curve Fit Assistant to optimize the curve to obtain a best fit that includes all points and optimizes Accuracy for the data points.

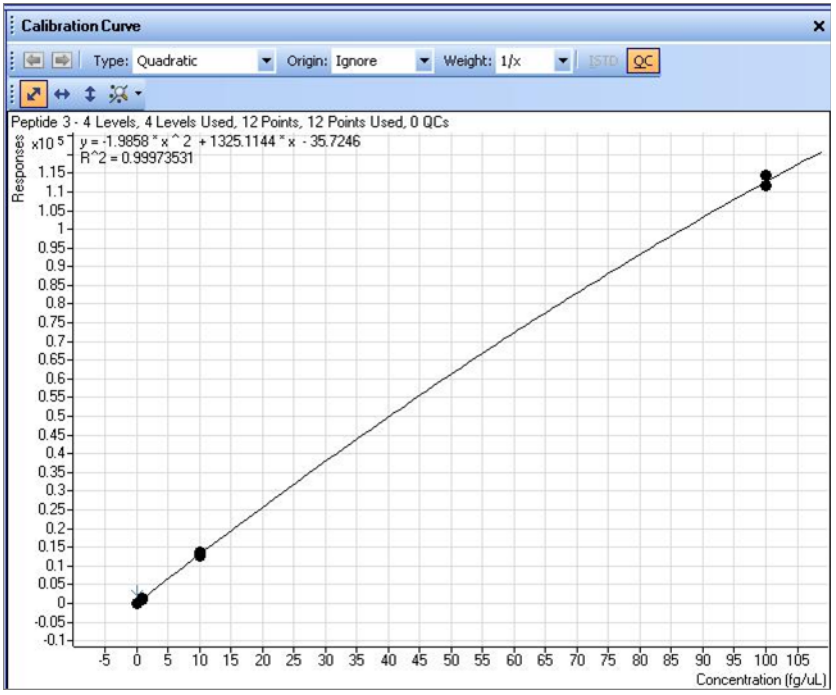


Figure 36 HSA Calibration Curve Example

You can use the Qualitative Analysis program to calculate the signal-to-noise ratio in Qualitative Analysis.

3 For 100 amol/µL in the 575.50 to 937.50 transition range, check that the minimum signal-to-noise ratio (defined from 0.5 to 3.0 minutes) is 100:1 (3x RMS noise).

