

ThermoFisher
S C I E N T I F I C

**2016 iQuan Series: Thermo Scientific™ TSQ
Endura™ and TSQ Quantiva™ Mass
Spectrometers Calibration and Maintenance**

Center of Excellence, Applied Markets

The world leader in serving science

Calibration

- What is Calibration?
 - Adjustment of quadrupole voltages for mass accuracy and resolution
 - Optimization of the electron multiplier sensitivity
- How often should I calibrate?
 - Quarterly
- What do I need?
 - Polytyrosine Calibration Solution P/N88325 Life Technologies™
 - Syringe P/N 365JLT41 Fisher Scientific™
 - LC/MS Grade Solvents from Fisher Scientific™
 - PEEK Tubing P/N 37005-100 Fisher Scientific™
 - Ion Transfer Tube from Thermo Scientific™
 - P/N 70005-20641 Quantiva MS
 - P/N 80100-20641 Endura MS

Good Calibration Practice Overview

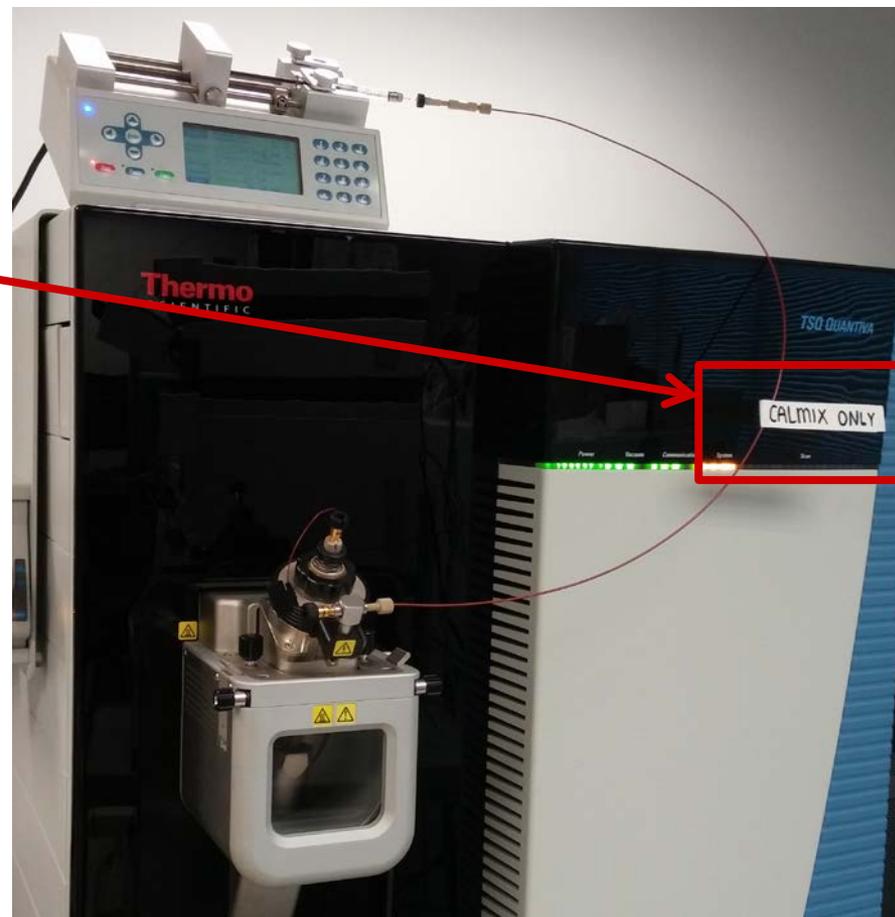
- Before Calibration...
 1. Stabilize the electronics if they were off
 2. Position the infusion setup
 3. Apply appropriate tune parameters for a 5 μ L/min flow rate
 4. Look for all calibrant masses in Tune software
 5. Make sure that spray is stable < 10% TIC Variation
- Calibrate Positive Mode Mass Precision/Resolution
- Calibrate Positive Mode Electron Multipliers
- Calibrate Negative Mode Mass Precision/Resolution
- Calibrate Negative Mode Electron Multipliers

Warm Up the Electronics If They Were Off:

- Instrument must be warmed up for 30-40 minutes if the electronics have been off, including:
 - Electronics in Service mode
 - Instrument shutdown
 - Placing the instrument in “Off” mode at the end of a sequence
 - Leaving the source open for more than 60 minutes
- Stabilize electronics by pressing  in the Tune software
- Calibrations can be performed immediately if the instrument is switched from “STAND BY” to “ON” mode.

