Agilent G1978A Multimode Source for 6300 Series Ion Trap LC/MS

Set-Up Guide

Agilent Technologies

Notices

Manual Part Number

G1978-90080

Edition

First Edition, December 2008

Printed in USA

Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95051 USA

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In This Guide

This guide explains how to install, maintain and troubleshoot your nanoelectospray ion source.

1 Installation

This chapter tells you how to install the multimode ion source. It also tells you how to verify the installation.

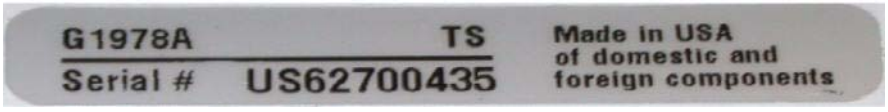
2 Performance Verification

This chapter includes the tasks that you need to do for performance verification of your ion trap with multimode source.

3 Methods

This chapter describes basic operation and maintenance for the multimode ion source.

Note that only multimode sources with thermal switches are supported on the Agilent 6300 Series Ion Trap LC/MS systems. These sources are identified with a seriel label that includes “TS”, as shown below:



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This chapter contains instructions to install the G1978A multimode source on the Agilent 6300 Series trap instrument and to change the source.

Installation

Step 1. Prepare to install

Before you install the multimode source, check that you have the appropriate parts and tools.

1 Check that you have these parts:

- Bundled Multimode ESI/APCI Source (p/n G1978A)
- Multimode ESI/APCI Source (p/n G1978-65439)
- Multimode HV Module Assembly (p/n G1978-60050)
- Trap MM ESI/APCI Enablement Kit (p/n G1978-60351)
- ChemStation B.01.03 or greater
- Trap Control Software 6.1

NOTE All 6300 series trap instruments have the necessary hardware components already installed. The 6300 series instruments contain the required GEPS-2 processor system and GELV-5 lens voltage board

2 Check that you have these tools, supplies and chemicals. The items in this list are not provided with your multimode source.

- Cloths and gloves, clean, lint-free
- Water and organics, such as acetone, methanol, acetonitrile or isopropyl alcohol, all HPLC grade
- ¼ inch open-end wrench
- Torx drive T10

Step 2. Turn off power to the electronics tub

- Do the steps in "To shut down and vent the instrument" on page 41.

NOTE

The power to the instrument electronics tub should be completely shut off if not a bundled 6300 series installation. This includes the SL, XCT, XCT Plus and XCT Ultra. These instruments would be considered upgrades for use with the G1978A source. These instruments also need identification code changes that will not be available at the release of G1978A Multimode on trap instruments.

Step 3. Replace any older GELV with GELV-5 and GEPS-1 with GEPS-2

The GELV-5 Lens voltage PCA assembly and the new GEPS-2 board are required electronics boards for use with the multimode source. You will need to upgrade the instrument boards if you have a trap instrument prior to the 6300 series, and if the trap model is not Ultra.

Figure 1 Complete GELV-5 with new piggy back board on the upper left side of board, and the GELV-5A revision next to the connector.

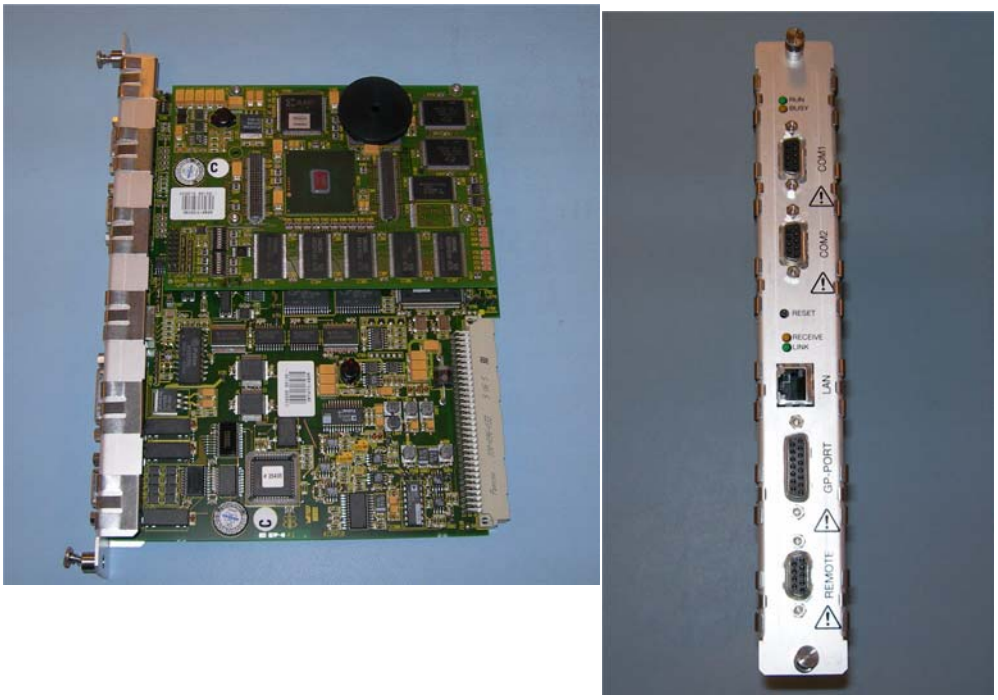


Figure 2 The new GEPS-2 with new face plate.

CAUTION The following steps should only be performed by an Agilent trained FCE. Damage to the chip can happen at power on if these steps are not performed properly.

1. Verify that the instrument electronics tub is turned off. Remove the top cover magent to cut power to the electronics tub.
2. Remove the older version GELV PCA board from the tub assembly.
3. With the GELV-5 PCA still out, install the 10 M APCI voltage cable in series with the exsisting APCI high voltage cable.
4. Install the GELV-5 and make all cable connections

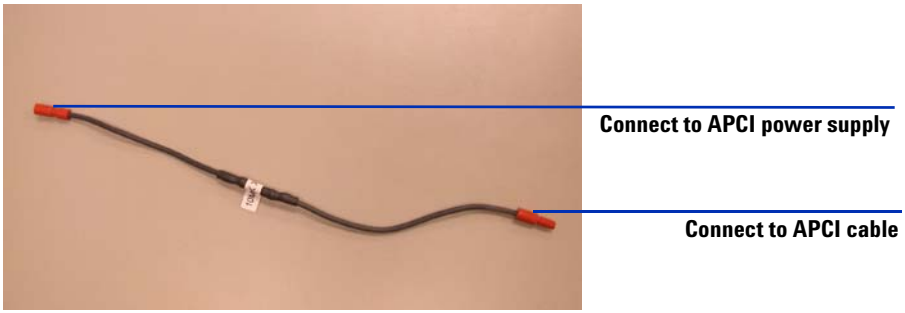


Figure 3 10 MΩ ACPI high voltage cable (p/n G1978-60806). In the next steps, make sure that the appropriate ends are connected to the APCI power supply and APCI cable.

5 Remove the GEPS-1 processor board from the instrument.

CAUTION Be careful when you install the new GEPS-2 that the edge connector is aligned properly with the GESI spectrometer interface connector.

6 Install the new GEPS-2 processor board in the instrument.

7 Replace all the foam and covers that you previously removed.

Step 4. Convert from ESI, APCI or APPI to multimode source

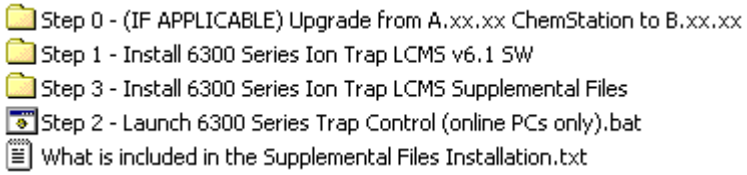
- Do the steps in "To convert from ESI, APCI or APPI to the multimode Source" on page 13.

Step 5. Install the Trap Control Software 6.1

The G1978A multimode source is supported on ChemStation version B.01.03 or later with the Trap Control Software 6.1.

1 Remove any previous version of the Trap Control Software using the Control Panel Add/Remove Software application.

2 Run the **setup.exe** program in each of the numbered folders and the .bat file, in order of the numbered steps.



Step 6. Verify performance of the multimode source

Before using your system, you should verify the performance of your system.

1 Start the ChemStation software.

2 Do the steps in "To verify the ion trap calibration" on page 46.

3 Do the steps in "To bake out the ion trap" in the *Maintenance Guide*.

4 Do the steps in "Step 1. Prepare the sample" on page 32.

NOTE These verification methods are to be used for sensitivity verification for bundled instruments shipped with a multimode source only.

Changing Sources

This section includes tasks that you will need to do change the source on your instrument.

To convert from ESI, APCI or APPI to the multimode Source

CAUTION If you are installing this source on this instrument for the first time, follow the steps in "Installation" on page 7.

1 From the Trap Control Software, select Shutdown to turn off these parameters:
   - Drying Gas (L/min)
   - Nebulizer Pressure (psig)
   - Drying Gas Temperature (°C)
   - Vaporizer Temperature (APCI source only)
   - Lamp Off (APPI source only)
2 Wait for the source to cool (until temperatures are at least below 100°C).
3 Disconnect the nebulizer gas tubing from the currently installed ion source.
4 Disconnect the Ion Trap LC/MS sample inlet tubing.
5 If the APCI or APPI source is installed, remove the APCI vaporizer heater cable and APCI high voltage cable.
6 If the APPI source is installed, remove the GEPS-2 processor system Com 2 RS-232 cable.
7 Remove the currently installed ion source.
8 Unscrew and remove the spray shield. See Figure 4.