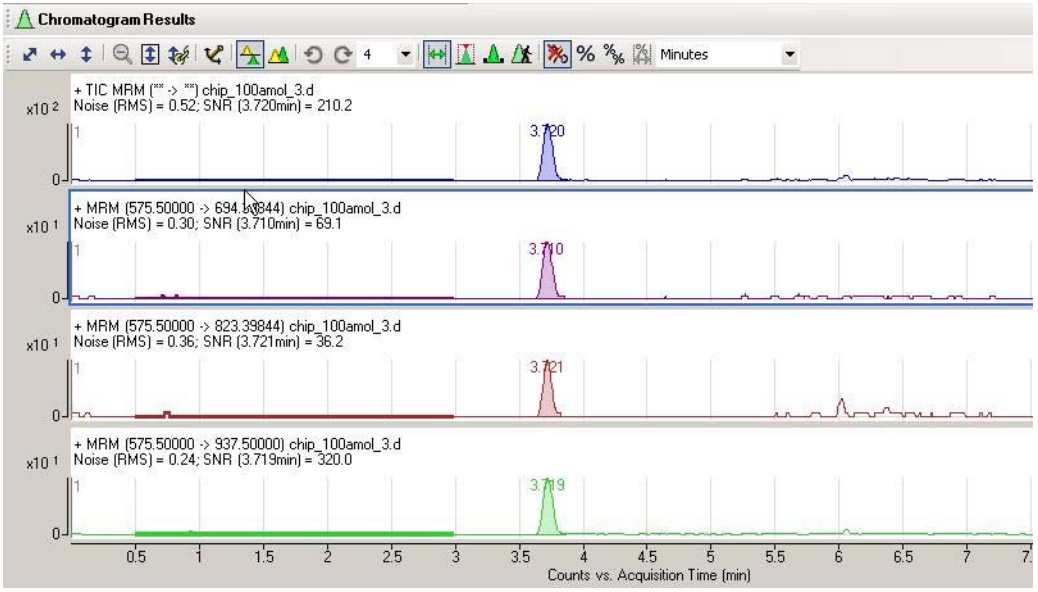


Figure 37 Calculated Signal-to-noise for 575.5 to 937.5 Transition

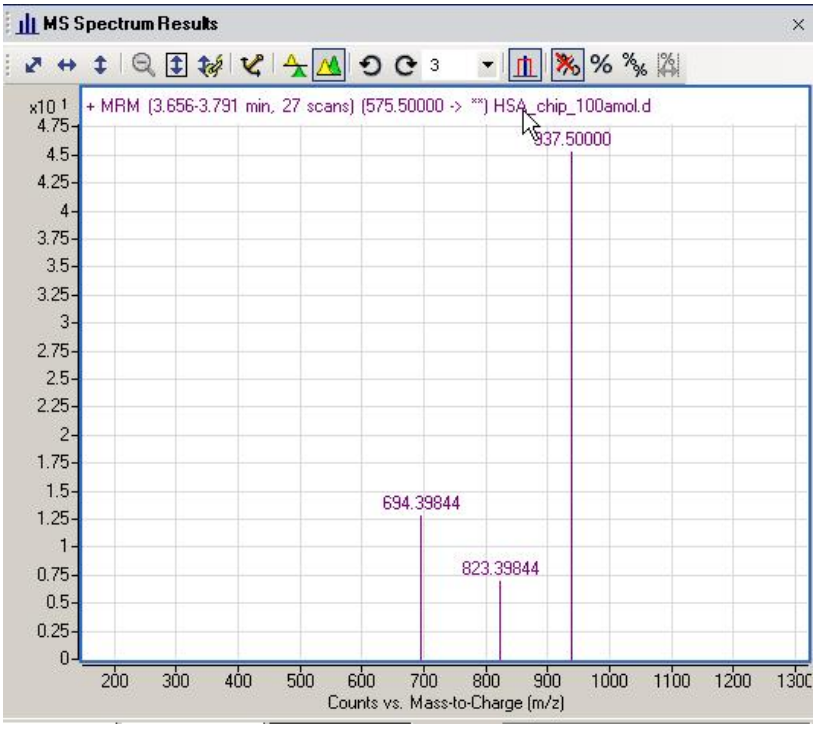


Figure 38 Mass Spectra for 575.5 *m/z*

5 Other Setup Tasks

This chapter contains tasks that are related to setting up your Triple Quad instrument.

For complete information on using the your Triple Quad instrument, refer to the list of resources found in the *Quick Start Guide*.

To start the Data Acquisition program

You can start the Data Acquisition program in one of two ways:

- Double-click the **Data Acquisition** icon in the Agilent MassHunter program group, or
- Click **Start > Programs > Agilent > MassHunter Workstation > Data Acquisition**.

To stop MassHunter Engines

1. Click **Programs > Agilent> MassHunter WorkStation > Launcher**.
2. Click **Stop** to stop the engines. All engines will become red.

To start the engines, click **Start**.

To stop MassHunter processes in case of unrecoverable errors

When you stop MassHunter processes, loss of data can occur.

- Click **Programs > Agilent > MassHunter Workstation > Acq Tools > Remove MassHunter Processes**.

To view the log book

- Double-click the **Acq System Logbook** icon in the **Agilent MassHunter** program group, or click **Start > Programs > Agilent > MassHunter Workstation> Acq System Logbook**.

You can also view the logbook from within the MassHunter Data Acquisition program.

To configure the network interface card

The PC shipped with the Triple Quad instrument is equipped with two network cards (LAN-cards). The first LAN-card is for connection to the “house network” (via 10/100/1000 Base-T network card). The IP address for this card can either be “static” or dynamically assigned (e.g. obtained by a DHCP server). By default, TCP/IP is configured as the network protocol for this card, which is built-in to the PC.

The second LAN-card (in slot #1) is dedicated for instrument communication with the Triple Quad instrument and the Agilent 1100/1200 Series LC system. *This network card should NOT be connected to the site LAN.*

To verify is the Instrument LAN network card settings are correct, follow these steps.

