



G4240 HPLC-Chip/MS Interface with 6510 Q-TOF or 6210 TOF

Internal Reference User Guide

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These instructions apply only to Agilent 6510 Q-TOF LC/MS and 6210 Time-of-Flight LC/MS systems with an HPLC-Chip/MS interface, not to the Agilent 6300 Series Ion Trap LC/MS systems configured with this interface.

When you do accurate mass measurements on a TOF or Q-TOF instrument, you may want 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 guide shows you how to do this on Agilent 6510 Q-TOF or 6210 TOF instruments with a G4240 HPLC-Chip/MS Interface installed 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.

Procedures

This section contains instructions to set up the instrument, and to create and use the IRM solution to obtain reference masses.

Before you begin

- 1 Make sure you have these parts.

Table 1

Parts	Description
G1982-60259	Modified top plate with brushes and wick (depending on when the system was shipped, your instrument may have this already)
G1982-85001	IRM high mass compound (HP-1221)
G1982-85002	IRM high mass solvent (Fluorinert™ FC-70)
G1982-85003	IRM low mass compound (methyl stearate)
G1982-20000	Replacement IRM wick (each) (optional)

- 2 Check that the drying gas supply to the MS system is altered so that oxygen is present in the drying gas and the flow out of the ionization region is restricted.

Refer to G1995-90000 *G1995A Low Background Site Preparation Kit Instructions*.

To operate successfully using this modified top plate, the drying gas supply to the 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 done but this modified top plate is installed, the spray from the Chip may be erratic and unstable. If you have not made this alteration to the drying gas supply, do not use the modified top plate.

Procedures

To install the modified top plate

To install the modified top plate

The major differences between a modified top plate and the standard top plate are the brushes on the top and an 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.

The modified top plate is shown below.



Figure 1 Modified top plate. Top view showing the brushes (left) and bottom view showing the wick (right). Your modified top plate may look slightly different, but it must have the brushes and wick to function effectively.

- 1 In the MassHunter Acquisition software, set the instrument to **Standby** mode.

WARNING

Do not continue with this procedure unless the TOF or Q-TOF LC/MS instrument is in Standby mode. Otherwise, high voltage is exposed when the top plate is removed.

- 2 Eject any Chip from the HPLC-Chip/MS interface, open the interface cover, and flip out the stages assembly.
- 3 Remove the mounting screw using a T10 Torx driver ([Figure 2](#)).

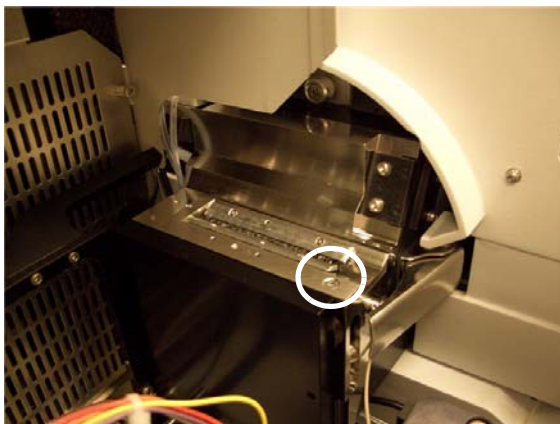


Figure 2 HPLC-Chip Cube interface assembly showing the location of the mounting screw for the top plate.

- 4 Remove the original top plate.
 - 5 Install the modified top plate in the same position and screw it in place.
- At this point, you may add the IRM solution.

To prepare IRM Solution

You can choose to use one or both internal reference mass standards. The high mass solvent (FC-70) is optional, but response from the IRM compounds will be more uniform and lasts longer when it is used.

CAUTION

ACN and FC-70 are immiscible and so should not be mixed together before applying them to the wick. 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.

- 1 Put 10 mg G1982-85003 methyl stearate into a container.
- 2 Add 10 mL of ACN to make a 1000 µg/mL of methyl stearate solution.
- 3 Shake to dissolve.
- 4 Refrigerate this stock solution if you want 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

- 1 Add 900 µL ACN to a vial.
- 2 Add 100 µL low mass IRM stock solution.
- 3 Add 20 µL G1982-85001 HP-1221.
- 4 Mix on a vortex mixer.

You must refrigerate this working solution between uses.

To apply the IRM Solution

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, 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 to 300 μL of solution before saturation occurs.

