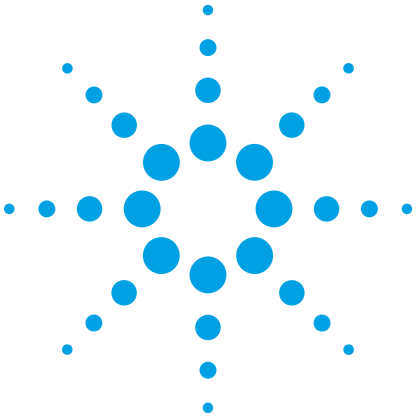

Agilent 6400 Series Triple Quad LC/MS System

Installation Guide

Agilent Technologies

Notices

Manual Part Number

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Software Revision

This guide is valid for the B.06.00 SP3 revision or higher of the Agilent MassHunter Workstation Software - Data Acquisition program and compatible Qualitative Analysis and Quantitative Analysis programs, until superseded.

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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, APPI, MultiMode, and HPLC-Chip Cube 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 in the lab.

   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 unpacked 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.

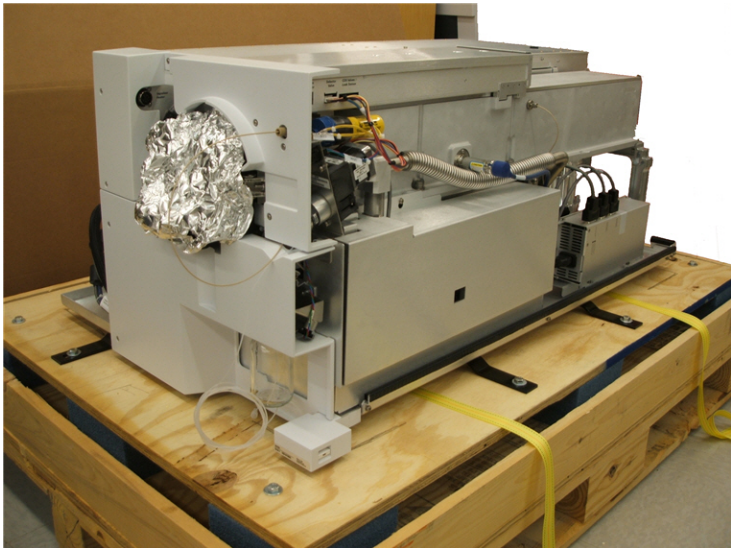


Figure 5 Triple Quad on shipping pallet with covers removed (6460 pictured)

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 3a. Set up the Agilent MS40+ pumps

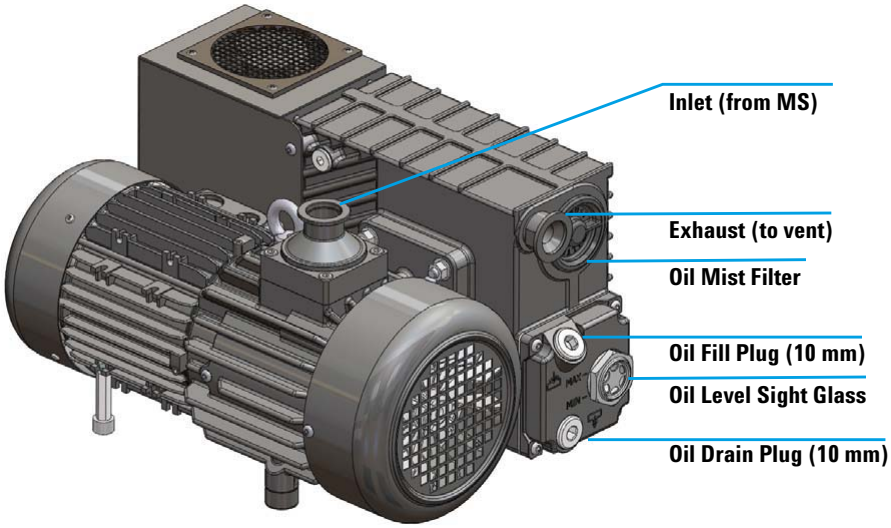
Do these steps if your instrument include one or more Agilent MS40+ pump.

Install two foreline pumps for 6490 and one foreline pump for 6420 or 6460C.

Before you begin, make sure that you have:

- Pump oil pan (p/n G1946-00034)
- Tygon 1/2-inch I.D. exhaust tubing (p/n G1946-80077)
- Agilent MS40+ foreline pump
- SW60 pump oil
- NW25 to 5/8-inch tube elbow (p/n G1960-20003)
- NW20/25 hinged clamp with wing nut (p/n 0100-1398)
- O-ring (p/n 0905-1592)
- pump power cord (p/n 8121-1753)

If the system is installed with G3215A Mass Spec Bench, follow the instructions in the Mass Spec Bench *User Guide* to install the pump or pumps inside of the Mass Spec Bench.



1 Unpack the Tygon 1/2-inch I.D. exhaust tubing (p/n G1946-80077) and Pump oil pan (p/n G1946-00034) from the shipping kit.

2 Remove the Agilent MS40+ foreline pump from its shipping container.

3 Set the Agilent MS40+ foreline pump into the Pump oil pan (p/n G1946-00034).

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

4 Cut an appropriate length of the Tygon 1/2-inch I.D. exhaust tubing (p/n G1946-80077).

5 Find the NW25 to 5/8-inch tube elbow (p/n G1960-20003) and the O-ring (p/n 0905-1592). Put the O-ring on the exhaust adaptor.

6 Locate one NW20/25 hinged clamp with wing nut (p/n 0100-1398), open the swing clamp, and use it to clamp the exhaust adaptor onto the oil mist filter. Connect the Tygon 1/2-inch I.D. exhaust tubing (p/n G1946-80077) to the exhaust adaptor.

7 Add the entire 1-liter bottle of SW60 pump oil to the foreline pump.

CAUTION Foreline pumps are shipped dry. Oil must be used for proper operation. Failure to add oil before a system is pumped down damages foreline pumps.

8 For 6420: Plug the pump power cord (p/n 8121-1753) into the power inlet of the foreline pump. Secure the pump power cord in place using the lock mechanism. Plug the other end into the LC/MS AC board port labeled **Foreline Pump**.

9 For 6460C: Plug the pump power cord (p/n 8121-1753) into the power inlet of the foreline pump. Secure the pump power cord in place using the retaining bale mechanism. Plug the other end into the Pump Expander assembly port labeled **Foreline**.

10 For 6490: Plug the two pump power cord (p/n 8121-1753) into the power inlets of the foreline pumps. Secure the pump power cords in place using the retaining bale mechanisms. Plug the other ends into the Pump Expander assembly ports. Connect the Ion Funnel Foreline Pump to the port labeled **Auxiliary** and connect the Foreline Pump to the port labeled **Foreline**. See Figure 7.

Figure 7 Pump expander box ports (6490 pictured)

Step 3b. Set up the Edwards E2M28 pump

Do these steps if your instrument includes an Edwards E2M28 pump.

Before you begin, check that you have:

- Edwards mist filter and instructions (p/n 3162-1056)
- Edwards oil drain kit and instructions (p/n 3162-1057)
- NW25 to 5/8-inch tube elbow (p/n G1960-20003)
- NW20/25 hinged clamp with wing nut (p/n 0100-1398)
- O-ring (p/n 0905-1592)
- metal foreline pump hose (p/n G1969- 20123)
- pump oil pan (p/n G1946-00034)
- Tygon 1/2-inch I.D. exhaust tubing (p/n G1946-80077)
- NW25 centering ring (p/n 0100-1551)

If the system is installed with a G3215A Mass Spec Bench, please follow the instructions to install the pumps inside the pump enclosure of the Mass Spec Bench.

1. Unpack the mist filter, exhaust hose, and pump oil drip from the shipping kit:
2. Remove the foreline 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 pan (p/n G1946-00034).

   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 foreline pump with Inland 45 fluid.

The foreline 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 foreline 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 foreline 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.

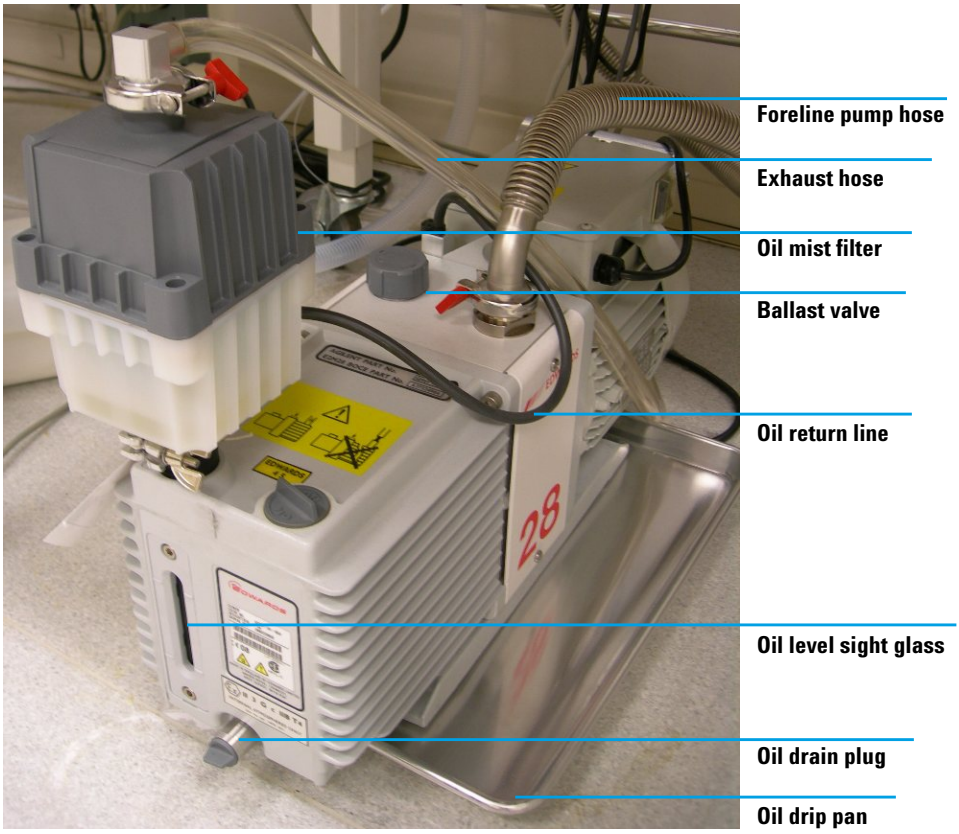


Figure 8 Example foreline pump. Note that your pump may look slightly different.

7 Check that the ballast valve is closed.

Closing the ballast valve prevents additional air from being introduced to the pump. This allows the foreline 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 NW25 to 5/8-inch tube elbow (p/n G1960-20003) and the O-ring (p/n 0905-1592). Put the O-ring on the exhaust adaptor.

9 Locate one NW20/25 hinged clamp with wing nut (p/n 0100-1398), open the swing clamp, and use it to clamp the exhaust adaptor onto the oil mist filter. Connect the Tygon 1/2-inch I.D. exhaust tubing (p/n G1946-80077) to the exhaust adaptor.

10 Attach one end of the metal foreline pump hose (p/n G1969- 20123) using one NW20/25 hinged clamp with wing nut (p/n 0100-1398) and one NW25 centering ring (p/n 0100-1551) to the LC/MS mainframe.

11 Connect the other end of the metal foreline pump hose to the inlet fitting on the foreline pump using one NW20/25 hinged clamp with wing nut (p/n 0100-1398) and one NW25 centering ring (p/n 0100-1551).

12 Plug the power cord from the foreline pump into the power outlet labeled **Foreline** on the AC board.

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 foreline pump hose connection on the back left of the instrument.
2. Connect the foreline pump hose to the KF-25 inlet adapter on the foreline 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, and then connect the other end of the tubing to a vent connection that is separate from the vent used for the foreline pump.

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

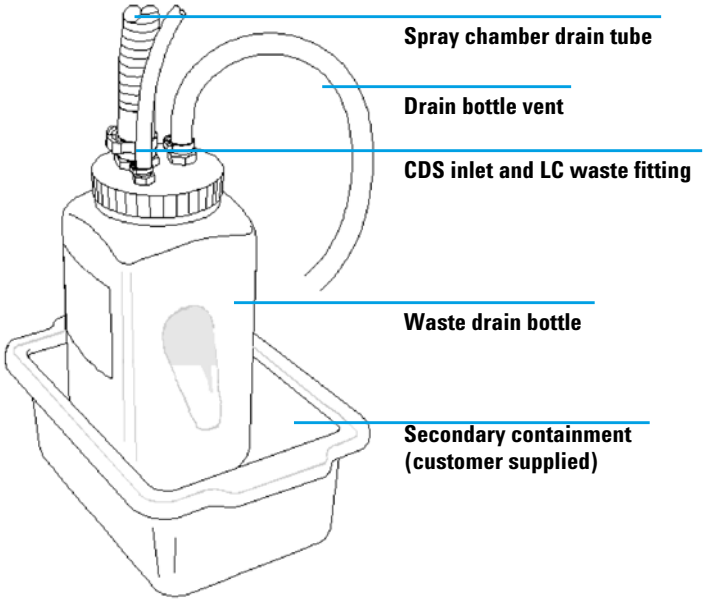


Figure 9 Drain bottle

7 Install the gas filter(s):

For Triple Quad instruments without AJS ESI source:

a Cut an appropriate length of the 1/4-inch PTFE 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.

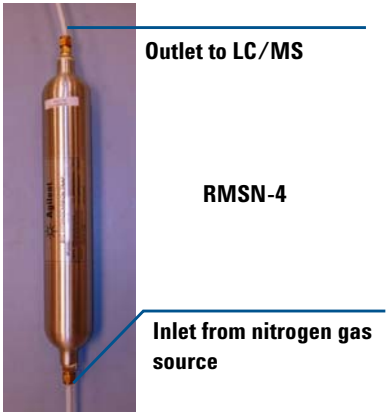


Figure 10 Gas filter connected in series

For 6460 and 6490 with AJS ESI source:

a Cut an appropriate length of the 1/4-inch PTFE 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 PTFE 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.

