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Lifetime Testing of Agilent RoHS Compliant Ion Injectors on Multiple Agilent LC/MS Triple Quadrupole Instruments to Test Their Robustness towards Heavy Matrix and a Novel Cleaning Technique

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Introduction

The ion injector on an Agilent LC/MS instrument is used to help transfer ions from the atmospheric pressure region into the vacuum system by applying voltages to the front of the ion injector to help draw in the ions via an opposite polarity voltage. The opposite end of the ion injector is also charged differentially (fragmentor voltage) to accelerate the ions into the focusing optics (Figure 1).

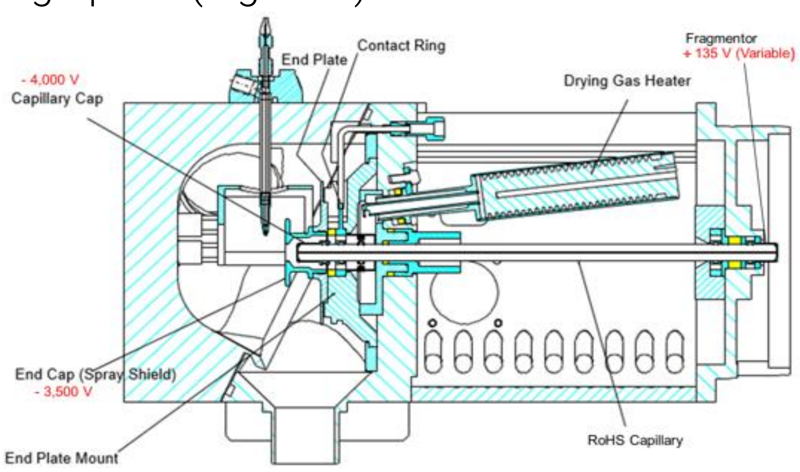

Figure 1. Diagram of the ion source and desolvation assembly showing where the RoHS ion injector is located and the typical applied voltages in positive mode.

The Restriction of Hazardous Substances (RoHS) in Electrical and Electronic Equipment Directive in the European Union has been driving efforts to reduce the use of hazardous materials. The legacy ion injectors were allowed in Agilent instruments under an exception. However, the new ion injectors (Figure 2 and 3) now meet full RoHS compliance without sacrificing previous performance.

Figure 2. FS Ion Injector, 0.6 mm ID, 180 mm (p/n G3911-30000)

Figure 3. Ultivo and MSD iQ Ion Injector, 90 mm (p/n G3911-30001)

A primary concern for routine analysis on LC/TQ systems is instrument stability; which can vary over time due to the soiling of crucial ion optics like the ion injector. Although cleaning of these injectors is easy with very minimal downtime, multiple cleanings with harsh surfactants can decrease their performance over time. This study presents lifetime testing of the new Agilent RoHS compliant ion injectors with a novel cleaning technique using citranox solution. Heavy instrument use was simulated through 13,000+ injections of bovine urine, which was specifically chosen due to the challenging endogenous components that may cause ion injector contamination, autotune failures and Early Maintenance Feedback (EMF) triggers.

Experimental

Chemicals and Reagents

Unfiltered bovine urine was obtained from BioIVT. LC/MS sulfa checkout standard, and LCMS-grade water, acetonitrile and formic acid were obtained from Agilent Technologies, Inc.

Sample Preparation and Instrument Set-up

Bovine urine was diluted in 50:50 acetonitrile/water at a 1:1 ratio and spun down at 4,500 x g for 10 min. Supernatant was injected into the TQ through a UHPLC guard column, Agilent Zorbax Extend-C18, 80Å 2.1 mm (p/n 821725-907). Experiments were carried out on two separate LC/TQ instruments to test the robustness of both new ion injectors. Pump flow was set to 0.8 mL/min isocratic flow of 50:50 acetonitrile/water + 0.1% formic acid. Fragmentor voltage on ion injectors was set to 100 V.

