Technical Overview

Agilent
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Evaluation of Agilent RoHS-Compliant Ion Injectors

Introduction

Recently, governments across the globe have started to reduce the use of hazardous materials in electronic components. Specifically, the Restriction of Hazardous Substances in Electrical and Electronic Equipment (RoHS) Directive in the European Union has been driving this effort. The Agilent resistive ion injector has been updated to a leaded glass–free, RoHS-compliant-without-exemption part for use in Agilent LC/MS quadrupole instruments that do not have an ion funnel. The ion injector acts to pull desolvated ions from the desolvation chamber into the MS (Figure 1). In doing so, the ions are culminated into a single, narrow beam, allowing the MS to separate the ions according to their mass-to-charge ratio (*m/z*). The ion injector is resistive, which allows for fast polarity mode switching, and has metal contacts at either end for electrical connection to the MS. In this technical overview, the performance of the Agilent RoHS ion injectors was compared to that of their respective legacy ion injector models (Table 1).

The RoHS-compliant-without-exemption ion injector

An ion injector is a metal-capped glass capillary. The RoHS-compliant Agilent FS ion injector (part number G3911-30000) is the 180 mm, long version of the capillary for use in most LC/MS systems (Figure 2). The RoHS-compliant Agilent Ultivo and MSD iQ ion injector (part number G3911-30001) is the 90 mm, short version of the capillary that is used in the Agilent Ultivo triple quadrupole LC/MS (LC/TQ; product number G6465B) and the Agilent InfinityLab LC/MSD iQ (product number G6160A) instruments (Figure 3). Both the 90 and 180 mm RoHS ion injectors are considered drop-in replacements for their respective legacy ion injectors (as shown in Table 1).

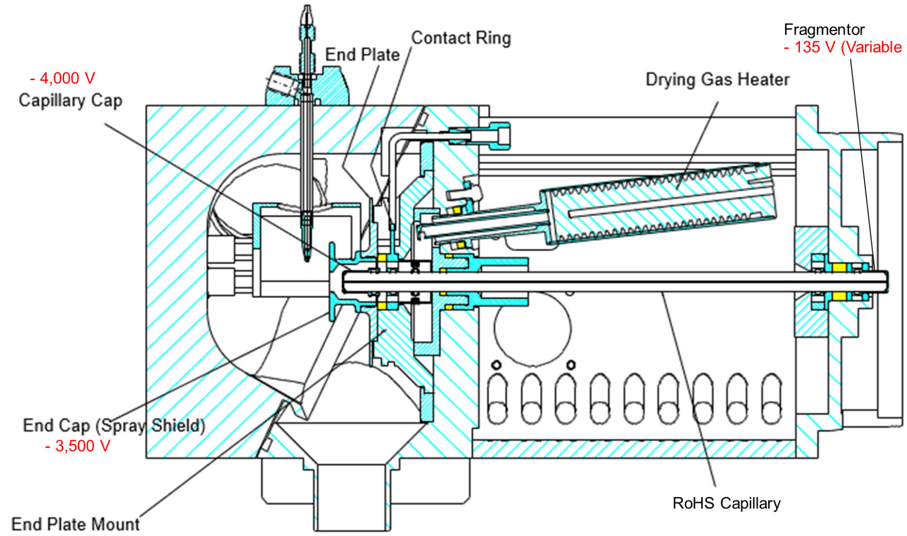

Figure 1. Diagram of the desolvation chamber showing where the Agilent RoHS ion injector is located inside the MS source and the applied voltages in positive mode. The ion injector on an Agilent LC/MS system is used as an entry point into the portion of the instrument under vacuum, to accelerate ions into the ion-focusing optics. The entrance of the ion injector is charged with the opposite electrical charge of the ionization mode. The opposite polarity pulls ions through the nitrogen drying gas and into the sample entry point of the ion injector. The opposite end of the ion injector is also charged differentially (fragmentor) to accelerate the ion into the focusing optics.

Table 1. Part numbers and descriptions of the Agilent legacy and RoHS ion injectors.

Legacy Part Number	Legacy Description	RoHS Part Number	New RoHS Description
G1960-80060	Capillary, fast switching (FS), 0.6 mm id, 180 mm	G3911-30000	FS ion injector, 0.6 mm id, 180 mm
G6301-80004	Ultivo ion injector, 90 mm	G3911-30001	Ultivo and MSD iQ ion injector, 90 mm

Figure 2. RoHS-compliant Agilent FS ion injector, 0.6 mm id, 180 mm (part number G3911-30000).

Figure 3. RoHS-compliant Agilent Ultivo and MSD iQ ion injector, 90 mm (part number G3911-30001).

For those laboratories that may need to undergo risk assessment due to the part number changes, a letter is available attesting that the new parts have the same form, fit, and function (Figures 4 and 5).

Agilent

Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808

www.agilent.com

June 1, 2024

Dear Customer,

G1960-80060 (Capillary, FS, 0.6MM) will be obsolete as of 01-October-2024 and replaced by the Agilent produced capillary G3911-30000 (FS Ion Injector, 0.6mm ID, 180mm). The new part number is a direct replacement and have the same form, fit, and function. The new part number is now ROHS compliant without requiring an exemption like the legacy Ion Injector.

Best Regards,

Paul Tripp
CSD Product Manager
GC Supplies, MS Supplies and Gas Management
paul_tripp@agilent.com

Figure 4. Customer letter about the RoHS-compliant Agilent FS ion injector (part number G3911-30000).

Agilent

Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808

www.agilent.com

June 1, 2024

Dear Customer,

G6301-80004 (Ultivo Ion Injector) will be obsolete as of 01-July-2024 and replaced by the Agilent produced capillary G3911-30001 (Ultivo and MSD iQ Ion Injector). The new part number is a direct replacement and have the same form, fit, and function. The new part number is now ROHS compliant without requiring an exemption like the legacy Ion Injector. Furthermore, the new part number is available at a new reduced price.

Best Regards,

Paul Tripp
CSD Product Manager
GC Supplies, MS Supplies and Gas Management
paul_tripp@agilent.com

Figure 5. Customer letter about the RoHS-compliant Agilent Ultivo and MSD iQ ion injector (part number G4911-30001).

Tune performance

The ion injector introduces ions into the ion optics of the MS. The tune performance of the RoHS ion injectors was evaluated against their respective legacy ion injectors. Tune abundances are influenced by multiple factors, including the age of the tune solution and the condition of the system. Therefore, to create a suitable comparison, all ion injectors were evaluated on the same day using the same tune mix. Ion abundances of all ion injectors were normalized to a reference legacy ion injector. Due to variability in the delivery system of the tune mix, acceptance criteria for comparison were that all ion injectors should show a > 80% relative abundance to the reference ion injector.

The RoHS Ultivo and MSD iQ ion injector was evaluated against the legacy Ultivo ion injector on the Ultivo LC/TQ. Figure 6 shows the comparison of three legacy ion injectors against four RoHS ion injectors. All ions are above the 80% acceptance criteria in both positive and negative mode. All tunes and instrument self-evaluations were passed in this test.

The RoHS FS ion injector was evaluated against the legacy FS ion injector on the Agilent 6470B LC/TQ. Figure 7 shows the comparison of five legacy ion injectors against five RoHS ion injectors. All ions are above the 80% acceptance criteria in both positive and negative mode. All tunes and instrument self-evaluations were passed in this test.