WARNING **Do not touch the multimode source or the capillary cap. They may be very hot. Let the parts cool before you handle them.**

WARNING **Do not insert fingers or tools through the openings on the multimode chamber. When in use, the capillary and capillary cap are at high voltages up to 4 kV.**

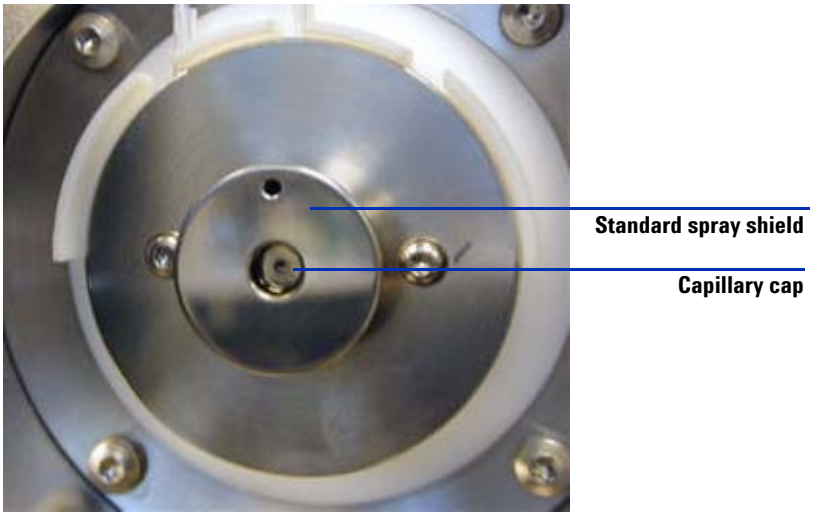


Figure 4 Standard spray shield and capillary cap for ESI or APCI

9 Remove the capillary cap. If needed, moisten a clean cloth with isopropyl alcohol and wipe the capillary cap. See Figure 5.

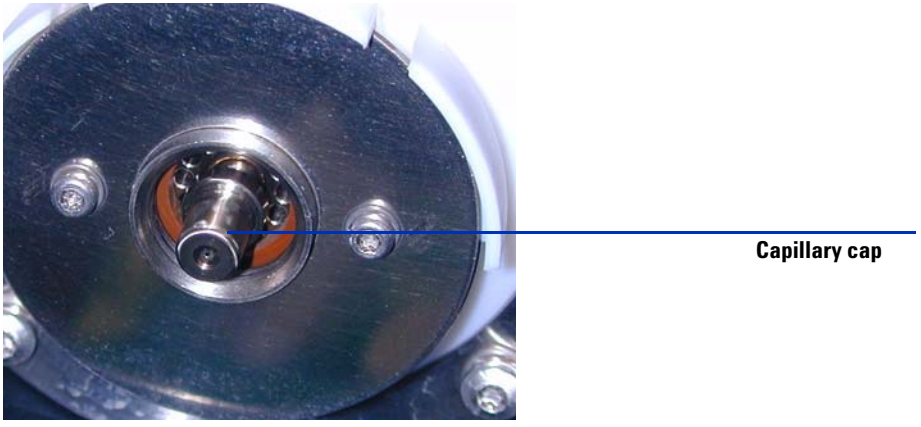


Figure 5 Spray shield removed.

10 Place the capillary cap back on the capillary.

11 Install the new spray shield with field shaping electrodes. See Figure 6.

Figure 6 Multimode spray shield

12 Screw the multimode spray shield into the holder for the spray shield. See Figure 7.

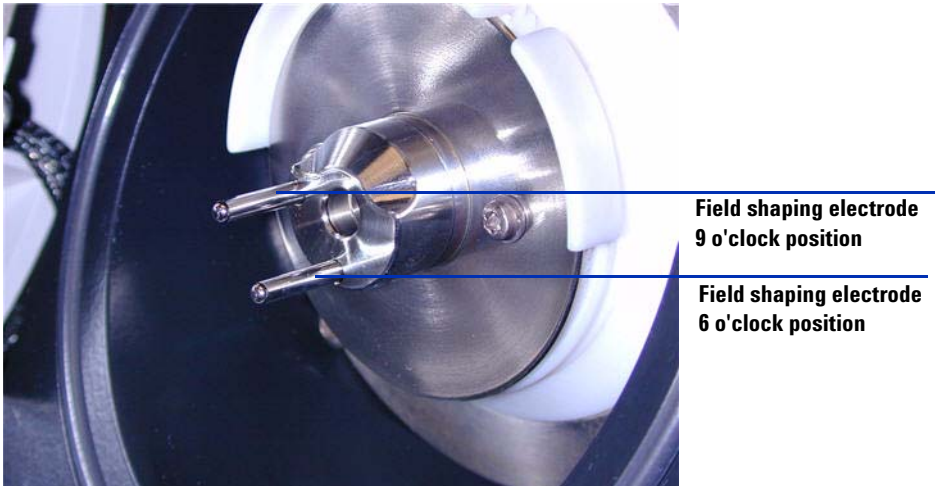


Figure 7 Multimode spray shield installed

NOTE The field shaping electrodes should be in the nine o'clock and the six o'clock position. Loosen the end plate screws on each side to adjust the field shaping electrodes position.

13 Remove the shipping cover from the multimode source spray chamber.

Figure 8 Multimode spray chamber with shipping cover

14 Install the spray chamber on the spray chamber mount.

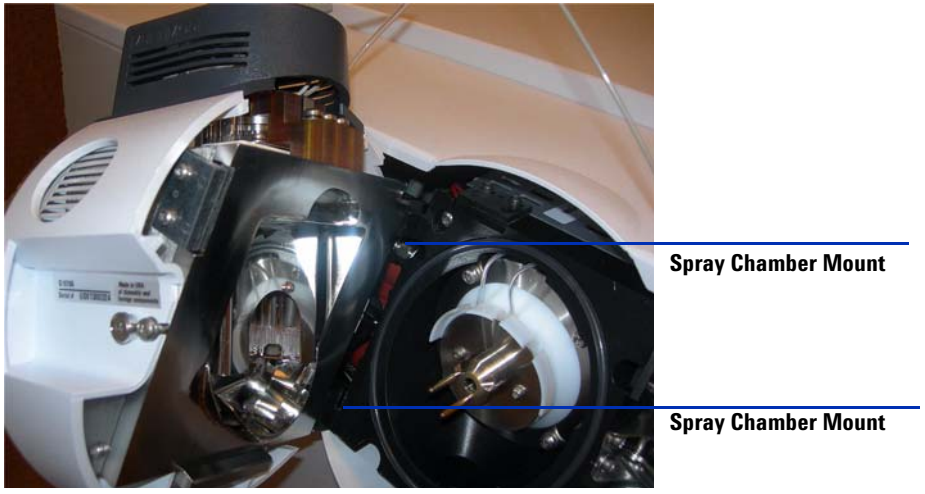


Figure 9 Multimode source installed on the spray chamber mount

15 Install the nebulizer on the multimode source spray chamber.

Never use a nebulizer spacer on the multimode source. The nebulizing space is used for the standard G1948A ESI source only.

Figure 10 No nebulizer on top of the multimode source

16 Connect the 1/8-inch nebulizer gas tubing from the Ion Trap LC/MS mainframe to the nebulizer gas fitting. See Figure 11.

Figure 11 Nebulizer with gas tubing connected

17 Connect the Ion Trap LC/MS sample tubing to the Ion Trap LC/MS diverter valve inlet filter. See Figure 12 on page 18.

WARNING **The Agilent 1100 and 1200 Series LC Liquid Chromatograph diverter valve is an integral part of the G1978A safety system. The LC mobile phase flow must always be connected to the diverter valve inlet filter. Never bypass the diverter valve and connect directly to the nebulizer. If the diverter valve is used in a manner not specified by Agilent Technologies, the protections provided by the diverter valve may be impaired.**

Figure 12 Ion Trap LC/MS sample tubing connected to inlet filter

18 If you are installing the multimode source for the first time, follow the steps in "To install the HV control PCA" on page 19.

19 Follow the steps in "To connect multimode source cables" on page 23.

To install the HV control PCA

1 Remover the cover from the source HV and control PCA power supply. See Figure 13.

Figure 13 Cover removed from the source HV and control PCA power supply

2 Attach the RS-232 cable to the HV and control PCA power supply RS-232 connector. See Figure 14.

Figure 14 Attaching the RS-232 cable

3 The instrument front cover, top cover, safety cover with magnet, and side panel access door should be removed.

4 Remove the plastic cable clamp from the desolvation heater cable See Figure 15.

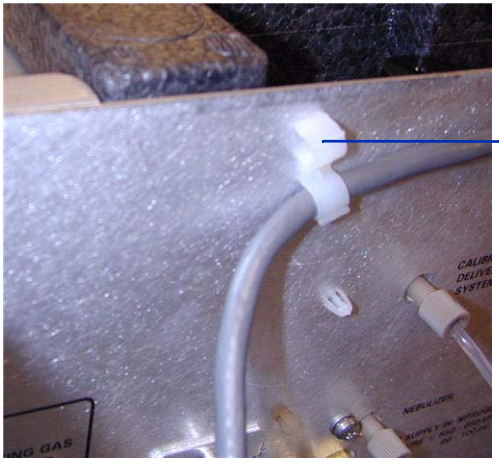


Figure 15 Cable clamp removal

5 Reroute the cable under the Calibrant Delivery System gas line. See Figure 16.

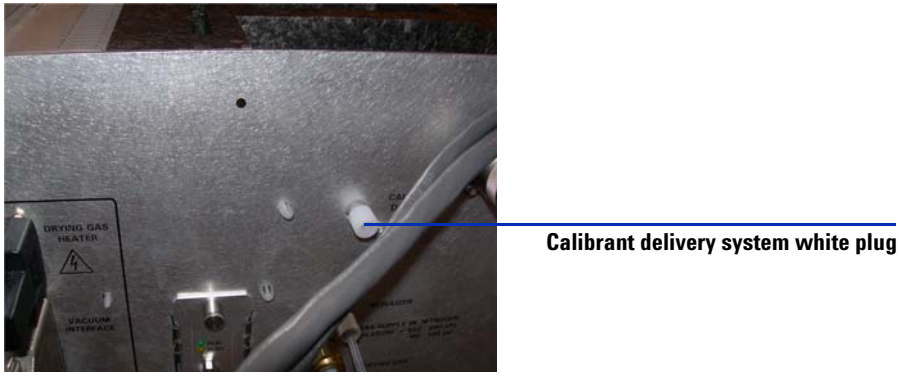


Figure 16 Calibrant delivery system gas line

6 Attach the HV and control PCA power supply to the tub with the self-trapping screw supplied. See Figure 17.

Figure 17 Attach the HV and control PCA power supply

7 Clamp the top cover of the HV and control PCA power supply with screws provided to the support bracket. See Figure 18.

Figure 18 Clamping to support brackets

8 If you are installing the HV control PCA as part of a conversion to the multimode source, return to **"To convert from ESI, APCI or APPI to the multimode Source"** on page 13.