1 Click **Start > Settings > Control Panel.**

2 Double-click **Network Connections.**

3 Right-click the icon for the network card for the house LAN, click **Properties**, then configure the network card as needed.

  Because this network card is independent from the card used for instrument control, it may be configured using a static IP address or using a DHCP server.

4 Right-click the icon for the network card for the Triple Quad instrument, click **Properties**, then check that these parameters are set:

  - Subnet Mask 255.255.255.0
  - Gateway - leave blank

NOTE Because network configurations vary widely from site to site, get assistance from an IT representative when you connect to the site network. Configuration by an Agilent Customer Engineer is on a best-effort basis only.

To confirm configuration of NICs

In this step, you confirm that the correct LAN configuration exists.

1 Click the **Start** button and click **Run**.

2 Type `CMD` for the command prompt. A command window appears.

3 At the command prompt, type `ipconfig/all`

See Figure 39 for an example screen.

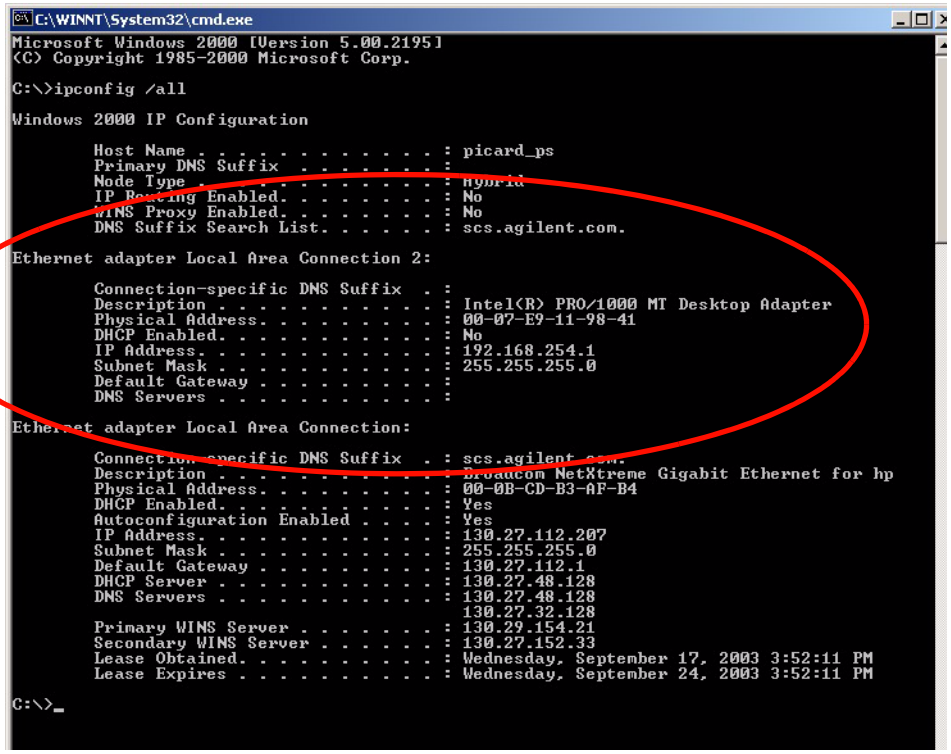


Figure 39 Example LAN Configuration

4 Check that the first *Local Area Connection* listed is the network card used to control the Triple Quad instrument.

5 The default address for the LAN card should be:

`192.168.254.1 - Default IP address`
`255.255.255.0 - Subnet Mask`

6 Check that the second *Local Area Connection* listed contains the information for house LAN. By default, this connection comes configured for DHCP.

7 If the network cards are not properly configured, modify their settings.

   a Right-click **My Network Places**, then click **Properties**.

   b Right-click the LAN card that is to be used to control the Triple Quad instrument, then click **Properties**.

   c Double-click the TCP/IP protocol.

   d Configure the card using the IP addresses from above, then click **OK** to apply the settings.

   e Click **Advanced**. Scroll down to the **Advanced Settings** menu.

   f Check that in the **Adaptors and Bindings** tab, the LAN card that is communicating to the Triple Quad instrument is listed first. If it is not, select the card, click the arrow on the right and move it to the top. Then click **OK** to apply the setting.