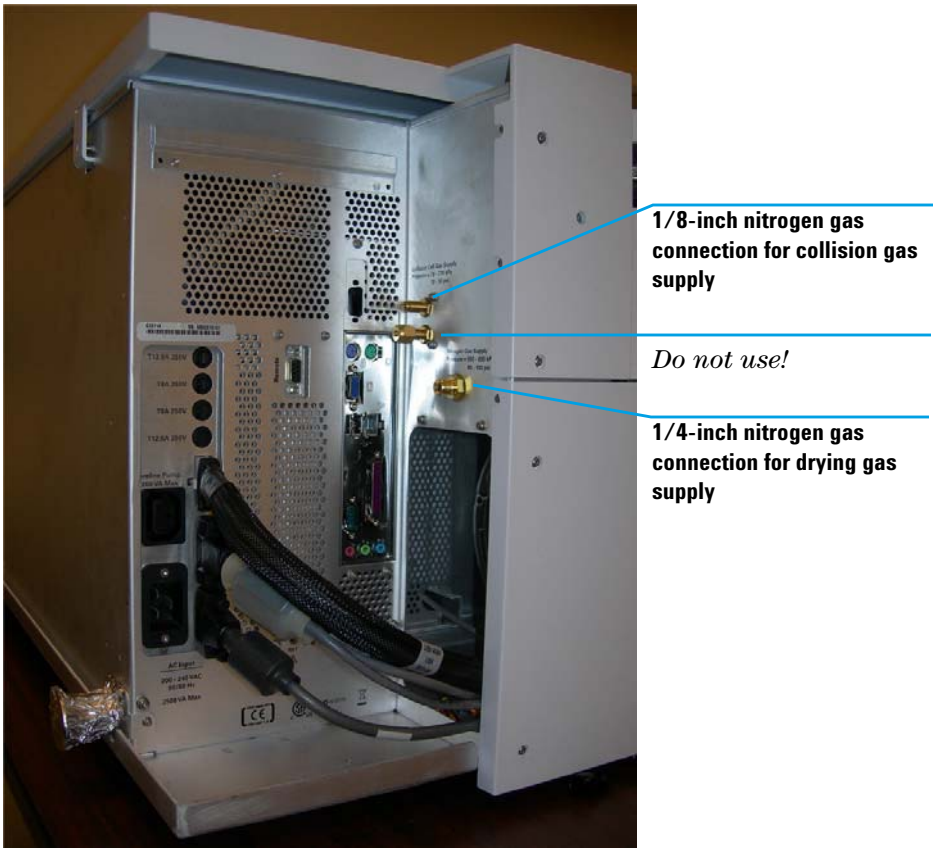


Figure 12 Power and gas connections on back left of Triple Quad

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, and 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.

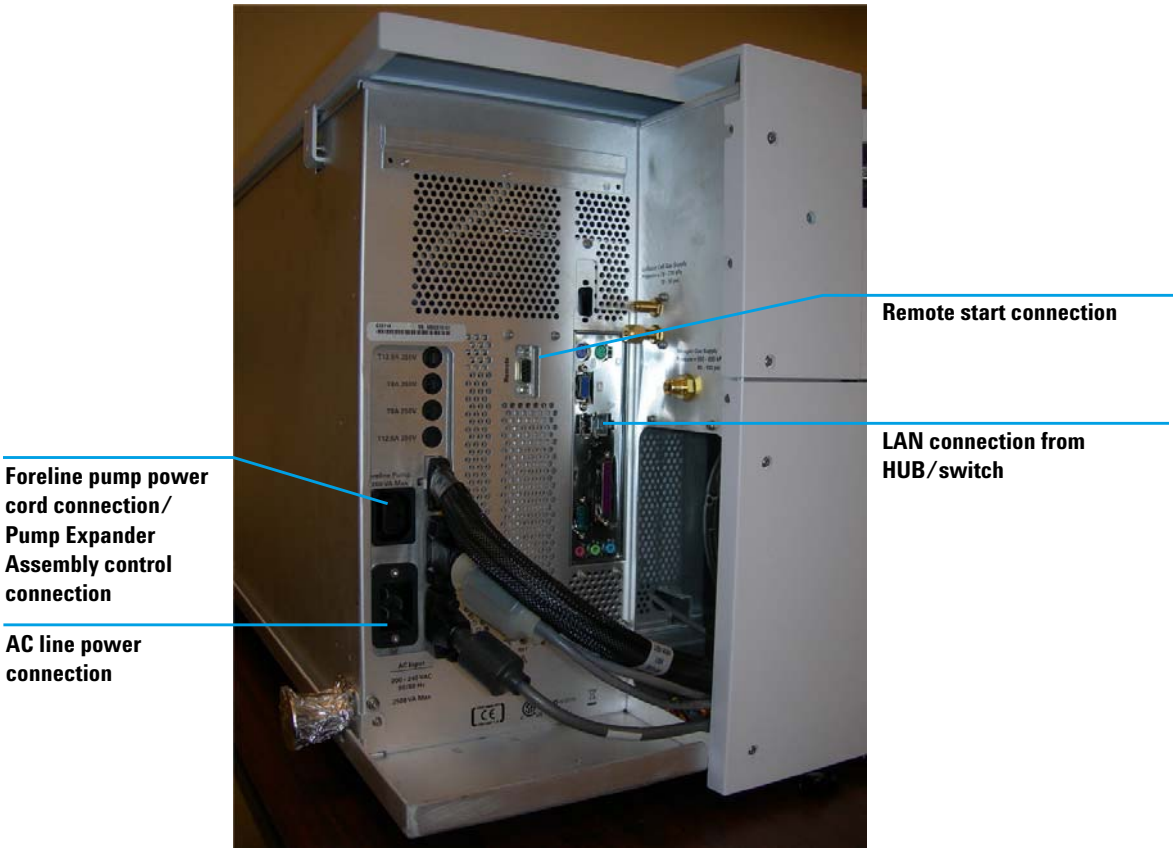


Figure 13 Power and electrical connection at back left side of Triple Quad

Figure 14 Power and electrical connection at back left side of Triple Quad (6460C)

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 or Figure 14.

12 Connect the foreline pump power cord into the foreline pump connector on the back left side of the Triple Quad. See Figure 13 or Figure 14.

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 or Figure 14

15 For 6460C and 6490: Plug the Pump Expander Assembly power cord into the wall outlet.

Figure 15 Pump Expander Assembly box

Step 5. Install the spray chamber

Agilent Jet Stream ESI Source (6460/6490 only)

Before you begin, check that you have these parts:

- Agilent Jet Stream (AJS) ESI source
- 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 AJS ESI source from the packaging inside the instrument chassis next to the e-Module. See Figure 16.

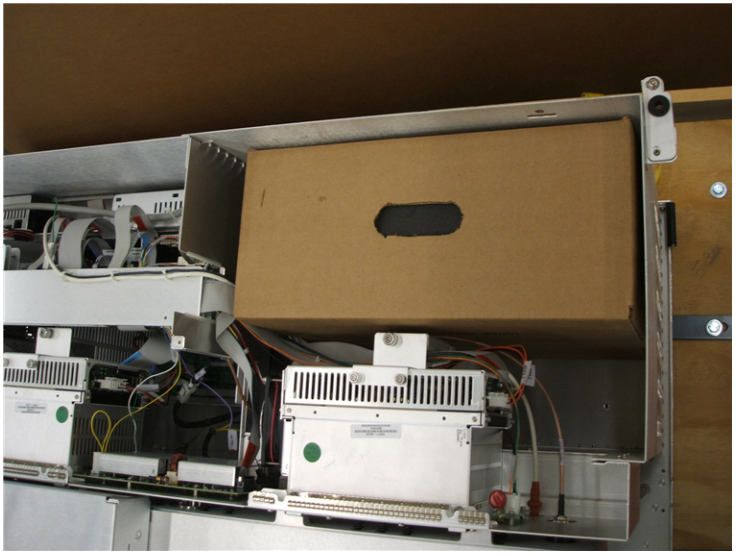


Figure 16 Packaging for AJS ESI source for 6460. For 6490, the AJS ESI is shipped outside of the instrument chassis.

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, and then adjust the latch to the proper fit, and 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 AJS ESI source.

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

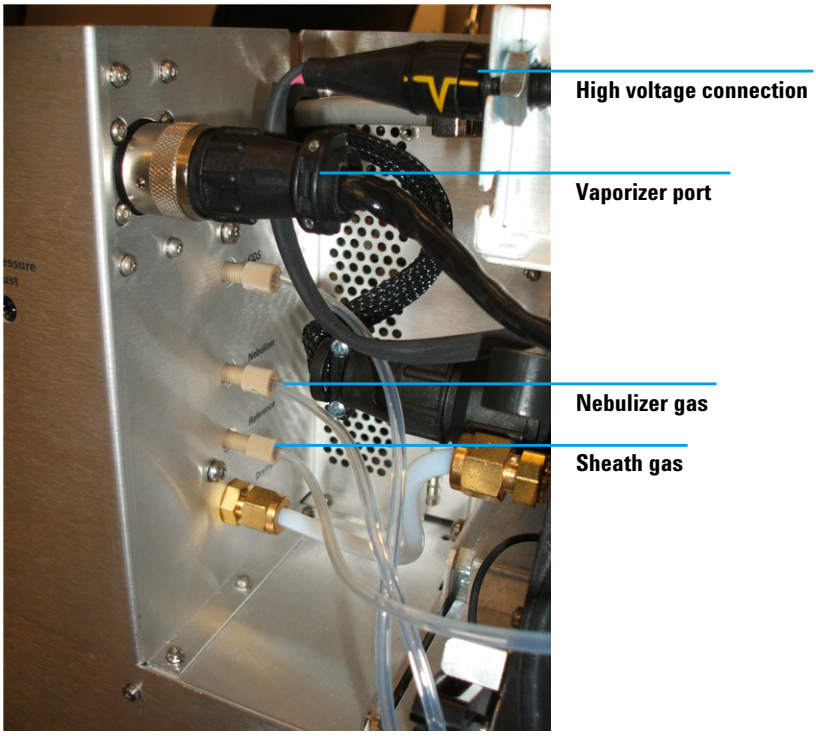


Figure 17 Connections for AJS ESI source

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 foreline pump(s) will become quieter within a few seconds.

Agilent G1948B Electrospray Source

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, adjust the latch to the proper fit, and 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 foreline pump(s) 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, adjust the latch to the proper fit, and 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 foreline pump(s) will become quieter within a few seconds.

Step 6. Install the Agilent 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 LC system. Follow its installation documents. Other LCs are not supported.

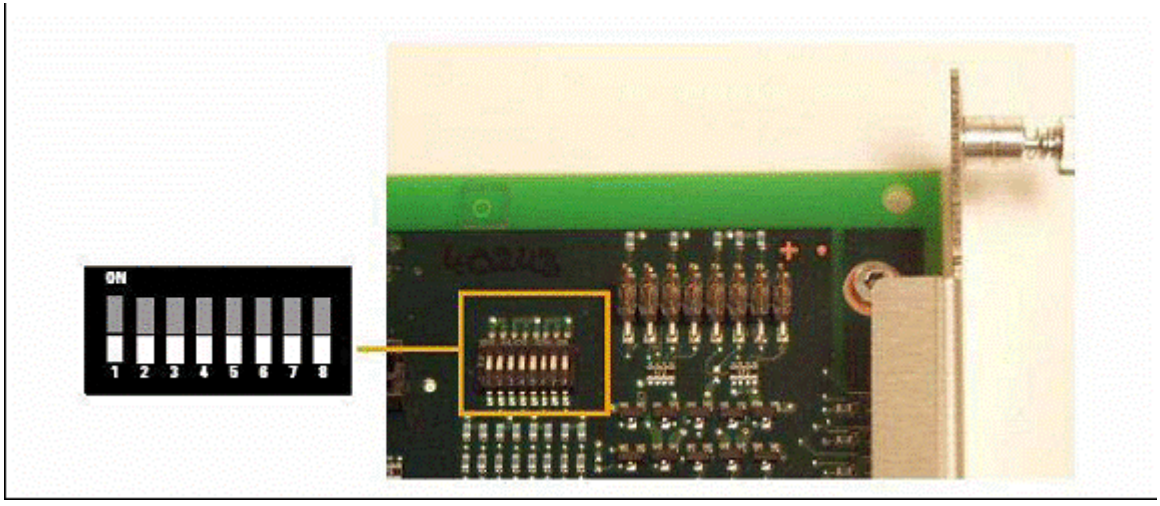
2 Connect the remote start cable from the Triple Quad to a Remote Start connector on the LC sampler, pump or other detector module only.

3 Install an Agilent G1369 LAN Interface card into the LC detector module if required. If a detector module is not present, install the Agilent G1369 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 G1369 (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" and complete the steps that remain.

Step 1. Set up the computer system

1. Set up the PC:
   a. Unpack the PC and put it on a suitable bench top or table.
   b. 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 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 installed with software and the appropriate drivers for the installed accessories.
9. When the computer starts, create a user account. This account will be used to acquire data. Do not use the Admin account for this purpose.

Step 2. Check PC network card configuration

1 Click **Start > Control Panel**, and then under **Network and Internet**, click **View network status and tasks**.

If Control Panel displays in Small icons view, click **Network and Sharing Centers**.

2 Click **Change adapter settings**.

3 From the list of network connections, double-click the network connection that corresponds to the instrument LAN connection.

4 Scroll down the list to **Internet IP Protocol 4 (TCP/IPv4)** (or **Internet IP Protocol**), select it, and then click **Properties**.

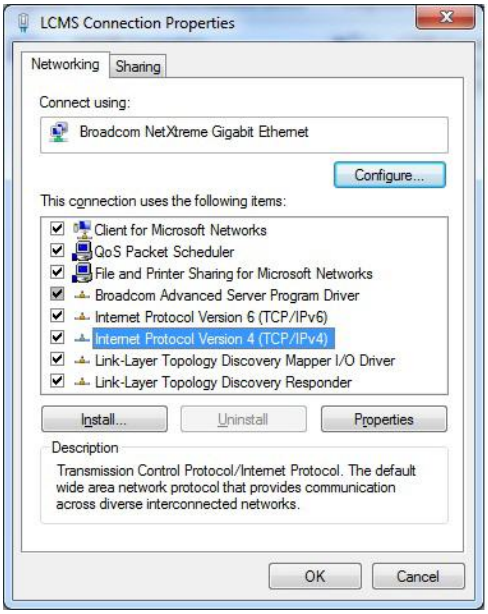


Figure 18 LCMS Connection Properties dialog box - Networking tab

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

IP address: 192.168.254.1

Subnet mask: 255.255.255.0

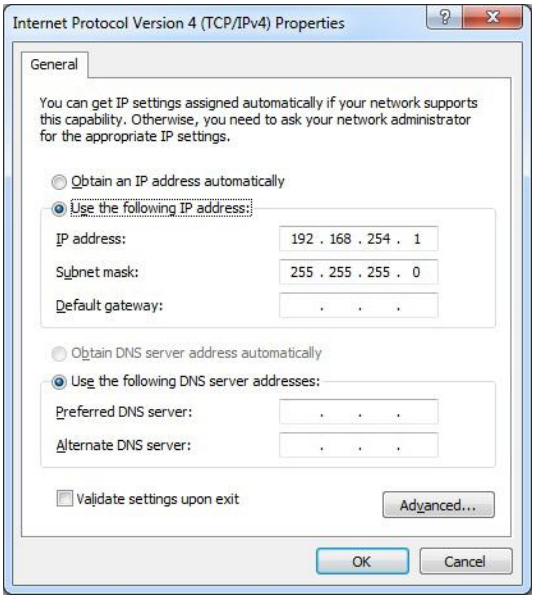


Figure 19 Internet Protocol Version 4 (TCP/IP) Properties dialog box

Step 3. Prepare for installation

1 Use the **Uninstall or change a programs** command in Control Panel to remove programs that you will be upgrading. *Do not remove programs for which you need only to install a service pack.*

- Excel, if you need to upgrade (only Excel 2010, Office 2010, Excel 2013, and Office 2013 [32-bit] are supported)
- Agilent MassHunter Workstation Qualitative Analysis
- Agilent MassHunter Workstation Quantitative Analysis
- Agilent MassHunter Workstation Quantitative Analysis Reporting
- Agilent MassHunter Workstation Quantitative Analysis Compliance

2 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 activated 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" on page 47.

