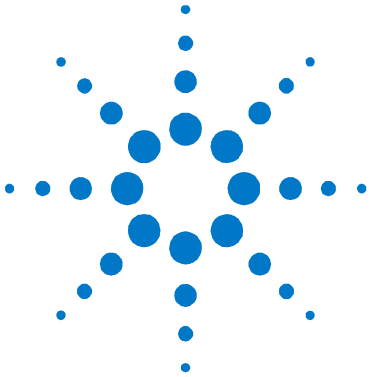

HPLC-Chip/Nanospray Upgrade Installation Guide

Agilent Technologies

Notices

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1 Introduction

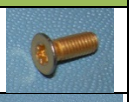
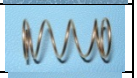
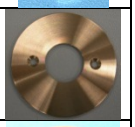
This document describes the installation of the HPLC-Chip/Nanospray Upgrade Kit. This upgrade is designed for all Agilent LC/MS instruments utilizing the HPLC-Chip interface in either the HPLC-Chip configuration or Nanospray configuration.

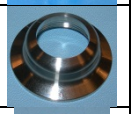
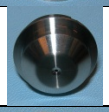
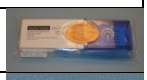
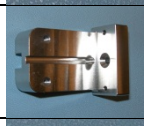
This document and procedure assumes that air is present in the drying gas supply. If no air is available, the HPLC Chips will charge which appears as unstable spray on the video capture device or black/white monitor.

Please note that this document is best viewed when printed in color.

Kit Contents

The HPLC-Chip/Nanospray Upgrade Kit includes the following items:

G1982-67000	**HPLC-Chip/Nanospray Upgrade Kit**		
Part Number	Part Description	Quantity	Part Picture
G1969-20148	End Plate Screw, Gold, M3 x 0.5 x 8mm long flat head screw	2	
2680-0217	Button head screw	1	
G1982-20005	Contact spring	1	
G1982-20006	Radial Gas Diverter	1	
G1946-20111	T-nut	2	
G1946-20156	End Plate, Counter Bored Holes	1	
G1964-20303	Capillary Insulator	1	

G1982-67000	HPLC-Chip/Nanospray Upgrade Kit Cont.		
Part Number	Part Description	Quantity	Part Picture
G1982-20008	Holey Counter Electrode	1	
G1982-20011	Wing Nut, Stainless Steel	1	
G1982-20111	End Cap Nano Spray Shield	1	
G1982-20012	Wave washer, Gold plated	1	
G1982-60004	Cover with flex steel	1	
GT430-20358	Electrode Spacing Tool	1	
59987-20040	Dielectric Capillary	1	
0515-1644	Screw, M3 x 0.5 x 12 mm	1	
G1982-20152	Emitter Cover	1	
5022-2145	ZDV Union	2	
9301-6477	Glass Tip Pkg – Nanospray Emitters	1	
0515-5373	M4 Thumb Screw	1	
G1982-20316	Needle Holder	1	
G1982-60025	Nano Nut Assy	1	
0100-2609	Tefzel Ferrule	2	
G1982-90000	Field Upgrade Installation Manual	1	
0100-2436	Ferrule	1	

G1982-67000	HPLC-Chip/Nanospray Upgrade Kit Cont.		
Part Number	Part Description	Quantity	Part Picture
0100-2437	PEEK Nut	1	
0100-2611	ZDV Union	1	
5065-9906	LC-MS Capillary Connector	1	
G1982-85001	Chip Cube High Mass Reference (HP-1221)	1	Not Shown
G1982-85002	Chip Cube High Mass Solvent (FC-70)	1	Not Shown
G1982-85003	Chip Cube Low Mass Reference Sample	1	Not Shown

Required Tools

The following tools are required for the installation of the HPLC-Chip/Nanospray Upgrade Kit. Not all of the required tools are supplied with the kit:

Included in Kit			
8710-2624	0.9 mm Allen Wrench	1	
8710-2623	1.5 mm Allen Wrench	1	
Not included in Kit			
8710-1623	Torx T-10 driver	1	
8710-1615	Torx T-20 driver	1	
8710-1029	Flat blade screwdriver	1	

Prepare for the upgrade

1. If installed, remove the HPLC-Chip Cube and HPLC-Chip Cube interface from the LC/MS instrument.
2. Remove the HPLC-Chip interface spray shield and gas diverter. Properly dispose these parts as they will no longer be used and are **not** compatible with the new gas diverter.
3. Before beginning the upgrade procedure, it is important to verify the current performance of the LC/MS System. With a standard source installed including the standard capillary cap and spray shield, perform a Positive and Negative ion mode autotune. Examine the tune reports to verify that the LC/MS system is meeting tune specifications.
4. Right-click on the LC/MS icon in the Status pane and select "Vent".
    - After the LC/MS system has completed the vent process, switch off the front switch and unplug the instrument.
    - It is suggested that since the instrument is being completely vented, that an Ion Optics Servicing be performed. This would involve removing the desolvation assembly and cleaning Skimmer 1 and possibly cleaning Lens 1 and Lens 2 of the Ion Optics Assembly.

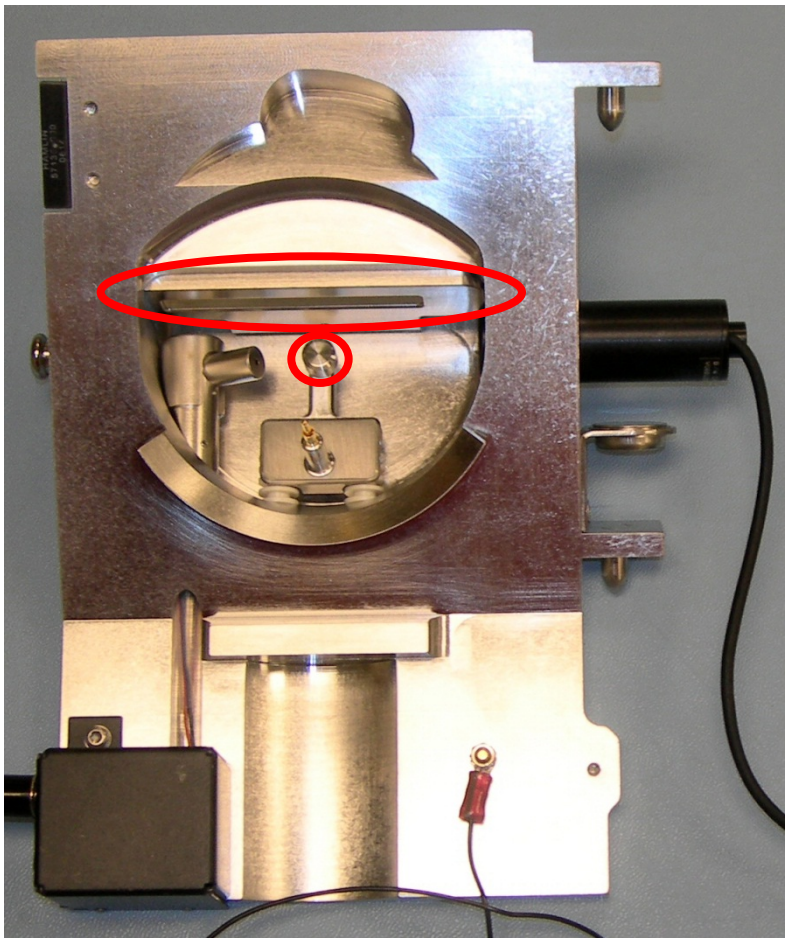
2 Installation

This section describes the installation of the HPLC-Chip/Nanospray Upgrade Kit hardware.