To connect multimode source cables

1 Connect the RS-232 cable to the GEPS-2 processor system Com 2, which is located on the left side of the instrument chassis. See Figure 19.

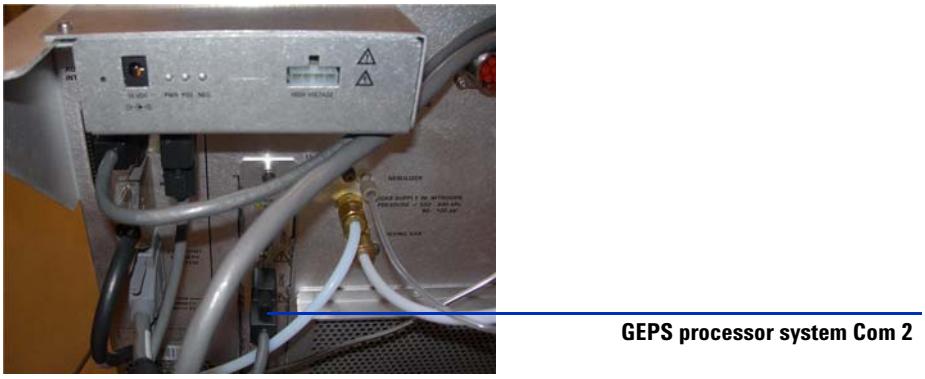


Figure 19 RS-232 cable connections

2 Connect the +15V DC power supply to the HV and control PCA. See Figure 20.

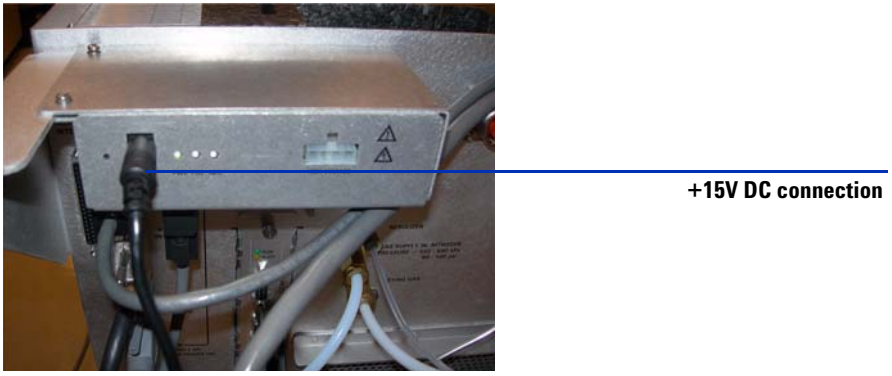


Figure 20 HV and control PCA

3 Connect the other end of the +15V DC power supply into an 110V AC outlet using the power cord supplied with the +15V DC power supply. See Figure 21.

Figure 21 Power cord and +15V DC supply

4 Use a cable-tie to +15V DCoutput power cable of the power supply (p/n 0950-4581) to the RS-232 cable of the the Multimode HV Module Assembly (p/n G1978-60050.)

The cable tie will secure the +15V DC cable to prevent the cable from being unplugged by accident.

5 Connect the vaporizer heater, APCI high voltage, and HV and control PCA cables. The APCI heater connector, APCI high voltage connector, and HV and control PCA connector are located on the left side of the instrument chassis. See Figure 22.

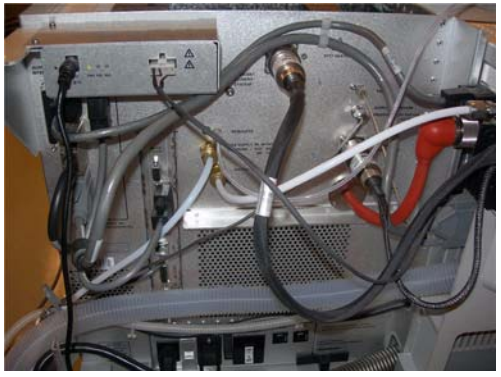


Figure 22 Multimode source cable connections

6 Close service panel door and validate all covers are in place. See Figure 23.

Figure 23 Multimode source with covers installed

To remove the multimode source

Do the following steps to remove the multimode source.

1 From the Trap Control Software, put the instrument in Shutdown mode. Shutdown will turn off all temperatures and gas flows.

WARNING **Do not touch the multimode source or the capillary cap. They may be very hot. Let the parts cool before you handle them.**

WARNING **Never touch the source surfaces, especially when you analyze toxic substances or when you use toxic solvents. The source has several sharp pieces which can pierce your skin including the APCI corona needle, vaporizer sensor and counter current electrode.**

WARNING **Do not insert fingers or tools through the openings on the multimode chamber. When in use, the capillary and capillary cap are at high voltages up to 4 kV.**

2 Wait around 20 minutes until the source is cool (below 100°C).

3 Open the service door on the left side of the MS to access the cables. See Figure 24.

Figure 24 Instrument with multimode source installed

4 Disconnect the ESI high voltage charging electrode cable. See Figure 25.

5 Disconnect the APCI heater (vaporizer) cable and APCI High voltage cable. See Figure 25.

6 Unplug the 15V DC connection to the multimode electronics module. See Figure 25.

NOTE When you switch to other source types, remove the +15V DC power to the multimode HV module. The new source will be identified as an unknown source if the +15V DC power is left connected to the HV module.

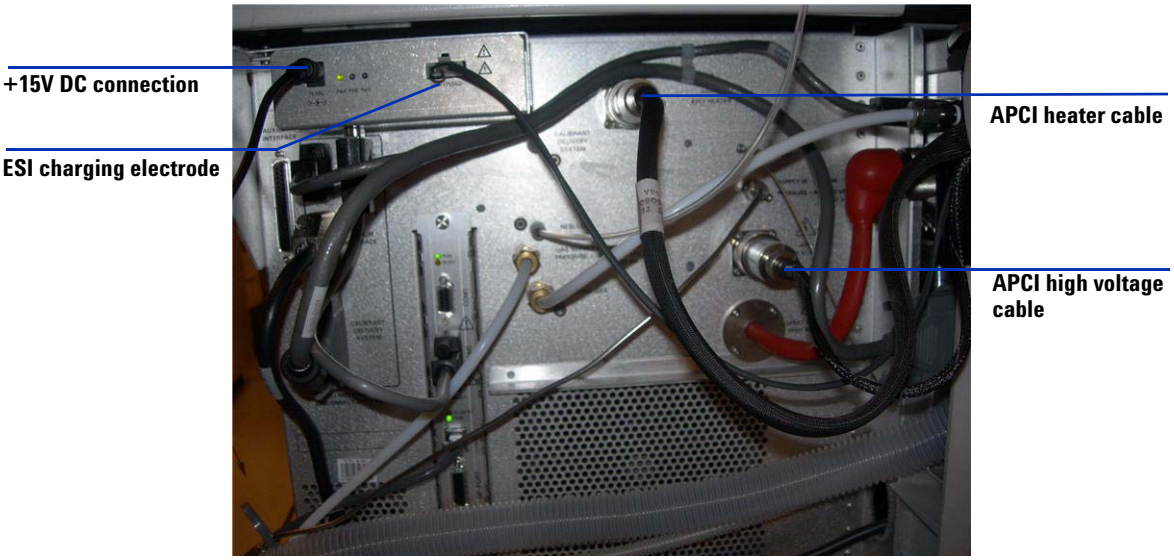


Figure 25 Instrument with service door open

7 Unscrew the nebulizer gas line from the nebulizer.

8 Unscrew the LC sample tubing from the nebulizer.

9 Open the latch on the source and open the source.

10 Remove the multimode source from the spray chamber mount.

11 Place the source shipping cover on the source.

12 If you are converting from a multimode source to another source type, continue in the section "To convert from multimode to ESI, APCI or APPI" on page 29.

13 If you are cleaning the multimode source, continue in the section "To clean the multimode source periodically" in the *Maintenance Guide*.

To convert from multimode to ESI, APCI or APPI

WARNING **Do not touch the multimode source or the capillary cap. They may be very hot. Let the parts cool before you handle them.**

WARNING **Never touch the source surfaces, especially when you analyze toxic substances or when you use toxic solvents. The source has several sharp pieces which can pierce your skin including the APCI corona needle, vaporizer sensor and counter current electrode.**

1. Do the steps in "To remove the multimode source" on page 26.
2. If the source to be installed is an APPI source, disconnect the multimode high voltage PCA RS-232 serial cable from the GEPS-2 Com 2.
3. Unscrew and remove the multimode spray shield with the field shaping electrodes.
4. Install the new source and the standard spray shield, making sure that the hole in the spray shield is in the 12 o'clock position.
5. For APCI and APPI ion source, connect the vaporizer heater cable and the APCI high voltage cable. For the APPI source, connect the RS-232 cable to the GEPS-2 Com 2 port.
6. For all sources, reconnect the nebulizer gas line tubing and the Ion Trap LC/MS sample tubing.

2
Performance Verification

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This chapter includes the tasks that you need to do for performance verification of your 6300 Series Ion Trap LC/MS with multimode source.

Proper solvent mixture for multimode performance verification

Solvent dilutions are given for all supported instruments with multimode source. The reserpine performance verification is needed on bundled 6310, 6320, 6330 and 6340 trap instruments shipped with a multimode source.