If the upgrade to the Data Acquisition program is for a service pack only, continue at "Step 5. Install the Qualitative Analysis program" on page 46.

The installation of the Data Acquisition program includes installation of Optimizer and Study Manager. Do not separately install these programs.

1. Check that no other program is running on your system, including Windows Update.
2. Put the Data Acquisition installation disc into the disc drive.

   The welcome screen appears.
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**, and then follow the instructions on the screen to install the software.
   - In the License Agreement screen, mark the **I accept the terms of the license agreement** check box, and then click **Next**.
   - In the Choose Destination Location screen, click **Next** to accept the default destination location.

     The default is **C:\Program Files (x86)\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, and then click **Next**. See Figure 20.

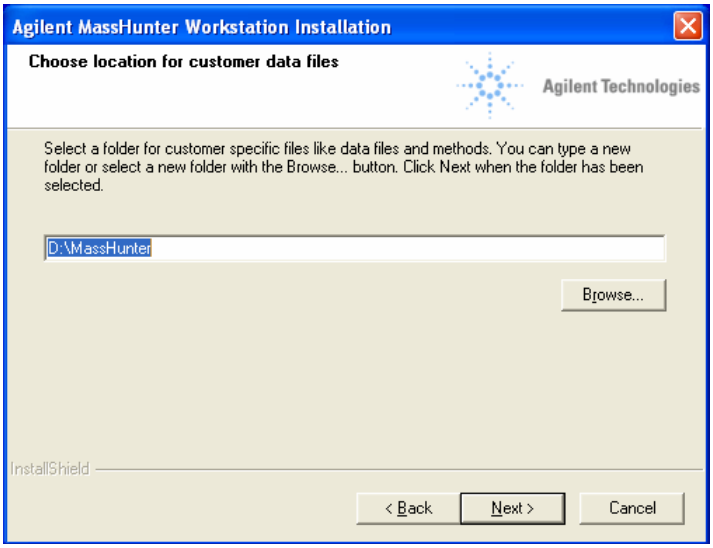


Figure 20 Choose location for customer data files screen

- In the Start Copying Files screen, review the settings and 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 resolution, click **OK**.
- 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**.

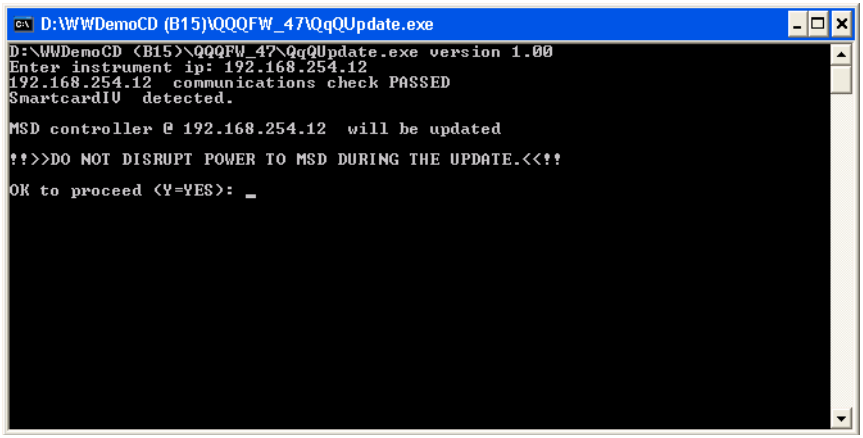
5 Click **Install Microsoft .NET 4.0** and follow the instructions, if any, to install Microsoft .NET Framework 4.0.

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.

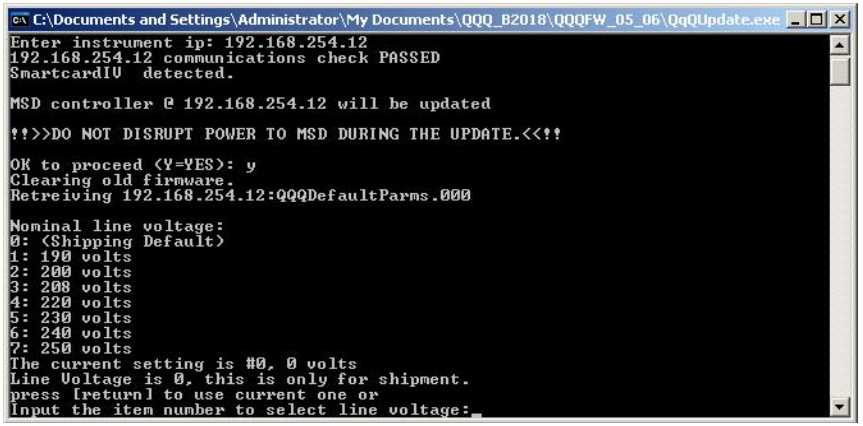


7 If the instrument is using an AJS ESI and this is a new installation:

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

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

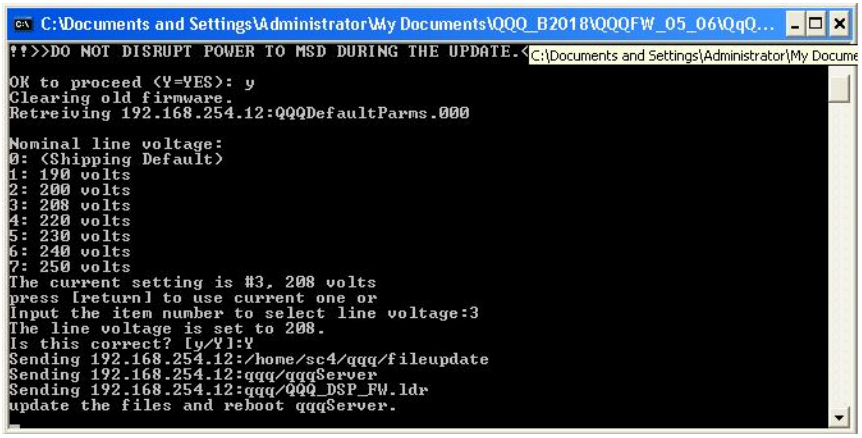
c When prompted, enter the country-specific nominal input voltage. Verify with a Digital Multi-Meter (DM) using the AC Voltage measuring function.



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 AJS ESI 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 are 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.

Step 5. Install the Qualitative Analysis program

- Refer to the *Offline Qualitative and Quantitative Analysis Installation Guide* to install the Qualitative Analysis program.

CAUTION You must use the **Run as administrator** option to install the program. Failure to do so can result in an incomplete installation, which can cause the system to run unreliably.

Do not install any other MassHunter program until instructed to do so.

Step 6. Install the Quantitative Analysis program

- Refer to the *Offline Qualitative and Quantitative Analysis Installation Guide* to install the Quantitative Analysis program.

CAUTION You must use the **Run as administrator** option to install the program. Failure to do so can result in an incomplete installation, which can cause the system to run unreliably.

Do not install any other MassHunter program until instructed to do so.

Step 7. Install and configure Microsoft Excel

If you are doing an upgrade installation, continue at "Step 8. Install Service Packs for Data Acquisition" on page 47.

The MassHunter Data Acquisition program supports:

- Excel 2010
- Office 2010
- Excel 2013
- Office 2013

- Refer to the *Offline Qualitative and Quantitative Analysis Installation Guide* to install and configure Excel.

Do not install any other MassHunter program until instructed to do so.

Step 8. Install Service Packs for Data Acquisition

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

If you received a separate Service Pack installation disc, insert that disc instead.

2 If the folder **Service Packs** exists on the installation disc, then:

a Open the **Service Packs** folder. If the folder contains another folder, such as **SP1**, open that folder.

b Locate the installation file, which ends with **SP *x*.exe**.

c Right-click the installation file and select **Run as administrator**.

d 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:

   Refer to the *Offline Qualitative and Quantitative Analysis Installation Guide* to install the Quantitative Compliance program.

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

1 Refer to the *Offline Qualitative and Quantitative Analysis Installation Guide* to install the Qualitative Analysis Reporting program.

CAUTION You must use the **Run as administrator** option to install the program. Failure to do so can result in an incomplete installation, which can cause the system to run unreliably.

Do not install any other MassHunter program until instructed to do so.

2 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.

3 Set the Microsoft Image Writer as the default printer:

a Click **Start > Devices and Printers**.

b 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.

Step 11. Check Excel Add-Ins for MassHunter

- Refer to the *Offline Qualitative and Quantitative Analysis Installation Guide* to verify the installation of Excel Add-Ins for MassHunter.

Step 12. Configure the instrument

If available, configure the device from the Device Configuration tool in the Data Acquisition program. Go to **Tools > Device Config**.

Some HPLC instrument setups can be configured only through the Device Configuration tool from the Data Acquisition program. The Capillary Pump and the Nano pump with the Chip Cube are examples.

In this step, you configure the Triple Quad instrument and the HPLC instrument.

1. Open the **Agilent MassHunter** folder on the desktop, and 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. Mark the **Mass Spectrometer** check box.
   c. Click **Agilent 6400 Series Triple Quadrupole**.
   d. Mark the **(1100/1200/1260/1290)/CE** check box.
   e. Click **Agilent LC System Access**.
   f. Click **Device Config** and configure the LC system.
   g. Click **OK**.

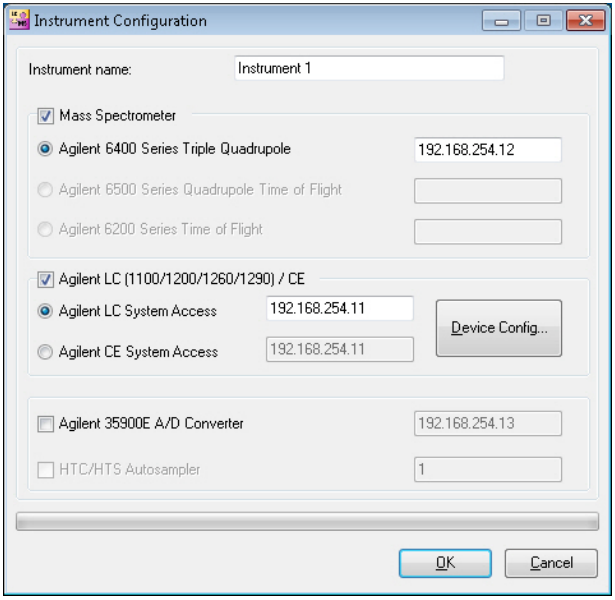


Figure 21 Instrument Configuration Dialog Box

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**.

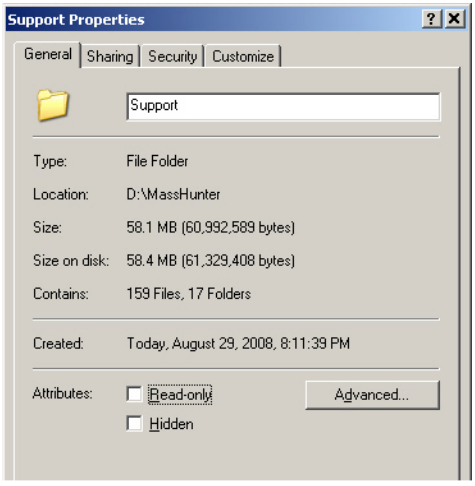


Figure 22 Support Properties dialog box

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

The Confirm Attribute Changes dialog box is displayed.

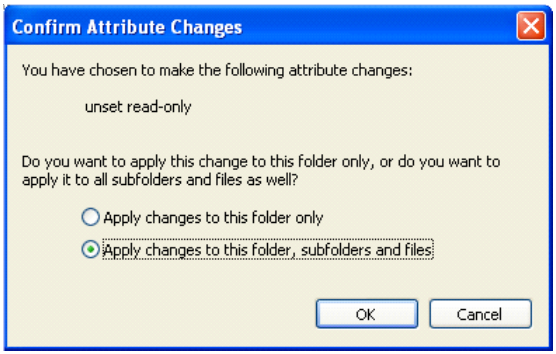


Figure 23 Confirm Attribute Changes dialog box

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

Step 14. Confirm the LC firmware revision

1. Start the Data Acquisition program.
2. Click **File > Print > Instrument Configuration Report**.
3. Click **Screen**.
4. Click **OK**.
5. Go to http://www.chem.agilent.com/_layouts/agilent/downloadFirmware.aspx?whid=69761 and check that you have the most recent firmware for your instrument.
6. 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.
7. 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 contact your Agilent representative.

Step 15. Finish the installation

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

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

2. Install or enable any other program that you will use with your MassHunter workstation. Refer to 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 the *Offline Qualitative and Quantitative Analysis Installation Guide* for more information.

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. Condition the Agilent LC instrument

Before you begin, check that you have:

- 18 MΩ, HPLC grade deionized water (p/n 8500-2236)
- high-purity HPLC grade isopropanol
- premixed LC flushing solvent (50:25:15:10 isopropanol/acetonitrile/cyclohexane/dichloromethane) (p/n G1969-85026)
- high-purity HPLC grade acetonitrile (p/n G2453-85050)
- high-purity HPLC grade methanol (p/n 8500-1867)
- high purity formic acid (p/n G2453-85060)

1 Flush the LC system.

This flushing procedure is very effective at reducing background contaminants from the LC systems when they are interfaced with a mass spectrometer. The flushing procedure must be done for each solvent channel to ensure contaminants are removed from separate degasser and pumping channels.

2 Remove any column(s) and bypass the flow cell for the UV detector, unless it is rated for high-pressure (400 bar) operation. Route the outlet capillary to a waste container. Don't try to re-use solvents used for the flushing procedure.

3 Use a handheld controller or the Data Acquisition program to set up the pump module to deliver deionized water for 15 minutes at 3 mL/minute to remove any salt buffers. Do 5 injections with deionized water to clean the injector path and sample loop.

4 Set up the pump module to deliver HPLC grade isopropanol for 5 minutes at 3 mL/minute. Do 5 injections with isopropanol to clean the injector path and sample loop.

5 Set up the pump module to deliver the premixed LC flushing solvent (50:25:15:10 isopropanol/acetonitrile/cyclohexane/dichloromethane) for 15 minutes at 3 mL/minute. Do 5 injections with the flushing solvent to clean the injector path and sample loop.

Alternatively, while the Triple Quad instrument pumps down, the flushing solvent can be pumped at a low flow rate (e.g. 0.1 mL/minute) overnight.

CAUTION Make sure that the flushing solvent does not stay in the LC for an extended period of time (for example, several days) because the cyclohexane may be harmful to the pump seals.

6 Set up the pump module again to deliver HPLC grade isopropanol for 5 minutes at 3 mL/minute. Do 5 injections with isopropanol to clean the injector path and sample loop.

7 Install the column used for installation verification (p/n 959757-902) and wet the column with 100% methanol.

8 Set up the pump module again to deliver HPLC grade methanol for at least 30 minutes at 0.5 mL/minute. Make sure that the solvent stream selector valve on the Triple Quad instrument is set to waste position or the outlet tubing from the column is *not* connected to the instrument.