- 1 In the MassHunter Acquisition software, set the instrument to **Standby** mode.
- 2 Eject any Chip from the HPLC-Chip/MS interface, open the interface cover, and flip out the stages assembly.
- 3 Briefly mix the IRM working solution on a vortex mixer to assure homogeneity.
- 4 Load a pipetter with the desired amount of IRM working solution (e.g., 200 μL).
- 5 Insert the pipette tip into the bushing in the brush closest to the mass spectrometer and dispense the IRM solution. Do not drip the IRM solution onto the brushes. See [Figure 3](#).

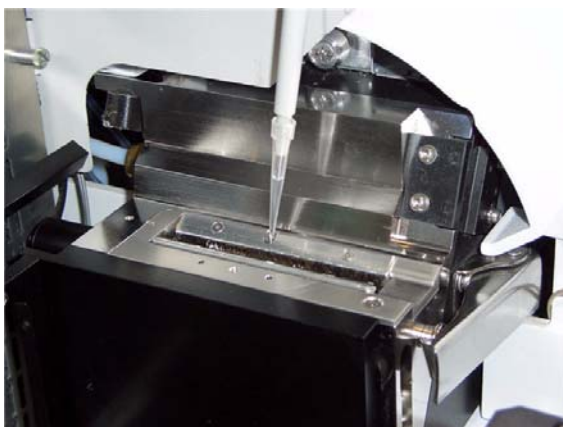


Figure 3 Applying an IRM solution through the bushing in the brush.

Procedures

To apply the IRM Solution

- 6 Wait 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.
- 7 Load a pipetter with the G1982-85002 FC-70 liquid (e.g., 200 µL), and slowly add it to the wick in the same manner as for the IRM working solution. Pipette slowly (10 to 20 seconds to transfer) to avoid overfilling and to allow time for the wick to soak up the viscous solution.
- 8 Close up the stages assembly and the HPLC-Chip/MS interface cover.
- 9 Load a Chip and turn on the instrument from the software.

You will not see any signals from the IRM compounds unless a spray is 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 325°C and flow of 5 L/min.

The accurate mass values are listed in [Table 2](#)

Table 2 Accurate Mass Values

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

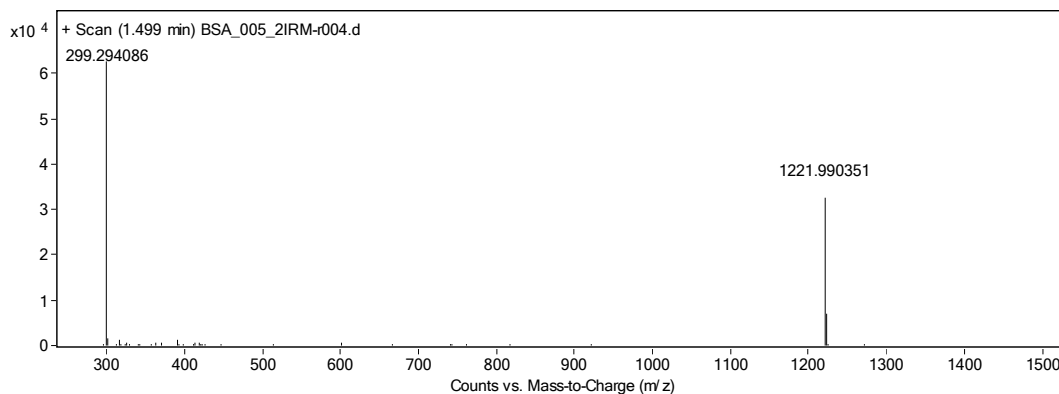


Figure 4 Example spectrum of the IRM compounds, 1 scan/second (9,816 transients/scan). Instrument: 6510 QTOF; IRM preparation: 200 μL of 100 $\mu\text{g}/\text{mL}$ methyl stearate and 2% HP-1221 in ACN, allow ACN to evaporate, followed by 200 μL FC-70; drying gas temperature: 325 $^{\circ}\text{C}$; 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

- 1 Enter the m/z value into the **Reference Mass** tab of the MassHunter Acquisition software and mark the check box in front of the entry. Repeat for the other IRM compound if it is used.
- 2 Mark the **Enable Reference Mass** check box.
- 3 Set the acquisition mass range in MS mode wide enough to include the reference masses selected.
- 4 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 approximately 5,000 to 10,000 transients per scan (see [Figure 4](#)). 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.

Procedures

To enable reference mass correction

Keep the abundances above 5,000 counts for all scan speeds to obtain adequate ion statistics. With current electronics (March 2007), keep the abundances below the value of $90 \times$ (number of transients per scan) to avoid saturation effects. For 2 scans/second (4,880 transients/scan), the desirable range is approximately between 5,000 to 440,000 counts abundance.