Position the Infusion Setup

- For calibration, use a **syringe** and **line** that are dedicated to Calmix only.
- Here the syringe and line are labeled “Calmix only”



Apply Appropriate Tune Parameters

The screenshot shows the Thermo TSQ Quantiva Tune Application interface. A central text box contains the following instructions:

Enter Tune values directly, or use saved settings:

1. Open the side bar using the arrow button
2. Click on “Favorites”
3. Single click to view the settings in:
 - a. System settings defaults or
 - b. Settings that were previously saved by users for calibration
4. Double click to load settings
5. Press “Apply”

Red circles with numbers 1 through 5 are placed on the interface to indicate the steps: 1 is on the side bar arrow, 2 is on the Favorites button, 3a is on the System Settings header, 3b is on the User Settings header, and 5 is on the Apply button.

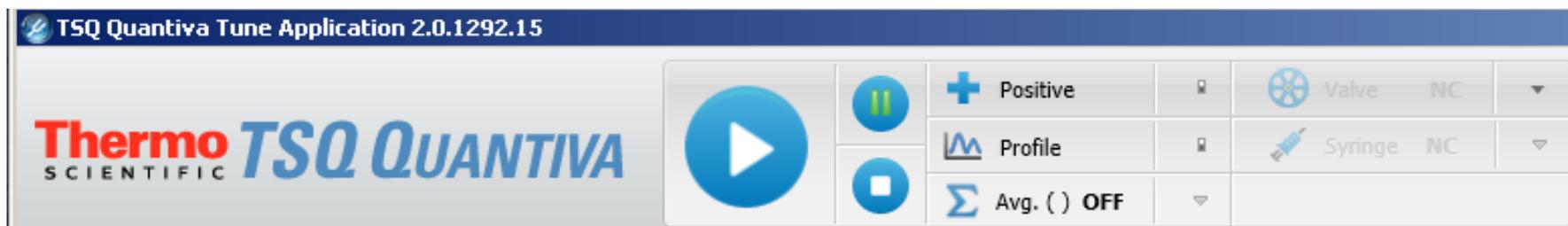
The interface includes a top navigation bar with 'ION SOURCE', 'DEFINE SCAN', and 'CALIBRATION' tabs. The left sidebar shows 'Ion Source' and 'Optimization' sections with various parameters like 'Current LC Flow', 'Ion Source Type', 'Pos Ion Spray Voltage', 'Neg Ion Spray Voltage', 'Sheath Gas', 'Aux Gas', 'Sweep Gas', 'Ion Transfer Tube Temp', and 'Vaporizer Temp'. The right sidebar shows 'FAVORITES' and 'SYSTEM SETTINGS' sections. The 'FAVORITES' section lists parameters such as 'Scan Type', 'Scan Range', 'Q1 Resolution', 'Scan Rate', 'Ion Source Type', 'Pos Ion Spray Voltage', 'Sheath Gas', 'Aux Gas', 'Sweep Gas', 'Ion Transfer Tube Temp', 'Vaporizer Temp', 'APPI Lamp', and 'Polarity'. The 'SYSTEM SETTINGS' section includes 'System Settings' and 'User Settings'.

Typical Source Settings for Calibration

Example for HESI-II probe and syringe pump infusion

Ion Source Type	HESI	
<input checked="" type="checkbox"/> Pos Ion Spray Voltage (V)	3500	→ Spray voltage is typically 2.7-3.4 kV pos mode, 2.4-3.0 kV for neg mode.
<i>Neg Ion Spray Voltage (V)</i>	2500	
<input checked="" type="checkbox"/> Sheath Gas (Arb)	5	→ Sheath gas flow is typically 5-30 for pos mode, 20-35 for neg mode. Aux gas flow is typically 0-7.
<input checked="" type="checkbox"/> Aux Gas (Arb)	0	
<input checked="" type="checkbox"/> Sweep Gas (Arb)	0	
<input checked="" type="checkbox"/> Ion Transfer Tube Temp (°C)	325	→ Capillary Temp is typically set to 325 (Endura MS) or 350 (Quantiva MS)
<input checked="" type="checkbox"/> Vaporizer Temp (°C)	0	→ Vaporizer temperature is typically 0-60 C.