Upgrade the HPLC-Chip Interface

1. While the LC/MS instrument is venting, remove and properly dispose of the following parts from the HPLC-Chip interface:

    - Counter Electrode – Use the supplied Hex Wrench (part number 8710-2623) to remove the counter electrode. The set screw only needs to be loosened to remove the counter electrode.
    - Existing Top Plate – The existing top plate may or may not contain the IRM wick used to introduce IRM compounds into the spray chamber. The new Top Plate will contain the IRM wick but also has been modified to provide additional standoff clearance for the high voltage spray shield.



The items circled in red are to be removed and discarded.

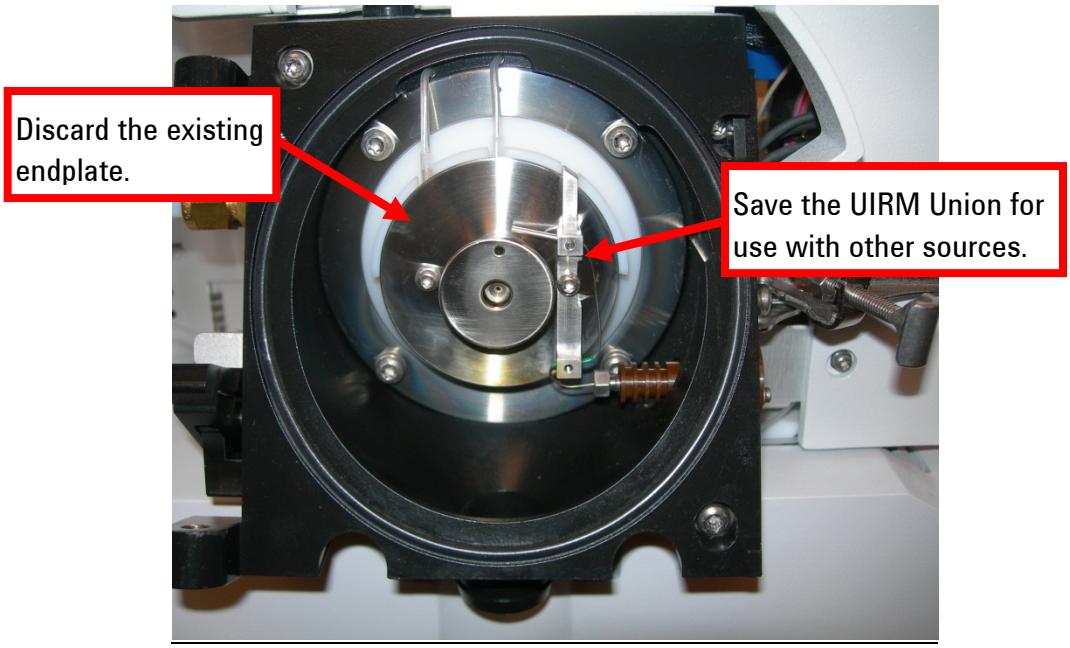
2. Locate the new Holey Counter Electrode (part number G1982-20008).

3. Using the hex wrench, install the new counter electrode. Set the Holey Counter Electrode flush against the Electrode Mounting Bracket. Temporarily tighten the set screw to keep the new counter electrode in place. The final spacing adjustment will be performed with the interface installed on the instrument.

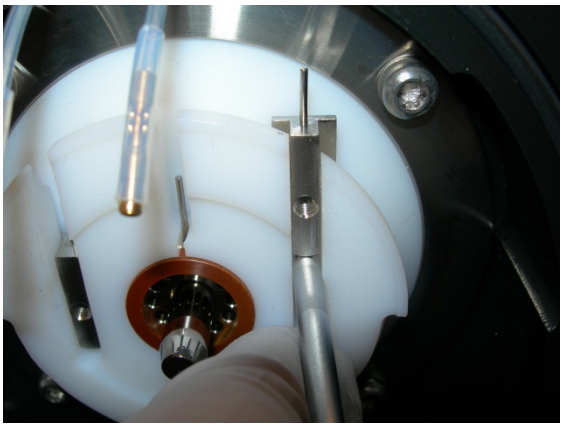
Replace the gas distributor inside the desolvation assembly

1. Once the instrument has vented, remove the spray shield and the existing endplate. The stainless steel endplate cannot be used with this upgrade kit. Please dispose it in the proper disposal container. The new endplate that is part of this upgrade kit is compatible with all source types.

   Please note: If the instrument type is a TOF or Q-TOF ***and*** the instrument has the UIRM, remove the UIRM hardware. Place this hardware in a clean bag as this hardware will no longer be used with the HPLC-Chip interface. However, it can still be used with the standard source types supported with the TOF or Q-TOF.



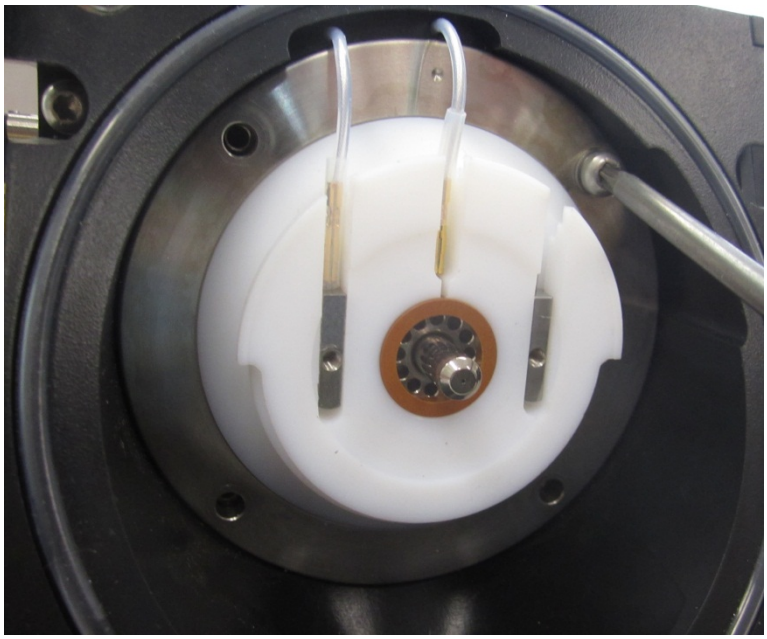
2. Remove the Chamber and Capillary High Voltage cables from the T-nut connectors.
3. Remove the two existing high voltage T-nut connectors that are used to secure the end plate and discard.



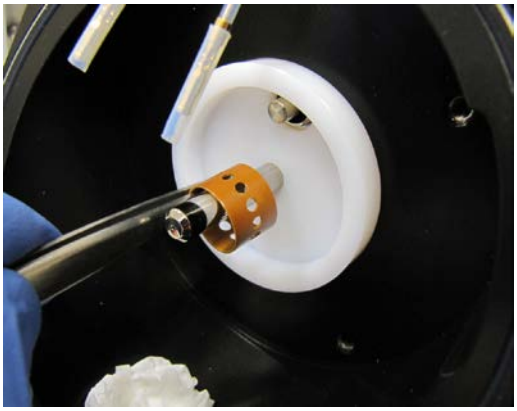
4. Remove the currently installed capillary. A new capillary is supplied as part of this upgrade kit.

   Please Note: This capillary will not be used after installation of this kit. Destroy this capillary so that it cannot be reused in the instrument or any other instrument.

5. Remove the four T-20 torx screws used to secure the metal ring that holds the Teflon spray shield support block.



6. Remove the Teflon endplate insulator block to access the vespel gas distributor. Remove and discard the old gas distributor.



7. Locate the new dielectric capillary (part number 59987-20040). Dip or wet the capillary with Isopropanol before installing to lubricate it. Install the new capillary.