Instrument 1 Conditions

- Multisampler G7167B
- Binary Pump G7120A
- **G6475A LC/TQ w/ Ion Injector G3911-30000**
- Injection Volume: **2 µL**
- Detector Gain: 4
- 10,000 injections without any injector cleaning; followed by 5,000 injections with ion injector cleanings at every 1,000 injections (**total 15,000 injections with 6 cleanings**)

Instrument 2 Conditions

- Vialsampler G7129C
- Binary Pump G1312B
- **Ultivo LC/TQ w/ Ion Injector G3911-30001**
- Injection Volume: **10 µL**
- Detector Gain: 3
- 10,000 injections without any injector cleaning; followed by 3,000 injections with ion injector cleanings at every 1,000 injections (**total 13,000 injections with 4 cleanings**)

Autotune was run before experiments were started. Checktunes were run at every 1,000 injections to ensure instrument performance. If a checktune was out of tolerance, an autotune was performed followed by another checktune. Tune ion abundances that were recorded during the checktune procedure were plotted to evaluate the effects of matrix and cleanings. TIC signals of sulfa-standard spiked bovine urine were recorded every 100 injections to ensure ions were still reaching the detector. **No cleaning of the ion source chamber, spray shield and capillary cap was carried out over the course of the injection series.**

Ion Injector Cleaning

Ion injector tips were inserted in pipette tips to protect the metal plating. The injectors were sonicated in 2% by volume Citranox solution for 15 minutes. This was followed by several rinses and three sonications in LCMS water to remove all the surfactants. Lastly, the injector was sonicated for 15 minutes in methanol.

Tune Ion Abundance Comparisons Relative to the Number of Cleanings

Checktunes were performed thrice around cleanings; a) before starting a cleaning, b) immediately after the cleaning (without performing an autotune) and c) following an autotune. The abundance percentage recovery at these three stages was calculated by comparing them to abundances from a suitable starting point of the experiment.

G6475A Abundance Recovery (Figure 4)

On the G6475A, cleaning just the ion injector did not help tune abundance recovery in the positive polarity. This might be due to ion suppression being caused in the positive polarity by other contaminated ion optics. However, abundance recovery was seen in the negative polarity. EMV voltage looked stable through all the post-cleaning autotunes, indicating no prominent effect on the detector. A minor repair had to be performed on the G6475A at 5000 injections due to an issue with tune solution delivery. The nebulizer needle and the tune bottle were replaced to restore tune solution delivery to the MS. Tune abundances increased considerably following this repair. Hence post-repair checktune abundances (at 5000 injections) were used to compare abundance recovery for cleanings.