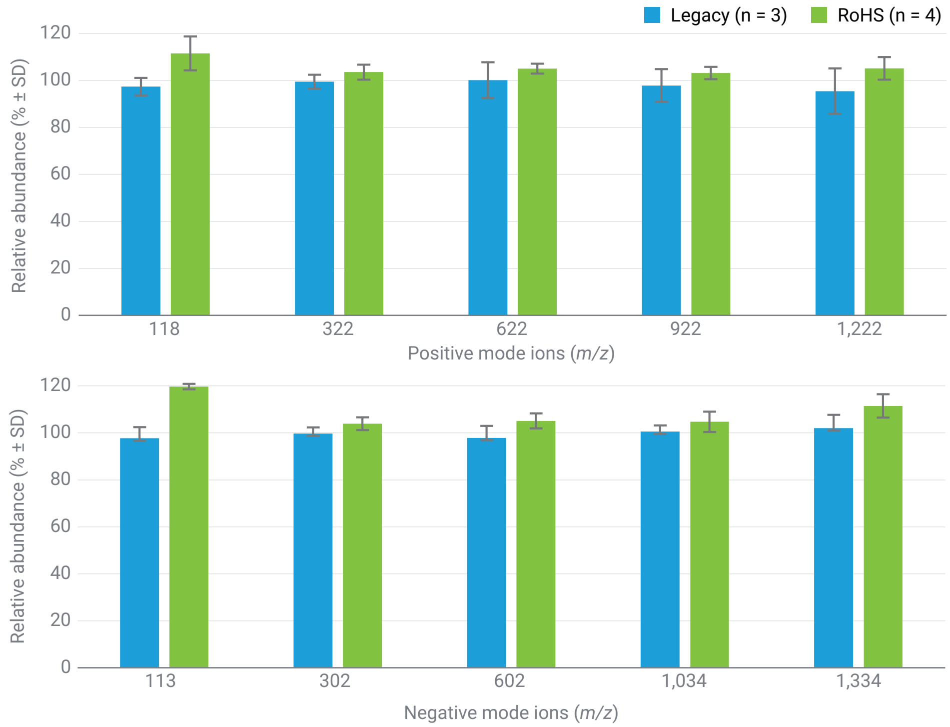

Figure 6. Comparison of the relative signal abundance (% ± SD) of tune ions on the 90 mm legacy Agilent Ultivo ion injector versus the 90 mm RoHS Agilent Ultivo and MSD iQ ion injector in positive and negative mode. The comparison was run on an Agilent Ultivo LC/TQ.

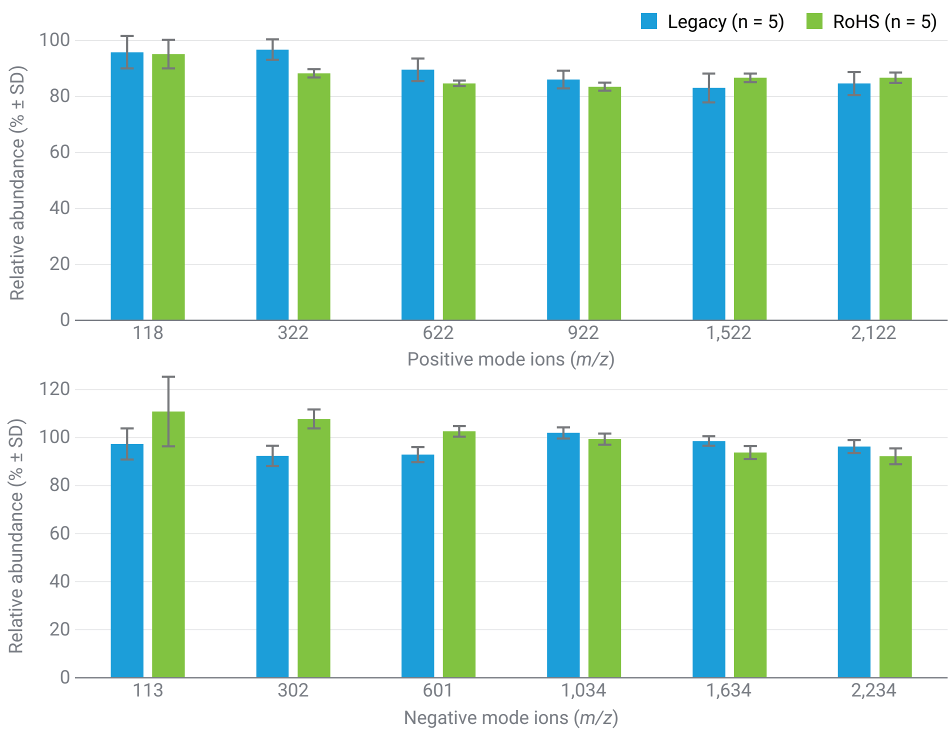

Figure 7. Comparison of the relative signal abundance (% ± SD) of tune ions on the 180 mm legacy Agilent FS ion injector versus the 180 mm RoHS Agilent FS ion injector, in positive and negative mode. The comparison was run on an Agilent 6470B LC/TQ.

Lifetime performance

Ion injectors are subjected to various conditions over the lifetime of use. Many kinds of mobile phases and buffers can be used, which subject the ion injector to a range of pH levels. Thus, a simulated lifetime performance was evaluated under acidic and basic conditions. RoHS ion injectors were subjected to a simulated year of use and referenced against a new legacy ion injector. The low-pH (acidic) simulation was performed by pumping 80 L of 0.1% formic acid in 50:50 acetonitrile:water into the MS at 1 mL/min. The high-pH (basic) simulation was performed by pumping 80 L of 0.2% ammonium hydroxide in 50:50 acetonitrile:water into the MS at 1 mL/min. Scans were run continuously on the instrument during each simulation. A reference autotune was performed at the beginning of each simulation. After running 80 L of acidic or basic mobile phase through the RoHS ion injector, tunes (n = 5) were performed and compared to the beginning reference tune on a new legacy ion injector.

The 90 mm RoHS Ultivo and MSD iQ ion injector was evaluated against the legacy Ultivo ion injector on the Ultivo LC/TQ. Figures 8 and 9 show the comparison under basic and acidic conditions, respectively. All relative abundances for the RoHS ion injectors in both acidic and basic conditions were above 80% after 80 L of simulated use.

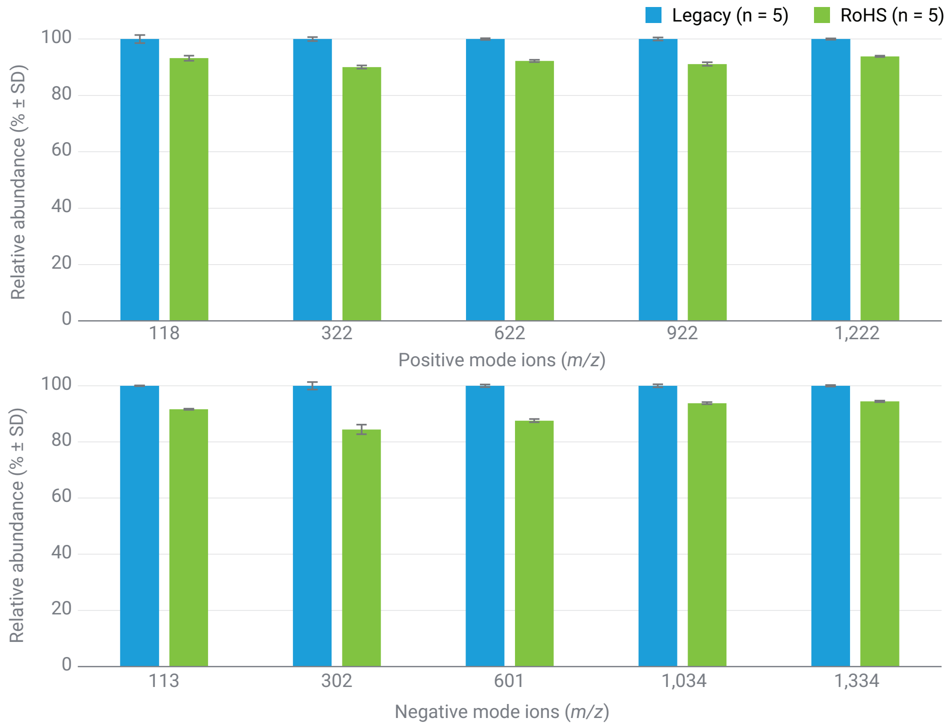

Figure 8. Comparison of the relative signal abundance (% ± SD) of tune ions on a reference 90 mm legacy Agilent Ultivo ion injector versus the 90 mm RoHS Agilent Ultivo and MSD iQ ion injector, in positive and negative mode, under basic conditions. The comparison was run on an Agilent Ultivo LC/TQ.