CAUTION	**Do not twist the capillary while installing it. Doing so will permanently damage the platinum coating of the capillary.**

Be careful not to damage the canted coiled spring located inside the capillary cap at the fragmentor end of the desolvation assembly. It is suggested to remove the desolvation assembly to ensure that the canted coiled spring has remained in place.

8. Locate the new gas distributor (part number G1964-20303). With the solid side facing the Teflon wall, slide the new gas distributor over the new capillary. Push it on until it is flush with the Teflon block.

G1964-20303
Gas Distributor

9. Reinstall the Teflon support block for the end plate with the stainless steel ring. Make sure that the new gas distributor is positioned properly before reinstalling the screws. Be careful during the reinstallation not to damage the URIM vespel union.
10. Reinstall the 4 T-20 torx screws to secure the metal ring in place.
11. Locate the two T-nut high voltage connectors (part number G1946-20111) and install them into the Teflon block.

12. Locate two gold pan head screws (part number G1969-20148) and the new end plate (part number G1946-20156). Install the new end plate and secure in place with the two pan head screws. Make sure that the screws are snug but not over-tightened.

13. Reinstall the standard spray shield and capillary cap. When installing the capillary cap, do not twist or the capillary can be permanently damaged.
14. Plug the instrument back into the wall outlet and begin the pump down procedure.
15. **For TOF and Q-TOF Instruments ONLY:** Locate the Button Head Cap Screw (part number 2680-0217). On the outside of the desolvation assembly, thread the plug into the UIRM union. Do not over-tighten or the Vespel union will crack.

16. **For TOF and Q-TOF Instruments ONLY:** Start Diagnostics and performed a 2 hour HV conditioning procedure.
17. Once the LC/MS instrument has pumped down and stabilized, perform a standard autotune. Compare the tune report to the one previously obtained. The results should be approximately the same when comparing reports.

Assemble the new spray shield and gas diverter assembly

1. While the instrument is tuning or pumping down, locate the following parts:

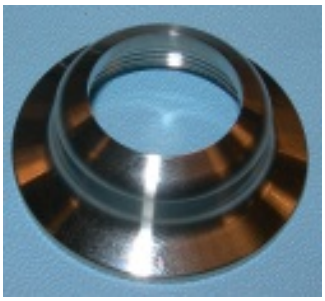
   - Winged Nut (part number G1982-20011)
   - End Cap Nano Spray (G1982-20111)
   - Radial Gas Diverter (part number G1982-20006)
   - Wave Spring, Gold Plated (part number G1982-20012)
   - Contact Spring (part number G1982-20005)

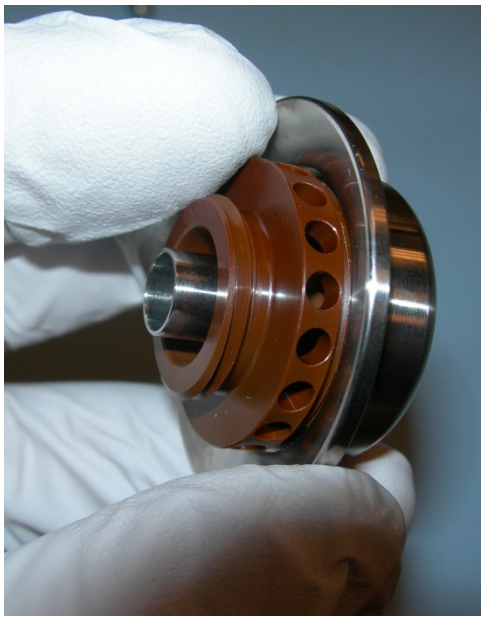
   When assembling these parts, wear powder free or lint free gloves. Treat all parts as ultra clean parts.

2. Start assembling the new spray shield by sliding the Wave Spring (part number G1982-20012) over the End Cap Nano Spray part (part number G1982-20111).

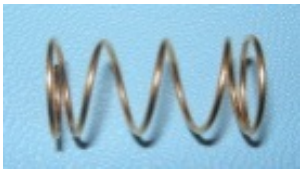
3. Locate the Radial Gas Diverter (part number G1982-20006) and insert into the End Cap Nano Spray. Make sure to align the pin in the Radial Gas Diverter with the notch on the End Cap.

4. Thread the vespel Radial Gas Diverter into Winged Nut (part number G1982-20011). Make sure to thread the Radial Gas Diverter completely into Winged Nut.

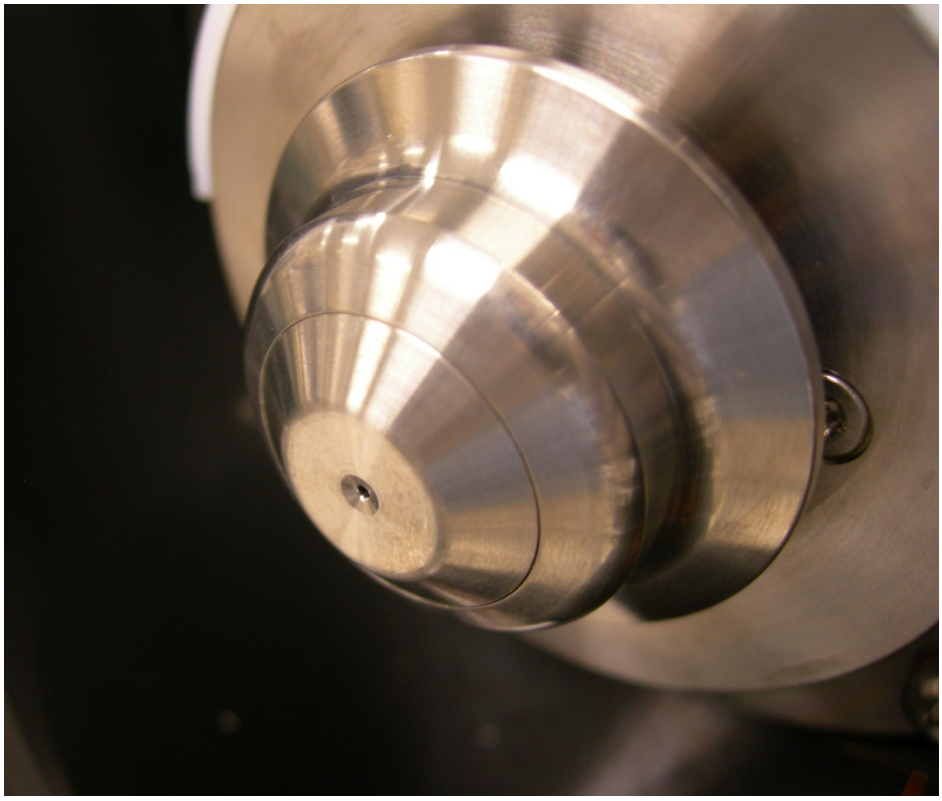




5. Once all of the parts have been assembled, rinse all of these parts with Isopropanol in a clean, glass beaker at least three times.
6. Once the parts have been rinsed, remove the parts from the beaker and place them on a lint free cloth.
7. Locate the Contact Spring (part number G1982-20005) and insert it into the cleaned spray shield assembly.