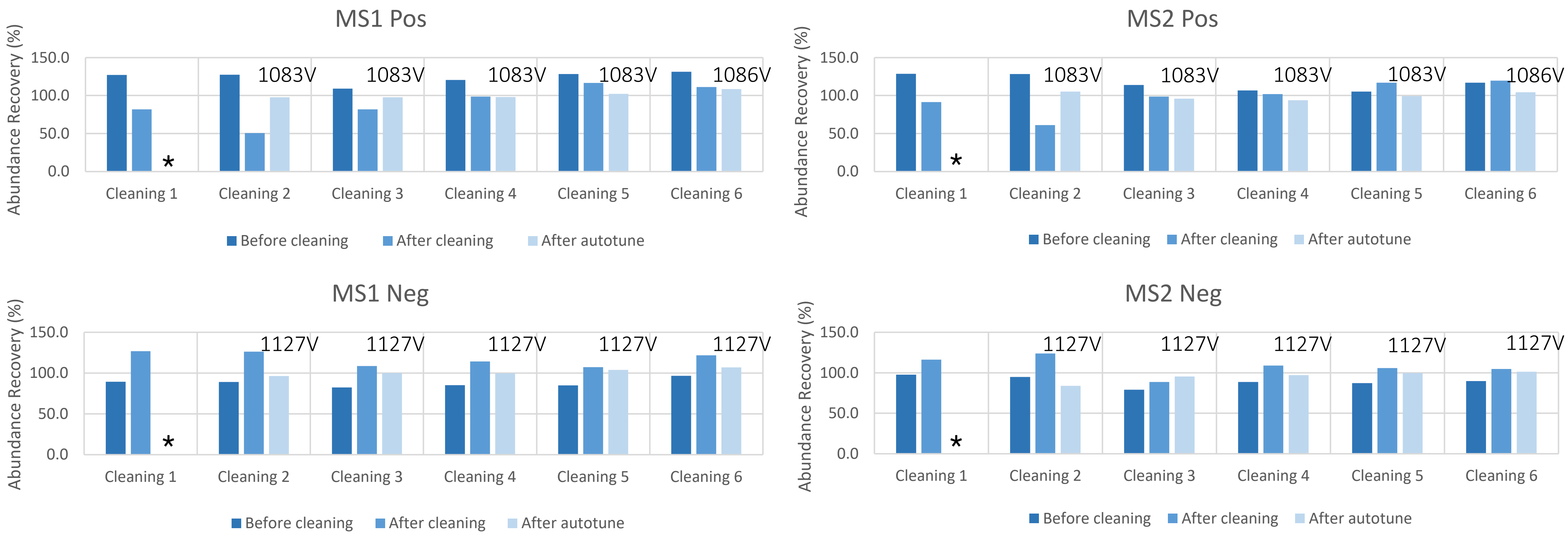

Figure 4. Total tune ions abundance recovery percentage from checktunes performed on the G6475A at cleanings performed every 1000 injections. EMV values displayed in graph were set at autotune. * No autotune was performed after Cleaning 1.

Ultivo Abundance Recovery (Figure 5)

On the Ultivo, cleaning the ion injector mostly helped recover abundances in both polarities. This shows that ion injector cleanliness had a major effect on the abundances. As seen in the graph, EMV voltage values consistently decreased with every post-cleaning autotune. This demonstrated that ion injector cleanliness might help detector health. Further investigation will be needed to explore this relationship. To calculate percentage recovery, the abundances from the cleanings were compared to the checktune abundances before the start of the experiment.