To remove the MassHunter Workstation software

1. If you have any data, methods, libraries, or other files or directories that you want to save, move or copy them to a directory outside of those to be deleted.
2. Click **Start > Settings > Control Panel**, then double-click **Add or Remove Programs**.
3. Remove these programs:
   - **Agilent MassHunter WorkStation Data Acquisition**
   - **Agilent MassHunter WorkStation Qualitative Analysis**
   - **Agilent MassHunter WorkStation Quantitative Analysis**
4. Close *all* applications except Explorer.
5. Reboot the computer. You are now ready to load new software.

To reconfigure the instrument

If you have more than one stack of HPLC modules from which to choose, or a new supported LC module is installed, the Instrument Configuration registry must be cleared for MassHunter Workstation.

1 Put the Triple Quad instrument into standby mode.

2 Close the MassHunter Acquisition program.

3 Stop MassHunter processes.

See "To stop MassHunter processes in case of unrecoverable errors" on page 108.

4 Start the Instrument Configuration program (**Programs > Agilent > MassHunter Workstation > Acq Tools > Instrument Configuration**).

5 Under **Mass Spectrometer**, click the Triple Quad instrument. Leave all check boxes cleared.

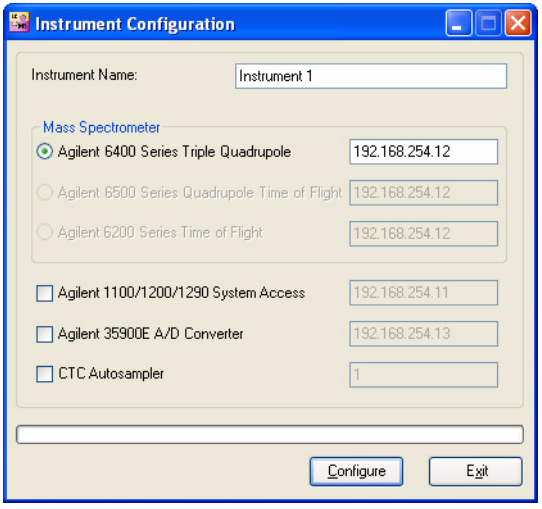


Figure 40 Instrument Configuration

6 Click **Configure**.

7 Start the MassHunter Workstation Acquisition program and confirm that only the MS instrument is available.

8 Close the Acquisition program.

9 Stop MassHunter processes.

10 Open Instrument Configuration again.

11 Click the Triple Quad instrument and mark the **Agilent 1100/1200/1290 System Access** check box.

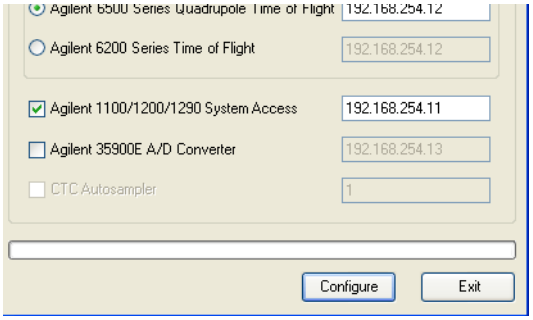


Figure 41 Instrument Configuration dialog box

12 Click **Configure**.

The registry has now been cleared and configured for any new LC module(s).

6
Reference

This chapter contains acquisition parameters for the HPLC-Chip and reserpine checkout method.

6460/6430 Reserpine Checkout Method Acquisition Parameters

The following graphics give the default acquisition parameters for the 6460 and 6430 POS MRM Reserpine Checkout method.