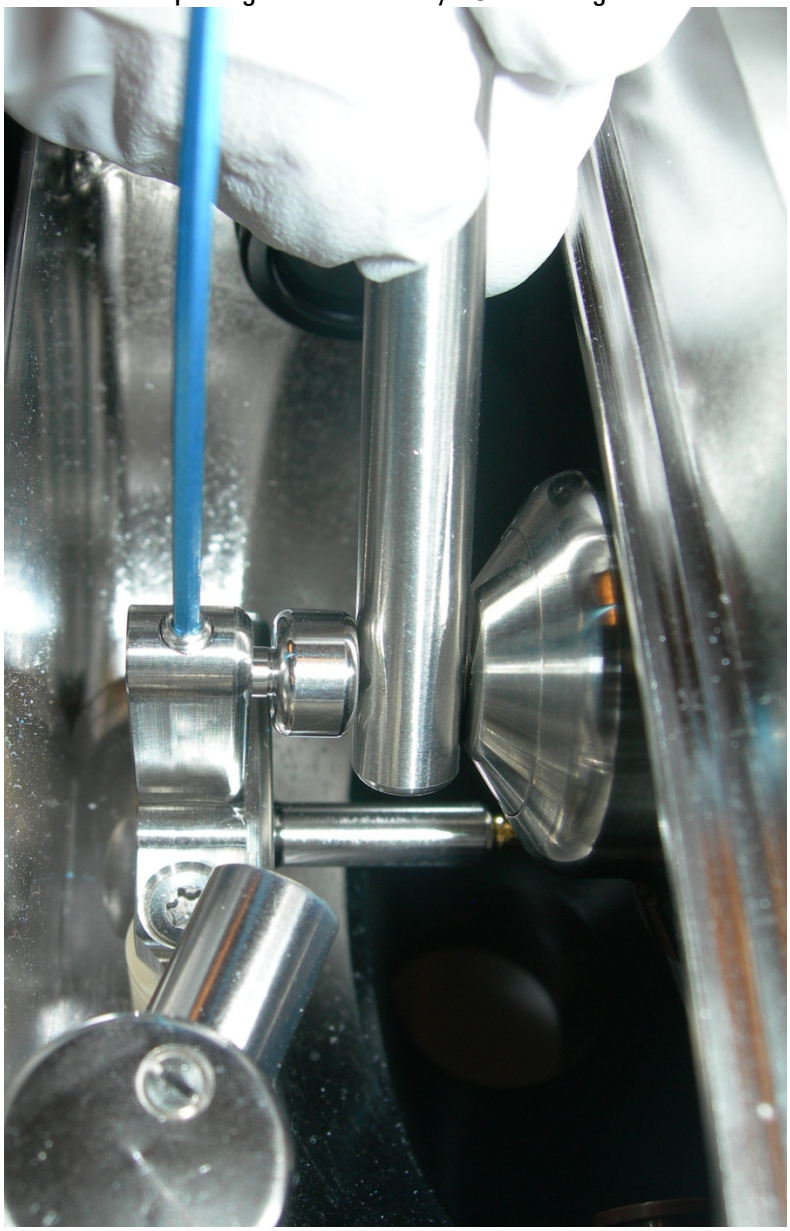
8. Allow the instrument to complete the High Voltage Conditioning and Check Tune process before proceeding. Once the tune process has completed, convert the system **back** to Chip Cube mode.
9. Remove the currently installed spray shield and capillary cap. Store them in a clean, lint free bag.
10. Before installing the new spray shield assembly, confirm that the two pan head screws securing the end plate are not loose. Once the new chip cube spray shield is installed, access to these two screws is not possible. Thread the spray shield assembly with contact spring onto the End Plate. Make sure to thread the spray shield completely onto the End Plate. Otherwise, the spacing between the Spray Shield and Counter-Electrode will not be correct.
11. The new spray shield assembly will now appear as the picture below.



Set the Holey Counter Electrode Distance

CAUTION	**This procedure will be performed with the HPLC Chip Cube Interface installed. Make sure that the instrument is in either Standby or Off. Otherwise, the Spray Chamber High Voltage Electronics will be energized and present a shock hazard.**

1. Install the HPLC Chip Interface onto the desolvation assembly. Please note that the Top Plate should not be installed at this time.
2. Connect the power cables for the illumination LED and video cable for the camera.
3. Locate the Electrode Spacing Tool (part number GT430-20358).
4. Loosen the set screw securing the Holey Counter Electrode. Be careful not to over-loosen the set screw or the Holey Counter Electrode will fall out of its holder
5. Insert the Electrode Spacing Tool between the counter electrode and the spray shield. The Electrode Spacing Tool **must** touch the Holey Counter Electrode **AND** the new spray shield assembly. This means the spacing is set correctly. See the figure below as an example.

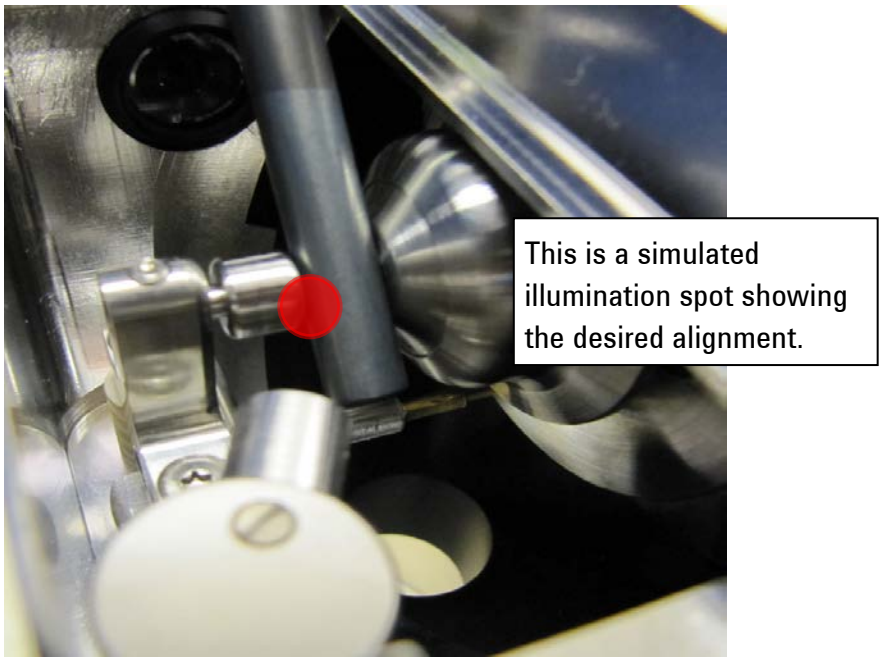


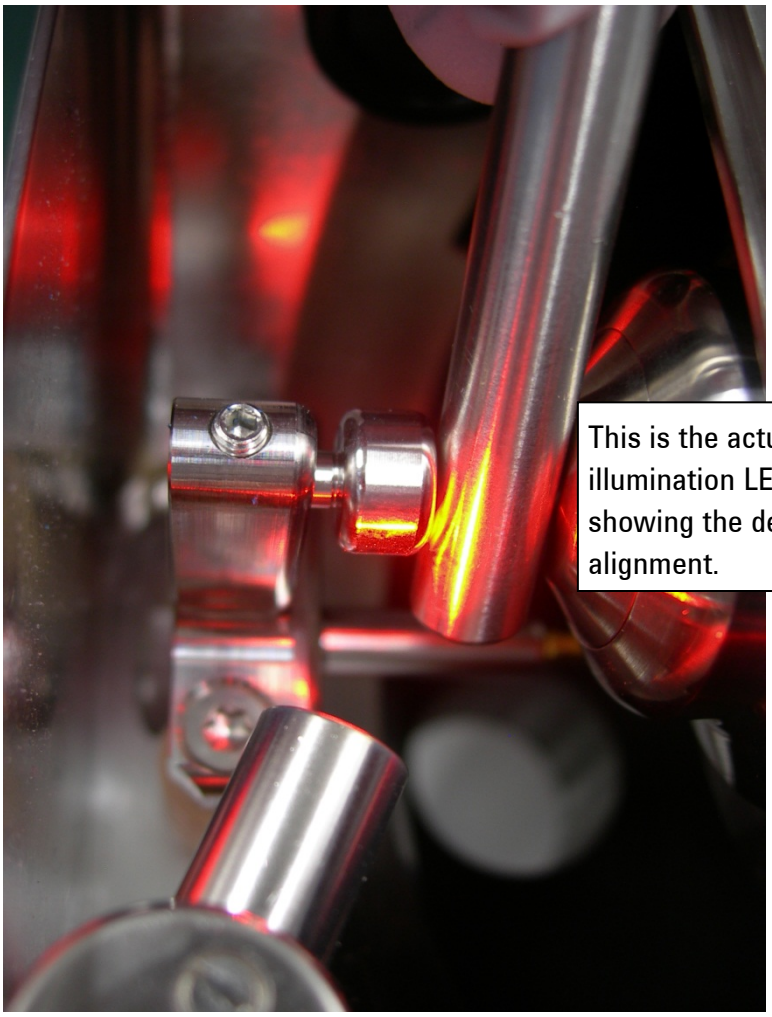
6. Once the spacing is set correctly, tighten the set screw that secures the Holey Counter Electrode in place. Once the set screw has been tightened, recheck the spacing to ensure that there is no extra space. Otherwise, the high voltage field will not be correct.