Step 1. Prepare the sample

For all 6300 series trap models prepare a 75:25 methanol/water with 5 mM ammonium formate as the mobile phase.

At least HPLC grade solvents should be used. Solvents that are acceptable for most LC applications may contain high levels of background that are detectable by the more sensitive LC/MS Ion Trap. LC solvents used with the LC/MS 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.

NOTE This verification method may only be used on a bundled instrument shipped with a multimode source.

Before you begin, check that you have:

- 1 mL graduated pipette, p/n 9301-1423
- 50 mL volumetric flask (two each), p/n 9301-1424
- 100 mL volumetric flask, p/n 9301-1344
- Positive ion performance evaluation sample, p/n G2423A
- Plastic bottles for storing dilutions, p/n 9301-1433

A bundled instrument will come with the supplies listed above.

The supplied performance evaluation samples must be diluted to concentrations required for the ion trap system checkout. Refer to the section "Proper solvent mixture for multimode performance verification" for more information.

NOTE Use the diluted samples within a day of dilution. Refrigerate the intermediate (first) dilution in the supplied bottles.

Tips

- Rinse the graduated pipettes and volumetric flasks thoroughly with deionized water before, in between, and after 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. Doing this minimizes any background contributed by the vials and caps. The vials may be run uncapped if the septa are found to be a source of background contamination.

For 6310 Ion Trap with multimode source, Positive SIM mode dilution

1. In the ChemStation software, load the sensitivity method **Res_6310.m**.
2. Use the 75:25 methanol/water with 5 mM ammonium formate as the dilution solvent.
3. Create a dilution of 1000:1 (5 pg/µL) in the elution solvent as follows:
   a. Transfer 1 mL of 5 ng/µL reserpine to 50 a mL flask.
   b. Dilute to 50 mL mark with 75:25 methanol/water with 5 mM ammonium formate as the dilution solvent.
   c. Transfer 5 mL of the first dilution to the 100 mL flask.
   d. Dilute to the 100 mL mark with the dilution solvent.
   e. Transfer 1 to 2 mL of the final 5 pg/µl reserpine dilution to a glass via and cap it.
4. Place the reserpine dilution vial in the autosampler position 2.
5. Transfer some of the 75:25 methanol/water with 5 mM ammonium formate to a second vial to inject as a blank.
6. Place the blank in position 1.

For 6320 Ion Trap with multimode source, Positive SIM mode dilution

1. In the ChemStation software, load the sensitivity method **Res_6320.m**.
2. Mix some of each LC reservoir solvent into another container at 75:25 methanol/water with 5 mM ammonium formate to be used for the dilution solvent.
3. Create a dilution of 5,000:1 (1 pg/µL) in the elution solvent as follows:
   a. Transfer 1 mL of 5 ng/µL reserpine to a 50 mL flask.
   b. Dilute to the 50 mL mark with 50:50 ACN/H_2O with 0.2% formic acid as the dilution solvent.
   c. Transfer 1 mL of the first dilution to the other 100 mL flask.

d Dilute to the 100 mL mark with the dilution solvent.

e Transfer 1 to 2 mL of the final 1 pg/µL reserpine dilution to a glass vial and cap it.

4 Place the reserpine dilution vial in the autosampler position 2.

5 Transfer some of the dilution solvent to a second vial to inject as a blank.

6 Place the blank in position 1.

For 6330 Ion Trap with multimode source, Positive SIM mode dilution

1 In the ChemStation software, load the sensitivity method **Res_6330.m**.

2 Mix some of each LC reservoir solvent into another container at 75:25 methanol/water with 5 mM ammonium formate to be used for the dilution solvent.

3 Create a dilution of 50,000:1 (100 fg/µL) in the elution solvent as follows:

a Transfer 1 mL of 5 ng/µL reserpine to a 50 mL flask.

b Dilute to the 50 mL mark with 75:25 methanol/water with 5 mM ammonium formate as the dilution solvent.

c Transfer 1 mL of the first dilution to the other 50 mL flask.

d Dilute to the 50 mL mark with the dilution solvent.

e Transfer 5 mL of the first dilution to the 100 mL flask.

f Dilute to the 100 mL mark with the dilution solvent.

g Transfer the 1 to 2 mL of the final 100 fg/µl reserpine dilution to a glass via and cap it.

4 Place the reserpine dilution vial in the autosampler position 2.

5 Transfer some of the dilution solvent to a second vial to inject as a blank.

6 Place the blank in position 1.

For 6340 Ion Trap with multimode source, Positive SIM mode dilution

1 In the ChemStation software, load the sensitivity method **Res_6340.m**.

2 Mix some of each LC reservoir solvent into another container at 50:50 ACN/H2O (0.2% formic acid) to be used for the dilution solvent.

3 Create a dilution of 50,000:1 (100 fg/µL) in the elution solvent as follows:

a Transfer 1 mL of 5 ng/µL reserpine to a 50 mL flask.

b Dilute to the 50 mL mark with 75:25 methanol/water with 5 mM ammonium formate as the dilution solvent.

c Transfer 1 mL of the first dilution to the other 50 mL flask.

d Dilute to the 50 mL mark with the dilution solvent.

e Transfer 5 mL of the first dilution to the 100 mL flask.

f Dilute to the 100 mL mark with the dilution solvent.

g Transfer the 1 to 2 mL of the final 100 fg/µl reserpine dilution to a glass via and cap it.

4 Place the reserpine dilution vial in the autosampler position 2.

5 Transfer some of the dilution solvent to a second vial to inject as a blank.

6 Place the blank in position 1.

Step 2. Acquire sample data

1 Confirm the following settings, and adjust for CapLC:

- ICC is ON
- Target = 30,000 for 6310 (500,000 for 6320, 6330 and 6340)
- Max. Acc. Time = 300ms
- Nebulizer pressure = 40 psi (1100/1200 LC)
- Nebulizer pressure = 20 psi (CapLC)
- Dry gas flow = 9 L/min (1100/1200 LC)
- Dry gas flow = 5 L/min (CapLC)
- Dry gas temperature = 350°C (1100/1200 LC)
- Vaporizer temperature = 250°C (1100/1200 LC)
- Dry gas temperature = 325°C (CapLC)
- Vaporizer temperature = 250°C (1100/1200 LC)
- Mass scan range = 150 to 650
- Scan resolution = Normal
- Averages = 3 (1100/1200 LC)
- Averages = 6 (CapLC)
- Rolling averages = 2

2 In Agilent ChemStation, verify an injector method with the following steps:

- 1 REMOTE Startpulse
- 2 DRAW def amount from vial 1
- 3 INJECT
- 4 REPEAT 6 times
- 5 WAIT 1.00 min
- 6 DRAW def. amount from vial 2
- 7 INJECT
- 8 END REPEAT

3 Check that the defined injection amount is 1 µl (2.5 µL for 6320, 6330 and 6340) and the column compartment temperature is 30°C.

LC flow rate for 1100 LC should be 400 µL/min (and for CapLC, 20 µL/min).

4 Specify the appropriate Sample Info.

5 Isolate mass 609.3 (width 2 amu).

6 Set the Frag Amp to between 0.8 and 1.2 volts. Use the value that provides the optimum signal-to-noise values.

7 Start the run of 7 injections and monitor the sum of the product ions 397 and 448.

Step 3. Process the results

The acquisition method automatically processes the data. The data file load automatically into the 6300 Series DataAnalysis program.

- If reprocessing is required:
  - a Load the data file into DA.
  - b Modify Method Parameters.
  - c Select Method > Run to run automation script.

The script generates a chromatographic trace of EIC 397;448 +All MSn, runs the command Find > Compounds - Integrate Only and then prints results to "Sensitivity Checkout Report (P).layout".

The sensitivity specifications are as follows for all six reserpine peaks (not averaged):

- 6310 - Calculated S/N >= 50:1, 5 pg on column
- 6320 - Calculated S/N >= 50:1, 1 pg on column
- 6330 - Calculated S/N >= 50:1, 250 fg on column
- 6340 - Calculated S/N >= 50:1, 250 fg on column

To run the Multimode Demo Sample

The ESI + APCI LC Demo Sample is required at installation for verification of the multimode source in all modes of operation multimode ESI, multimode APCI and multimode ESI + APCI.

1 In ChemStation, load the **Multimode_Checkout.m** method.

2 Transfer the ESI + APCI LC Demo Sample (P/N G1978-85000) to a vial (no dilution needed).

3 Place the vial with the ESI + APCI LC Demo Sample in position 1.

4 Create Solvent A: 65/35 Methanol /Water with .2 % Acetic acid flow .400 mL/min.

5 Check the Injector program for FIA demo sample run.

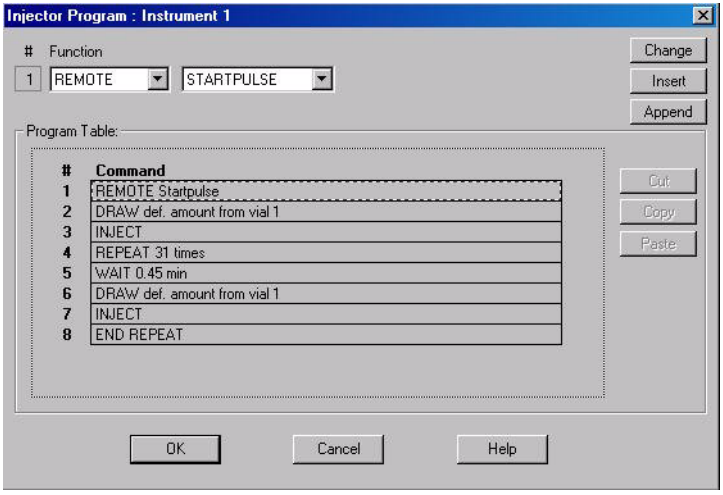

Figure 26 Injector program

6 Make sure that you have a Zorbax column, SB-C 18, 2.1mm x 30mm, 3.5um, (p/n 873700-902, provided with G2440CA/DA) installed on the LC/MS trap.