The ion signals from the IRM compounds decay quickly at first but 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.)

With 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 you no longer need these IRM signals, you may clean the interface assembly as usual 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°C to 360°C and 10 to 12 L/min drying gas flow for several days).

Maintenance

To clean the modified top plate

- Wipe the modified top plate with a dry absorbent cloth or tissue only.

CAUTION

Do not expose it to or immerse it in any organic solvent. These solvents will dissolve the binder used for the brushes and will destroy them.

To clean the wick

Over time, an increase in background signals may result from material being adsorbed on or deposited in the wick in the top plate.

- 1 Remove the top plate.
- 2 Remove the wick using tweezers.
- 3 Clean the wick by soaking it in a suitable organic solvent such as ACN.
- 4 Allow the wick to dry completely.
- 5 Reinstall the wick in the top plate.
- 6 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.
- 7 Remove any stray fibers with tweezers, or blow them off with compressed air or nitrogen.
- 8 Reinstall the top plate. A replacement wick is available if needed (p/n G1982-20000).

Troubleshooting

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

If the spray is unstable

(It is assumed that air is being added to the drying gas, so this is not the problem.)

- ✓ 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 flowpath 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 flowrate; check that the pressures and flows for the air and nitrogen supplies are set correctly.
- ✓ Particulate matter in any of the flowpaths may cause instability. Check that the flowpaths are clear.

If you do not get a recognizable signal or spectrum

If you turn on the MS, the capillary current goes to a high level (several microamps) and stays there, but you do not get recognizable signal or spectrum, check for these issues.

- ✓ 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 wick touching or close to the capillary cap.
 - 1 Put the instrument in Standby mode.
 - 2 Eject the Chip and remove the HPLC-Chip/MS interface.
 - 3 Open the interface assembly.
 - 4 Inspect for and clean off any loose bristles or fibers.
- ✓ Check for contamination on the spray cap or spray cap insulator. Remove these, clean, and reinstall.

If you see flashes of light on the video display of the ionization region when you turn on the MS

If you see flashes of light on the video display of the ionization region when you turn on the MS

- ✓ 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 you continue.
- ✓ Remember to wait a few minutes for the ACN to evaporate after applying the IRM working solution but before turning on the instrument.

If you have flat-topped peaks

You may have 2 million counts abundance for the internal reference masses, and they have flat-topped peaks.

- ✓ The signal is at complete saturation due to a large amount of internal reference mass compound in the interface assembly. Complete saturation occurs at $256 * (\text{number of transients per scan})$. (This value is correct as of March 2007.)
 - Remove the Chip cube and clean the interface assembly to remove most of the IRM compounds, or
 - Eject the Chip, turn on the MS, temporarily set the temperature to 350°C and drying gas flow to 10 L/min, and bake out the interface assembly for a few hours or overnight.

If you see a high baseline offset from the IRM compounds

When you plot a TIC or BPC, you may see a high baseline offset from the IRM compounds and little peaks or no peaks from your sample.

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

If you see IRM peaks, but the mass assignments for the IRM compounds and your sample are off by a large error

- ✓ 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. The experimental value also may be averaged over several scans (also specified in this tab) to improve its stability over time. If the average experimental reference mass value is outside the error window, no correction occurs for that scan. 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.
- ✓ The mass range during acquisition might not include the mass peak from the IRM compound. Change the mass range to fix this.

If your system has a piece of translucent Teflon tubing in the spray cap insulator

You may find a piece of translucent Teflon tubing, 3 mm OD x 100 mm long, sticking out of the spray cap insulator.

- ✓ You do not need this tubing, although there is no harm in leaving it in the system. The brushes on the modified top plate minimize the drying gas flow out of the top, so there is little disturbance of the spray if this piece of tubing is eliminated.

If you don't have a hose or drain bottle on the bottom of the interface assembly.

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The hose and drain bottle are required.

- ✓ The hose and drain bottle need to be connected, and 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 G1995-90000 *G1995A Low Background Site Preparation Kit* instructions) for details.

If you get background contaminants

If after you add the IRM solutions, you may see masses in your spectrum that are from background contaminants: 281, 297, 371, 391, 445, and 519 m/z .

- ✓ 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.

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In this guide

This guide describes how to use reference masses in your analysis in real time so that the mass values in the acquired spectra may be corrected for short-term variations.

This guide contains instructions to install a modified top plate for your HPLC-Chip/MS interface on a 6510 Q-TOF or 6210 TOF system, to create and use the reference mass solution, and to troubleshoot your system.

If you have comments about this guide, send an e-mail to feedback_lcms@agilent.com.

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