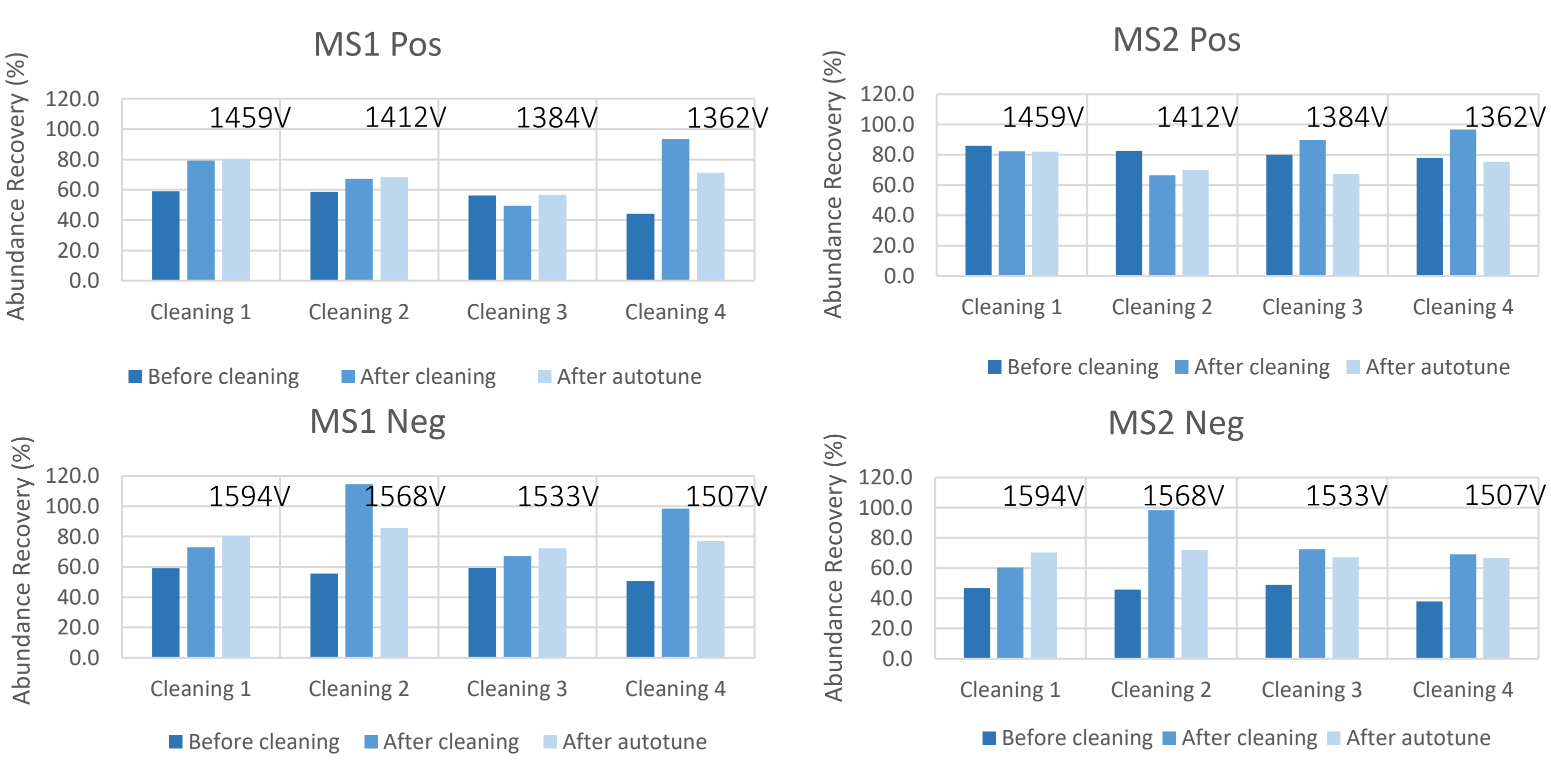

Figure 5. Abundance recovery percentage at checktunes performed on the Ultivo at cleanings performed every 1000 injections. EMV values displayed in graph were set at autotune.

No ion injector EMFs were triggered on either system.

Effect of Autotune on Ion Tolerances Related to Matrix Contamination and Ion Injector Cleaning

The data below is representative example of the effect of autotune post-cleaning on mass calibration (*m/z* drift) delta and peak width (FWHM) delta of tune ions. The graphs in orange represent the delta values from a checktune run immediately after cleaning. The graphs in blue represent delta values from a checktune which was preceded by an autotune after cleaning. In all the graphs below, the red dash lines represent the tolerances on mass calibration and peak width.

G6475A Tune Tolerances

To meet the tolerances for *m/z*<1,000, *m/z* assignment and peak width must both remain within ±0.14 Da. For the G6475A, all 6 checktunes that were run directly after the cleanings did not show any "Out of Tolerance" events. However, the autotunes did rein in the delta values close to 0 to improve system performance. Similar behavior was observed for MS2 tolerances.