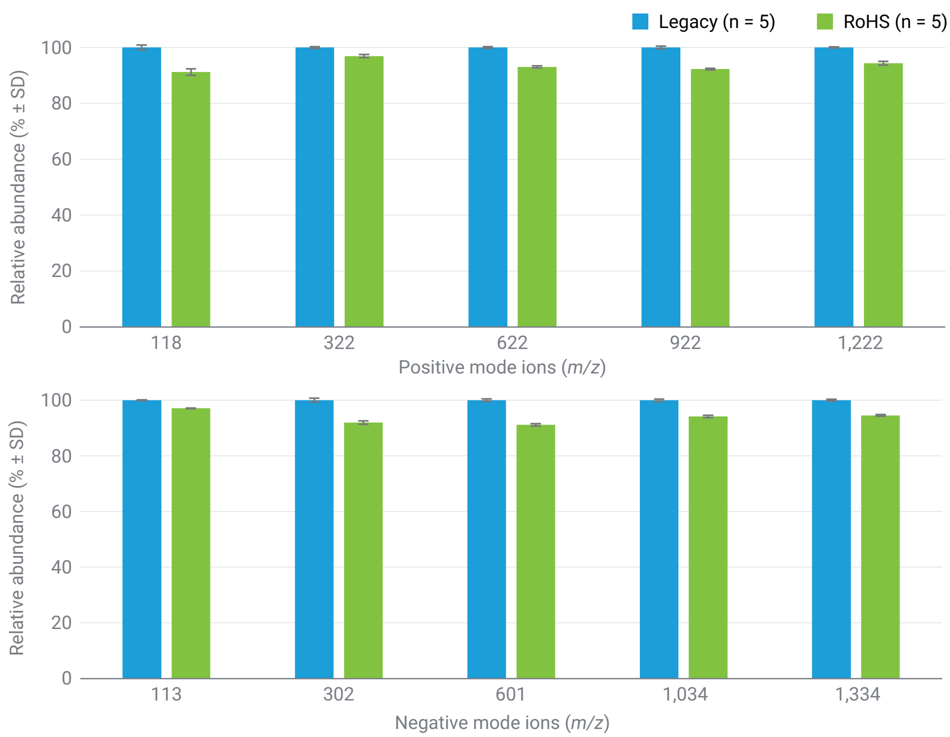

Figure 9. Comparison of the relative signal abundance (% ± SD) of tune ions on a reference 90 mm legacy Agilent Ultivo ion injector versus the 90 mm RoHS Agilent Ultivo and MSD iQ ion injector, in positive and negative mode, under acidic conditions. The comparison was run on an Agilent Ultivo LC/TQ.

The 180 mm RoHS FS ion injector was evaluated against a reference legacy FS ion injector on the 6470B LC/TQ. Figures 10 and 11 show the comparison under basic and acidic conditions respectively. To further test the high mass capabilities of the 180 mm ion injector, a subsequent test was performed on the Agilent 6545XT AdvanceBio quadrupole time-of-flight LC/MS (LC/Q-TOF).

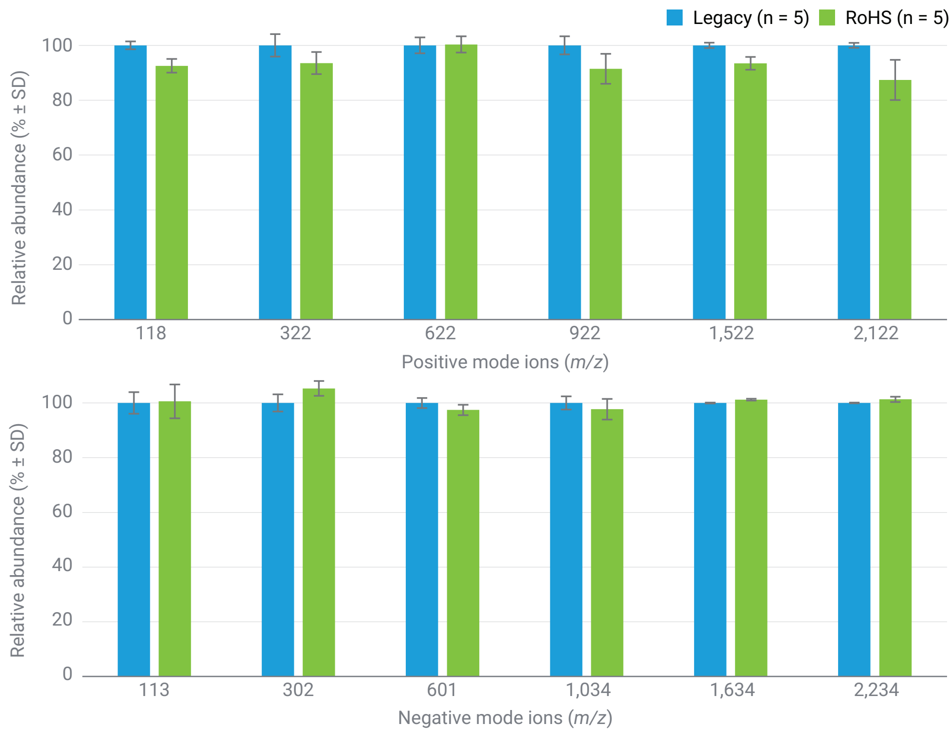

Figure 10. Comparison of the relative signal abundance (% ± SD) of tune ions on a reference 180 mm legacy Agilent FS ion injector versus the RoHS Agilent FS ion injector, in positive and negative mode, under basic conditions. The comparison was run on an Agilent 6470B LC/TQ.

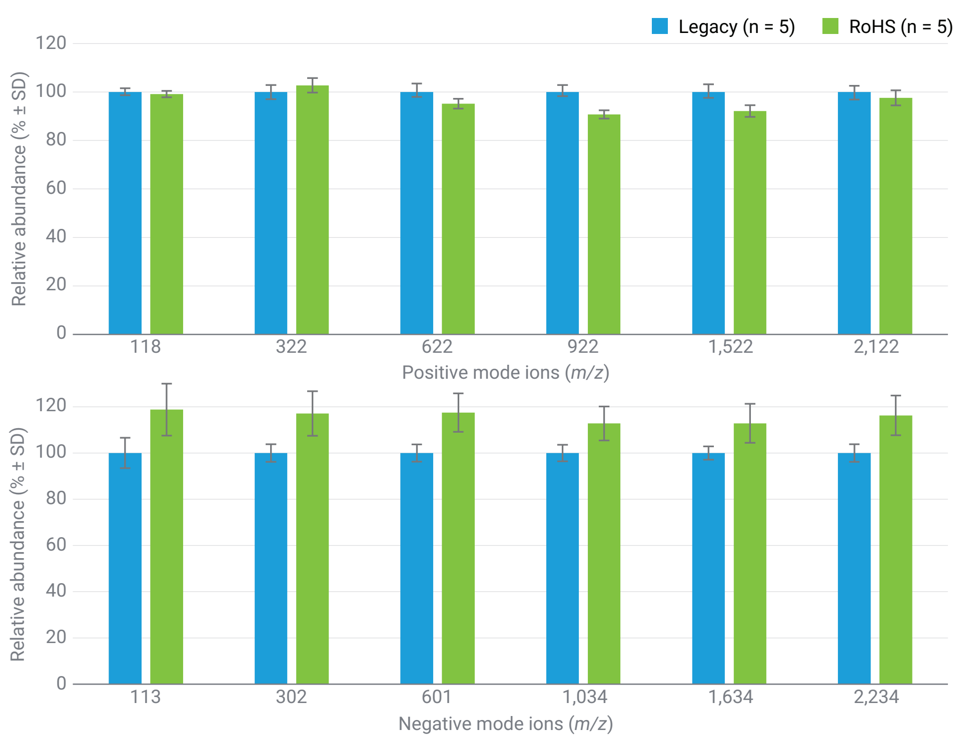

Figure 11. Comparison of the relative signal abundance (% ± SD) of tune ions on a reference 180 mm legacy Agilent FS ion injector versus the RoHS Agilent FS ion injector, in positive and negative mode, under acidic conditions. The comparison was run on an Agilent 6470B LC/TQ.

Figure 12 shows the comparative results of the same RoHS FS ion injectors under acidic and basic conditions. All relative abundances for the RoHS FS ion injectors under both acidic and basic conditions were above 80% after 80 L of simulated use on all instruments. The comparability of the RoHS FS ion injector on another MS instrument (the 6545XT LC/Q-TOF) to demonstrate the higher mass capabilities of the 180 mm ion injector further supports the use of the RoHS FS ion injector as a direct replacement for the legacy FS ion injector.

Instrument detection limits and signal-to-noise ratio

Signal-to-noise ratio (S/N) specifications and, more recently, instrument detection limits (IDLs) have been used as a checkout specification for installed instruments. To ensure performance of the RoHS ion injectors, both metrics were evaluated on the 90 and 180 mm ion injectors. The compound used for positive mode S/N and IDL was reserpine (CAS 50-55-5) and the compound used for negative mode S/N and IDL was chloramphenicol (CAS 56-75-7). The specification for S/N is 20,000:1 and for IDL is < 50 fg on the Ultivo LC/TQ. The specification for S/N is 350,000:1 and IDL is < 4 fg on the 6470B LC/TQ.