Realign the Illumination LED and Camera

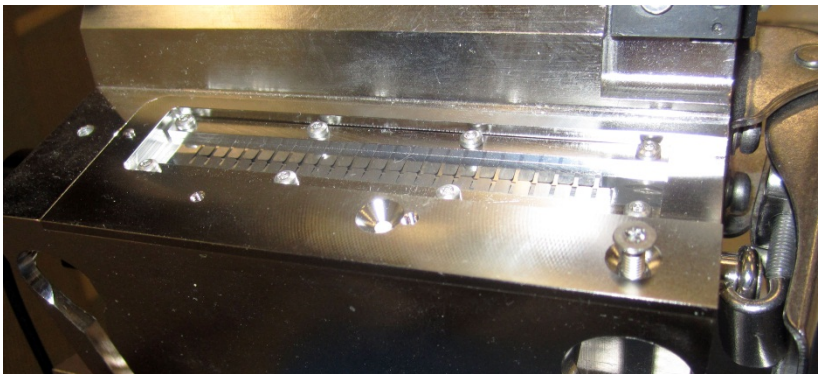
Because the Holey Counter electrode has different depth dimensions, the illumination LED will not be pointing at the right location. This will result in poor illumination of the spray from the chip. Therefore, the illumination LED position will need to be adjusted. This procedure must be performed with the HPLC Chip Cube Interface **not** installed on the LC/MS instrument. If this adjustment procedure is performed while the interface is mounted on the instrument, the interface can be pushed off of the instrument by the I-button reader. This results in the interface dropping on the floor which will damage the interface.

1. Open the spray chamber and place on a flat surface.
2. Locate the set (grub) screw that secures the illumination LED assembly in place.
3. Using the supplied 0.9 mm Allen wrench (part number 8710-2624), loosen the set screw slightly.
4. Use the spacing tool between the Holey counter electrode and the spray shield to make visualizing the LED easier.
5. Rotate the LED assembly to where 1/3 of the beam is striking the edge of the Holey Counter Electrode. See the figure below.

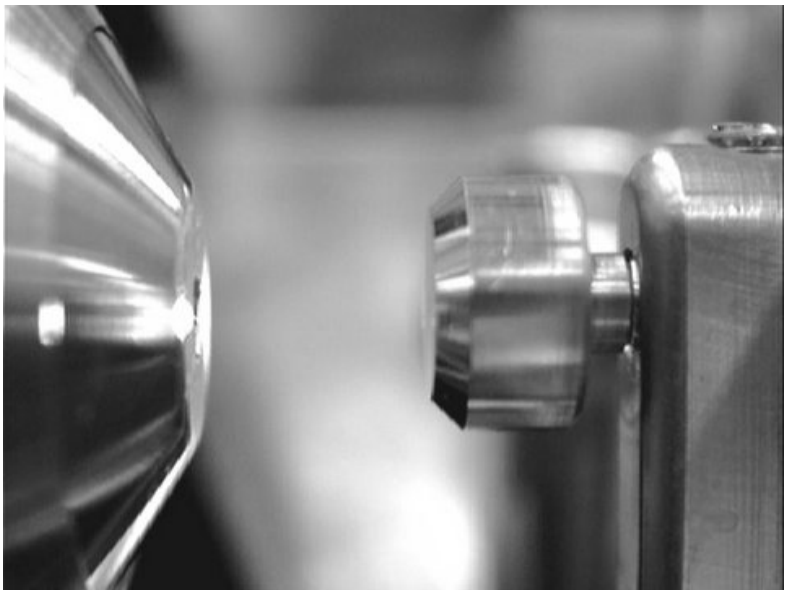




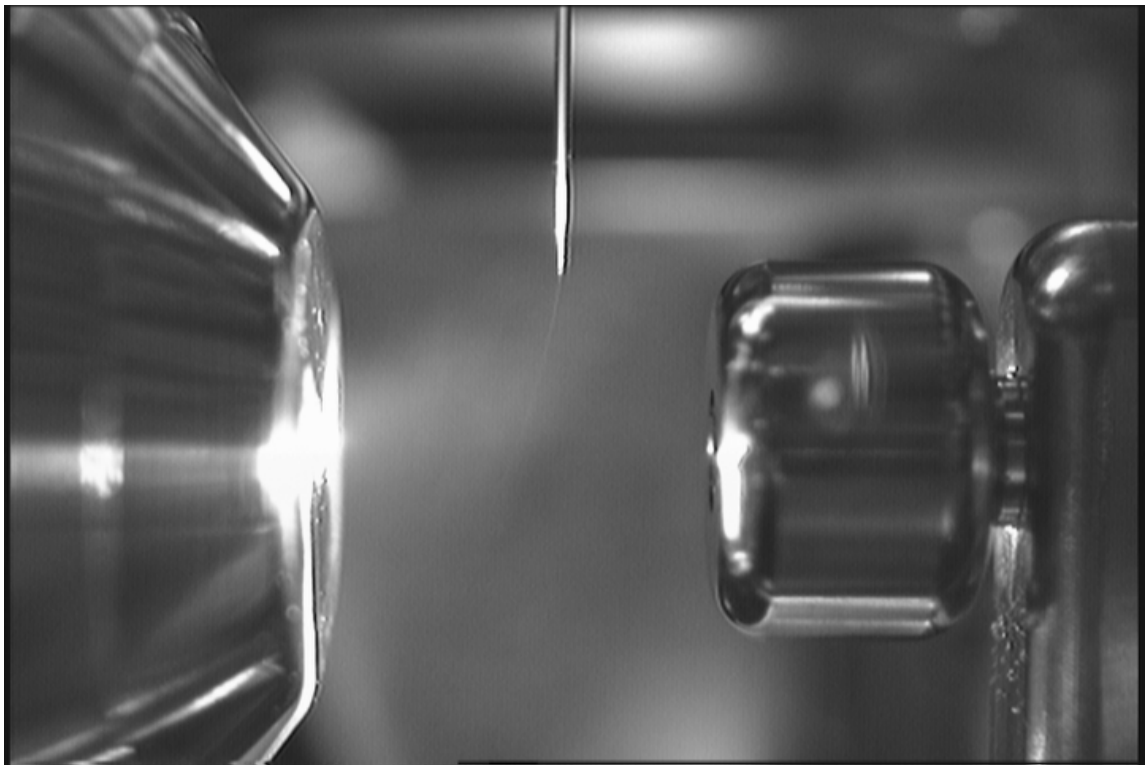
6. Once the best position has been achieved, tighten the set (grub) screw.
7. If the camera power cable and video cable have been disconnected, reconnect those cables.
8. Reinstall the HPLC Chip Cube Interface on the LC/MS instrument.
9. The new top plate can now be installed.
10. Locate the new top plate (part number G1982-60004) and install it on the HPLC interface. Use the new screw (part number 0515-1644) to secure the top plate in place.