Start!

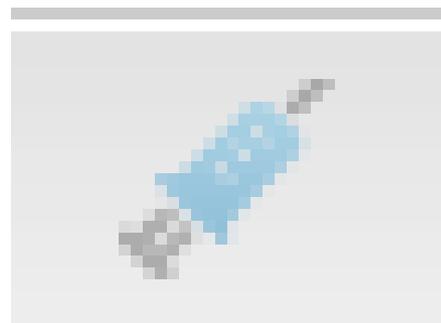


- Start the MS scanning



- Choose positive mode or negative mode using 

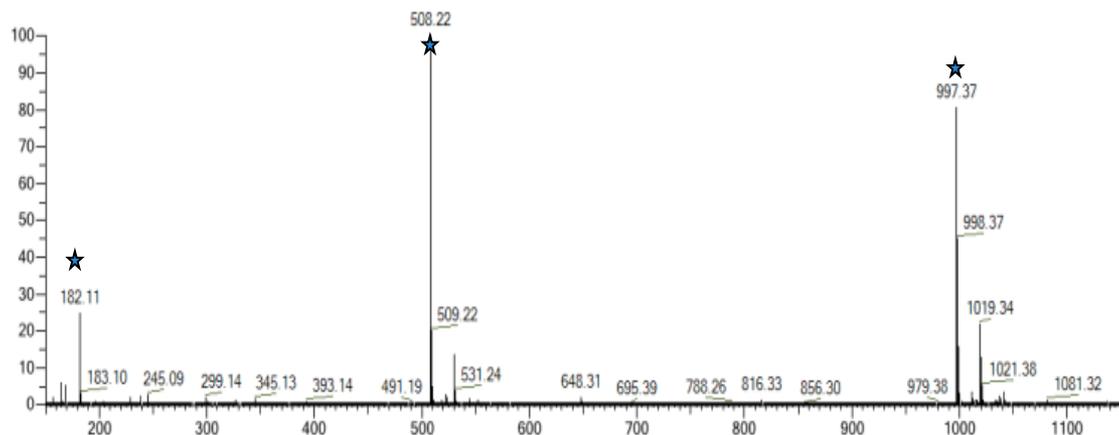
- Start the syringe pump flowing



- In positive mode 3-5 $\mu\text{l}/\text{min}$
- In negative mode 10-20 $\mu\text{l}/\text{min}$

Look for All Calibrant Masses in the Tune Software

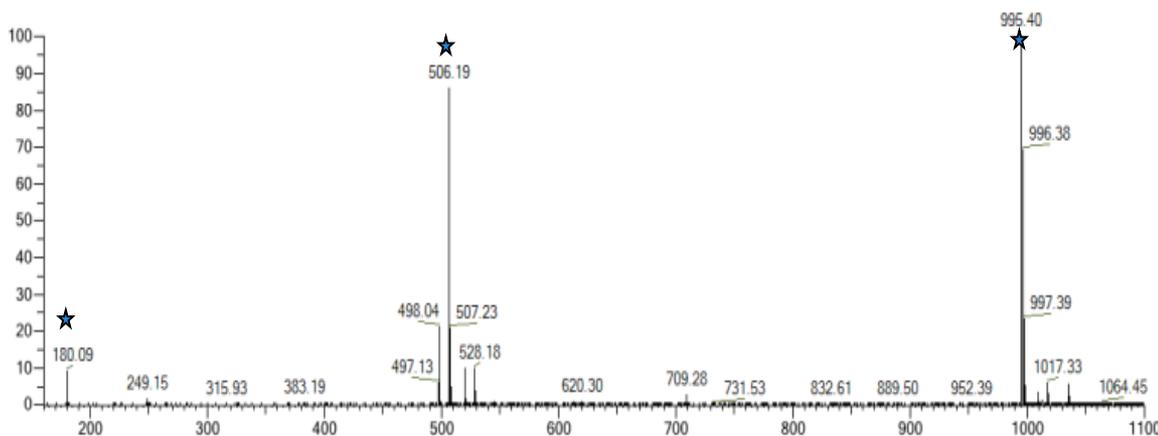
Positive Mode NL: $2^{\text{e}7}$ (Quantiva MS) $2^{\text{e}6}$ (Endura MS)