7 Set up the Trap Control Software to do a six segment run:

Table 1

Segment	Mode
1	Multimode ESI positive Crystal violet Ion 372.2
2	Multimode ESI negative 1-Heanesulfonic acid Ion 165.1
3	Multimode APCI positive Carbozol Ion 168.1
4	Multimode APCI negative 9-Phenathrol Ion 193.1
5	Multimode ESI + APC positive Ion 372.2 and 168.1
6	Multimode ESI + APCI negative 165.1 and 193.1

8 Set **Trap Drive** to **Smart** and target mass **165** for all segments.

9 Set the trap Chromatogram EIC for all ions of interest: 372.2, 165.1, 168.1, and 193.1.

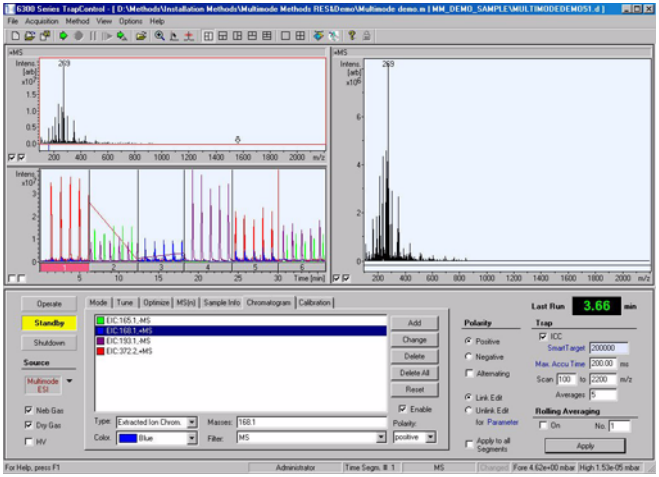

Figure 27 Trap Control Multimode six segment run

10 From data analysis load the Multimode six segment data file.

11 Run the script below:

- Analysis.Compounds.Clear
- Analysis.Chromatograms.Clear

- Analysis.Chromatograms.Add daEIC, daAllMS, "372.2", daPositive
- Analysis.Chromatograms.Add daEIC, daAllMS, "165.1", daNegative
- Analysis.Chromatograms.Add daEIC, daAllMS, "168.1", daPositive
- Analysis.Chromatograms.Add daEIC, daAllMS, "193.1", daNegative
- Analysis.Chromatograms.IntegrateOnly
- Analysis.Save
- Form.Close

The first EIC is 372.1 in ESI positive and mixed mode. You should see at least 20% recovery for mixed mode.

The second EIC is 168.1 in APCI Positive and mixed mode. You should see at least 20% recovery.

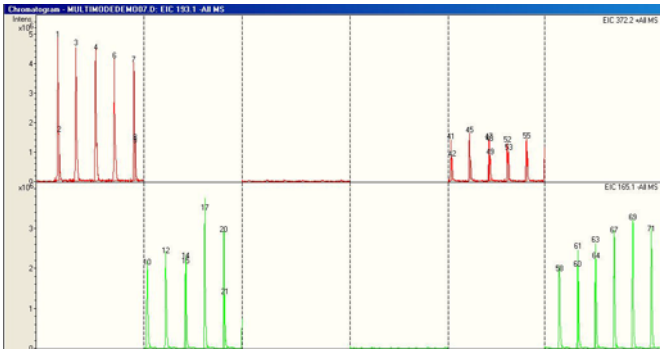

Figure 28

The third EIC is 165.1 in APCI positive and mixed mode. You should see at least 20% recovery for mixed mode.

The fourth EIC is 193.1 in APCI Negative and mixed mode. You should see at least 20% recovery.

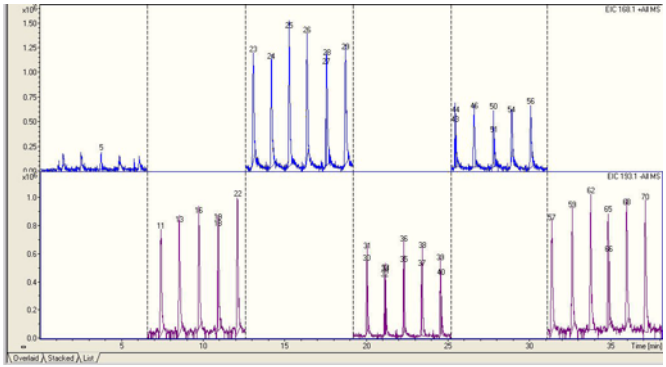

Figure 29

To shut down and vent the instrument

Follow these steps to shut down, vent, and completely turn off the instrument.

With Trap Control Software revision 6.0 and above, vent and pump-down can be done from the Internet Explorer.

1 Open Internet Explorer and go to http:\ 192.168.254.10.

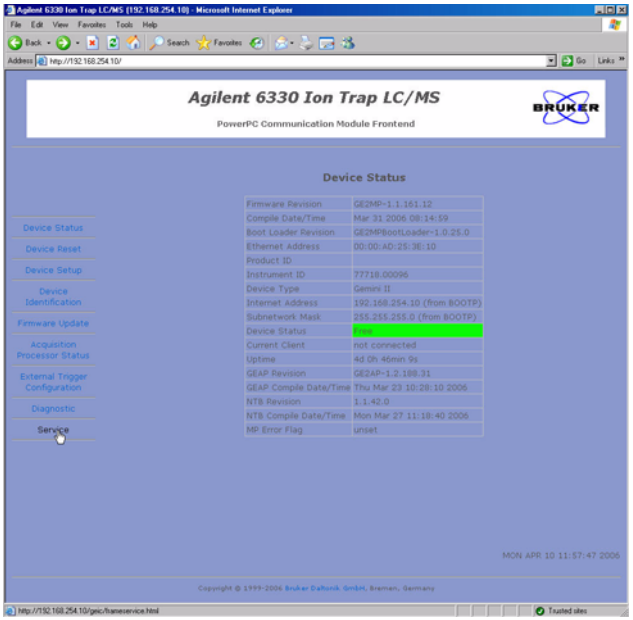

Figure 30

2 Click **Service > Vacuum System**.

Figure 31

3 From the Vacuum System page, click **Vent VacSys**.

Figure 32 Unvented instrument

4 Wait until both turbos are below 10% speed before you continue.

WARNING **The drying gas heater and APCI vaporizer heater will also still be hot. Wait for the instrument to cool off before you continue.**

WARNING **Do not touch the multimode source or the capillary cap. They may be very hot. Let the parts cool before you handle them.**

WARNING **Never touch the source surfaces, especially when you analyze toxic substances or when you use toxic solvents. The source has several sharp pieces which can pierce your skin including the APCI corona needle, vaporizer sensor and counter current electrode.**

5 After both turbos are below 10%, turn off the power switch on the front and the side of the LC/MS.

The instrument automatically pumps down when the side and front power switched are turned back on. Launch the Internet Explorer and monitor the pump down status.

To verify the ion trap calibration

Calibrations are not necessary at installation. The instruments come already calibrated. The current calibration file is stored on the instrument GEPS-2 processor system. When the Trap Control Software is installed, the GEPS-2 processor system downloads the calibration yep file to the **C:\BDalSystemData\Data\Instruments** directory.

If you need to calibrate the system, note that:

- The Scan, Fragmentation and Isolation calibrations on 6300 series Traps with the multimode source are done in APCI mode.
- The calibration is done from the same Calibration Tab view for all source types.
- The calibration mix used for multimode source is called G2432A APCI\APPI tuning mix**.**

To calibrate the source:

1 Select the appropriate mass list as positive or negative for the G2432A calibrant.

  If no positive or negative calibration mass list for the APCI masses exist, create positive and negative APCI mass lists for multimode calibrations.

  - Click **Options > Edit Mass List**.
  - For each mass list that you need to create, click the Sample Name, then add the appropriate masses to the list (see Table 2 for Positive ions and Table 3 for Negative ions).
  - Click **Save**, then **Close**.
  - All modes are calibrated the same as with the standard G1948A ESI source, except for the calibrant being used. The ionization mode has changed from ESI to APCI for the calibrations with the G1978A multimode source installed.

2 Choose the appropriate calibration type:

  - The Fragmentation and Isolation calibration are for the Ultra Scan mode only.
  - Scan calibration can be used for all modes (including Ultra Scan).

3 Load the methods for the appropriate mode (Ultra Scan, Standard-Enhanced, Standard-Maximum or Extended). The methods are located in the **D:\Methods\Instalation Methods** folder.

4 Click the **Report** radial button in the Calibration Tab menu. Whenever a calibration is completed the current calibration data is stored in the **C:\BDalSystemData\Data\Instruments\Agilent 6330 Ion Trap LC MS** folder. The current calibration is located in the folder named by the instrument number, and the previous calibration is stored in the **Backup** folder.

5 Click **Show Spectra** to view the spectra. (See Figure 35).

6 Click **Print** to print the calibration report.

The calibration report can be used to validate the Scan calibration for correct mass assignment within 0.2 AMU of the actual mass when calibration is complete. The Actual Mass and the Observed Mass is displayed when you do a Scan calibration. See Figure 2. The Scan calibration is available for Ultra Scan, Std-Enhanced, Std- Maximum or Extended scan modes.

Other available calibration modes are Detector, Ejection Factor, Ejection Phase, Isolation, and SPS parameter.