11. With the top plate installed, examine the image on the video monitor. If the image is not clear or appears blurry, the camera is not focused correctly.
12. To refocus the camera, using the supplied 1.5 mm Allen Wrench (part number 8710-2623) loosen the set (grub) screw that secures the camera in place.
13. In order to focus the camera, examine the image on the monitor while either inserting or pulling out the camera relative to the HPLC Chip Cube Interface. When the camera is properly focused, the image on the monitor should appear as below.



14. Once the camera has been focused, tighten the set (grub) screw. Recheck the image on the video monitor to ensure that the camera has not moved while tightening the set screw.
15. Reinstall the HPLC Chip Cube on the instrument. Be careful not to damage any of the camera or video cables.
16. Restart the MassHunter Acquisition software if needed to have the HPLC Chip Cube recognized.
17. Insert a chip into the HPLC Chip Cube. The image on the video monitor should resemble the image below.



3 New Starting Set Points and Default Method Set Points

Before the upgraded HPLC-Chip Interface can be used, the drying gas flow, temperature, and capillary voltages must be adjusted from previous values. The drying gas flow behavior within the interface is significantly different from the previous design. The heat is more evenly distributed while the gas turbulence is significantly lower. All methods must be adjusted during this time. Otherwise, old methods with inappropriate set points will exist which will lead to new customer satisfaction issues.

Please note that this design still requires the use of clean, dry air for optimal performance.

New Default Drying Gas Temperature, Drying Gas Flow, and Capillary Voltage Set Points for Single Bore Capillary LC/MS Systems

The default Drying Gas Temperature for all applications shall be:

- 350°C for MassHunter Acquisition for TOF and Q-TOF B.02.01 Service Pack 2 or lower.
- 365°C for MassHunter Acquisition for TOF and Q-TOF B.02.01 Service Pack 3 or higher.
- 350°C for MassHunter Acquisition for QQQ B.02.01 Service Pack 2 or lower.
- 365°C for MassHunter Acquisition for QQQ B.03.02 Service Pack 3 or higher.

The default Drying Gas Flow for all applications shall be:

- 4 L/min for all applications. Do not exceed 5 L/min. Higher flows will lead to over drying of the Nanospray.

The default Capillary Voltage for all applications shall be:

- 1600 V starting point. With flow from the Nano Pump, increase the Capillary Voltage in steps of 25 V to obtain stable spray. Once spray has been established, increase the voltage by another 50 V. This will provide margin to account for ionization differences when the HPLC solvent gradient changes. However, do not exceed 2100 V or the tip of the HPLC Chip will be permanently damaged by the formation of an APCI Coronal discharge. This results in poor sensitivity for the application.

New Default Drying Gas Temperature, Drying Gas Flow, and Capillary Voltage Set Points for iFunnel Enabled (Multi-Bore Capillary) LC/MS Systems

The default Drying Gas Temperature for all applications shall be:

- 290°C for MassHunter Acquisition for TOF and Q-TOF B.05.00 or higher (still under development and may change with final release).
- 150°C for MassHunter Acquisition for QQQ B.04.01 or higher.

The default Drying Gas Flow for all applications shall be:

- 6490 iFunnel QQQ – 11 L/min for all applications. Higher flows will lead to over drying of the Nanospray and spray stability issues. Lower gas flows will lead to increased chemical/droplet noise
- 6550 iFunnel Q-TOF – 9 L/min for most applications. Still under development and may change with final release.

The default Capillary Voltage for all applications shall be:

- 1600 V starting point. With flow from the Nano Pump, increase the Capillary Voltage in steps of 25 V to obtain stable spray. Once spray has been established, increase the voltage by another 50 V. This will provide margin to account for ionization differences when the HPLC solvent gradient changes. However, do not exceed 2100 V or the tip of the HPLC Chip will be permanently damaged by the formation of an APCI Coronal discharge. This results in poor sensitivity for the application.

4 New Reference Mass Introduction Procedure

Reference Mass Solution Operation with a new Top Plate – For Use with TOF or Q-TOF LC/MS Instruments ONLY!

PLEASE NOTE: These instructions apply only to 6200 Series TOF and 6500 Series Q-TOF LC/MS systems with an HPLC-Chip/MS interface. Do not use the reference mass solution with 6400 Series Triple Quad or 6300 Series Ion Trap LC/MS Systems.

When performing accurate mass measurements on a TOF or Q-TOF instrument, it is desirable to add compounds which ionize under the analysis conditions and provide known accurate mass values ("reference masses") in real time so the mass values in the acquired spectra may be corrected for short-term variations. This document provides a description for performing this function on Agilent TOF or Q-TOF instruments outfitted with a G4240 HPLC-Chip/MS Interface and operated in positive ion polarity. You need a modified top plate for the HPLC-Chip/MS interface assembly and chemicals to make solutions of reference mass compounds. After applying the solutions to an absorbent wick on the underside of the top plate, the IRM compounds slowly evaporate and are ionized, providing signals for correction of the mass assignments.

In order to operate successfully using this modified top plate, the drying gas supply to the LC/MS system must be altered so that air (oxygen) is present in the drying gas and the flow out of the ionization region is restricted. If this alteration has not been performed but this modified top plate is installed, the spray from the Chip will be erratic and unstable. The complete instructions for this alteration are in part number G1995-90000 (instructions for G1995A Low Background Site Preparation kit). If you have not made this alteration to the drying gas supply, it is not recommended to use the modified top plate.

The major differences from the previous generations of top plates are the replacement of the conductive brushes on the top and the addition of a contained absorbent wick on the bottom. The brushes close off the slot in the top plate while still allowing for insertion and ejection of the HPLC-Chip. This minimizes the entry of outside air and contaminants into the ionization region, lowering the abundance of background ions produced and detected by the instrument. The wick holds the IRM solution. You do not need to dismount the Chip cube in order to install the modified top plate.

Preparation of the IRM solutions

The example below assumes you wish to use two internal reference mass standards; however, it is possible to omit either one if desired. The solution of internal reference mass standard(s) in acetonitrile (ACN) is applied to the wick and allowed to dry. Then the high mass solvent (FC-

70) is applied. While the use of FC-70 is optional, and signals from the IRM compounds will be obtained without its use, the response is more uniform and lasts longer when it is used. Note that ACN and FC-70 are immiscible and so should not be mixed together before applying them to the wick. (However, they may be applied sequentially to the wick.) The amounts given below are intended as a guideline but may need adjustment for your instrument or need.

Low Mass IRM stock solution: 1000 µg/ml methyl stearate in ACN

Use reagent grade ACN or higher purity to minimize the presence of unwanted background ions due to contaminants. Weigh out a suitable amount of G1982-85003 methyl stearate, e.g., 10 mg, into a container, and add 10 mL of ACN to make a 1000 µg/mL of methyl stearate solution. Shake to dissolve. Refrigerate this stock solution if it is desired to keep it for more than a few days.