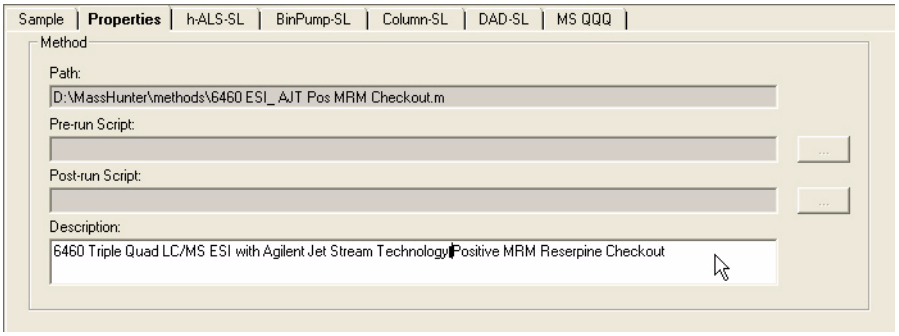


Figure 42 Properties tab

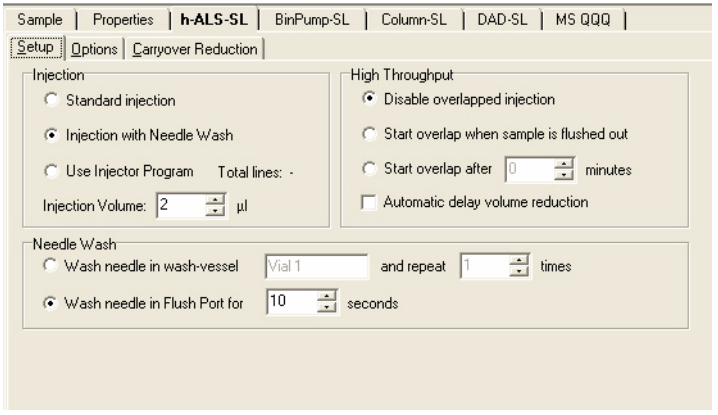


Figure 43 ALS tab

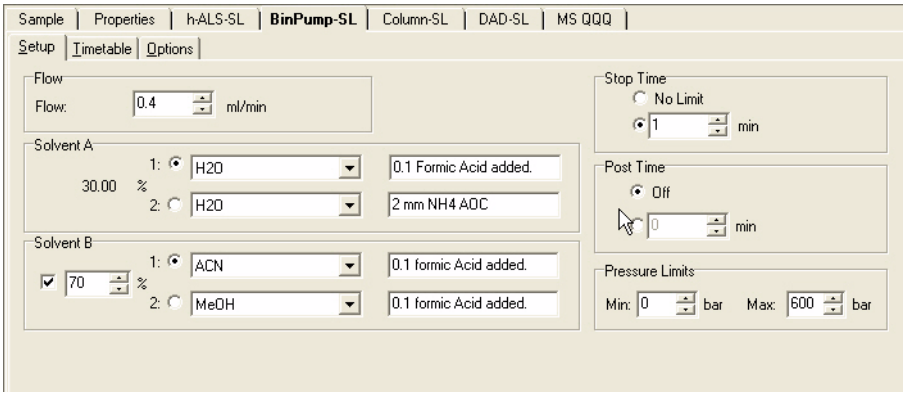


Figure 44 Bin Pump tab

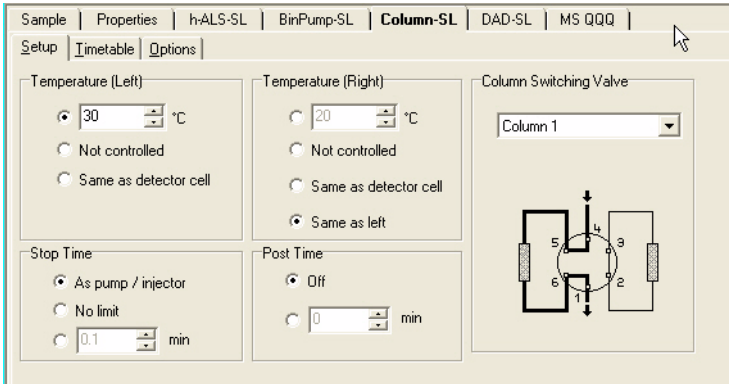


Figure 45 Column tab

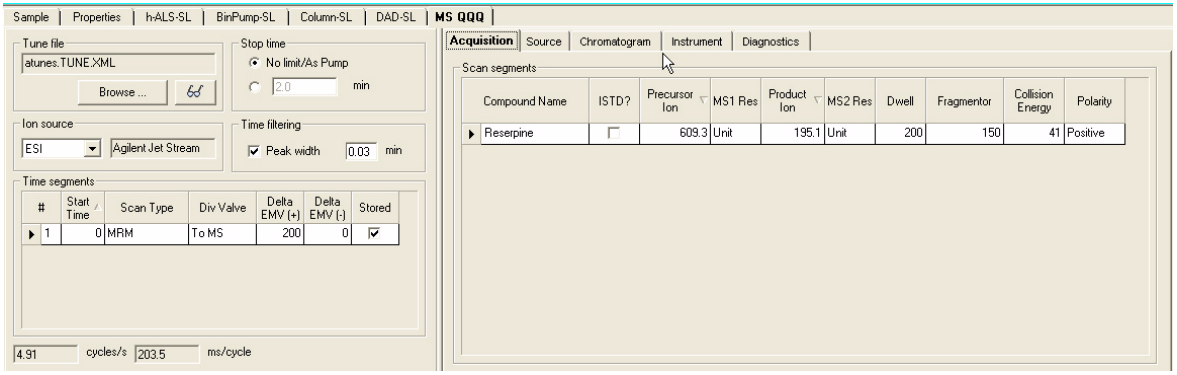


Figure 46 MS QQQ tab (6460 only)

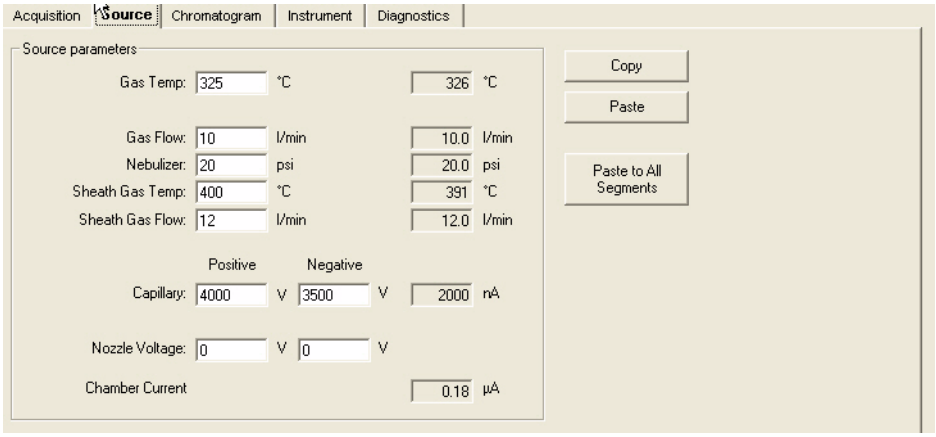