Pierce Triple Quadrupole MS Calibration Solution



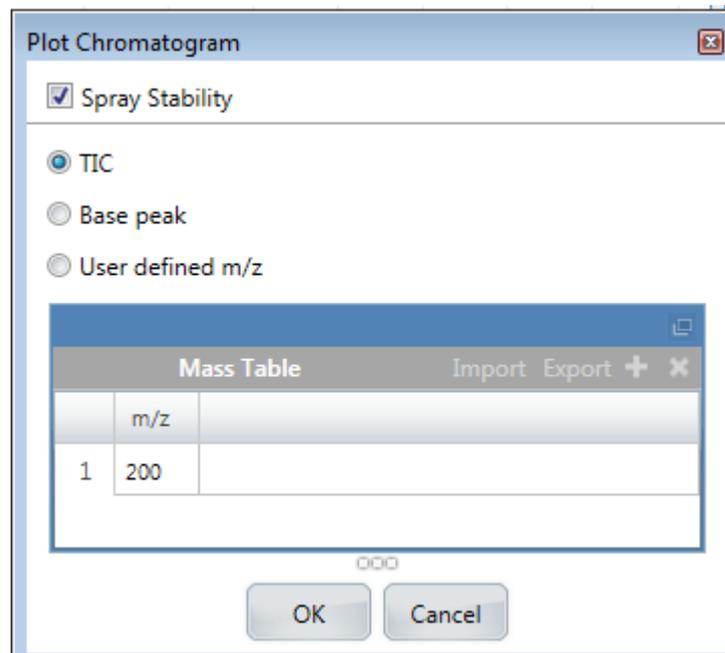
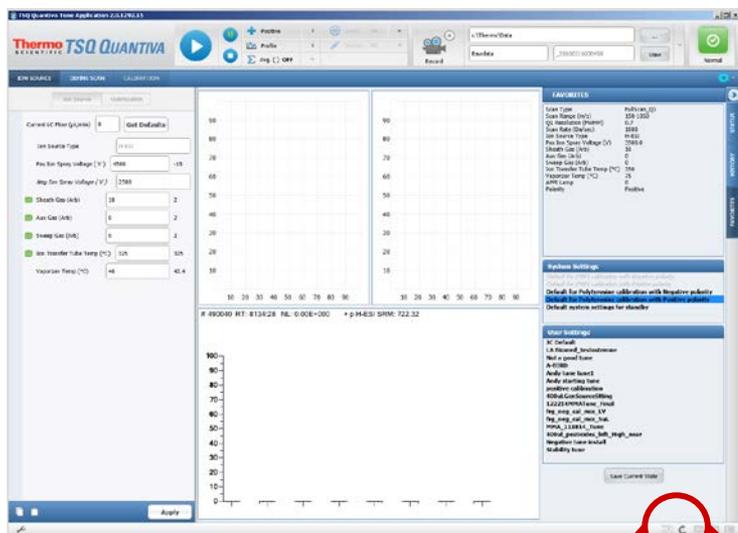
Negative Mode NL: $4^{\text{e}6}$ (Quantiva MS) $7^{\text{e}5}$ (Endura MS)



Part # 88325
In the case of
contamination or
degradation,
use new Calmix

★ Starred peaks
indicate calibration ions

Make Sure Spray is Stable



Click on the “Plot Chromatogram” icon



Plot “Spray Stability” and “TIC” or a user-defined calibrant m/z

- Adjust source parameters until stability is <10% TIC variation
- Scan for 100-200 scans to be sure spray is stable

Calibrate (*Ready, Set, Go!*)

- Navigate to the “Calibration” tab, then press “Calibrate...”
- Select, then press Calibrate in order:
 - Calibrate Mass Position and Resolution
 - Calibrate Electron Multiplier
- Use the polarity button  in the taskbar to switch polarities, assess the spray stability, and repeat calibrations
 - Calibrate Mass Position and Resolution
 - Calibrate Electron Multiplier
- Never skip spray stability
- All successful calibration procedures are automatically updated
- Failed calibrations are discarded