Table 2 shows the IDL results, in positive and negative mode, from eight of each the RoHS FS ion injector and the RoHS Ultivo and MSD iQ ion injector. All IDL values were below specification.

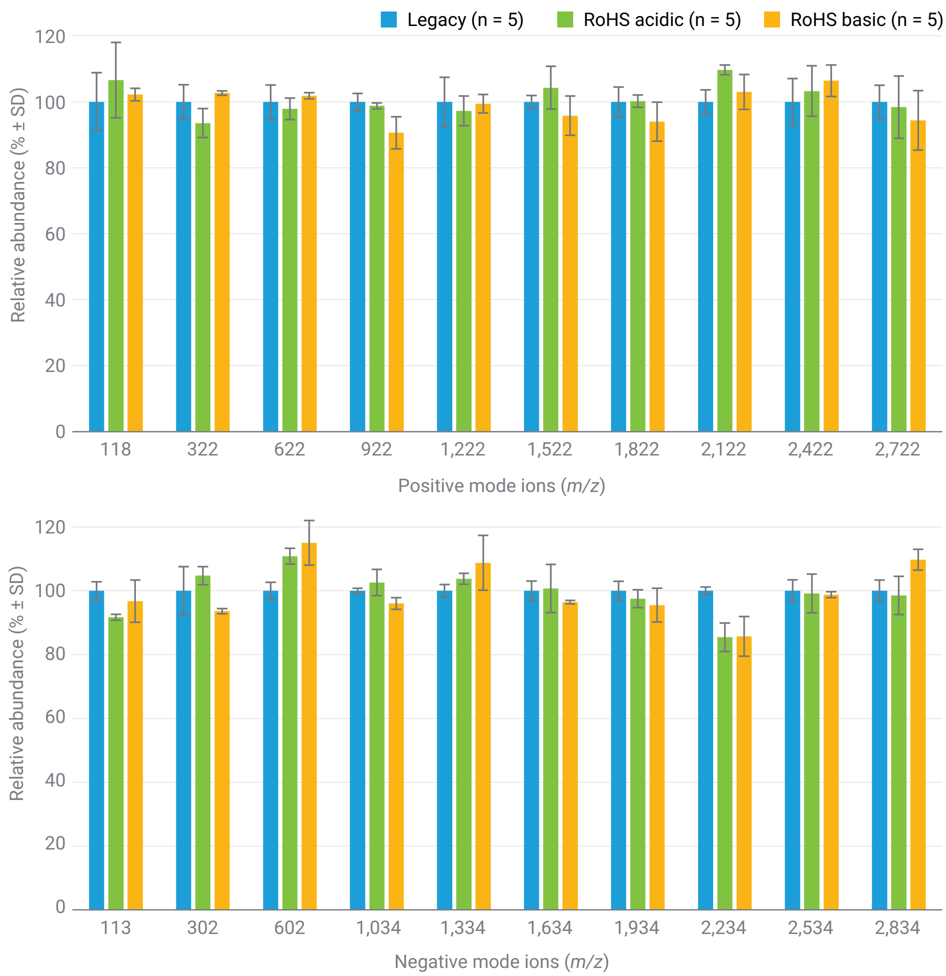

Figure 12. Comparison of the relative signal abundance (% ± SD) of tune ions on a reference 180 mm legacy Agilent FS ion injector versus the 180 mm RoHS Agilent FS ion injector, in positive and negative mode, under acidic and basic conditions. The comparison was run on an Agilent 6545XT AdvanceBio LC/Q-TOF.

Table 2. IDLs in positive and negative mode on Agilent Ultivo and 6470B LC/TQ instruments using the 90 mm RoHS Agilent Ultivo and MSD iQ ion injector (n = 8). The IDL specification is < 50 fg for the Ultivo LC/TQ and < 4 fg for the 6470B LC/TQ.

	Agilent Ultivo LC/TQ (90 mm Ultivo Ion Injector)		Agilent 6470B LC/TQ (180 mm FS Ion Injector)	
Ion Injector	**Positive (fg)**	**Negative (fg)**	**Positive (fg)**	**Negative (fg)**
RoHS 1	29.48	24.9	2.33	0.76
RoHS 2	15.42	23.34	1.82	1.01
RoHS 3	9.96	29.96	2.44	0.79
RoHS 4	10.77	28.3	3.14	0.97
RoHS 5	21.98	31.73	1.97	1.55
RoHS 6	12.8	24.54	2.31	1.18
RoHS 7	16.46	18.57	1.95	0.80
RoHS 8	35.49	42.07	2.48	0.70

Figure 13 shows the S/N results, in positive and negative mode, of the 90 mm RoHS Ultivo and MSD iQ ion injector (n = 8). Figure 14 shows the S/N results, in positive and negative mode, of the 180 mm RoHS FS ion injector (n = 8). All S/N values were above specification.

Robustness

MS front ends are often subjected to heavy matrices and extended use. Even in adverse sample conditions, it is imperative that the ion injector can transmit ions to the optics reliably over time. The robustness of the RoHS ion injectors was tested by simulating heavy instrument use through 10,000 sample injections (20 µL) of dilute bovine urine. Bovine urine was selected as a challenging matrix due to the large amount of solutes present in the matrix. Samples were diluted on a 1:1 basis and centrifuged before injection to simulate a minimal sample preparation. Initially, an autotune was run to collect base tune parameters. Then, checktunes were conducted every 1,000 injections over the 10,000-injection experiment. A passing robustness was considered to be a mass calibration delta within 0.1 Da and a peak width delta within 0.14 Da throughout the 10,000 injections.

Figure 13. Comparison of S/N measurements on the 90 mm legacy Agilent Ultivo ion injector and 90 mm RoHS Agilent Ultivo and MSD iQ ion injector (n = 8), in positive and negative mode, on the Agilent Ultivo LC/TQ. A passing specification on the Ultivo LC/TQ is 20,000:1 (marked by the red, dashed line).

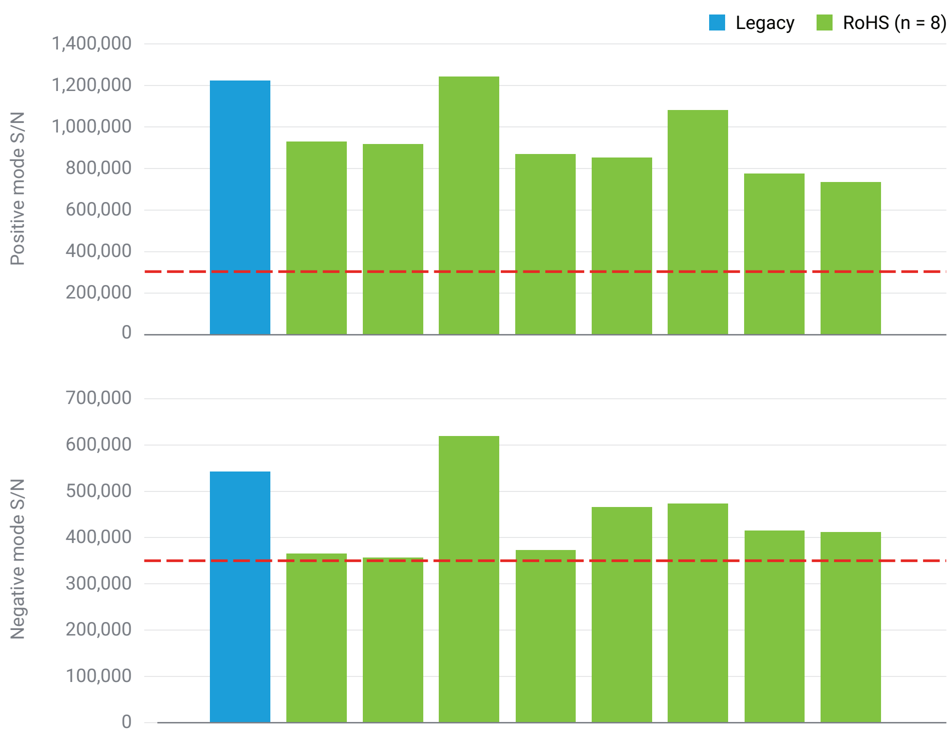

Figure 14. Comparison of S/N measurements on a 180 mm legacy Agilent FS ion injector versus a 180 mm RoHS Agilent FS ion injector (n = 8), in positive and negative mode, on an Agilent 6470B LC/TQ. A passing specification on the 6470B LC/TQ is 350,000:1 (marked by the red, dashed line).