Table 2 Positive ions - G2432A APCI Calibration Ions Empirical Formulas for High Resolution MS

	Positive Ion (m/z)	Empirical Formula
Purine+H	121.051421	C5.H5.N4
HP-0321+H	322.048699	C6.H19.O6.N3.P3
HP-0622+H	622.029499	C12.H19.O6.N3.P3.F12
HP-0921+H	922.010300	C18.H19.O6.N3.P3.F24
fragment	1307.969049	C25.H17.O6.N3.P3.F40
HP-1521+H	1521.971900	C30.H19.O6.N3.P3.F48
fragment	1807.937049	C35.H17.O6.N3.P3.F60
HP-2121+H	2121.933500	C42.H19.O6.N3.P3.F72

Table 3 Negative Ions - G2432A APCI Calibration Ions Empirical Formulas for High Resolution MS

	Negative Ion (m/z)	Empirical Formula
Purine-H	119.035771	C5.H3.N4
fragment	556.001426	C10.H15.O6.N3.P3.F10
fragment	805.985476	C15.H15.O6.N3.P3.F20
fragment	1305.953400	C25.H15.O6.N3.P3.F40
fragment	1805.921399	C35.H15.O6.N3.P3.F60

Fragment ion signals are progressively stronger at higher m/z by increasing CID energy.

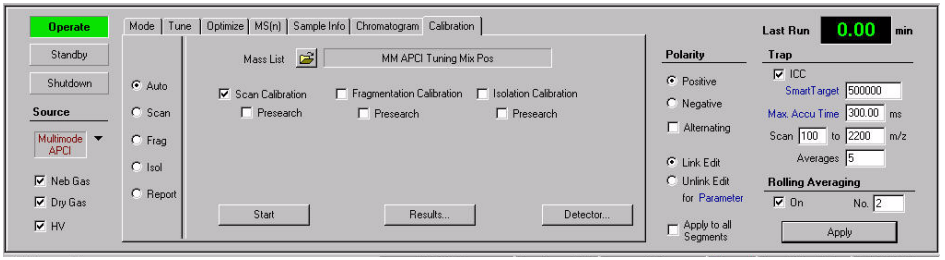

Figure 33 Calibration window in standard customer mode

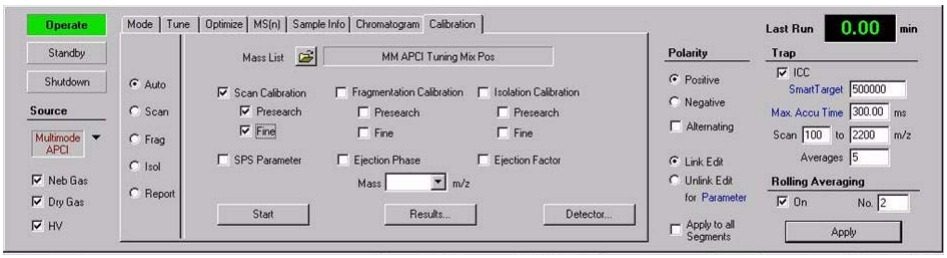

Figure 34 Calibration window shown in service mode. Note that Service mode should be done by an Agilent FSE.

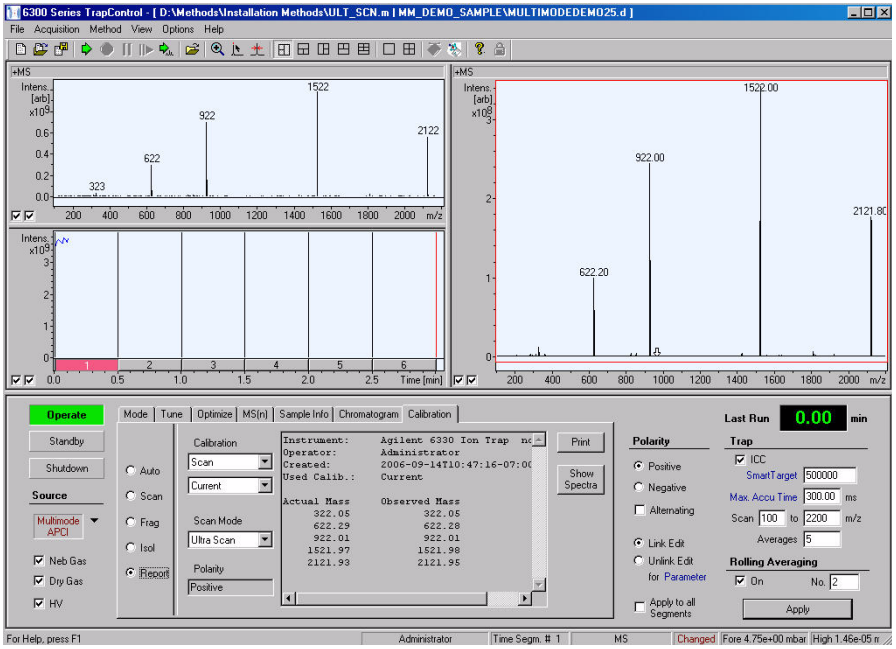

Figure 35 After the calibrations have completed from the Calibration Tab, either Print or Show Spectra can be selected.

Scan Calibration Report

Instrument	Agilent 6330 Ion Trap no 00096
Operator	Administrator
Created	2006-09-14T10:47:16-07:00
Used Calib.	Current
Scan Mode	Ultra Scan
Polarity	Positive

Actual Mass	Observed Mass
322.05	322.05
622.29	622.28
922.01	922.01
1521.97	1521.98
2121.93	2121.95

Figure 36 An example report that is created when Print is selected.

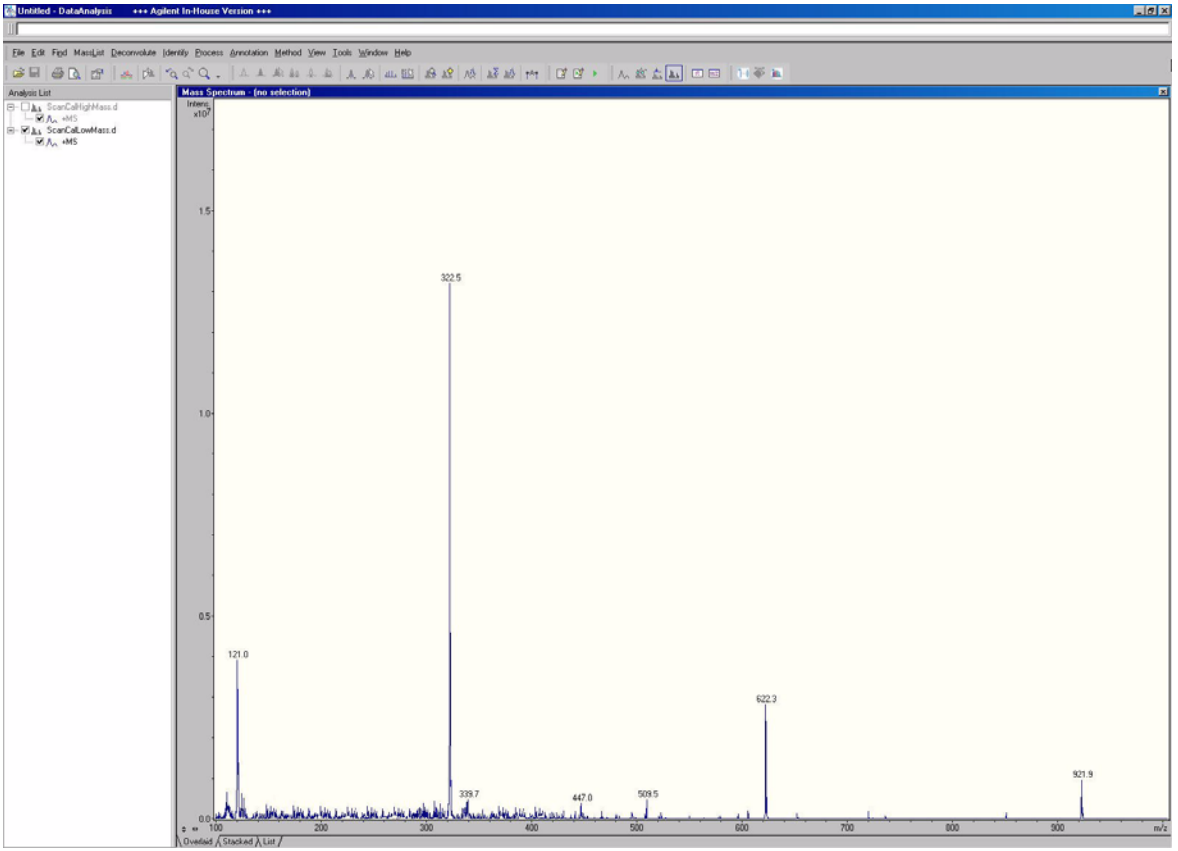

Figure 37 Ultra Scan Spectra from Multimode source calibration in APCI mode for Low mass ions. The Show Spectral Tab was selected and the data appears in data analysis.