   If the HPLC Stack has a Thermostatted Column Compartment (TCC), set the temperature of the column compartment to 60°C to reduce the back pressure and to help remove any contaminants on the column.

CAUTION For the 6460C, do both the Negative Ion Mode checkout and the Positive Ion Mode checkout *in that order*. The mobile phase used in the Positive Ion Mode checkout contains formic acid, which interferes with Negative Ion Mode and requires significant flushing to remove.

9 For 6460C, negative mode checkout:

   a For Channel A, install 1 liter of 100% water.

   b For Channel B, install 1 liter of 100% methanol.

   c Make sure flow rate is set to 0.5 mL/minute with 70:30 water:methanol to condition column with checkout mobile phase.

   d Do 5 injections with the blank premixed mobile phase (70:30 water: methanol) to clean the injector path and sample loop.

   e Continue at "Step 2. Prepare the performance evaluation samples" on page 61 to prepare the Negative Ion Mode checkout sample.

10 For 6460C, positive mode checkout:

   a For Channel A, install 1 liter of water with 0.1% formic acid.

   b For Channel B, install 1 liter of 100% acetonitrile.

c Do 5 injections with the blank premixed mobile phase (70:30 water: methanol) to clean the injector path and sample loop.

NOTE Up to 0.1 percent formic acid can be added to the solvent that you just prepared to help ionize the reserpine checkout sample. Do not add the formic acid until after you prepare the Reserpine checkout sample.

11 For all other models:

a Install 1 liter of 70:30 acetonitrile:water with 0.1% formic acid.

b Make sure the flow rate is set to 0.4 mL/minute to condition the column with checkout mobile phase.

c Do 5 injections with the blank premixed mobile phase (70:30 water: methanol) to clean the injector path and sample loop.

NOTE Use solvents that are at a minimum HPLC grade. Solvents that are acceptable for most LC applications may contain high levels of background that are detectable by the more sensitive Q-TOF instrument. LC solvents used with the Q-TOF instrument should be rated for both HPLC and pesticide, environmental, or GC/MS analyses. Use the highest purity solvents you can obtain. Acceptability of solvents must be empirically determined.

Step 2. Prepare the performance evaluation samples

In this step, you dilute the supplied performance evaluation sample (reserpine) to the concentrations needed for the Triple Quad system checkout.

For the 6460C, you prepare an additional performance evaluation sample (chloramphenicol) for negative mode checkout. See "Negative Mode (for 6460C only)" on page 64.

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.

Positive Mode (for all 6400 Series LC/MS)

Required Parts

- 1 mL graduated pipette (p/n 9301-1423)
- 2 × 100 mL volumetric flasks (p/n 9301-1344)
- ES/APCI Positive Ion Performance Standard (p/n 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 reserpine amount that is needed for performance verification.

Table 1 6490 Performance Verification Summary, MRM Mode

	6490 with AJS ESI 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 6460C Performance Verification Summary, MRM Mode

	6460C with AJS ESI Positive MRM Mode	**6460C 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	40:60 water:acetonitrile	40:60 water:acetonitrile
Method	6460C ESI_AJT Pos MRM Checkout.m	6460C ESI Pos MRM Checkout.m
Worklist	6460C ESI_AJT Pos MRM Checkout.wkl	6460C ESI Pos MRM Checkout.wkl
Performance specifications	5000:1 Auto-RMS	1500:1 Auto-RMS

Table 3 6430 and 6460A Performance Verification Summary, MRM Mode

	6460A with AJS ESI Positive MRM Mode	6430, 6460A 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
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 4 6410/6420 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)

Negative Mode (for 6460C only)

Required Parts

- 1 mL graduated pipette (p/n 9301-1423)
- 2 × 100 mL volumetric flasks (p/n 9301-1344)
- ESI Negative Ion Performance Standard (p/n 5190-0591)
- Plastic bottle for storing first dilution (p/n 9301-1433)

1. Transfer 1 mL of 5 ng/µL chloramphenicol (p/n 5190-0591) to a 100 mL volumetric flask. Use a clean graduated pipette.
2. Dilute to the 100 mL mark with 70:30 water:methanol. Transfer 1 mL of the first dilution to the other 100 mL volumetric flask. Use a clean graduated pipette.
3. Dilute to the 100 mL mark with 70:30 water:methanol.

   This provides 500 fg/µL at 2 µL injection volume, which equals the final 1 pg chloramphenicol amount that is needed for performance verification.
4. Go back to "Step 1. Condition the Agilent LC instrument" on page 58 and repeat in Positive Ion Mode.

Table 5 6460C Performance Verification Summary, MRM Mode

	6460C with AJS ESI Negative MRM Mode	**6460C ES G1948B Negative MRM Mode**
Sample	Chloramphenicol, 5 ng/µL	Chloramphenicol, 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)	5190-0591	5190-0591
Solvent	70:30 water:methanol	70:30 water:methanol
Method	6460 ESI_AJT Neg MRM Checkout.m	6460 ESI Neg MRM Checkout.m
Worklist	6460 ESI_AJT Neg MRM Checkout.wkl	6460 ESI Neg MRM Checkout.wkl
Performance specifications	5000:1 Auto-RMS	1500:1 Auto-RMS

Step 3. Review methods and run worklist

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

Table 6 Methods and worklists to copy

If you have...	Copy...	From...	To...
6490A with AJS ESI	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
6460C with AJS ESI	6460 ESI_AJT Pos MS2 Scan.m 6460C ESI_AJT Pos MRM Checkout.m	D:\MassHunter\Support\Checkout\6460 Methods and Worklist\Pos	D:\MassHunter\Methods
	6460C ESI_AJT Pos MRM Checkout.wkl	D:\MassHunter\Support\Checkout\6460 Methods and Worklist\Pos	D:\MassHunter\Worklists
	6460 ESI_AJT Neg MS2 Scan.m 6460 ESI_AJT Neg MRM Checkout.m	D:\MassHunter\Support\Checkout\6460 Methods and Worklist\Neg	D:\MassHunter\Methods
	6460 ESI_AJT Neg MRM Checkout.wkl	D:\MassHunter\Support\Checkout\6460 Methods and Worklist\Neg	D:\MassHunter\Worklists
6460C with ESI	6400 ESI Pos MS2 Scan.m 6460C ESI Pos MRM Checkout.m	D:\MassHunter\Support\Checkout\6460 Methods and Worklist\Pos	D:\MassHunter\Methods
	6460C ESI Pos MRM Checkout.wkl	D:\MassHunter\Support\Checkout\6460 Methods and Worklist\Pos	D:\MassHunter\Worklists
	6400 ESI Neg MS2 Scan.m 6400 ESI Neg MRM Checkout.m	D:\MassHunter\Support\Checkout\6460 Methods and Worklist\Neg	D:\MassHunter\Methods
	6400 ESI Neg MRM Checkout.wkl	D:\MassHunter\Support\Checkout\6460 Methods and Worklist\Neg	D:\MassHunter\Worklists

Table 6 Methods and worklists to copy

If you have...	Copy...	From...	To...
6460A with AJS ESI	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
6410B, 6420A, 6430A, 6460A with 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 - AJS ESI, Positive MRM Mode" on page 67
- "For 6460C - AJS ESI, Negative MRM Mode" on page 70
- "For 6460C - AJS ESI, Positive MRM Mode" on page 73
- "For 6460C - G1948B ESI, Negative MRM Mode" on page 76
- "For 6460C - G1948B ESI, Positive MRM Mode" on page 79
- "For 6460A - AJS ESI, Positive MRM Mode" on page 82
- "For 6410/6420/6430/6460A - G1948B ESI, Positive MRM Mode" on page 84
- "For 6410/6420 - G1978B Multimode Interface, Positive MRM Mode" on page 86.

For 6490 - AJS ESI, 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 × 10^{-5} Torr.

To view the high vacuum gauge reading, start the Data Acquisition program, change the **Context** to **Tune**, and then click the **Manual Tune > Cell** tab. Check that the high vacuum gauge reading is within range. If not, see "To reset the Collision Cell gas flow rate" on page 97.

2 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.

Table 7 6490 ESI_AJT Pos MRM Checkout.m parameters

Parameter	Value
Injection volume	2 µL
Autosampler temperature	4°C
Needle wash	flushport (25:75 water:methanol, 0.1% formic acid), 10 seconds
Mobile Phase	A = water, 0.1% formic acid B = acetonitrile, 0.1% formic acid
Gradient (for 1290)	**Time** **%B** 0.2 10 0.8 98 1.0 98 1.1 98 Stop time: 2 minutes

Table 7 6490 ESI_AJT Pos MRM Checkout.m parameters (continued)

Parameter	Value
Gradient (for 1260)	**Time** **%B** 0.2 10 1.0 98 2.0 98 2.1 98 Stop time: 2 minutes
Flow rate	0.4 mL/minute
Gas Temp	200°C (can be optimized, typically between 130°C to 200°C)
Gas Flow	12 L/minute (can be optimized, typically between 12 to 15 L/minute)
Nebulizer	20 psi (can be optimized, typically between 20 psi to 25 psi)
Sheath Gas Temp	400°C
Sheath Gas Flow	12 L/minute
Capillary	3500 V
Nozzle Voltage (AJS ESI)	500 V (can be optimized, typically between 0 to 500 V)
Collision Energy	41 V (can be optimized, typically between 37 to 42 V)
Fragmentor	fixed; determined by iFunnel and autotune
Accelerator voltage	3 V cell (can be optimized, typically between 1 to 3 V)
Delta EMV	200 V (can be optimized, typical between 200 to 350 V)
MS1 Res	Wide
MS2 Res	Unit

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

5 Click **Method > Open** 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 Click **Worklist > Open**, 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, and 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 6460C - AJS ESI, Negative 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 × 10^{-5} Torr.

To view the high vacuum gauge reading, start the Data Acquisition program, change the **Context** to **Tune**, and then click the **Manual Tune > Cell** tab. Check that the high vacuum gauge reading is within range. If not, see "To reset the Collision Cell gas flow rate" on page 97.

2 Start an **Autotune (negative polarity)**.

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 **Method > Open** and then load the method **6460 ESI_AJT Neg MRM Checkout.m**. The checkout method includes the following acquisition parameters:

Table 8 6460 ESI_AJT Neg MRM Checkout.m parameters

Parameter	Value
Injection volume	2 µL
Flow rate	0.5 mL/minute
Mobile phase	A = water B = methanol
Gradient (for 1260 or 1290)	**Time** **%A** **%B** Initial 90 10 0.10 90 10 1.00 2 98 2.00 2 98 2.01 90 10 Stop time: 3.7 minutes
Gas Temp	200°C
Gas Flow	6 L/minute
Nebulizer	35 psi
Sheath Gas Temp	400°C

Table 8 6460 ESI_AJT Neg MRM Checkout.m parameters (continued)

Parameter	Value
Sheath Gas Flow	12 L/minute
Capillary	2500 V
Nozzle Voltage	1000 V
Collision Energy	14 V
Fragmentor	130 V
Delta EMV	200 V
MS1 Res	Unit
MS2 Res	Unit
Compound	Chloramphenicol
Polarity	Negative
Time Filtering	0.03 minutes
Precursor Ion	321.2 *m/z*
Product Ion	152.0 *m/z*
Dwell	200 ms
Cell Accelerator Voltage	3 V

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

4 Click **Method > Open** and then load the method **6460 ESI_AJT Neg 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 chloramphenicol sample (500 fg/µL)

6 Click **Worklist > Open,** and then open the worklist: **6460 ESI_AJT Neg MRM Checkout.wkl**.

The worklist is set up to do one injection of the solvent blank using the **6460 ESI_AJT Neg MS2 Scan.m** method in order to collect background ion data, and then using the **6460 ESI_AJT Neg 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 chloramphenicol sample.

For 6460C - AJS ESI, 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 × 10^{-5} Torr.

To view the high vacuum gauge reading, start the Data Acquisition program, change the **Context** to **Tune**, and then click the **Manual Tune > Cell** tab. Check that the high vacuum gauge reading is within range. If not, see "To reset the Collision Cell gas flow rate" on page 97.

2 Start an **Autotune (positive polarity)**.

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 **Method > Open** and then load the method **6460C ESI_AJT Pos MRM Checkout.m**. The checkout method includes the following acquisition parameters:

Table 9 6460C ESI_AJT Pos MRM Checkout.m parameters

Parameter	Value
Injection volume	2 µL
Stop time	4 minutes
Flow rate	0.4 mL/minute
Mobile phase	A = water with 0.1% Formic Acid B = acetonitrile
Gradient (for 1260 or 1290)	**Time** **%A** **%B** Initial 90 10 0.10 90 10 1.00 2 98 2.00 2 98 2.01 90 10 Stop time: 4.00 minutes
Gas Temp	325°C
Gas Flow	10 L/minute
Nebulizer	20 psi

Table 9 6460C ESI_AJT Pos MRM Checkout.m parameters (continued)

Parameter	Value
Sheath Gas Temp	400°C
Sheath Gas Flow	12 L/minute
Capillary	4000 V
Nozzle Voltage	0 V
Collision Energy	41 V (can be optimized, typically between 37 to 41 V)
Fragmentor	150 V (can be optimized, typically between 140 to 250 V)
Delta EMV	200 V (can be optimized, typical between 200 to 350 V)
MS1 Res	Unit
MS2 Res	Unit
Compound	Reserpine
Polarity	Positive
Time Filtering	0.03 minutes
Precursor Ion	609.3 *m/z*
Product Ion	195.1 *m/z*
Dwell	200 ms
Cell Accelerator Voltage	7 V

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

4 Click **Method > Open** 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 Click **Worklist > Open,** and then open the worklist: **6460C 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, and then using the **6460C 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 6460C - G1948B ESI, Negative 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 × 10^{-5} Torr.

To view the high vacuum gauge reading, start the Data Acquisition program, change the **Context** to **Tune**, and then click the **Manual Tune > Cell** tab. Check that the high vacuum gauge reading is within range. If not, see "To reset the Collision Cell gas flow rate" on page 97.

2 Start an **Autotune (negative polarity)**.

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 **Method > Open** and then load the method **6400 ESI Neg MRM Checkout.m**. The checkout method includes the following acquisition parameters:

Table 10 6400 ESI Neg MRM Checkout.m parameters

Parameter	Value
Injection volume	2 µL
Flow rate	0.5 mL/minute
Mobile phase	A = water B = methanol
Gradient (for 1260 or 1290)	**Time** **%A** **%B** Initial 90 10 0.10 90 10 1.00 2 98 2.00 2 98 2.01 90 10 Stop time: 3.7 minutes
Gas Temp	310°C
Gas Flow	13 L/minute
Nebulizer	25 psi
Capillary	3000 V

Table 10 6400 ESI Neg MRM Checkout.m parameters (continued)

Parameter	Value
Collision Energy	13 V
Fragmentor	100 V
Delta EMV	200 V
MS1 Res	Unit
MS2 Res	Unit
Compound	Chloramphenicol
Polarity	Negative
Time Filtering	0.03 minutes
Precursor Ion	321.2 *m/z*
Product Ion	152.0 *m/z*
Dwell	200 ms
Cell Accelerator Voltage	3 V

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

4 Click **Method > Open** and then load the method **6400 ESI Neg 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 chloramphenicol sample (500 fg/µL)

6 Click **Worklist > Open,** and then open the worklist: **6400 ESI Neg MRM Checkout.wkl**.

The worklist is set up to do one injection of the solvent blank using the **6400 ESI Neg MS2 Scan.m** method in order to collect background ion data, and then using the **6400 ESI Neg 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 chloramphenicol sample.

