Application Note

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Application Robustness of the 6495D Triple Quadrupole LC/MS System for Nonstop Pesticide Analysis in Black Tea Matrix

Authors

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Abstract

System robustness is of utmost importance, especially when analyzing samples for routine, in-production types of analyses. Additionally, when evaluating samples for meaningful scientific results, the analysis of a large population of samples is necessary for good population statistics. This application note details the results obtained from nonstop injection of sample in a dirty matrix, spanning over 2,000 injections, and 16 days of nonstop operation of an Agilent 6495D LC/MS system. During the period of data acquisition, analyte response statistics remained within RSD < 10%. At the end of the experimentation period, the system successfully passed instrument detection limit checkout specifications and passed all standard procedures carried out during the automated instrument Checktune. These results indicate that the instrument is highly robust and suitable for high-throughput analysis of complex samples.

Introduction

Repeat sample introduction introduces the effects of sample accumulation and matrix deposition on the ion optics, as well as aging of components over extended periods of time. It is typical that instrument performance suffers if the system is not maintained in a timely manner. The 6495D triple quadrupole LC/MS system is equipped with VacShield and iFunnel technology that enables high sensitivity and high-performance analyses while being robust and rugged enough to withstand the effects of deposition from complex and dirty sample matrices.

- **VacShield:** ion injector capillary removal mechanism without instrument venting, which enables quick routine-maintenance, reduces downtime, and preserves system stability.
- **iFunnel technology:** a dual-staged stacked ring ion funnel used to compress and concentrate the ion beam to provide ultimate sensitivity. Innovations within the iFunnel evacuate matrix components while maintaining injection-to-injection MRM precision.
- **Instrument intelligence:** built into the overall system architecture to monitor and ensure that the instrument performs optimally during operation.

To properly demonstrate system robustness and reliability, the experiments defined in this application note are designed to simulate a production analytical environment where continuous, nonstop injection of samples is performed over an extended period without the need to carry out any type of system maintenance.

At the end of the stress period, an evaluation of the system was carried out to ensure that chemical performance specifications could still be met, the system was still within operable tolerance, and the effects of outer sample matrix deposition did not cause any detrimental effects to the instrument.

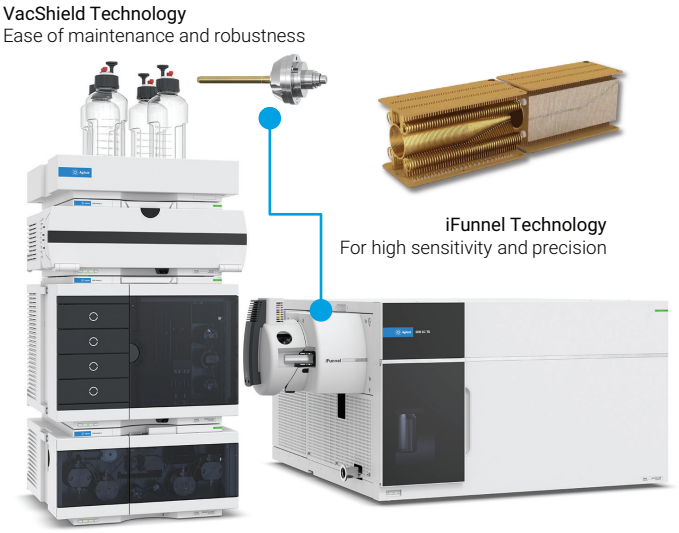


Figure 1. Compared to non-iFunnel systems, the Agilent 6495D triple quadrupole LC/MS system provides about 10x improvement in signal while also providing superior injection-to-injection measurement robustness and precision at sub-millisecond dwell times.

Methods

Sample preparation

Organic black tea was steeped in room temperature water for 2 hours. After incubation, one pouch of Agilent EN extraction salt (p/n 5982-6650) was added to the mixture, shaken, then centrifuged at 3,000 × g for 5 minutes. The black tea mixture was added into the Agilent QuEChERS Dispersive SPE for high pigment (10 mL of acetonitrile per 2 g of tea leaves), followed by shaking and centrifugation as prior.