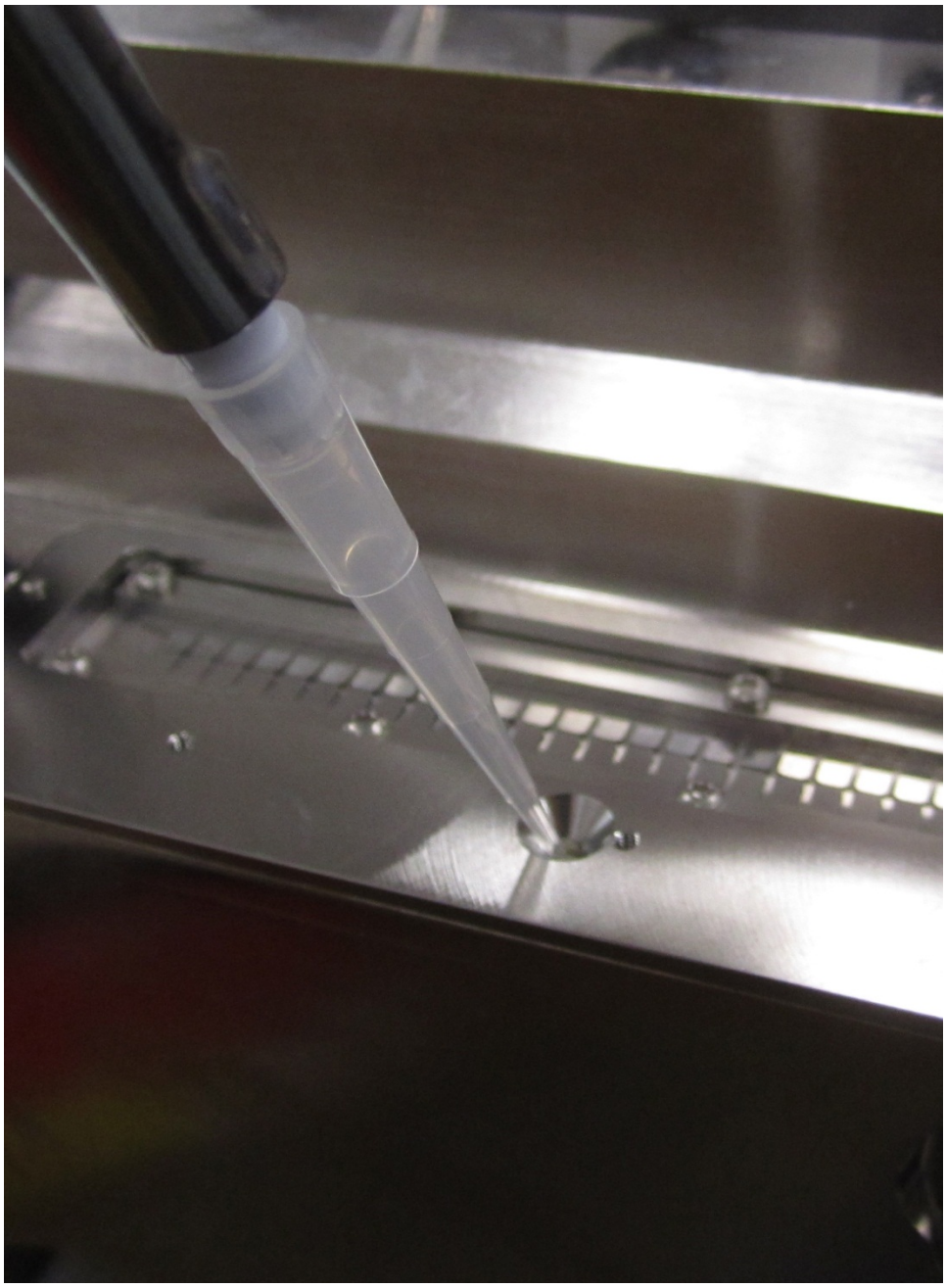
IRM Working Solution: 100 µg/ml methyl stearate and 2% (v/v) HP-1221 in ACN

Add 900 µL ACN to a vial. Add 100 µL low mass IRM stock solution, then 20 µL G1982-85001 HP-1221. Vortex to mix. This working solution must be refrigerated between uses.

Use of the IRM Solutions

The amount of solution to apply or its concentration may need adjustment to suit your needs. These compounds are designed to persist in the ionization region and are difficult to remove completely. Therefore, for the first few applications, it is advisable to apply smaller amounts until the response of your system under your analysis conditions is known. In typical use, 200 µL of the IRM working solution is applied and allowed to evaporate; then 200 µL of FC-70 is applied. The wick holds approximately 250—300 µL of solution before saturation occurs.

- Using the Acquisition software, set the instrument in Standby mode.
- Eject any Chip from the Chip cube, open the Chip cube cover, and flip out the stages assembly.
- Briefly vortex the IRM working solution to assure homogeneity.
- Load a pipetter with the desired amount of IRM working solution (e.g., 200 µL).
- Insert the pipette tip into the IRM port on the top plate. It is located closest to the HPLC Chip Cube and very slowly dispense the IRM solution into the wick. Avoid filling the port too quickly or the IRM solution will spread across the top plate. See the figure below for an example.



- ***Allow about five minutes for the ACN to evaporate at a typical standby source temperature of 300 °C. This pause is important to minimize the chances of arcing when the instrument is turned on.***
- Load a pipetter with the G1982-85002 FC-70 liquid (e.g., 200 µL), and add it to the wick in the same manner as for the IRM working solution. Pipette slowly (10-20 seconds to transfer) to avoid overfilling and to allow time for the wick to soak up the viscous solution.
- Close up the stages assembly and the Chip cube cover.
- Load a Chip and turn on the instrument from the software.

You will not see any signals from the IRM compounds unless there is a spray present and the mobile phase contains electrolytes. The IRM signals will be unstable during the startup and stabilization of the spray, which may take ten minutes or longer at a drying gas temperature of 350 °C and flow of 4 L/min.

The accurate mass values are:

Compound	*m/z* value for proton adduct (positive mode)
Methyl stearate	299.294457
HP-1221	1221.990637

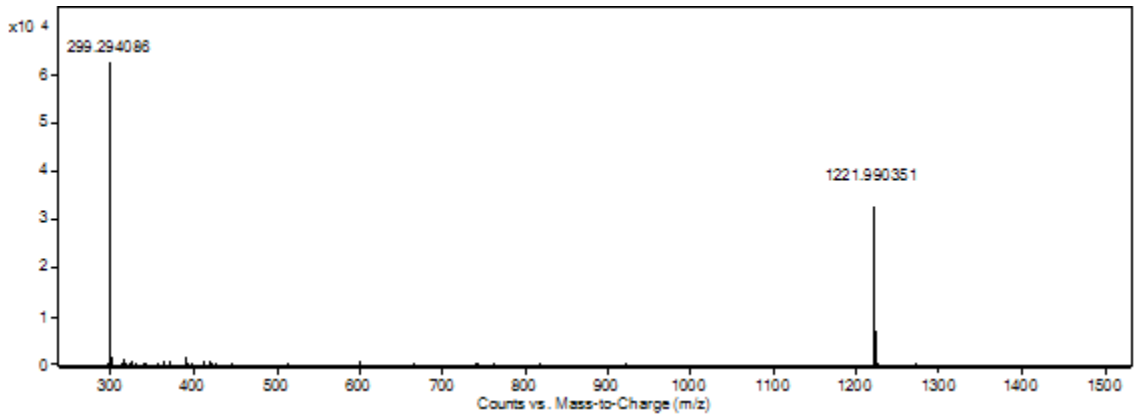


Figure 4. Example spectrum of the IRM compounds, 1 scan/second (9,816 transients/scan). Instrument: 6510 QTOF; IRM preparation: 200 µL of 100 µg/mL methyl stearate and 2% HP-1221 in ACN, allow ACN to evaporate, followed by 200 µL FC-70; drying gas temperature: 325 °C; gas flow: 5 L/min; mobile phase: 300 nL/min of 0.1% formic acid in 97/3 water/ACN.

To enable reference mass correction:

- Enter the *m/z* value into the Reference Mass tab of the Acquisition software and check the box in front of the entry. Repeat for the other IRM compound if it is used.
- Check the box "Enable Reference Mass".
- The acquisition mass range in MS mode must be set wide enough to include the reference masses selected.
- Save the method.