For 6460C - G1948B ESI, 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 × 10^{-5} Torr.

To view the high vacuum gauge reading, start the Data Acquisition program, change the **Context** to **Tune**, and then click the **Manual Tune > Cell** tab. Check that the high vacuum gauge reading is within range. If not, see "To reset the Collision Cell gas flow rate" on page 97.

2 Start an **Autotune (positive polarity)**.

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 **Method > Open** and then load the method **6460C ESI Pos MRM Checkout.m**. The checkout method includes the following acquisition parameters:

Table 11 6460C ESI Pos MRM Checkout.m parameters

Parameter	Value
Injection volume	2 µL
Flow rate	0.5 mL/minute
Mobile phase	A = water B = methanol
Gradient Timetable (for 1260 or 1290)	**Time** **%A** **%B** Initial 90 10 0.10 90 10 1.00 2 98 2.00 2 98 2.01 90 10 Stop time: 3.70 minutes
Gas Temp	350°C
Gas Flow	13 L/minute (can be optimized, typically between 10 to 13 L/minute)
Nebulizer	60 psi

Table 11 6460C ESI Pos MRM Checkout.m parameters (continued)

Parameter	Value
Collision Energy	41 V (can be optimized, typically between 37 to 41 V)
Fragmentor	150 V (can be optimized, typically between 140 to 250 V)
Delta EMV	200 V (can be optimized, typical between 200 to 350 V)
MS1 Res	Unit
MS2 Res	Unit
Compound	Reserpine
Polarity	Positive
Time Filtering	0.03 minutes
Precursor Ion	609.3 *m/z*
Product Ion	195.1 *m/z*
Dwell	200 ms
Cell Accelerator Voltage	7 V

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

4 Click **Method > Open** and then load the method **6400 ESI 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 Click **Worklist > Open,** and then open the worklist: **6460C 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, and then using the **6460C 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 6460A - AJS ESI, 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 × 10^{-5} Torr.

To view the high vacuum gauge reading, start the Data Acquisition program, change the **Context** to **Tune**, and then click the **Manual Tune > Cell** tab. Check that the high vacuum gauge reading is within range. If not, see "To reset the Collision Cell gas flow rate" on page 97.

2 Start an **Autotune (positive polarity)**.

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 **Method > Open** and then load the method **6460 ESI_AJT Pos MRM Checkout.m**. The checkout method includes the following acquisition parameters:

Table 12 6460 ESI_AJT Pos MRM Checkout.m parameters

Parameter	Value
Injection volume	2 µL
Stop time	1 minute
Flow rate	isocratic from channel A and B 30:70, at 0.4 mL/minute
Gas Temp	325°C
Gas Flow	10 L/minute
Nebulizer	20 psi
Sheath Gas Temp	400°C
Sheath Gas Flow	12 L/minute
Capillary	4000 V
Nozzle Voltage	500 V
Collision Energy	41 V (can be optimized, typically between 37 to 41 V)
Fragmentor	150 V (can be optimized, typically between 140 to 250 V)

Table 12 6460 ESI_AJT Pos MRM Checkout.m parameters (continued)

Parameter	Value
Delta EMV	200 V (can be optimized, typical between 200 to 350 V)
MS1 Res	Wide
MS2 Res	Unit

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

4 Click **Method > Open** 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 Click **Worklist > Open,** and then open 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, and 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 6410/6420/6430/6460A - G1948B ESI, 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 × 10^{-5} Torr for two turbo systems (6410 with G5273A, 6430 or 6460) and 2.7 to 3.3 × 10^{-5} torr for single turbo systems (6410 *without* G2573A, 6420).

To view the high vacuum gauge reading, start the Data Acquisition program, change the **Context** to **Tune**, and then click the **Manual Tune > Cell** tab. Check that the high vacuum gauge reading is within range. If not, see "To reset the Collision Cell gas flow rate" on page 97.

2 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 **Method > Open** and then load the method **6400 ESI Pos MRM Checkout.m**. The checkout method includes the following acquisition parameters:

Table 13 6400 ESI Pos MRM Checkout.m parameters

Parameter	Value
Injection volume	2 µL
Stop time	1 minute
Flow rate	isocratic from channel A and B 30:70, at 0.4 mL/minute
Gas Temp	325°C
Gas Flow	10 L/minute (can be optimized, typically between 10 to 13 L/minute)
Nebulizer	35 psi
Capillary	4000 V
Collision Energy	37 V (can be optimized, typically between 37 to 41 V)
Fragmentor	240 V (can be optimized, typically between 140 to 250 V)
Delta EMV	200 V (can be optimized, typical between 200 to 350 V)

Table 13 6400 ESI Pos MRM Checkout.m parameters (continued)

Parameter	Value
MS1 Res	Wide
MS2 Res	Unit

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

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 **Method > Open** 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 Click **Worklist > Open**, 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, and 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 6410/6420 - 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 1.9 to 2.3 × 10^{-5} Torr for two turbo systems (6410 with G5273A, 6430 or 6460) and 2.7 to 3.3 × 10^{-5} Torr for single turbo systems (6410 *without* G2573A, 6420).

To view the high vacuum gauge reading, start the Data Acquisition program, change the **Context** to **Tune**, and then click the **Cell** tab. Check that the high vacuum gauge reading is within range. If not, see "To reset the Collision Cell gas flow rate" on page 97.

2 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 **Method > Open** and then load the method **MMI-ES Pos MRM Reserpine Checkout.m**. The checkout method includes the following acquisition parameters:

Table 14 MMI-ES Pos MRM Reserpine Checkout.m parameters

Parameter	Value
Injection volume	2 µL
Stop time	1 minute
Flow rate	isocratic from channel A, at 0.4 mL/minute
Gas Temp	250°C
Gas Flow	10 L/minute (can be optimized, typically between 10 to 13 L/minute)
Nebulizer	60 psi
Capillary	2000 V
Collision Energy	37 V (can be optimized, typically between 37 to 41 V)
Fragmentor	240 V (can be optimized, typically between 140 to 250 V)
Delta EMV	400 V (can be optimized, typical between 200 to 400 V)

Table 14 MMI-ES Pos MRM Reserpine Checkout.m parameters (continued)

Parameter	Value
MS1 Res	Wide
MS2 Res	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 **Method > Open** 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 Click **Worklist > Open**, 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, and 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 4. Review methods and run worklist for bid specification (add-on service)

The G6061A Performance Specification Service is available only at the time of original Agilent 6400 Series Triple Quad LC/MS System bundle purchase.

The steps in this topic are done by an Agilent representative if the G6061A service was purchased.

For 6490 with G6061A - AJS ESI, Positive MRM Mode, do the steps in "For 6490 - AJS ESI, Positive MRM Mode" on page 67.

For 6460C with G6061A - AJS ESI, Positive MRM Mode, do the steps in "For 6460C - AJS ESI, Positive MRM Mode" on page 73.

For 6460C with G6061A - ESI, Positive MRM Mode, do the steps in "For 6460C - G1948B ESI, Positive MRM Mode" on page 79

For 6420, 6430, or 6460A with G6061A - ESI, Positive MRM Mode (AJS ESI), do the steps in this topic.

1 Verify that the Collision Cell gas flow rate produces a high vacuum gauge reading in the range of 1.9 to 2.3 × 10^{-5} torr for two turbo systems (6430 or 6460) and 2.7 to 3.3 × 10^{-5} torr for single turbo system (6420).

   To view the high vacuum gauge reading, start the Data Acquisition program, change the **Context** to **Tune**, and then click the **Manual Tune > Cell** tab. Check that the high vacuum gauge reading is within range. If not, see "To reset the Collision Cell gas flow rate" on page 97.

2 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 **Method > Open**, and then load the method **6061 ESI Pos MRM Checkout.m**. The checkout method includes the following acquisition parameters. Edit if needed.

Table 15 6061 ESI Pos MRM Checkout.m parameters

Parameter	Value
Injection volume	2 µL
Autosampler temperature	4°C
Needle wash	flushport (25:75 water:methanol, 0.1% formic acid), 10 seconds
Mobile Phase	A = water, 0.1% formic acid B = acetonitrile, 0.1% formic acid
Gradient (for 1290)	**Time** **%B** 0.2 10 0.8 98 1.0 98 1.1 98 Stop time: 2 minutes
Gradient (for 1260)	**Time** **%B** 0.2 10 1.0 98 2.0 98 2.1 98 Stop time: 4 minutes
Flow rate	0.4 mL/minute
Gas Temp	350°C (can be optimized, typically between 325°C to 350°C)
Gas Flow	10 L/minute (can be optimized, typically between 10 to 12 L/minute)
Nebulizer	35 psi (can be optimized, typically between 20 psi to 35 psi)
Sheath Gas Temp (AJS ESI)	400°C
Sheath Gas Flow	12 L/minute (AJS ESI)
Capillary	4000 V
Nozzle Voltage (AJS ESI)	500 V (can be optimized, typically between 0 to 500 V)
Collision Energy	41 V (can be optimized, typically between 37 to 42 V)
Fragmentor	150 V (can be optimized, typical between 150 V to 240 V)

Table 15 6061 ESI Pos MRM Checkout.m parameters (continued)

Parameter	Value
Cell Accelerator Voltage	4 V cell (can be optimized, typically between 2 to 5 V)
Delta EMV	200 V (can be optimized, typical between 200 to 350 V)
MS1 Res	Wide
MS2 Res	Unit

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

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

5 Click **Method > Open,** and then load the method **6061 ESI Pos MS2 Scan.m**.

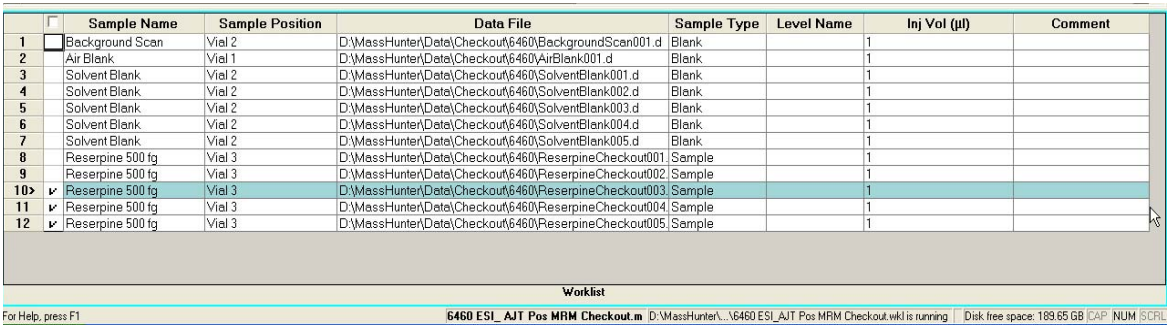
6 Put the vials into the LC autosampler.

- 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 Click **Worklist > Open**, and then load the worklist: **6061 ESI Pos MRM Checkout.wkl**.

The worklist is set up to do one injection of the solvent blank using the **6061 ESI Pos MS2 Scan.m** method in order to collect background ion data, and then using the **6061 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. After that, it runs the **6061ESI Pos MRM Checkout.m** method to run five more injections of the reserpine sample with 0.1 µL injection volume.

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 24 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.

   For G6460C with Agilent Jet Stream Technology, run the **6460 ESI_AJT Neg MRM Checkout.wkl** worklist first. After completion, change solvents (to A = Water w/0.1% Formic Acid and B = Acetonitrile w/0.1% Formic Acid for Positive Polarity), equilibrate the column for 1 hour at 30:70 A:B and run the **6460C ESI_AJT Pos MRM Checkout.wkl** worklist.

   For G6460C with ESI, run the **6400 ESI Neg MRM Checkout.wkl** worklist first. After completion, change solvents (to A = Water w/0.1% Formic Acid and B = Acetonitrile w/0.1% Formic Acid for Positive Polarity), equilibrate the column for 1 hour at 30:70 A:B and run the **6460C ESI Pos MRM Checkout.wkl** 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.

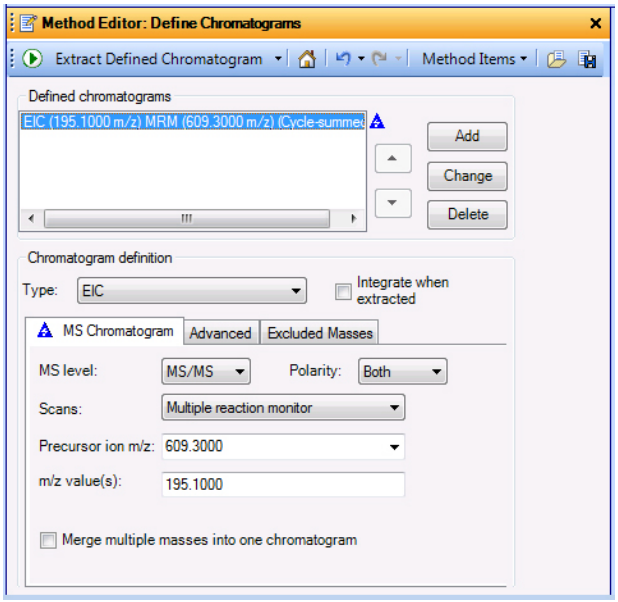
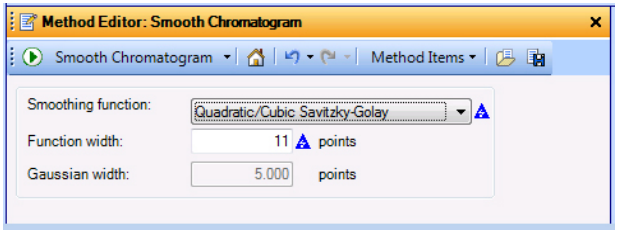


Figure 25 Extract Chromatogram dialog box

c Smooth the extracted chromatogram by setting **Smoothing 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 *For sensitivity verification with a standard checkout on 6410, 6420, 6430, 6460A, or 6490:*

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

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

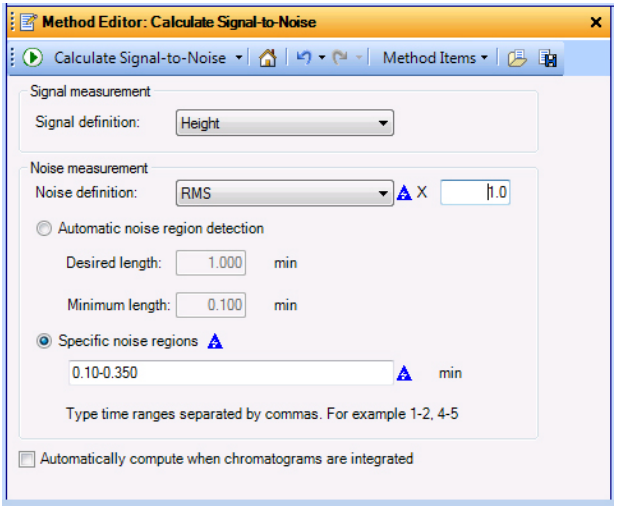


Figure 26 Calculate Signal-to-Noise dialog box (for standard checkout) on 6410, 6420, 6430, 6460A, or 6490

g *For sensitivity verification with a 6460C, or performed under G6061A for 6420, 6430, 6460A or 6490:*

Under Noise Measurement, for **Noise definition**, select **Auto-RMS** (available in MassHunter Qualitative Analysis B.05.00 and higher).