ION SOURCE	DEFINE SCAN	CALIBRATION
Polarity: Positive		
Calibration Options		
<input type="checkbox"/> Skip Spray Stability Evaluation		
Quad Selection		
<input type="radio"/> Q1MS <input type="radio"/> Q3MS <input checked="" type="radio"/> Both		
System Tune and Calibration Options		
<input type="radio"/> System Tune and Check		
<input type="radio"/> Check Mass Position and Resolution		
<input checked="" type="radio"/> Calibrate Mass Position and Resolution		
<input type="radio"/> Electron Multiplier Calibration		

Maintenance

Recommended Maintenance Schedule

Weekly:

- Replace LC solvents to maintain low background levels.
- Check sensitivity (using CalMix or a QC)
- Clean the ion transfer tube if sensitivity has declined
- Ballast pumps one at a time for 30 minutes bi-weekly

Quarterly:

- Evaluate, and if necessary, calibrate unused polarity modes.
- Clean the fan filters in the ventilation slots on the back of the instrument.
- Back up data and defragment the PC.

Semiannual/Yearly:

- Schedule a Preventative Maintenance visit (every 6 -12 months).
- Replace pump oil (every 6 months).

How to Clean an Ion Transfer Tube

1. Set the Vaporizer Temp to 30° C & Ion Transfer Tube (ITT) temperature to 150° C. Wait 5-10 min until temperature is reached.
2. Put the TSQ into “Off” Mode. (Use the stop button at top left)
3. Using protective gloves, remove the IonMax source housing.
4. Using additional protective paper towel, remove the Sweep Cap.
5. Using the ITT removal tool, unscrew counterclockwise the ITT; 90° for Quantiva or 4-5 full rotations for Endura.
6. Grab the ITT with the open end of the ITT removal tool; pull straight out until ITT is completely removed from manifold.
7. **Sonicate the ITT for 30 minutes in a 50:50 methanol/water containing 20% formic acid. Other solvents may be used depending on the deposit matrix solubility. Use only LCMS grade solvents.**
8. Rinse the ion transfer tube thoroughly with water, sonicate in water 15 minutes.
9. Rinse the ion transfer tube with methanol, sonicate in methanol 15 minutes.
10. Dry the ion transfer tube with a stream of oil-free nitrogen gas (i.e. from a tank, not a generator). If nitrogen is not available, air dry.
11. With the ITT tool attached to the front side, insert the ITT straight back to prevent bending/denting it
 - Try rotating slightly while inserting the ITT, or add a drop of MeOH to the back side (low pressure side) for lubrication.
12. When fully inserted, rotate ITT clockwise: 90° for Quantiva MS or 4-5 full rotations for Endura MS
 - Make sure the Quantiva MS ITT is inserted with the “letterbox” slot in the horizontal position, then rotate to be vertical.
13. Put TSQ into “Standby” mode (Pause button); Set ITT temperature (300 C for Endura; 325 C for Quantiva)

Endura RF-Lens or Quantiva EDIF Cleaning

- The RF-Lens/EDIF only need to be cleaned when cleaning the ion transfer tube doesn't improve sensitivity, usually less than once per year.
 1. Inspect the RF-Lens for any lint, particulates, sample buildup or coatings.
 2. Sonicate in either 50:50 methanol:water or a 1% solution of Liquinox™ in water for 10 – 15 minutes.
 3. Rinse the RF-Lens thoroughly with LCMS grade water then methanol.
 4. Blow the ion transfer tube with a stream of oil-free nitrogen gas.
 5. Inspect the RF-Lens with magnification for any lint or particulates that may have been introduced by this cleaning procedure. Use tweezers or similar tools to remove the lint or particulates.
- Do not sonicate the RF-Lens and skimmer in acidic solutions
- Do not disassemble the RF-Lens lenses

Endura MS: Venting and Establishing Vacuum

- To vent: Turn the MS off in the tune window, turn off electronics switch and turn off power switch. There is no damage to the instrument during power outage.
- To re-establish vacuum: Turn on the power switch, wait at least an hour to pump down to pressure, and then turn on the electronics.
- A mass calibration is required after venting, which should be done 15-24 hours after re-establishing vacuum.
- Dip the RF-Lens and Ion Guide after re-installing the RF-Lens and ion optics by following the Dipping instructions.