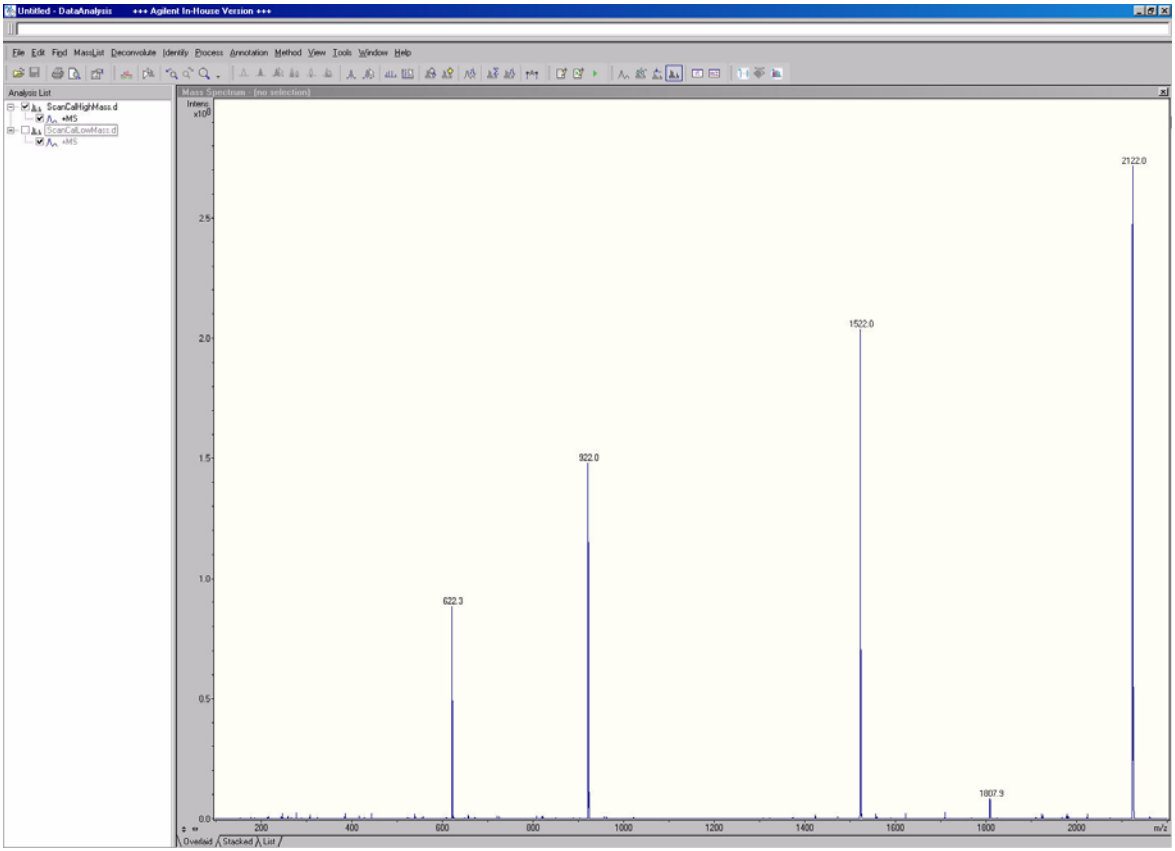

Figure 38 Ultra Scan Spectra from Multimode source calibration in APCI mode for high mass ions. The Show Spectral Tab was selected and the data appears in data analysis.

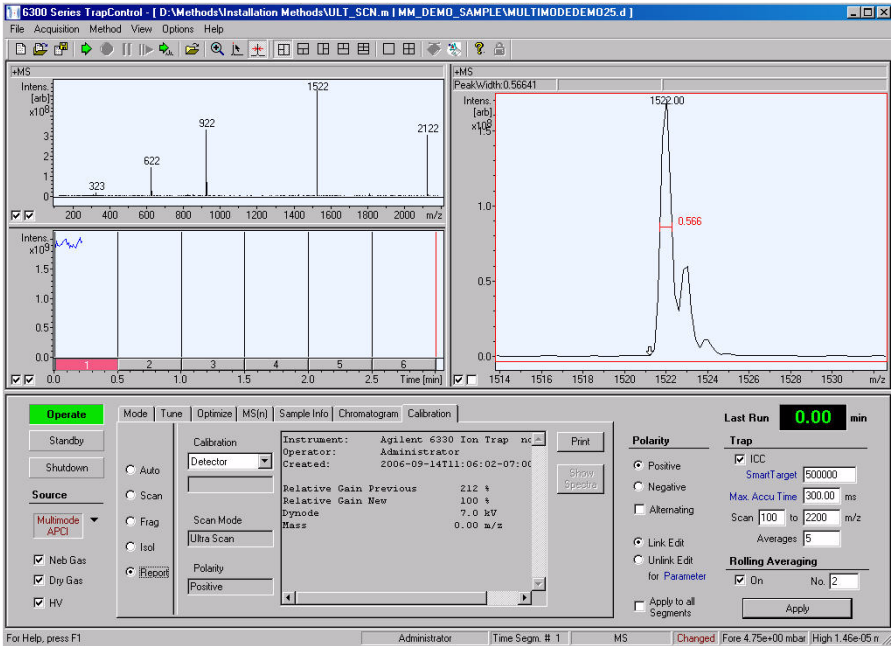

Figure 39 The Detector gain calibration should be done after the initial scan calibration. The report can be printed.

Detector Calibration Report

Instrument	Agilent 6330 Ion Trap no 00096	
Operator	Administrator	
Created	2006-09-14T11.0:02-07:00	
Polarity	Positive	
Mass	0.00	m/z
Dynode	7.0	kV
Relative gain Previous	212	%
Relative Gain New	100	%

Figure 40 A typical report when Print report is selected for Detector calibration.

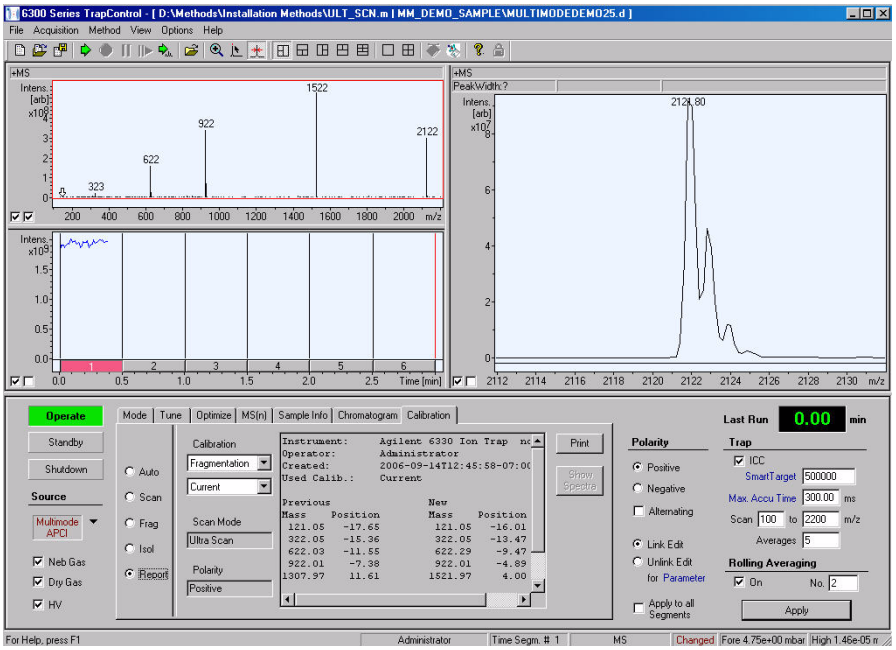

Figure 41 The Fragmentation gain calibration should be done after the Scan calibration. The report can be printed.

Fragmentation Calibration Report

Instrument Agilent 6330 Ion Trap no00096

Operator Administrator

Created 2006-09-14T12:45:58-07:00

User Calib. Current

Scan Mode Ultra Scan

Polarity Positive

Previous		New	
Mass	Position	Mass	Position
121.05	-17.65	121.05	-16.01
322.05	-15.36	322.05	-13.47
622.03	-11.55	622.29	-9.47
922.03	-7.38	922.01	-4.89
1307.97	11.61	1521.97	4.00
1521.97	0.99	2121.93	12.21
1807.94	8.37		
2121.93	11.11		

Figure 42 A typical report when Print report is selected for Fragmentation calibration.

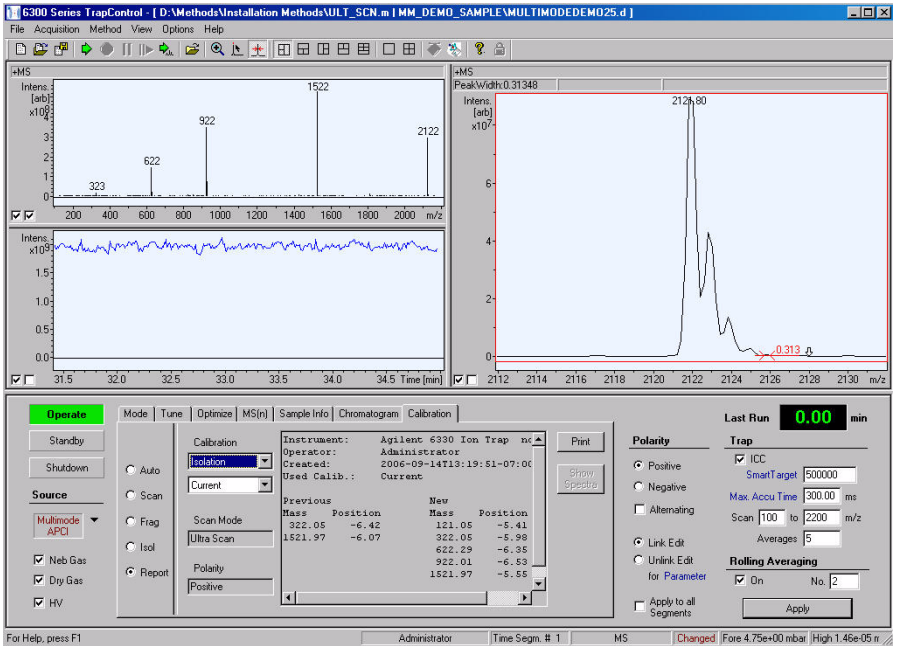

Figure 43 The Isolation calibration should be done after the Fragmentation calibration. The report can be printed.

Isolation Calibration Report

Instrument Agilent 6330 Ion Trap no00096

Operator Administrator

Created 2006-09-14T13:19:51-07:00

User Calib. Current

Scan Mode Ultra Scan

Polarity Positive

Previous		New	
Mass	Position	Mass	Position
322.05	-6.42	121.05	-5.41
1521.97	-6.07	322.05	-5.89
		622.29	-6.35
		922.01	-6.53
		1521.97	-5.55
		2121.93	-4.75

Figure 44 A typical report when Print report is selected for Isolation calibration.

3 Methods

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To create a method for alternating ESI and APCI operation 62

This chapter describes the tasks set up methods for the multimode source and to do maintenance steps that are specific to the instrument.

For more maintenance steps, see the *G1978A Multimode Source Maintenance Guide*.