Set the **Noise region boundary** to **Start time 0 minute** and **End time 1 minute**. (Make sure that the noise region does not include the reserpine peak.)

Set the **Noise region width** to **0.100 minute**.

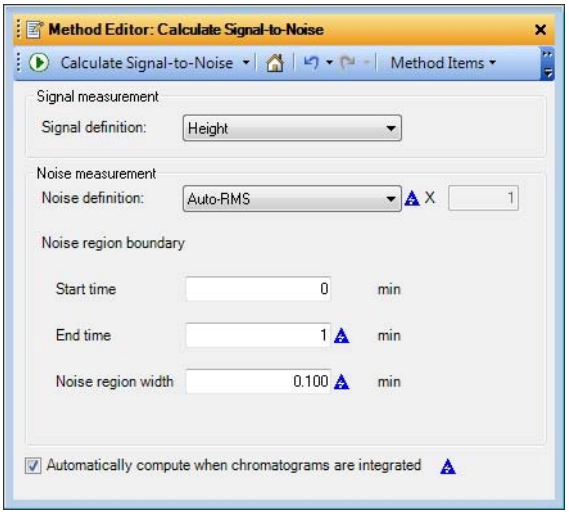


Figure 27 Calculate Signal-to-Noise dialog box (for a standard checkout with 6460C or G6061A Performance Specification Service checkout)

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 16.

Table 16 Minimum average signal-to-noise (standard installation checkout)

Instrument Model	Source	Average Signal-to-Noise (1 x RMS) (Positive Mode)	Average Signal-to-Noise (Auto-RMS) (Negative Mode)
6490	AJS ESI	10,000:1	
6460C	AJS ESI	5000:1 (Auto-RMS)	5000:1
6460C	ESI	1500:1 (Auto-RMS)	1500:1
6460A	AJS ESI	1000:1	
6460A	ESI	300:1	
6430	ESI	300:1	
6420	ESI	150:1	
6420	Multimode	150:1	

Table 17 Minimum average signal-to-noise for bid specification (sold as part of G6061A)

Instrument Model	Source	Average Signal-to-Noise (Auto-RMS)
6490	AJS ESI	50,000:1
6460	AJS ESI	10,000:1
6430	ESI	3,000:1
6420	ESI	1,500:1

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						

For models other than G6460C, if verification in negative mode is required, verify the instrument with the parameters listed in Table 18. Use the Agilent ES Negative Ion Performance Standard (p/n G1946-85005) that is included with your instrument.

Table 18 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 5 to 5.8 × 10^{-5} torr for a 6490, 1.9 to 2.3 × 10^{-5} for a 6460/6430 (two turbo pumps) and 2.7 to 3.3 × 10^{-5} for 6420/6410 (one turbo pump), 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 × 10^{-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, and then open the appropriate .xml file with Notepad:

6490	G6490AHWParams.xml
6460	G6460AHWParams.xml
6430	G6430AHWParams.xml
6420	G6420AHWParams.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. (Right-click the **Engine Launch** icon in the system tray, and then click **Shutdown Engines**.)

c 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:

a Put the nebulizer into the nebulizer adjustment fixture that is supplied in the shipping kit. Check that the nebulizer needle is properly adjusted. Make sure that the nebulizer needle is even with the end of the nebulizer nozzle.

b Install the nebulizer in the spray chamber.

WARNING **Surfaces can be extremely hot.**

c Remove the previously installed spray chamber. Install the standard spray shield if not already installed.

d Orient the standard spray shield so the off-axis hole is at the 12 o'clock position by loosening the two T10 screws that secure the end plate, orienting the spray shield, and re-tightening the two T10 screws (do not over-tighten).

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

f Connect the APCI corona and vaporizer heater cables to the connector on the left side panel of the instrument.

g Connect the 1/8-inch nebulizing gas tubing from the LC/MS mainframe to the nebulizer gas fitting.

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

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, run a manual tune with the G2432A APCI/APPI Calibration Solution.

1 Install the G2432A APCI/APPI Calibration Solution.

2 Check the tuning of the APCI source:

a In Data Acquisition program, set **Context** to **Tune**.

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

c In the **Manual Tune** tab, mark the **Calibrant** check box, and 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.

If needed, click **Adjust Gain & Offset** to establish the Mass Axis and Peak Width values. Repeat these for both MS1 and MS2 tabs.

3 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:

- 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 can damage the AJS ESI. 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:

a Put the nebulizer into the nebulizer adjustment fixture that is supplied in the shipping kit. Check that the nebulizer needle is properly adjusted. Make sure that the nebulizer needle is even with the end of the nebulizer nozzle.

b Install the nebulizer in the spray chamber.

WARNING **Surfaces can be extremely hot.**

c Remove the previously installed spray chamber. Install the multimode spray shield if not already installed.

d Orient the standard spray shield so the field-shaping electrode is at the 6 o'clock and 9 o'clock positions by loosening the two T10 screws that secure the end plate, orienting the spray shield, and re-tightening the two T10 screws (do not over-tighten).

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

f Connect the multimode high voltage, APCI corona, and vaporizer heater cables to the connector on the left side panel of the instrument.

g Connect the 1/8-inch nebulizing gas tubing from the LC/MS mainframe to the nebulizer gas fitting.

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

To verify the operation of the G1978B multimode source

1. Install the G1969-85020 MMI-L Low Concentration Tuning Mix.
2. Check the tuning of the multimode source:
   a. In Data Acquisition program, set **Context** to **Tune**.
   b. Open the autotune file that was generated with the electrospray or multimode source.
   c. In the **Manual Tune** tab, mark the **Calibrant** check box, and 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.

      If needed, click **Adjust Gain & Offset** to establish the Mass Axis and Peak Width values. Repeat these for both MS1 and MS2 tabs.
3. Save the tune file.

G1971B APPI Source

To install the G1971B APPI source

1 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.

2 Remove the currently installed source and install the APPI source:

a Put the nebulizer into the nebulizer adjustment fixture that is supplied in the shipping kit. Check that the nebulizer needle is properly adjusted. Make sure that the nebulizer needle is even with the end of the nebulizer nozzle.

b Install the nebulizer in the spray chamber.

WARNING **Surfaces can be extremely hot.**

c Remove the previously installed spray chamber. Install the standard spray shield if not already installed.

d Orient the standard spray shield so the off-axis hole is at the 12 o'clock position by loosening the two T10 screws securing the end plate, orienting the spray shield, and re-tightening the two T10 screws (do not over tighten).

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

f Connect the vaporizer heater cable to the connector on the left side panel of the instrument.

g Connect the 1/8-inch nebulizing gas tubing from the LC/MS mainframe to the nebulizer gas fitting.

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

Do *not* use the APPI USB to Serial Converter Cable (p/n 8121- 1013).

3 Connect the APPI power supply cable to the APPI DB9 power connector and screw it in. This connector contains both serial and power interfaces.

4 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.

5 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 G1971 APPI source on the Triple Quad instrument. To verify its proper operation, run a manual tune with the G2432A APCI/APPI Calibration Solution.

1 Install the G2432A APCI/APPI Calibration Solution.

2 Check the tuning of the APPI source:

a In Data Acquisition program, set **Context** to **Tune**.

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

c In the **Manual Tune** tab, mark the **Calibrant** check box, and 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.

If needed, click **Adjust Gain & Offset** to establish the Mass Axis and Peak Width values. Repeat these for both MS1 and MS2 tabs.

3 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 19:

a In a conical bottom polypropylene autosampler vial, put the volume of standard in Table 19.

b Add the volume of 15:85 acetonitrile:water with 0.1% formic acid solvent in Table 19.

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.

Sample E is optional and is used for collecting information.

Table 19 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/µL

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, and 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 20.

Table 20 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 28.

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	

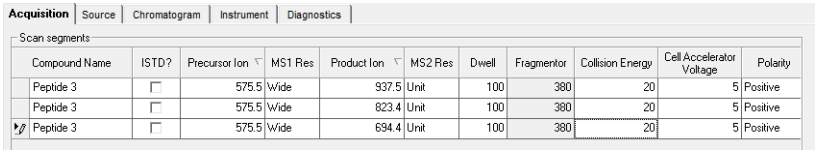
Figure 28 HPLC-Chip/MS Worklist

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 to 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	Cell Accelerator Voltage	Polarity
Peptide 3	☐	575.5	Wide	937.5	Unit	100	380	20	5	Positive
Peptide 3	☐	575.5	Wide	823.4	Unit	100	380	20	5	Positive
Peptide 3	☐	575.5	Wide	694.4	Unit	100	380	20	5	Positive

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.

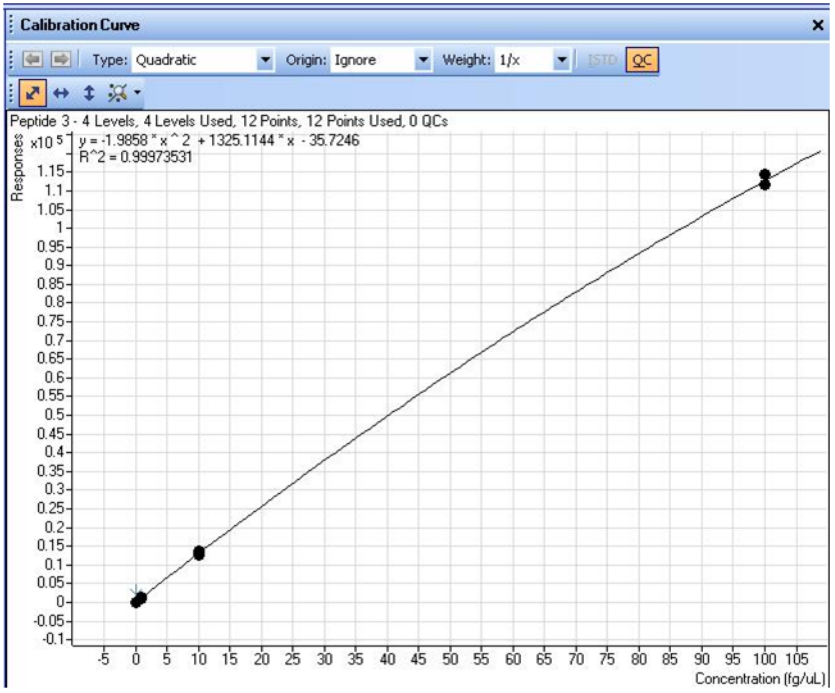


Figure 29 HSA Calibration Curve Example

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

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).

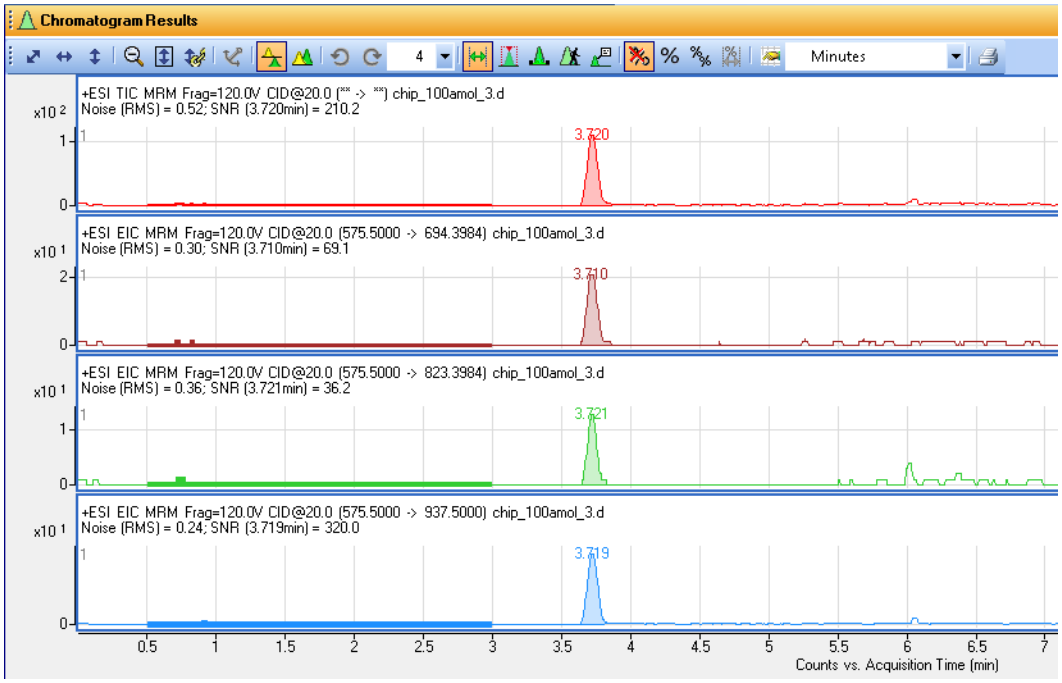


Figure 30 Calculated Signal-to-noise for 575.5 *m/z* -> ** transitions and a TIC MRM.

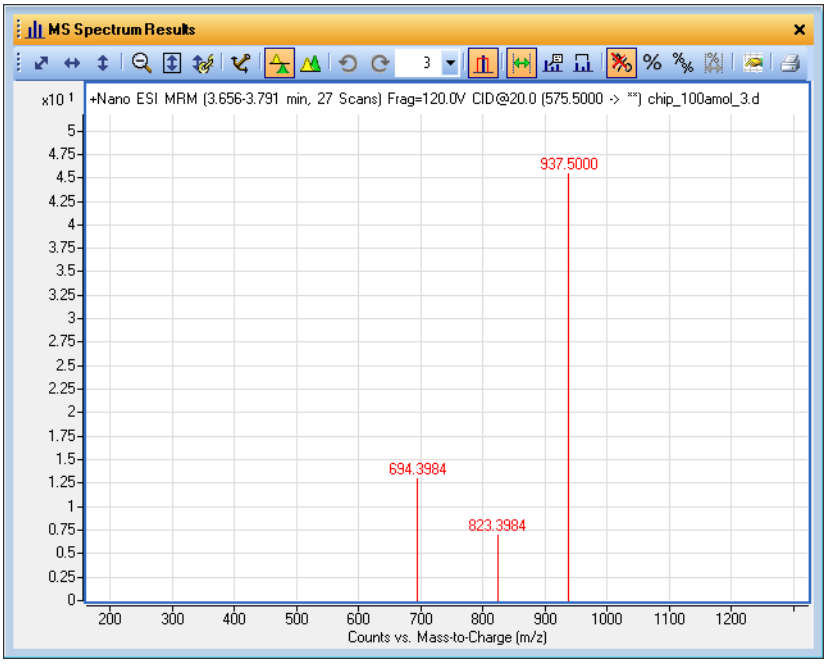


Figure 31 Mass Spectrum for MRM transition 575.5 *m/z* -> **

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**.