Supernatant was passed through a 0.45 µm syringe filter, then spiked with 100 ng/g in relation to 8 g of black tea of LC TOF/QTOF/QQQ Pesticide Test Mix (p/n 5190-0469) and three isotope-labeled compounds (atrazine-d_5, diazinon-d_{10}, and dimethoate-d_6) as internal standards (IS) for a final concentration of 20 pg/µL per analyte.

The resulting preparation shown in Figure 2 was partitioned into smaller vials, then injected with no further cleanup.

Figure 2. Black tea extract after QuEChERS Dispersive SPE cleanup. The solution remained highly pigmented, providing considerable opportunity for matrix deposition and contamination.

LC/MS method

The HPLC method was configured to be a standard chromatography method to provide adequate separation of analytes and to include a suitable column wash-out period in preparation for the next injection. The time for each chromatography run (injection-to-injection) was 8 minutes per injection.

The mass spectrometry method was developed to use ion source parameters typical for multiresidue pesticide oriented analysis (i.e., suitable enough to provide good desolvation and ionization through heating and gas flow). MRM transitions for the analytes of interest were obtained via the available dMRM Pesticide Database and fine-tuned to maximize abundance.

HPLC method

LC Parameter	Agilent 1290 Infinity LC
Guard Cartridge	Agilent Poroshell EC C18, 2.1 × 5 mm, 2.7 µm (p/n 821725-911)
Analytical Column	Agilent Eclipse Plus C18 RRHD, 2.1 × 50 mm, 1.8 µm (p/n 959757-902) at 30 °C
Injection Volume	2 µL
Mobile Phase	A) H_2O + 5 mM NH_4-formate + 0.1% formic acid B) MeOH + 5 mM NH_4-formate + 0.1% formic acid
Gradient Flow Rate	0.6 mL/min
Gradient	Time (min) %B 0.27 2 0.33 50 1.10 55 2.00 65 3.00 85 3.10 100 5.40 100 5.41 2
Stop Time/Post Time	5.50 min/2.50 min

MS ion source parameters

MS Source Parameters	Agilent Jet Stream Source
Drying Gas	15 L/min at 290 °C
Sheath Gas	12 L/min at 325 °C
Nebulizer	40 psi
Capillary Voltage	4,000 V (+), 3,000 (–)
Nozzle Voltage	1,500 V (±)

MS MRM and compound-specific parameters

Compound Name	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)
Atrazine	216.1	174.1	16
Atrazine-d_5	221.1	179.1	16
Diazinon	305.1	169.0	32
Diazinon-d_{10}	315.1	170.0	32
Dimethoate	230.0	199.0	0
Dimethoate-d_6	236.0	205.0	0

The following parameters were kept constant for all monitored MRM transitions:

Parameter	Value
MS1/MS2 Resolution	Unit/unit (0.7 Da FWHM)
Fragmentor	166 V
Collision Cell Acceleration Voltage	5 V
Dwell Time	15 ms
iFunnel Mode	Standard (high pressure/low pressure ion funnel RF voltages = 100 V/100 V)

Results and discussion

Analysis of robustness test data

Samples were injected in a back-to-back and nonstop manner, resulting in the following number of MS experiments:

- 2,000 Black tea sample injections
- 400 Blank injections: four blanks every 20 sample injections to prevent blockage and accumulation on the chromatography system
- 100 MS1 precursor scans and 100 MS2 full scans: Precursor and full scan every 20 sample injections between blanks to monitor MS1 and MS2 calibration

A total volume of 4 mL of black tea matrix was injected and delivered to the mass spectrometer with continuous LC flow. No MS-specific maintenance events such as cleaning, retuning, or mass calibration during the 16-day uninterrupted experiment were required. The experiment was concluded after a set number of injections and set time limit, not because of instrument failure.

Figure 3A shows raw peak areas for each analyte plotted as a function of injection number + every 100th injection.

Figure 3B shows a relative response plot for atrazine, diazinon, and dimethoate corrected against their isotopically labeled IS. To improve clarity in the figure, a slight offset was added to the relative responses to avoid overlapping of the data points.