Figure 47 MS QQQ Source tab for ESI with Agilent Jet Stream Technology (6460 only)

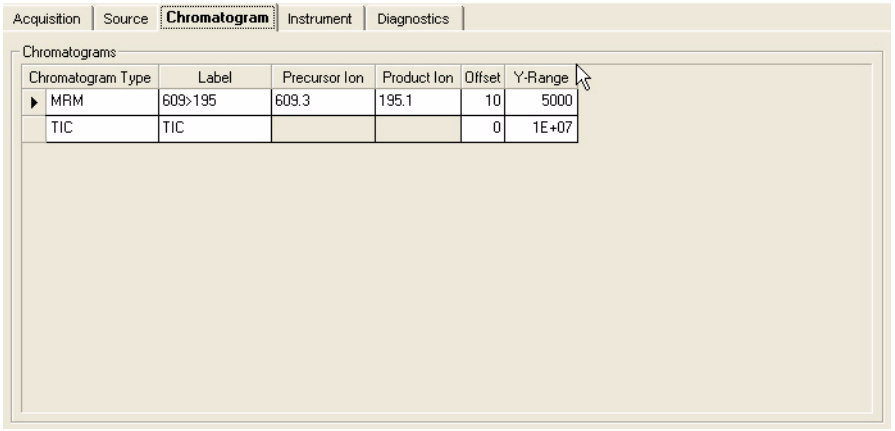


Figure 48 MS QQQ-Chromatogram tab

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In This Book

This book contains installation, configuration, verification, and start-up tasks to operate your Triple Quad LC/MS System.

Revision A1, November 2010

G3335-90096

Agilent Technologies