Shortly after loading the IRM solutions, the ion signals should stabilize at around 20,000 to 150,000 counts abundance when the instrument is operated at ca. 5,000—10,000 transients per spectrum. Actual values depend on the amount that was loaded, cleanliness of the interface assembly, operating parameters used for the MS, mobile phase composition, number of transients per scan, and other factors. It is desirable to keep the abundances above 5,000 counts for all scan speeds to obtain adequate ion statistics. With current electronics (March

2007), it is recommended to keep the abundances below the value of 90 * (number of transients per scan) to avoid saturation effects. For 2 scans/second (4,880 transients/scan), the desirable range is between ca. 5,000—440,000 counts abundance. The ion signals from the IRM compounds decay quickly at first but should stabilize at a lower usable level for days. Increasing the drying gas temperature increases the abundances for the IRM compounds but also leads to a faster rate of decline of the IRM abundances. The IRM response may be reduced when using mobile phase compositions containing high organic content, particularly high amounts of ACN. (This is especially true for methyl stearate.) Upon repeated use of the IRM solutions, a residual amount of the IRM compounds builds up in the interface assembly itself, requiring less frequent addition of the IRM solutions to maintain usable abundances.

The IRM compounds specifically have been selected to persist in the interface assembly and yield signals for an extended period of time. If the presence of these IRM signals no longer is desired, the interface assembly may be cleaned in the usual fashion using wipes and organic solvents (e.g., ACN and isopropyl alcohol) to remove the IRM compounds. However, traces of them will remain and be removed only by prolonged heating (350-365 °C and 10-12 L/min drying gas flow for several days).

If it is necessary to clean the modified top plate, remove the IRM wick from the holder. Then, soak the entire top plate in the appropriate organic solvent.

Over time, an increase in background signals may result from material being adsorbed on or deposited in the wick in the top plate. To clean the wick, remove the top plate from the HPLC Chip Cube Interface. Turn the plate over to access the two screws which hold the wick retainer in place. Remove the two screws using a T-6 driver. Remove the wick using tweezers. Clean the wick by soaking it in a suitable organic solvent such as ACN. Allow the wick to dry completely before reinstalling it in the top plate. Reinsert the wick into the slot of the top plate using tweezers, or press it firmly into place while wearing clean gloves. The wick is longer and wider than the slot and so must be compressed to fit. No portion should bulge out of the slot (see Figure 1), and the surface of the wick should barely be visible above the plate surface. Remove any stray fibers with tweezers, or blow them off with e.g., compressed air or nitrogen, and reinstall the top plate. A replacement wick is available if needed (part number G1982-20000).

Troubleshooting Tips

The following observations are not common but may help in some situations.

Observation (O): The spray from the chip tip is unstable.

Answer (A): There are multiple causes for this situation. This document assumes that air is being added to the drying gas. If air is not being added to the drying gas, the chip is charging

and this is the cause of the unstable spray. Otherwise, the flow from the nanopump may be unstable. The spray will fluctuate if there is a leak in a connection or a bubble in the flow path from the nanopump. Make sure the connections are leak-free, or purge the nanopump.

The air addition to the drying gas may not be at the optimum flow rate. Check that the pressures and flows for the air and nitrogen supplies are set correctly. Remember that the air addition is being added by a flow mass controller and ***not*** a regulator. The adjustment knob on the air addition manifold controls flow. The bubble will always be at 100% with 1 l/min of flow. At this rate, it is not enough to prevent charging of the chip.

Particulate matter in any of the flow paths or clogged gas traps will cause instability also.

Q: When I turn on the MS, the capillary current goes to a high level (several micro-amps) and stays there. I see a spray (or may not see a spray), but no recognizable signal or spectrum.

A: There is a leakage path from the spray cap to ground. This is most likely due to loose bristles from the brushes or fibers from the previous top plate Put the instrument in Standby mode, eject the Chip, remove the Chip cube, open the interface assembly, and inspect for and clean off any loose bristles or fibers.

Another cause is contamination on the spray cap or spray cap insulator. Remove these, clean, and reinstall.

Q: When I turn on the MS, I see flashes of light on the video display of the ionization region.

A: The instrument is arcing from the spray cap to ground. Do not continue to operate the instrument under these conditions! Immediately set the instrument in Standby mode. The cause is most likely the same as above, loose bristles or fibers near the spray cap, which need to be removed from the instrument before continuing.

Also, remember to wait a few minutes for the ACN to evaporate after applying the IRM working solution but before turning on the instrument.

Q: I have 2 million abundance counts for the internal reference mass ions and they have flat-topped peaks.

A: The signal for the reference mass ions is at complete saturation due to a large amount of internal reference mass compound in the interface assembly. Complete saturation occurs at 256 * (number of transients per scan). Remove the Chip cube and clean the interface assembly to remove most of the IRM compounds. Alternatively, eject the Chip, turn on the MS,

temporarily set the temperature to 365 °C and drying gas flow to 10 L/min, and bake out the interface assembly for a few hours or overnight.

Q: When I plot a TIC or BPC, all I see is a high baseline offset from the IRM compounds and little peaks or no peaks from my sample.

A: In the data analysis program, use a mass range that does not include the IRM masses; for example, plot the BPC from 302—1221 *m/z*.

Q: I can see the IRM peaks, but the mass assignments for the IRM compounds and my sample are off by a large error.

A: A mass error window is used to detect the reference masses, typically 100 ppm (+/-50 ppm about the true value) and specified in the Reference Mass tab of the method. Calibrate the instrument and the problem should disappear. The use of reference masses does not eliminate the need to calibrate the instrument on a regular basis. Data that has been collected already may be recalibrated using the <recalibrate> feature in the Data Analysis portion of the software.

Another cause of this effect is that the mass range during acquisition does not include the mass peak from the IRM compound. Change the mass range to fix this.

Q: There is no hose and drain bottle on the bottom of the interface assembly. Do I need this?

A: Yes. The hose and drain bottle need to be connected. The drain bottle needs to have a restrictor added to its vent. These additions close off the ionization region from the laboratory air or vent line, which often has contaminants contributing to background signals in the MS. See part number G1995-90000 (instructions for G1995A Low Background Site Preparation kit) for details.

Q: After I add the IRM solutions, I see masses in my spectrum that are from background contaminants: 281, 297, 371, 391, 445, and 519 *m/z*.

A: The modified top plate, IRM solutions, and/or their method of preparation may have some of these contaminants from their manufacture, packaging, or preparation. These signals normally decay rapidly during the first hour or so of operation.

5 Calibration or Auto Tuning Using CDS and Infusion Chip

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6 Static Needle Holder Installation and Use

With the upgraded HPLC-Chip Interface, a second top plate has been included to provide users an alternative to the HPLC Chip Cube Interface so that "classic" Nanospray emitters can be used. This top plate includes a universal needle holder, top plate, and optimized grounding path for the needle holder.

The needle holder is designed to accommodate the standard 5 cm fixed length Nanospray emitters. Any needles used with this holder should have tip sizes of 5 to 10 µm. Smaller tips can be used however the user must lower the capillary voltage significantly relative to the voltages used with the HPLC Chips. The only adjustment users should have to make is in one axis (up or down). The best position vertically must be finalized by the user. No other position adjustments are required by the user.

The static needle holder can be used with integrated nano columns/sprayers, static needles (no HPLC flow), and commercially available Nanospray emitters (New Objective, Proxeon (ThermoFisher)).

Use of Proxeon (Thermo Fisher) Nanospray Emitters

This section is still under development.

In This Book

The manual describes the following:

- Installation of the HPLC Chip Cube Spray chamber upgrade hardware.
- New instructions for IRM Solution

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