The 90 mm RoHS Ultivo and MSD iQ ion injector was run on the Ultivo LC/TQ. Throughout the experiment, the same ion injector was used. However, the mass calibration delta at injection 3,000, *m/z* 118, in positive mode, was out of tolerance, so an autotune was performed. The mass calibration delta in positive and negative modes was within ± 0.1 Da criteria for all injections after autotune was used (Figure 15). The peak width delta in positive and negative modes was within ± 0.14 Da criteria for all 10,000 injections (Figure 16).

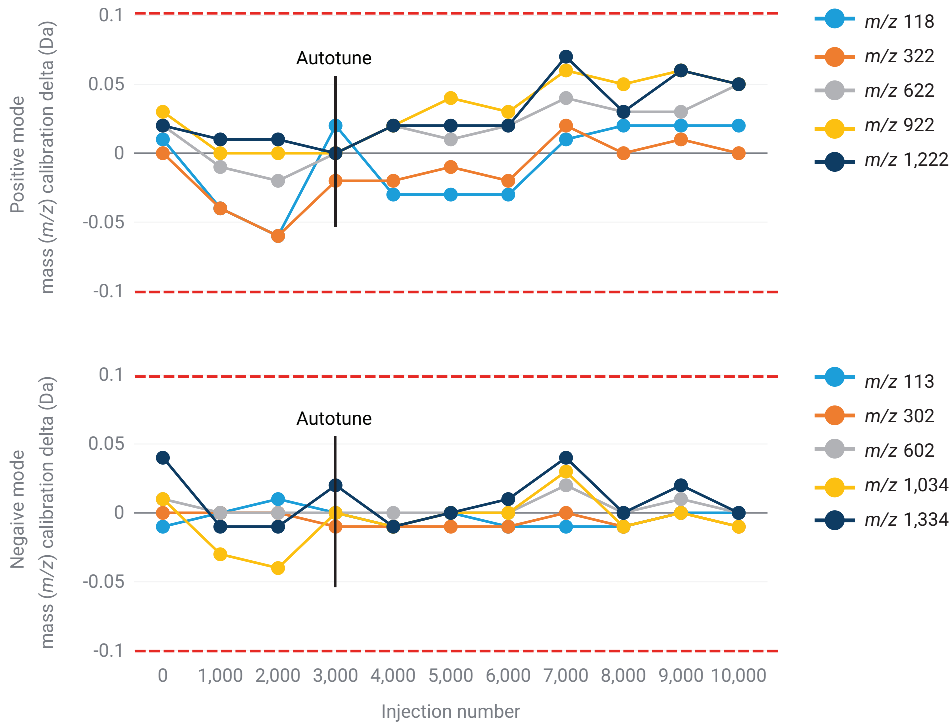

Figure 15. Mass calibration delta (Da) of the positive and negative mode tune ions, on an Agilent Ultivo LC/TQ, through 10,000 injections of dilute bovine urine. The tolerance for the mass calibration delta was ± 0.1 Da (marked by the dashed, red line).

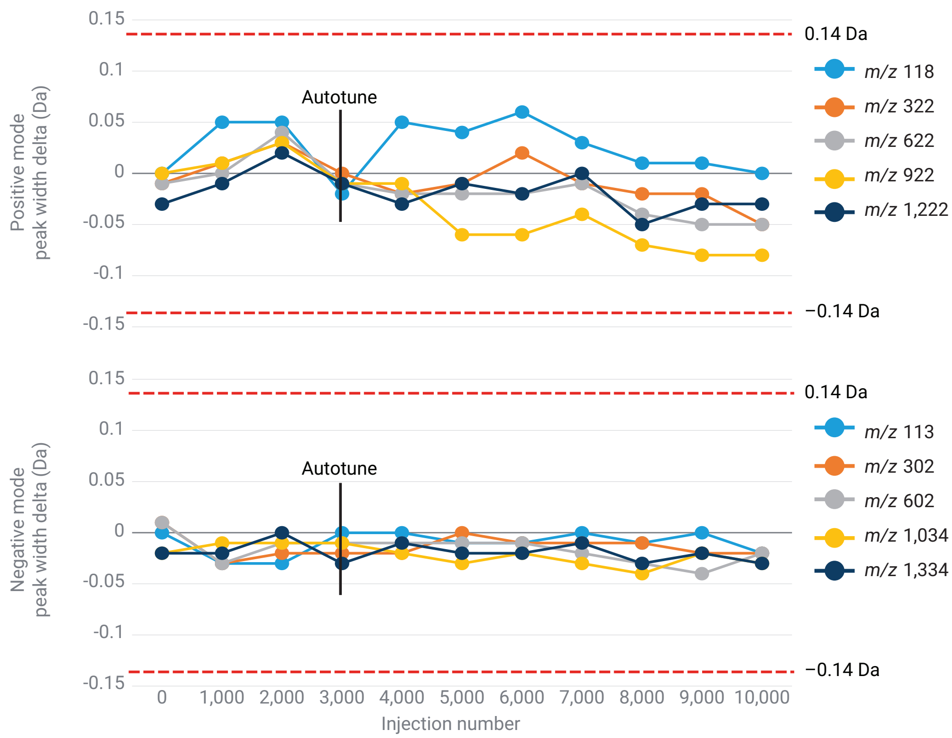

Figure 16. Peak width delta (Da) of the positive and negative mode tune ions, on an Agilent Ultivo LC/TQ, through 10,000 injections of dilute bovine urine. The tolerance for peak width delta was ± 0.14 Da (marked by the dashed, red line).

The relative abundance of tune ions in both positive and negative modes showed a downward trend with increasing injections (Figure 17). It was expected that tune ion abundance would decline over time as the front end and ion injector became dirty. However, throughout the experiment, the checktunes were able to pass criteria, showing the rugged nature of the ion injection.

The 180 mm RoHS FS ion injector was run on the 6470B LC/TQ. Throughout the experiment, the same ion injector was used. Mass calibration delta in positive and negative modes was within ± 0.1 Da criteria for all 10,000 injections (Figure 18).

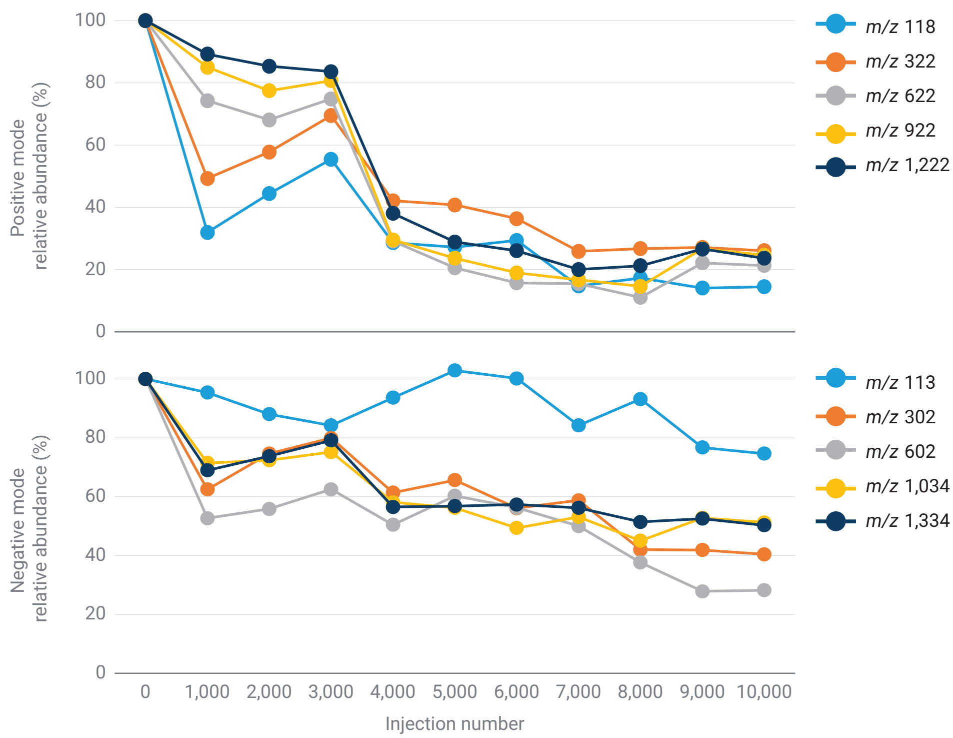

Figure 17. Relative abundance (%) of the positive and negative mode tune ions, on an Agilent Ultivo LC/TQ, through 10,000 injections of dilute bovine urine.