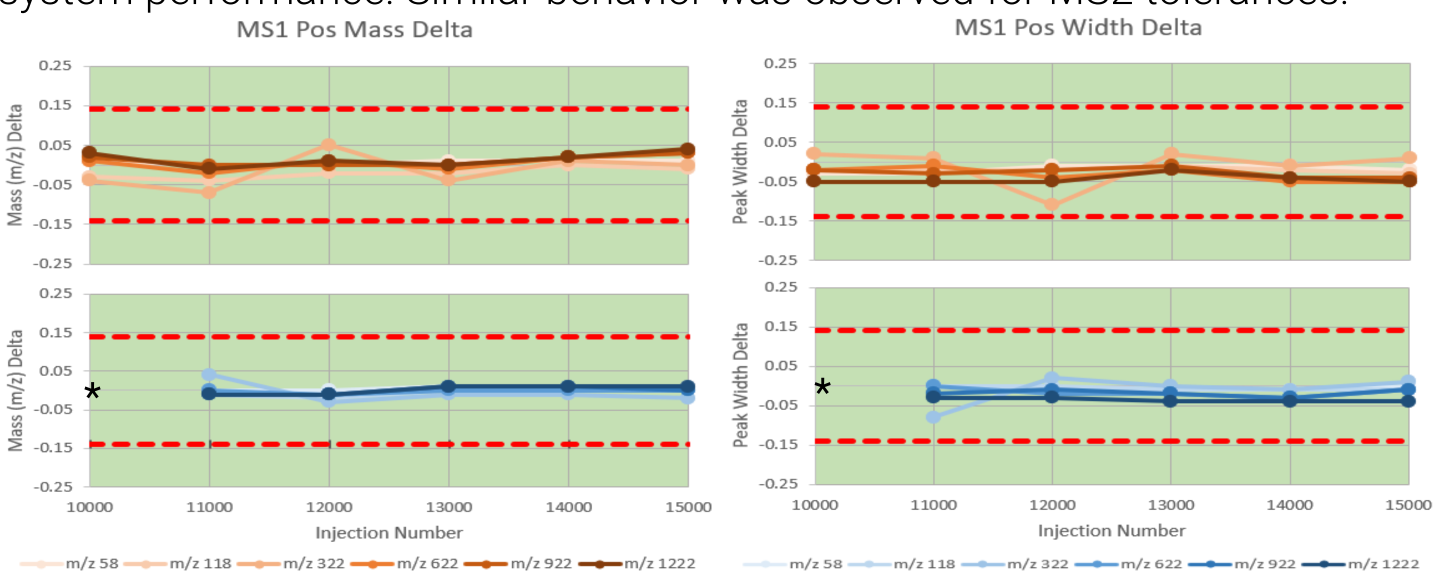

Figure 6. Mass delta and peak width delta plotted against the number of injections on the G6475A. Orange plots – data from checktunes run w/o autotune. Blue plots – data from checktunes run after autotune. Red dashes are tolerances. * No autotune was performed at injection # 10,000.

Ultivo Tune Tolerances

To meet tolerances for *m/z*<1,000, *m/z* assignment and peak width must remain within ±0.1 Da and ±0.14 Da respectively. For the Ultivo, 2 checktunes that were run directly after the cleanings showed "Out of Tolerance" events. Running an autotune brought the delta values within the tolerance limits. Similar behavior was observed in MS1 negative polarity. However, MS2 showed behavior similar to that of G6475A above in both polarities.