CAUTION Do not start Data Acquisition as administrator. (*Do not* right-click **Data Acquisition** and select **Run as administrator**.)

To stop MassHunter Engines

1. Close the **Data Acquisition** program.
2. Right-click the **Engine Launcher** icon in the system tray.
3. Click **Shutdown Engines** to stop the engines.

To start the MassHunter Engines, restart the Data Acquisition program. Alternatively, to start the MassHunter Engines without starting the Data Acquisition program, click **Start Engines**.

To stop MassHunter processes in case of unrecoverable errors

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

1. Click **Programs > Agilent > MassHunter Workstation > Acq Tools > Remove MassHunter Processes**.
2. Click **Yes** when you are asked whether you want to remove all MassHunter processes.

Only the Data Acquisition processes are stopped.

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 start the Logbook Viewer program within the MassHunter Data Acquisition program.

To configure the network interface card

The computer that is 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**, and 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**, and 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 remove the MassHunter Workstation software

1. If you have any data, methods, libraries, or other files or directories that you want to save, then move or copy them to a folder outside of those to be deleted.
2. Close all MassHunter Workstation programs, including the Data Acquisition, Qualitative Analysis and Quantitative Analysis programs.
3. Click **Start > Control Panel > Programs and Features**.
4. Remove these programs:
   - **Agilent MassHunter Workstation Data Acquisition**
   - **Agilent MassHunter Workstation Qualitative Analysis**
   - **Agilent MassHunter Workstation Quantitative Analysis**
   - **Agilent MassHunter Workstation Quantitative Analysis Reporting**
5. Close *all* applications.
6. Reboot the computer. You are now ready to install 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 118.

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

5 Mark the **Mass Spectrometer** check box, and then click the Triple Quad instrument. Leave all other check boxes cleared.

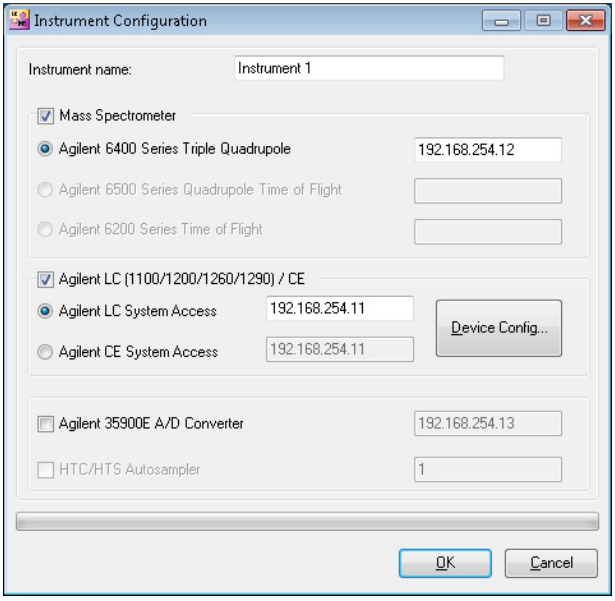


Figure 32 Instrument Configuration

6 Click **OK**.

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

8 Close the Data Acquisition program.

9 Stop MassHunter processes.

10 Open Instrument Configuration again.

11 Click the Triple Quad instrument and mark the **Agilent LC System Access** check box.

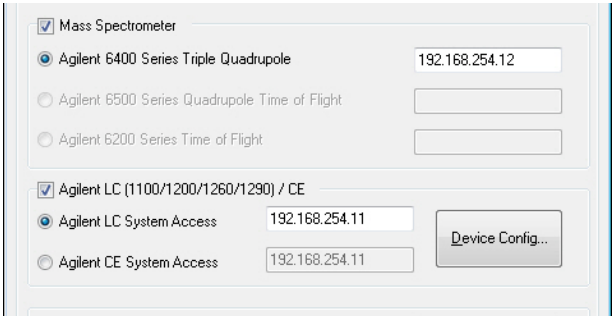


Figure 33 Instrument Configuration dialog box

12 Click **OK**.

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

Additional configuration steps may be required to set up LC devices.

6 Reference

This chapter contains acquisition parameters for the 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.

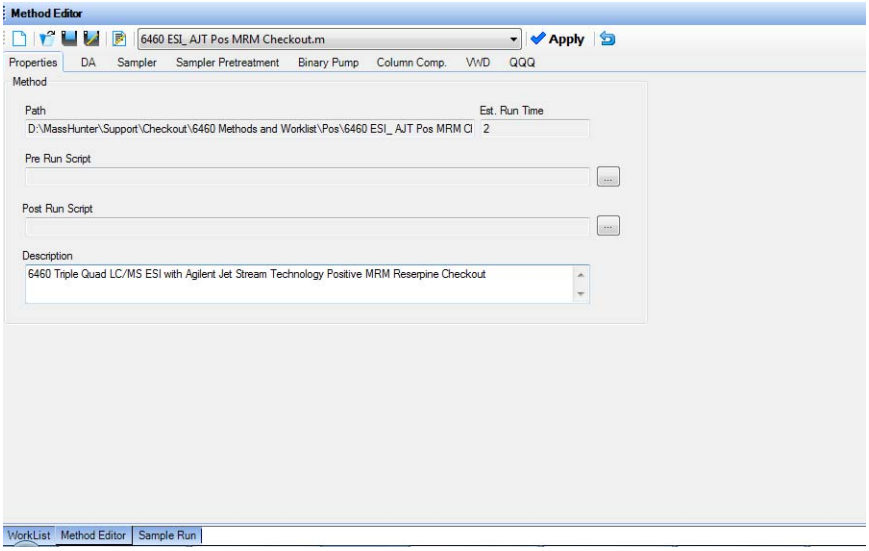


Figure 34 Properties tab

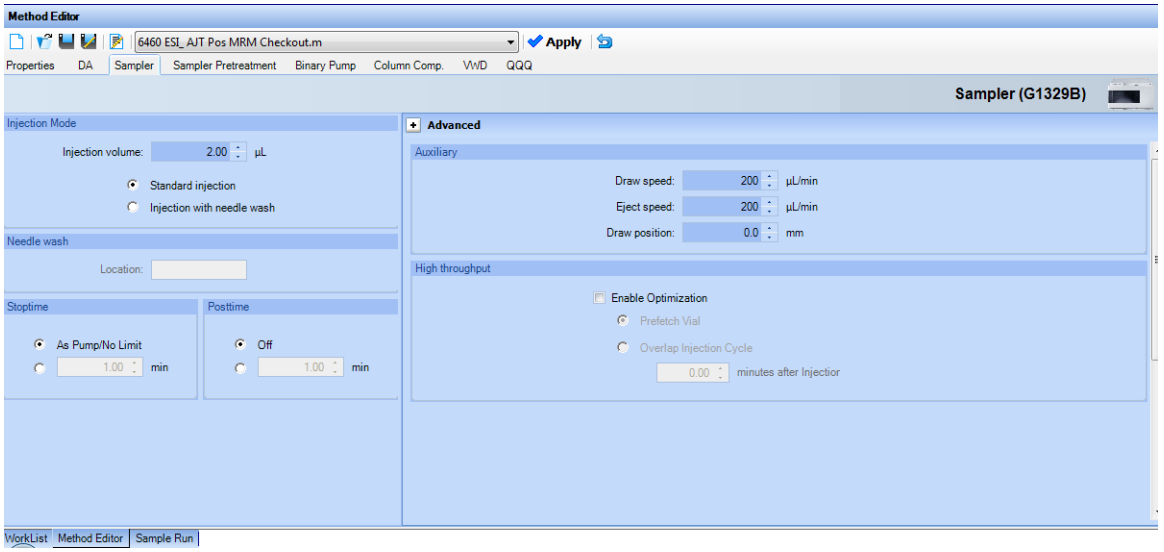


Figure 35 HiP Sampler tab

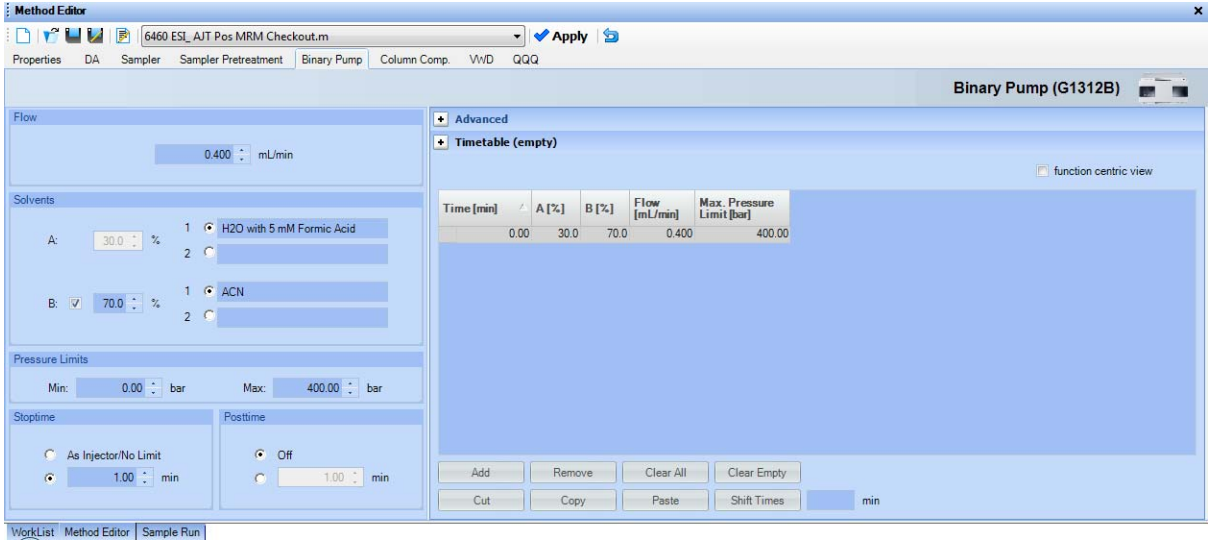


Figure 36 Binary Pump tab

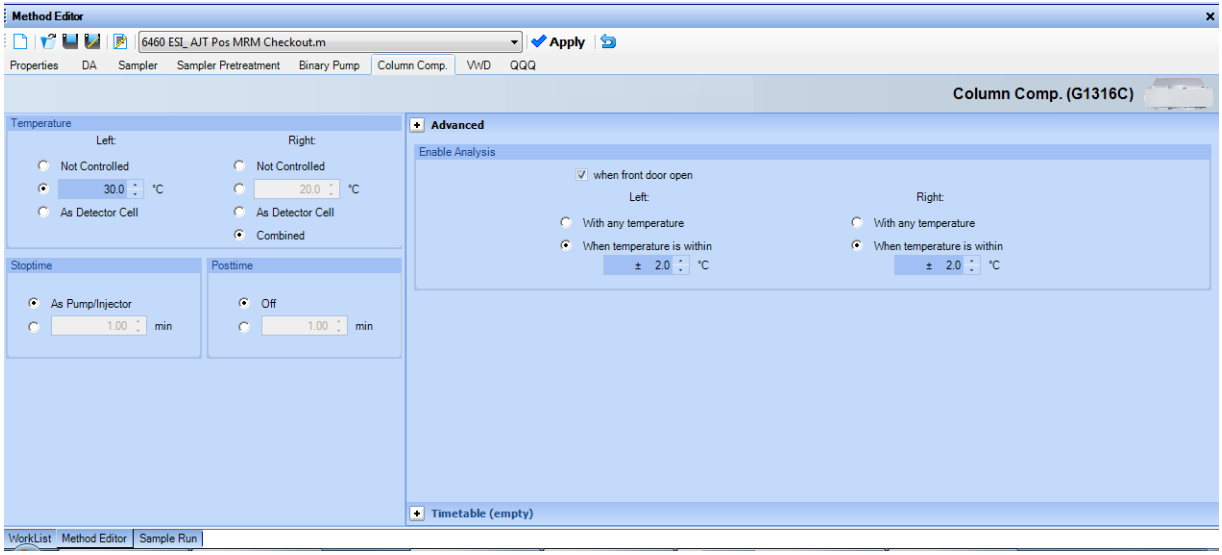


Figure 37 Column Comp. tab

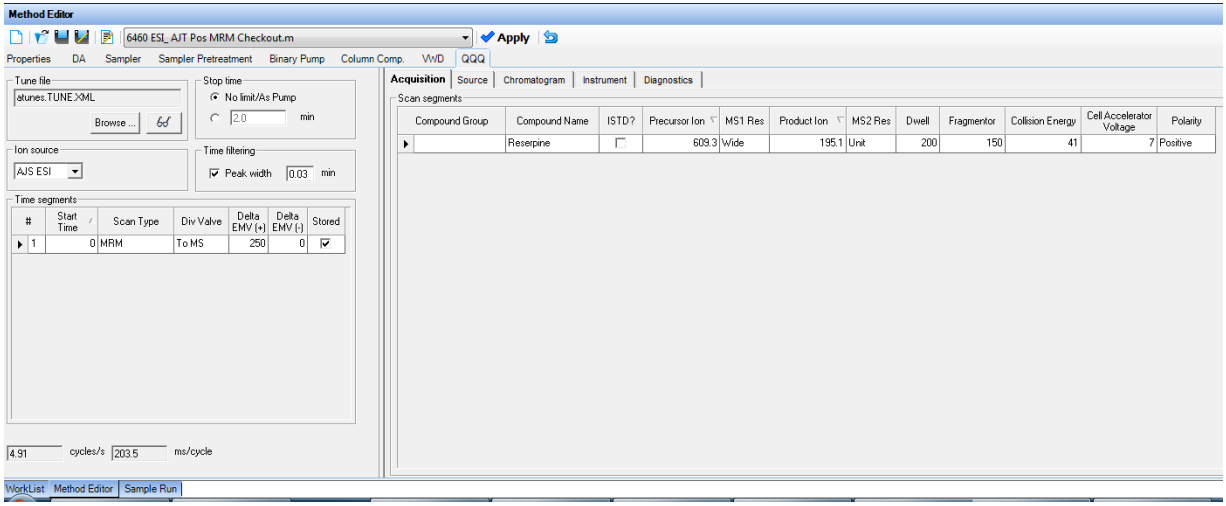


Figure 38 QQQ > Acquisition tab (6460 only)