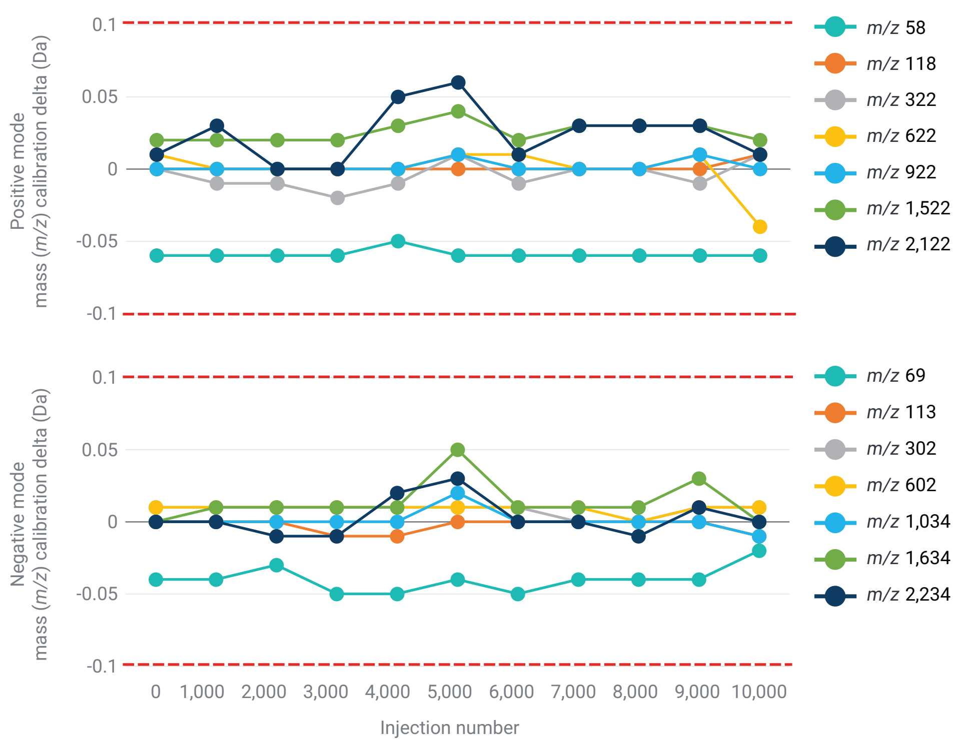

Figure 18. Mass calibration delta (Da) of the positive and negative mode tune ions, on an Agilent 6470B LC/TQ, through 10,000 injections of dilute bovine urine. The tolerance for mass calibration delta was ± 0.1 Da (marked by the dashed, red line).

The peak width delta in positive and negative modes was within ± 0.14 Da criteria for all 10,000 injections (Figure 19). The relative abundance of tune ions in both positive and negative modes showed a downward trend with increasing injections (Figure 20). Similar to the Ultivo and MSD iQ ion injector, this result showed the robustness of the FS ion injector through repeat injections of a worst-case-scenario matrix.

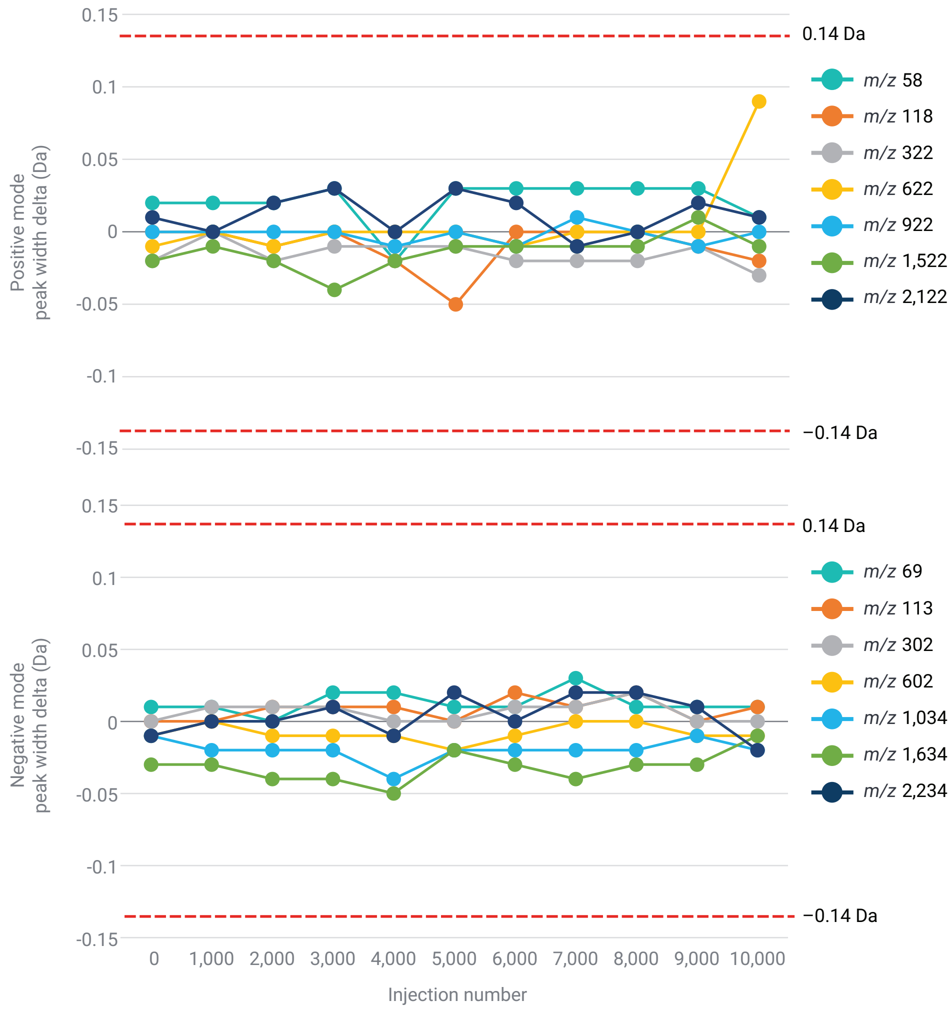

Figure 19. Peak width delta (Da) of the positive and negative mode tune ions, on an Agilent 6470B LC/TQ, through 10,000 injections of dilute bovine urine. The tolerance for peak width delta was ± 0.14 Da (marked by the dashed, red line).

Application performance

A comparison of full system application performance was executed, using all the same parameters and hardware except the ion injector. Nine model pharmaceutical compounds (Table 3) were chosen to create applications on the InfinityLab LC/MSD iQ and the Ultivo LC/TQ using the 90 mm legacy Ultivo ion injector and the 90 mm RoHS Ultivo and MSD iQ ion injector. Typically, the LC/MSD is used to look at product, purity, or process chemistry in pharmaceutical manufacturing. Thus, the samples run on the InfinityLab LC/MSD iQ were standards in neat solvent. As a challenge, a multiple reaction monitoring (MRM) method was developed to detect pharmaceuticals in human urine, diluted 10:1 with 0.1% formic acid in water, on the Ultivo LC/TQ.

Table 3. Pharmaceutical compounds used for comparison of a legacy Agilent Ultivo ion injector versus the RoHS Agilent Ultivo and MSD iQ ion injector. Pharmaceutical standards were obtained from Cerilliant (Round Rock, TX, U.S.).

Analyte Name	Abbreviation	CAS Number
Amitriptyline	AMI	549-18-8
Carbamazepine	CAR	298-46-4
Diltiazem	DIL	42399-41-7
Diphenhydramine	DIP	58-73-1
Fluoxetine	FLU	54910-89-3
Haloperidol	HAL	52-86-8
Lorazepam	LOR	846-49-1
Methylphenidate	MET	113-45-1
Sertraline	SER	79617-96-2

To effectively compare the legacy versus RoHS ion injectors, a robust and highly repeatable methodology was required. On both the single and triple quadrupole instruments, chromatographic separation was as expected (Figure 21). Weak base target analytes were chosen for analysis in positive mode because they perform well in the mobile phase conditions selected and are not subject to transformation or sorption under current chromatographic conditions.