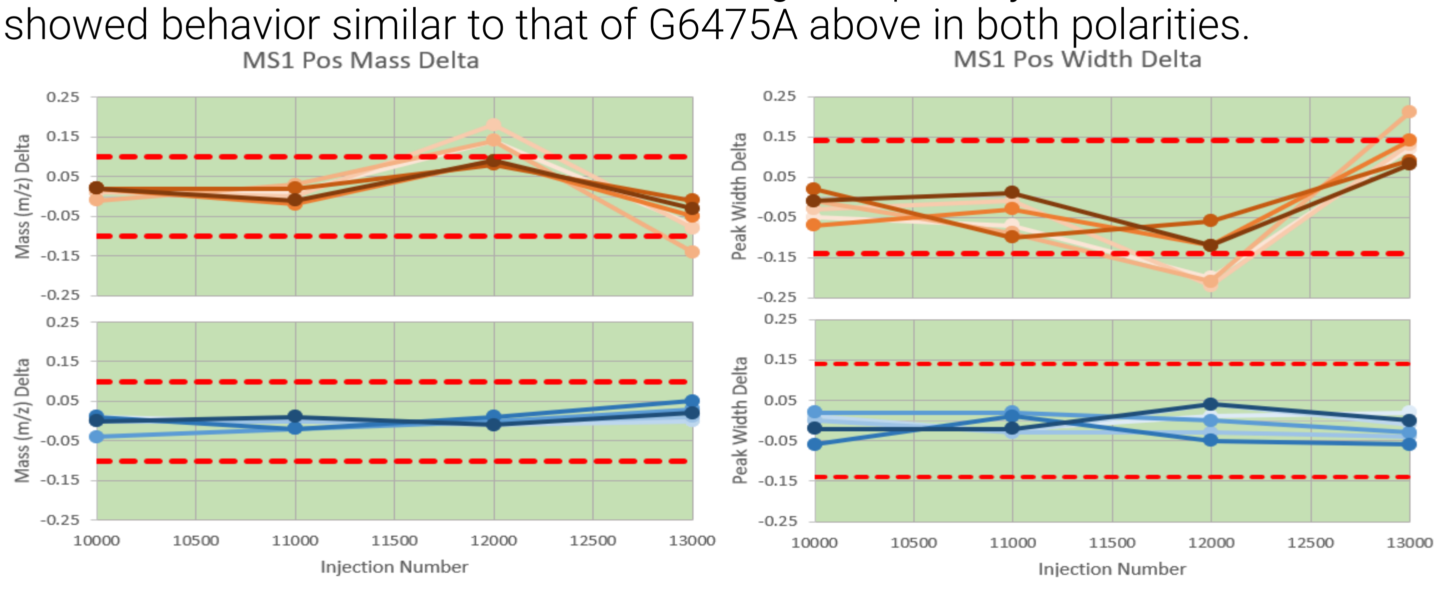

Figure 7. Mass delta and peak width delta plotted against the number of injections on the Ultivo. Orange plots – data from checktunes run w/o autotune. Blue plots – data from checktunes run after autotune. Red dashes are tolerances.

In summary, an autotune after cleaning benefits the instrument performance.

Pre-experiment to Post-experiment Physical Attributes

MS inlet on both systems before and after the injections. Heavy contamination seen on front end (Figure 8 and 9).

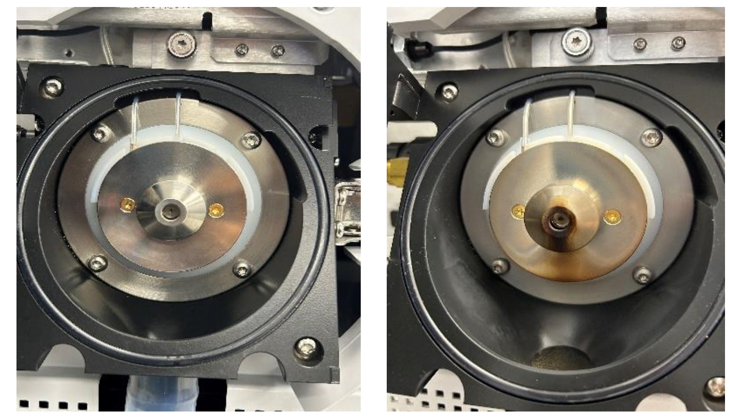

Figure 8. Before and after images on the G6475A inlet after 15,000 bovine urine injections of 2 µl each.

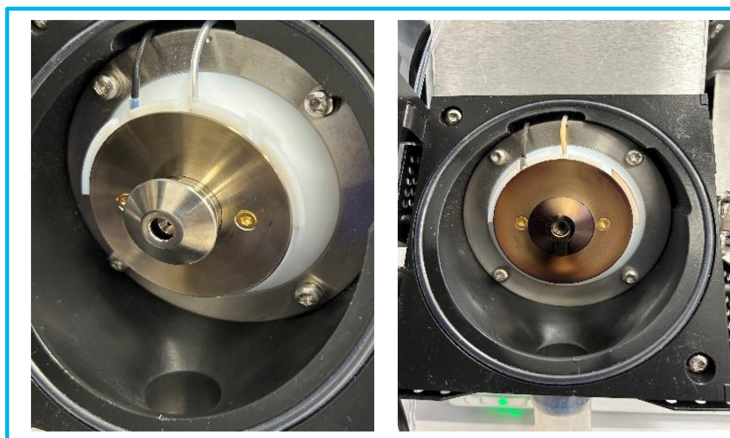

Figure 9. Before and after images on the Ultivo inlet after 13,000 bovine urine injections of 10 µl each.

Conclusions

- The robustness of the new Agilent RoHS compliant ion injectors, as well as the instrumentation, was demonstrated using multiple injections (13,000+) with a heavy matrix (bovine urine) sample and multiple cleanings with Citranox solution.
- Ion injector cleanings were found to benefit abundance recovery.
- Performing autotunes after injector cleanings greatly benefited the instrument performance, especially on the Ultivo.

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