Peak area statistics for each monitored analyte over 2,000 sample injections are summarized in Table 1 demonstrating that in all cases, the analytes of interest express < 10 %RSD when measuring raw peak area responses and < 5 %RSD when measuring IS-corrected responses.

Table 1. Signal response statistics represented by raw peak area and internal standard (IS) corrected peak area ratio.

Analyte	Raw Peak Area %RSD	IS Corrected Peak Area Ratio %RSD
Atrazine	3.7%	1.8%
Diazinon	9.1%	2.9%
Dimethoate	7.4%	4.4%

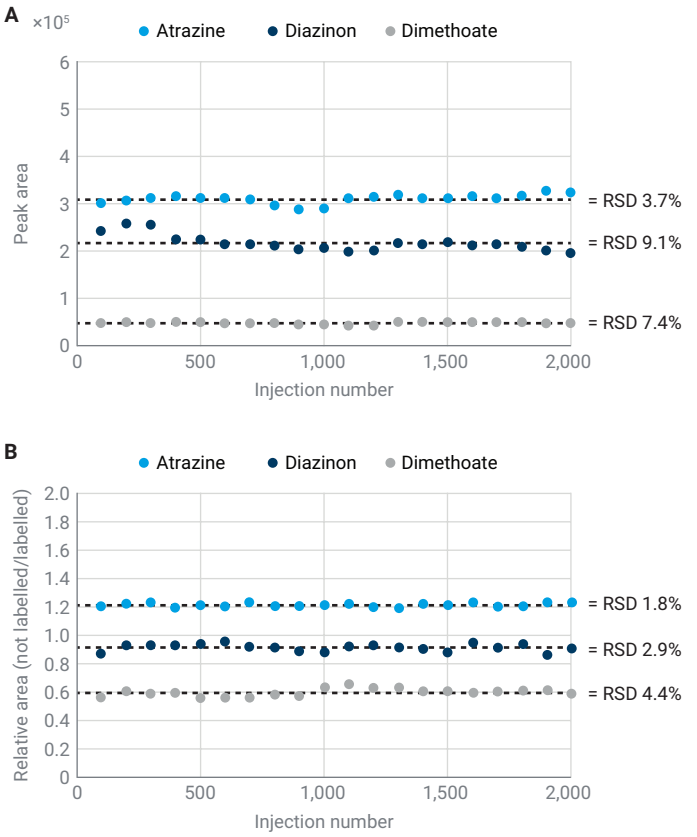


Figure 3. Signal response plots for each analyte of interest (injection number + every 100th injection). (A) Raw peak areas. (B) IS-corrected relative peak areas.

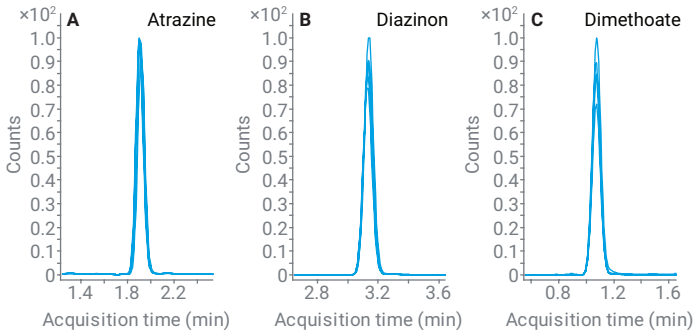


Figure 4. Replicate MRM chromatograms every 500 injections for each analyte of interest.

Physical examination of the desolvation assembly sample inlet before and after testing (Figure 5) demonstrates considerable deposition, charring, and accumulation of sample and matrix material around the inlet. Despite severe matrix deposition, instrument performance remained stable throughout the course of testing and evaluation.

Evaluation of instrument fitness

In-process mass axis calibration check

Built-in to the robustness testing through pesticides spiked into black tea matrix, precursor and full scans, performed between blanks every 20 sample injections, were included to monitor the possibility of mass axis drift from calibration tolerance. ESI-L Tune Mix ions in positive mode (*m/z* 118, 322, and 622) and negative mode (*m/z* 113, 302, and 602) were monitored covering the effective *m/z* range for the analytes of interest. Figures 6 and 7 demonstrate the stability of the target calibrant ions for the duration of the experiment. Results shown are the mass axis values as measured by the centroided mass spectral peak, showing minimal drift from the calibrated target value.