Quantiva MS: Venting and Establishing Vacuum

- To vent: Turn the MS off in the tune window, turn off electronics switch and turn off power switch. There is no damage to the instrument during power outage.
- To re-establish vacuum:
 - Remove source housing and sweep cone
 - Plug the end of the ion transfer tube either with the ion transfer tube removal tool or remove the ion transfer tube to let the tungsten ball drop
 - Wait 10 minutes to establish fore pressure
 - Take the tool off or put the ion transfer tube back in
 - Put source housing back on
 - Turn on electronics switch
 - Wait 15-24 hours until “Analyzer pressure” in the Tune Software is below 7E-6 before starting to scan (Important for the safety of your instrument!)
- Dipping and a mass calibration are required.

Dipping

- After re-installing the RF-Lens and ion optics, it is necessary to Dip the RF-Lens and ion Guide.
- Click on the Wrench icon, via the calibration page
- Run the Dip Source and Dip the Ion Guide procedures.

The image shows two screenshots of the Thermo Scientific TSQ Quantiva software interface. The left screenshot shows the 'CALIBRATION' tab with the 'Wrench' icon highlighted in a red box at the bottom left. A large blue arrow points from this icon to the right screenshot. The right screenshot shows the 'Diagnostics' menu expanded, with 'RF' checked and 'Dip Quantiva Source RF' and 'Dip Ion guide 1' also checked, all enclosed in a red box. The interface includes tabs for 'ION SOURCE', 'DEFINE SCAN', and 'CALIBRATION', and a 'Start' button at the bottom right.

When to Toggle the Electronics

- When:
 - Lost communication between MS and software
- Procedure:
 - Stop MS scanning (“Pause” in the Tune Software)
 - Turn off electronics switch
 - Wait 30 seconds or until maintenance is done
 - Turn on electronics
 - Return to “Pause” state in the Tune Software

Where Can I Find Software Update Information?

- Contact your local field service engineer, your sales representative, the CoE, or technical support.
- For information on how to obtain software updates contact your local sales representative, your field service engineer or technical support.
- Upgrading layered application software (Tune Software, Thermo Scientific™ Compound Discoverer™ Software, Thermo Scientific™ TraceFinder™ Software etc.)? Contact your local sales representative for upgrade paths and options.

Tech support phone number within the US:

(800) 532-4752

Tech support phone numbers outside the US:

<https://www.thermofisher.com/us/en/home/technical-resources/contact-us.html>

Troubleshooting

Troubleshooting Overview

Problem	Origin	Troubleshoot
<ul style="list-style-type: none">• Chromatographic peak shape• Carry-over• Contamination• Quantitation inconsistency	Upstream of the mass spec	<ul style="list-style-type: none">• Reduce injection volume/amount or change reconstitution solvent type• Fresh solvents• Flush lines with 100% Methanol or 50% isopropanol• Prepare fresh standards• Change column• Evaluate retention time and pump pressure reproducibility
<ul style="list-style-type: none">• Sensitivity• Spray stability• Contamination	Source	<ul style="list-style-type: none">• Re-optimize tune parameters• Replace needle
<ul style="list-style-type: none">• Sensitivity• Low pressure	Mass spec	<ul style="list-style-type: none">• Calibrate• Clean ion transfer tube• Clean RF-Lens/Exit Lens

Assessing Instrument Performance Using Calmix

- **Diagnostics**

- Calmix can serve as a back-up QC to evaluate month-to-month changes in sensitivity. During calmix infusion, “Record” or take screenshots of the spectrum to document the quality.

- **Hardware**

- The hardware performance is assessed by calibration and will indicate if it passes.
- Check the vacuum pressures regularly and record other hardware readback values in a log to track instrument performance

- **Contamination**

- Lower sensitivity in SRM mode could be due to contamination peaks, which can be seen in Full Scan mode by infusing fresh solvent or Calmix
- Infusing Calmix will demonstrate if contamination is before the source (upstream in the LC) or is it still present in infusion setup?
- Remaining contamination of the peek tubing and needle can be cleaned more thoroughly: Remove the source housing with the infusion setup intact and wash with water, methanol, acetone, methanol and water successively. Infuse Calmix again to assess the contamination improvement.

Thank you!