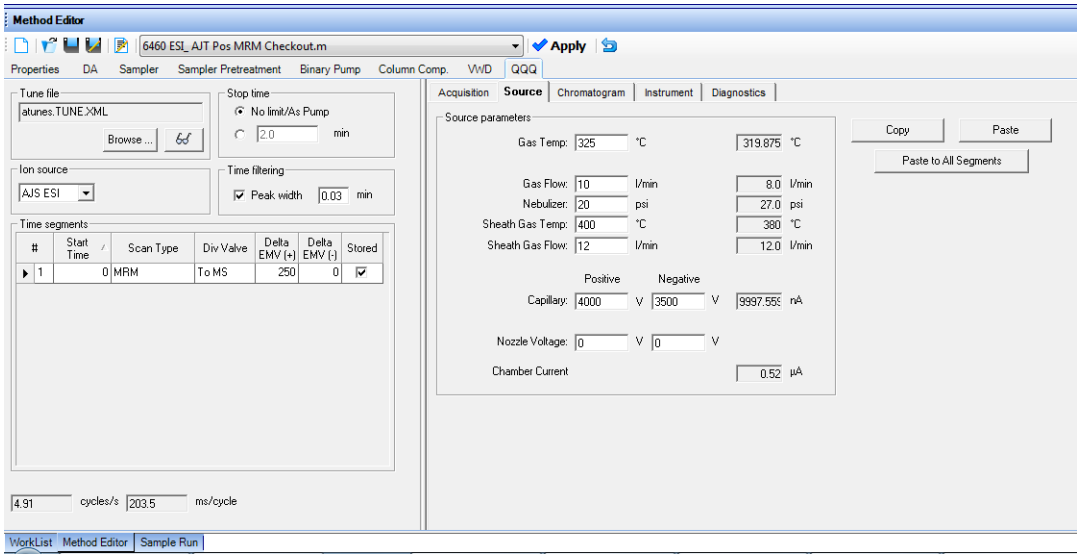


Figure 39 QQQ > Source tab for AJS ESI (6460 only)

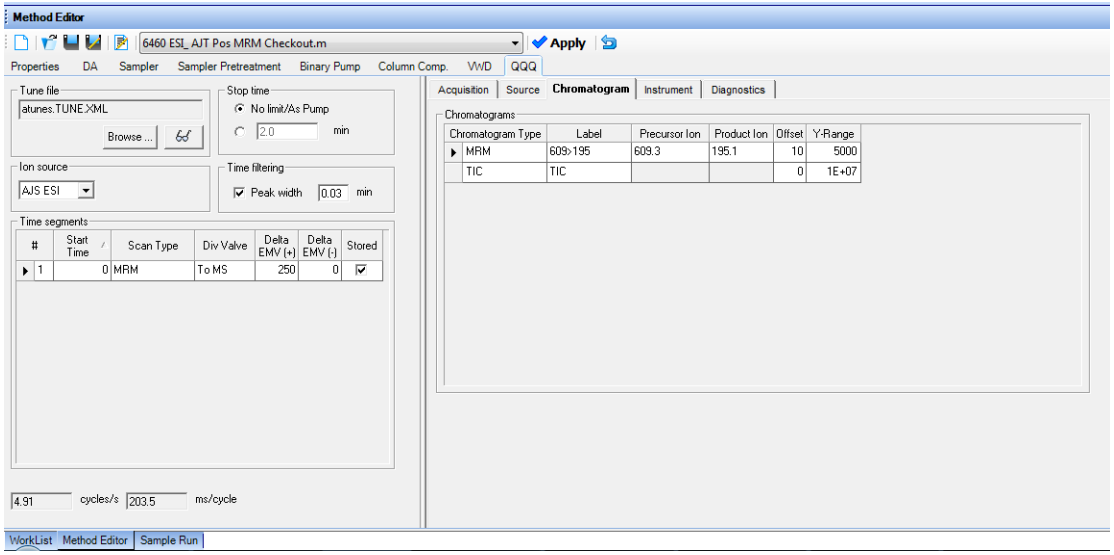


Figure 40 QQQ > Chromatogram tab

6490 Reserpine Checkout Method Acquisition Parameters

The following graphics give the default acquisition parameters for the 6490 POS MRM Reserpine Checkout method.

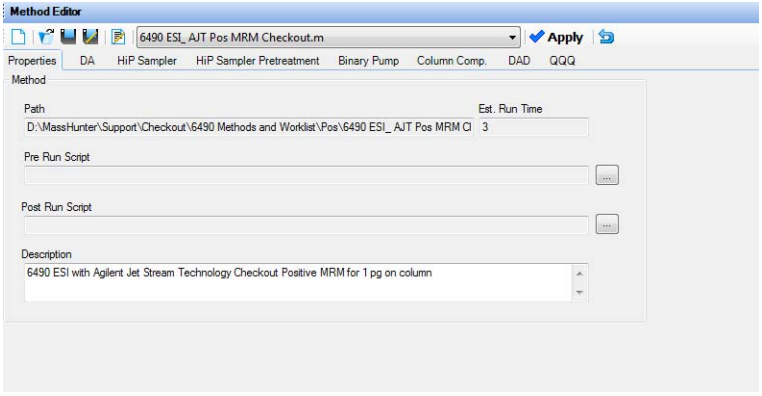


Figure 41 Properties tab

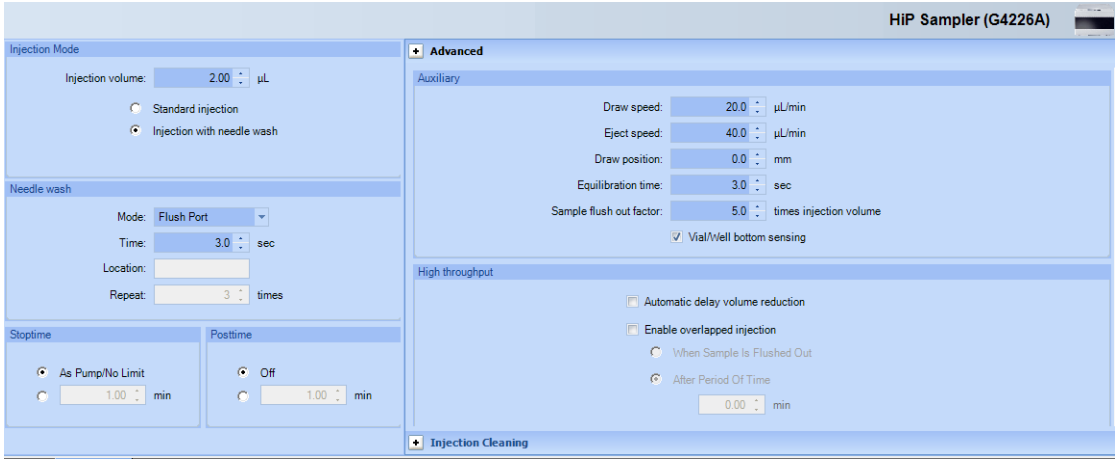


Figure 42 HiP Sampler tab

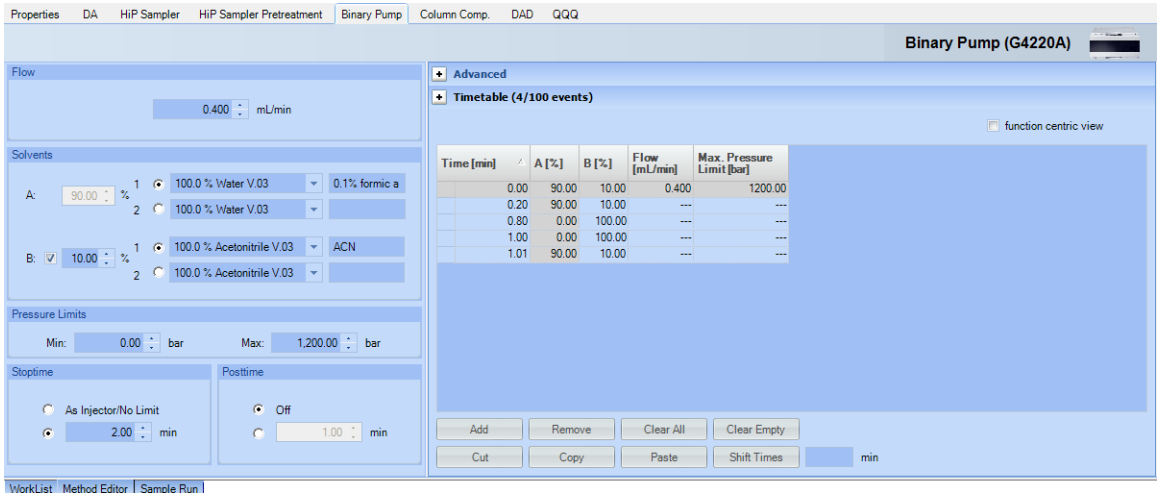


Figure 43 Binary Pump tab

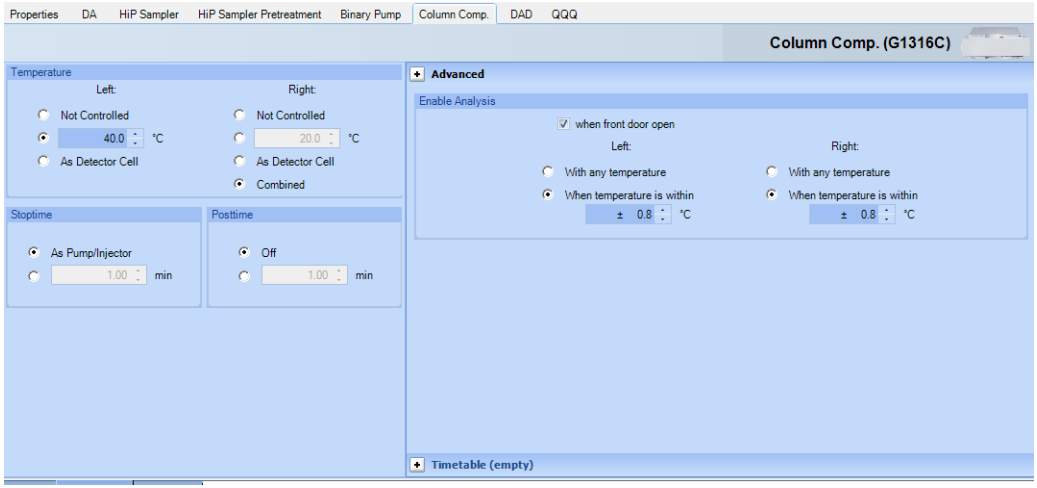


Figure 44 Column Comp. tab

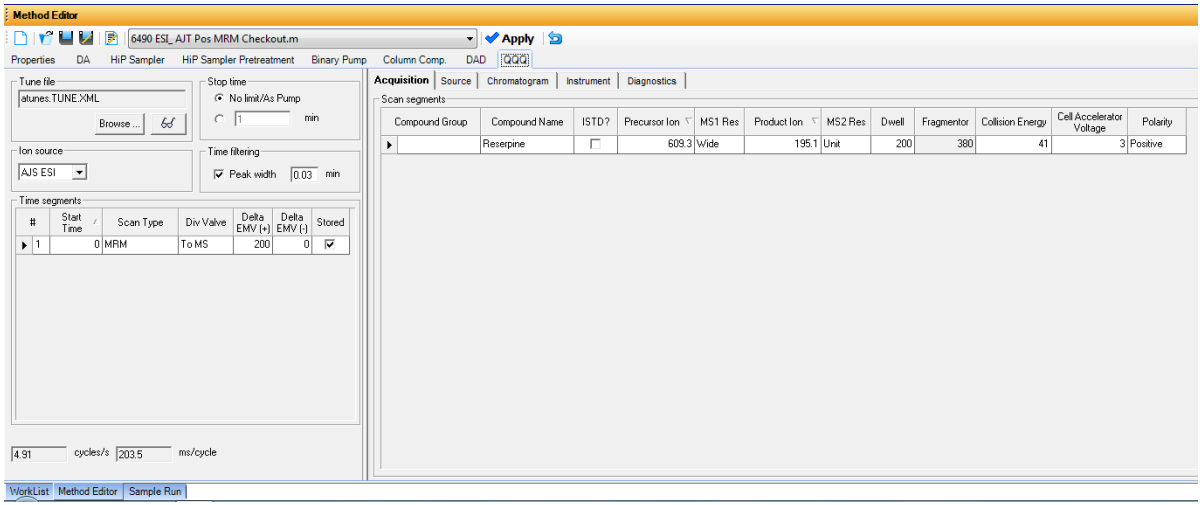


Figure 45 QQQ > Acquisition tab

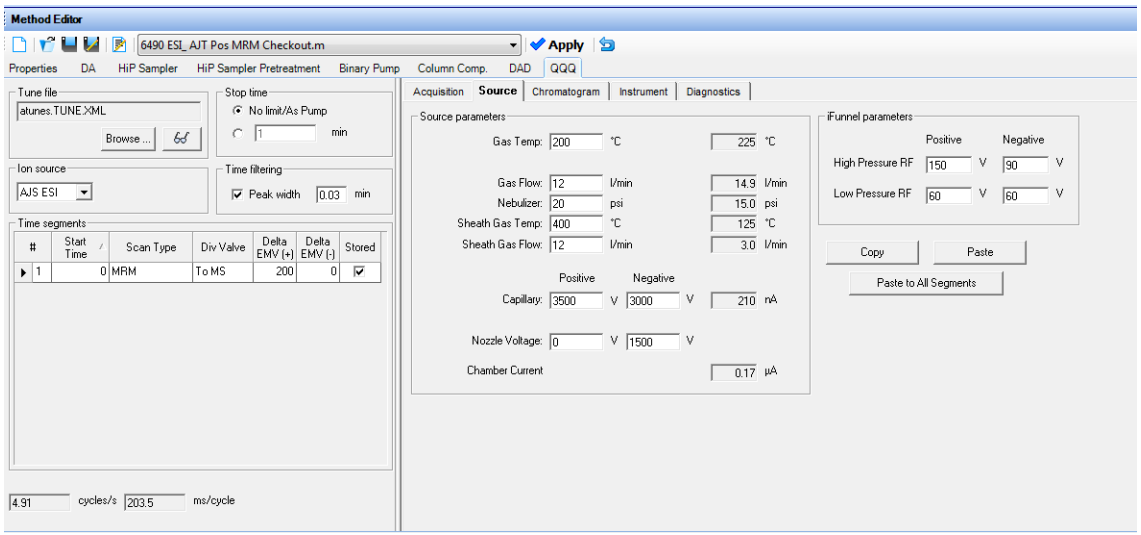


Figure 46 QQQ > Source tab for AJS ESI

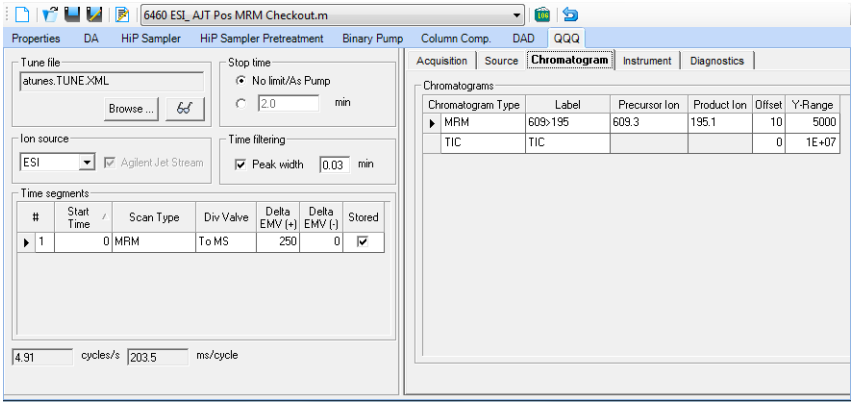


Figure 47 QQQ > Chromatogram tab

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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.

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