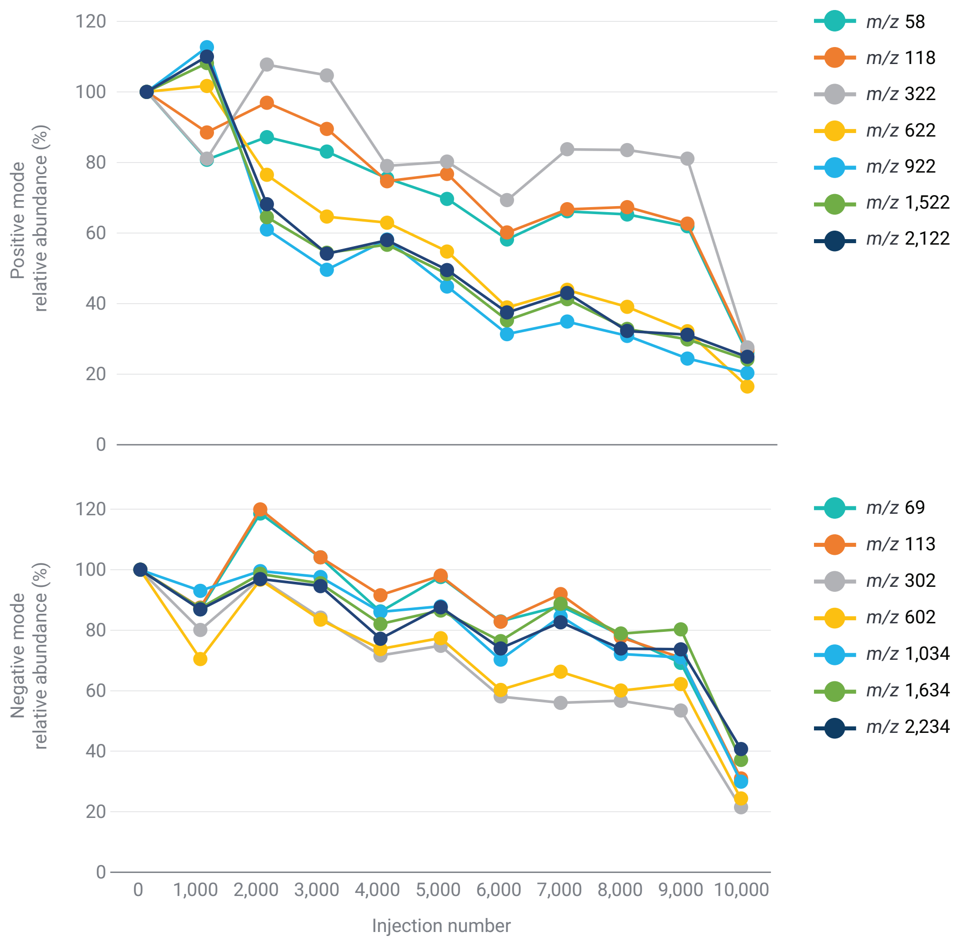

Figure 20. Relative abundance (%) of the positive and negative mode tune ions, on an Agilent 6470B LC/TQ, through 10,000 injections of dilute bovine urine.

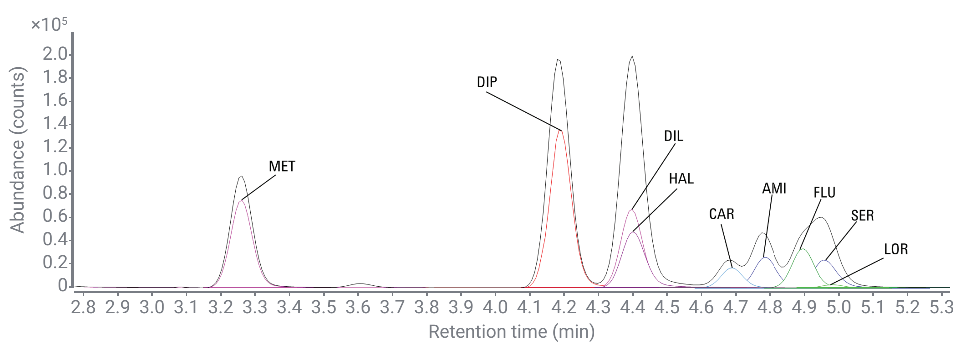

Figure 21. Total ion chromatogram and extracted ion chromatogram of target pharmaceuticals at 1 ppb, from the Agilent Ultivo LC/TQ.

Performance on the InfinityLab LC/MSD iQ

Before application work was performed on the InfinityLab LC/MSD iQ, 10 autotunes were performed on both the RoHS Ultivo and MSD iQ ion injector and legacy Ultivo ion injector. All tunes passed and showed no significant differences in how the tune algorithm applied physical parameters to the instrument. An eight-point calibration curve was made ranging from 5 to 500 ppb, and all R^2 values were > 0.995 on both ion injectors. Accuracy at the 10 and 100 ppb levels was assessed, and showed passing criteria on both ion injectors. Passing criteria for accuracy was from 80 to 120% of target concentration. Precision at the 10 and 100 ppb levels also passed acceptance criteria, which was < 5% RSD. IDLs at the 10 ppb spiking level were comparable and of the same order of magnitude (Figure 22). The only exception was that FLU had an IDL of 0.83 on the RoHS Ultivo and MSD iQ injector versus 3.87 on the legacy Ultivo ion injector.