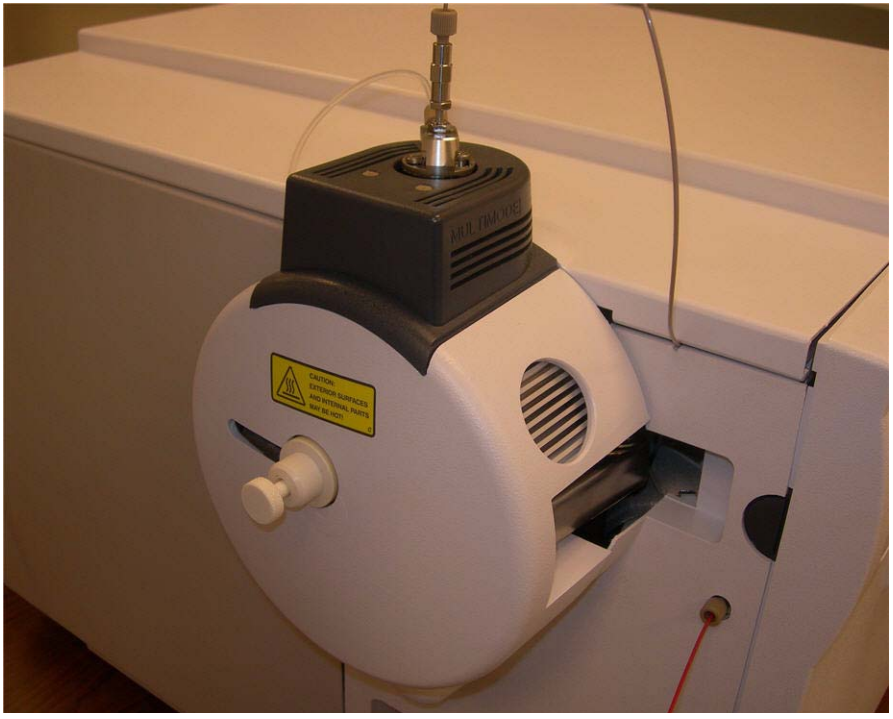
MULTIMODE
CAUTION
EXTERIOR SURFACES
AND INTERNAL PARTS
MAY BE HOT

Figure 45

To setup a method to use the multimode source

1 From the Trap Control Software, select **Shutdown**, then select the source type as Multimode.

There are three different modes for the multimode source:

- Multimode ESI
- Multimode APCI
- Multimode ES+APCI

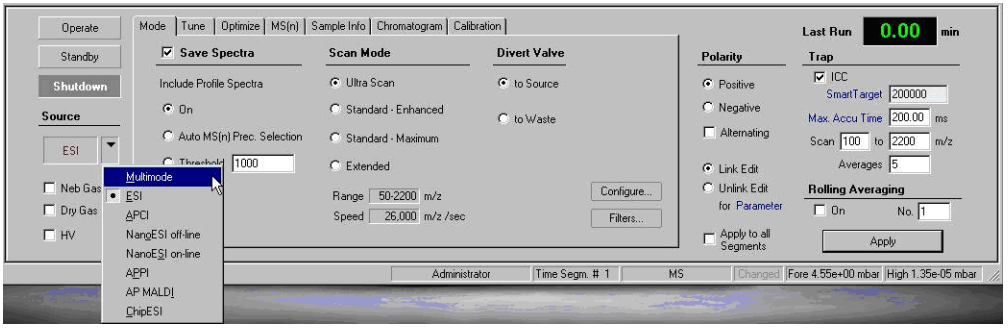

Figure 46

2 Select the mode for the analysis of interest. In the example below Multimode ESI is selected. The trap can be in Standby while selecting ESI, APCI or ESI +APCI.

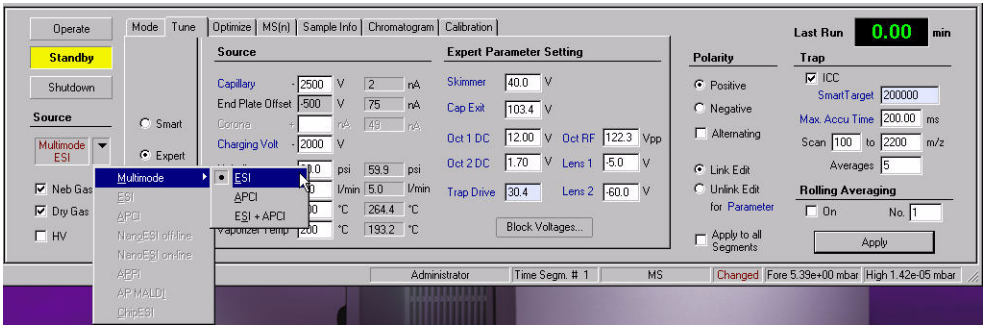

Figure 47

3 Set the tune parameters:

- Adjust the Nebulizer pressure, Drying gas flow, and Drying gas temperature, depending on the LC mobile phase and flow.
- In Multimode ESI, APCI or Multimode ESI + APCI mode, set the Vaporizer temperature appropriately for the analysis. The vaporizer temperature is on during Multimode ESI operation as well as APCI and ESI +APCI.

  You may want to use the default multimode source settings for Multimode ESI, Multimode APCI and Multimode ESI + APCI as starting parameters.

4 From Tune Expert make any other changes necessary for your method.

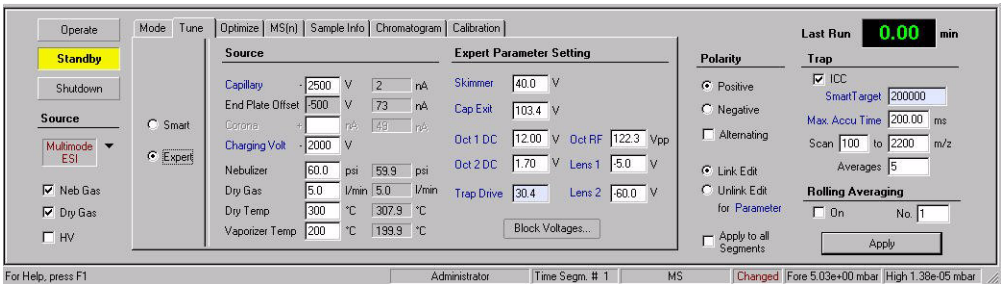

Figure 48

5 From the **Method** pull-down list, click **Save-6300 Series Trap Control Part**.

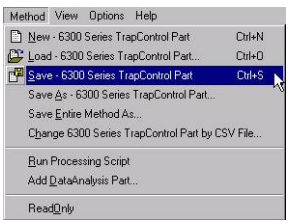

Figure 49

WARNING **The Liquid Chromatograph diverter valve on the Agilent 6300 Series traps is an integral part of the G1978A safety system. The LC mobile phase flow must always be connected to the diverter valve inlet filter. Never bypass the diverter valve and connect directly to the nebulizer. If the diverter valve is used in a manner not specified by Agilent Technologies, the protections provided by the diverter valve may be impaired.**

To create a method for positive/negative mixed mode operation

1 From the Trap Control Software, select the multimode source analysis type as **Multimode ES+APCI**.

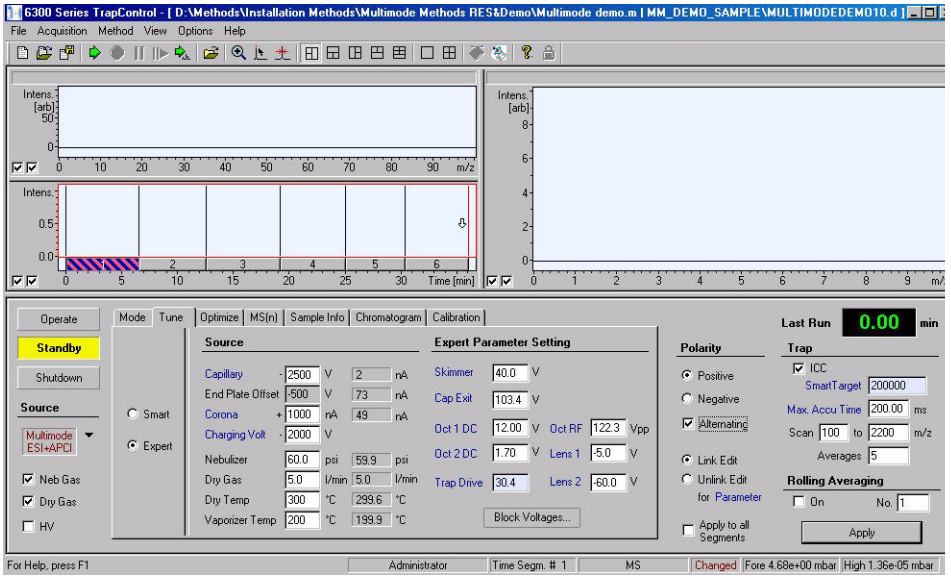

Figure 50

2 Select polarity to **Alternating**.

3 Select **Tune Expert** and set all other **Source** parameters necessary for the type of acquisition.

4 Save the Trap Control part of method.

Polarity switching is a very useful technique but it requires time for the ion chemistry to be established and the optics path to refill with ions. The gas density plays a role in the speed of refilling the ion path. The gas density is affected by source temperature. For a method running positive/negative switching, use a lower vaporizer temperature (150 to 200°C) and a lower Vcap (approximately 1000 V). These will greatly affect the quality of the results in positive/negative switching experiments.

To create a method for alternating ESI and APCI operation

1. From the Trap Control Software, select the multimode source analysis type as **Multimode ESI**.
2. To use alternating Multimode ESI and Multimode APCI, set each segment to either **Multimode ESI** or **Multimode APCI**.
3. Select **Tune Expert** and set all other **Source** parameters necessary for the type of acquisition.
4. Make any other changes that are necessary for your method.
5. Save the Trap Control part of the method.

NOTE In general, use mixed mode operation (MM-ES+APCI) instead of alternating Multimode ESI and Multimode APCI mode segments. Rarely do you need to know whether a compound responds purely in ESI or APCI modes on a chromatographic time scale.

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In This Book

This book contains installation, operation, maintenance and troubleshooting instruction for the Multimode Source for 6300 Series Ion Trap LC/MS.

Printed in USA
First Edition, December 2008

G1978-90080

Agilent Technologies