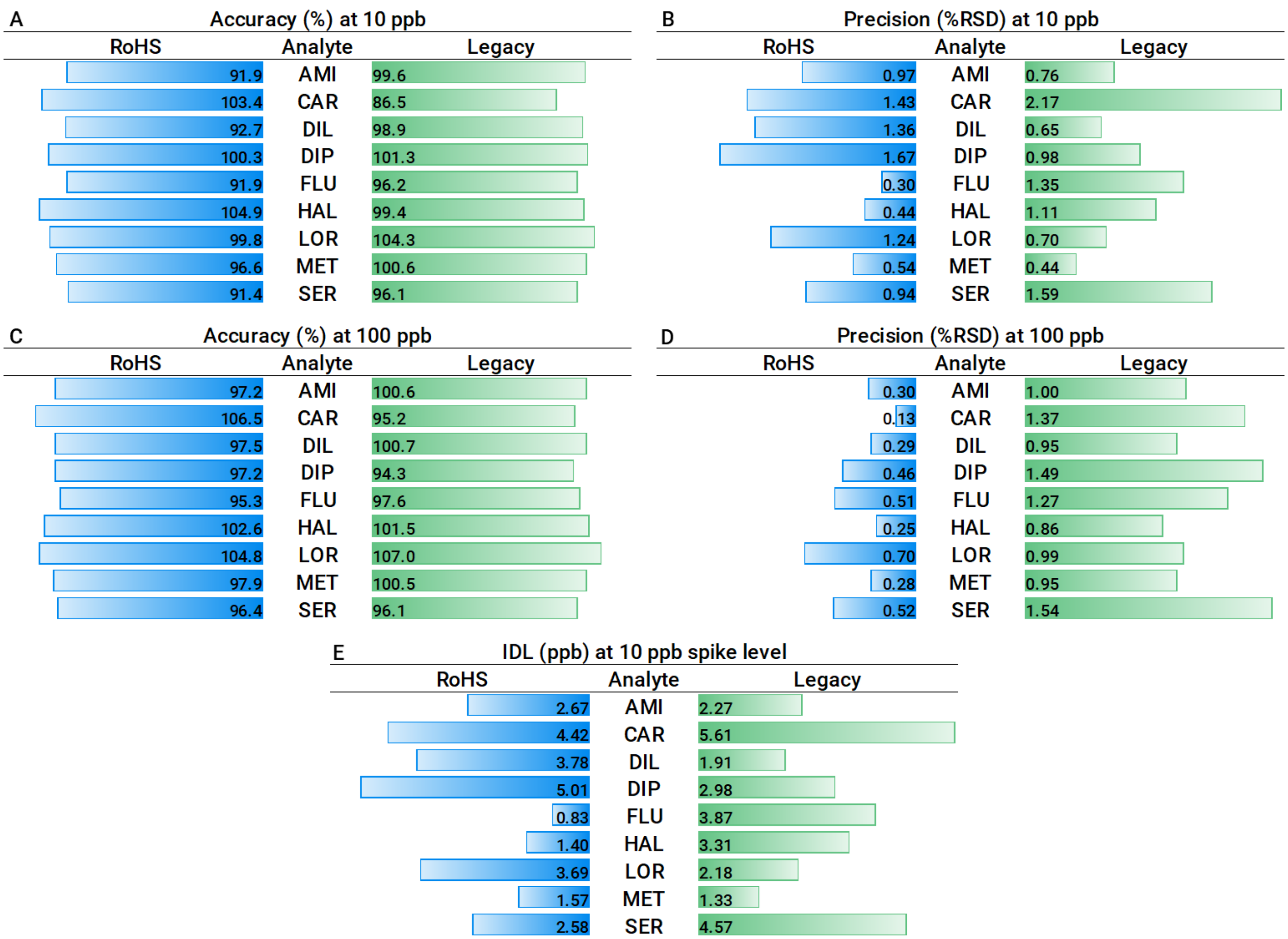

Figure 22. Comparison of the RoHS Agilent Ultivo and MSD iQ ion injector and the legacy Agilent Ultivo ion injector method validation parameters on the Agilent InfinityLab LC/MSD iQ. (A) Accuracy (%) at 10 ppb. (B) Precision (%RSD) at 10 ppb. (C) Accuracy (%) at 100 ppb. (D) Precision (%RSD) at 100 ppb. (E) IDLs (ppb) at the 10 ppb spiking level.

Performance on the Ultivo LC/TQ

Ten autotunes were run on both the legacy Ultivo ion injector and RoHS Ultivo and MSD iQ ion injector, and showed no significant difference in how the tune algorithm applied physical parameters to the instrument. Human urine is a complex matrix that was chosen to challenge the instrument and ion injector. The Ultivo LC/TQ is a sensitive instrument, so a trace-level calibration range that is relevant to current applications was chosen. An eight-point calibration curve ranging from 5 to 1,000 ppt was made, and all R^2 values were > 0.995 on both ion injectors. Limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision were examined to compare the RoHS and legacy ion injectors (Figure 23). LOD values were < 2.6 ppt for all analytes, which was below the calibration range. LOD was highly comparable between both ion injectors, showing that the urine matrix did not affect detection limits on either injector. LOQ was < 7.7 ppt for all compounds and, in several cases, below the calibration range. Similarly, LOQ values were comparable between both ion injectors. For this experiment, LOD and LOQ were determined statistically using the standard deviation of replicate injections. The low-level LOD and LOQ values show a high level of reproducibility from injection to injection. This is also seen in the precision at 10 and 100 ppt where all RSD values are < 10 %RSD, which was the criteria of acceptability given the low level of detection expected. Further accuracy at the 10 and 100 ppt level was comparable and reproducible on both ion injectors. The acceptance criteria for accuracy were 80 to 120%; however, in the current study, accuracy on both injectors fell between 90 to 110%.

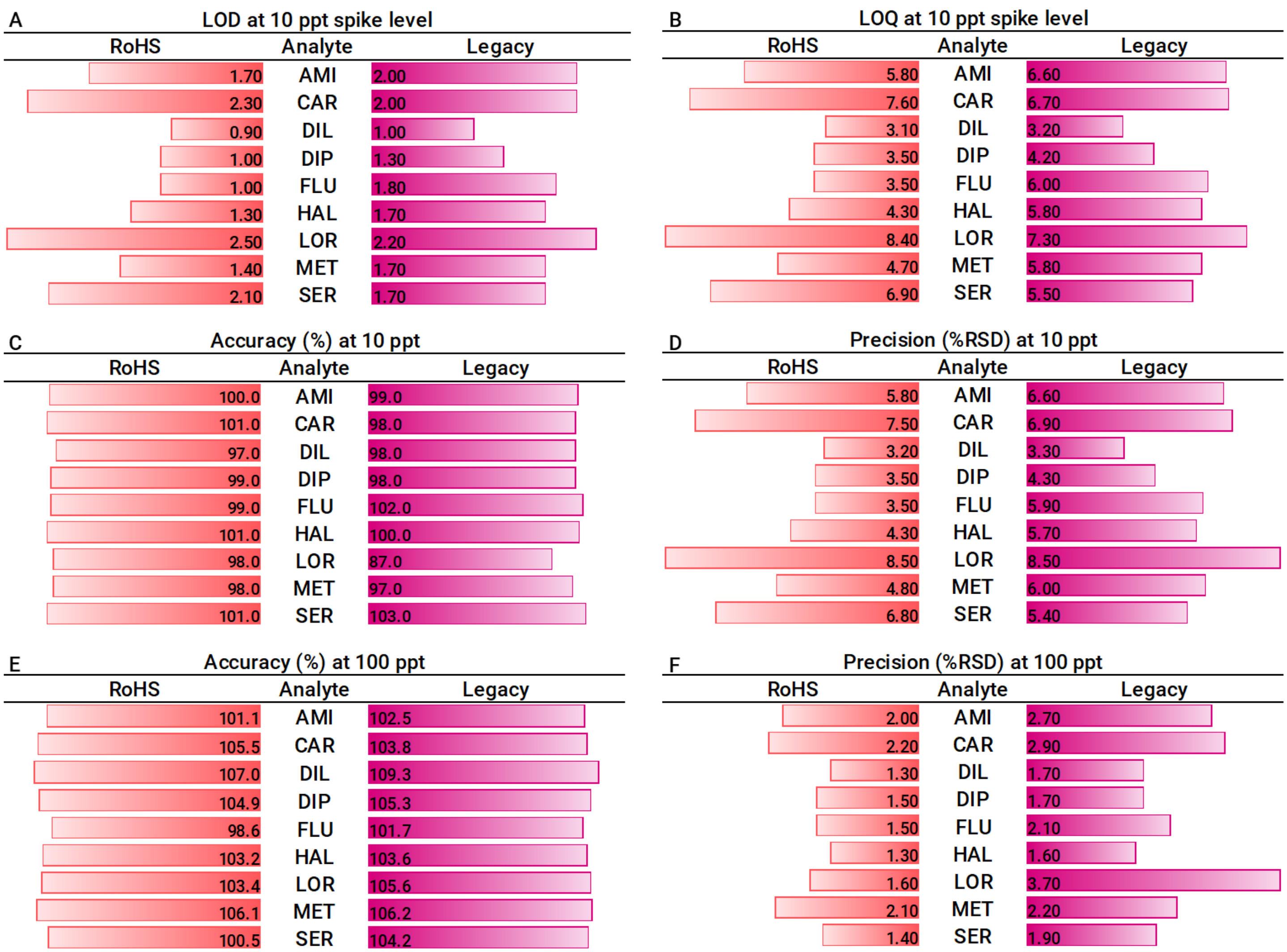

Figure 23. Comparison of the RoHS Agilent Ultivo and MSD iQ ion injector and legacy Agilent Ultivo ion injector method validation parameters on the Agilent Ultivo LC/TQ. (A) LOD (ppt) at the 10 ppt spiking level. (B) LOQ (ppt) at the 10 ppt spiking level. (C) Accuracy (%) at 10 ppt. (D) Precision (%RSD) at 10 ppt. (E) Accuracy (%) at 100 ppt. (F) Precision (%RSD) at 100 ppt.

Conclusion

In this technical overview, the Agilent RoHS-compliant ion injectors were compared to their respective Agilent legacy ion injectors. The studies presented in this technical overview demonstrate equivalent functionality between the legacy and RoHS ion injector models. Specifically, tune performance was shown to be equivalent between RoHS and legacy ion injectors, and lifetime performance under acidic and basic conditions was also shown to be comparable between RoHS and legacy ion injections, including at high mass on the Agilent 6545XT AdvanceBio LC/Q-TOF. Moreover, IDLs and S/N were within specifications on multiple of the tested RoHS ion injectors. Furthermore, the RoHS ion injectors held up to 10,000 injections of bovine urine and maintained tune criteria throughout, demonstrating excellent robustness. Finally, no differences in performance were observed on two different MS platforms when running target pharmaceuticals, both in neat samples and diluted human urine. In conclusion, the RoHS Agilent FS and Agilent Ultivo and MSD iQ ion injectors are suitable drop-in replacements for the legacy Agilent FS and Agilent Ultivo ion injectors.

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