Post-robustness test IDL check

Once the robustness test was concluded, a negative mode sensitivity checkout was immediately executed on the system to test for negative mode instrument detection limit (IDL) based on an injection of 1 fg of chloramphenicol on-column. This experiment was carried out because it is generally considered challenging to execute on a "dirty" system due to the deposition of acids and other contaminants that may cause ion suppression, poor sensitivity, and prefragmentation. Additionally, this is the standard negative-mode IDL performance checkout method that may be carried out during the installation of a new system.

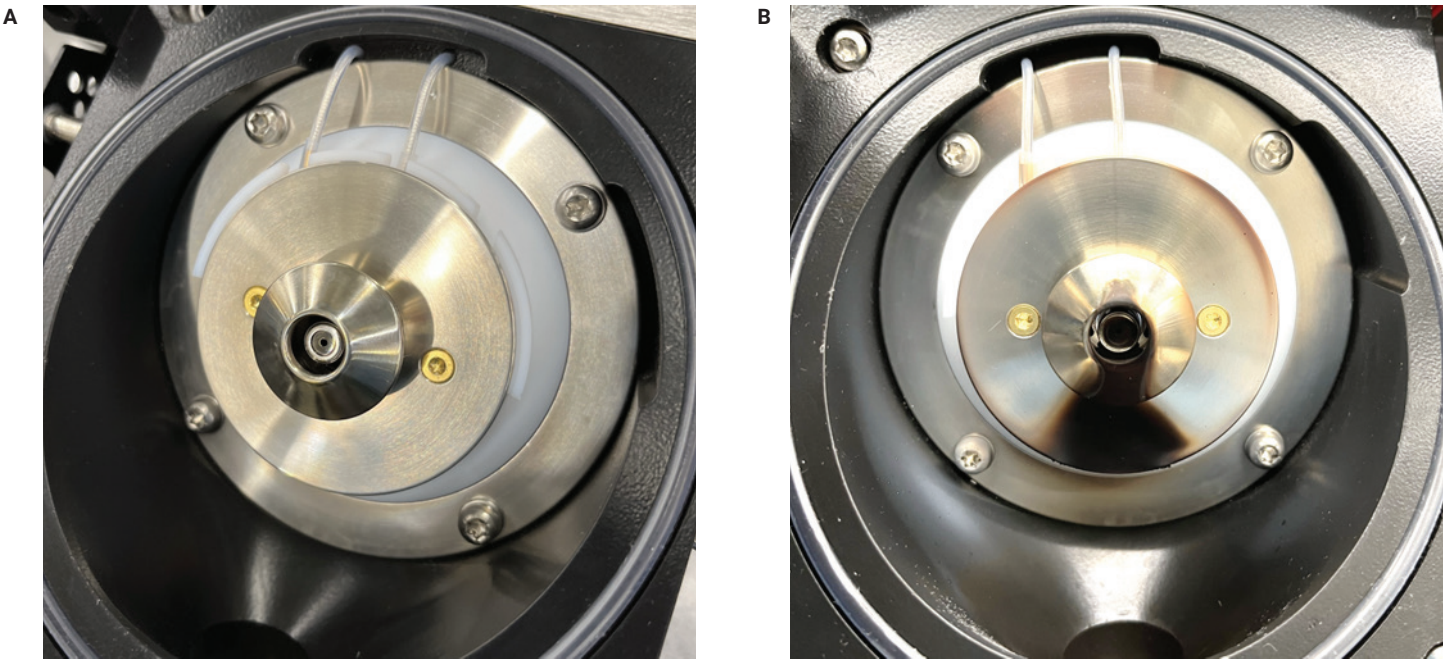


Figure 5. Photos of the MS sample inlet desolvation chamber assembly before (A) and after (B) robustness testing, showing the nebulizer spray profile from accumulation/deposition and charring of material found in the sample.

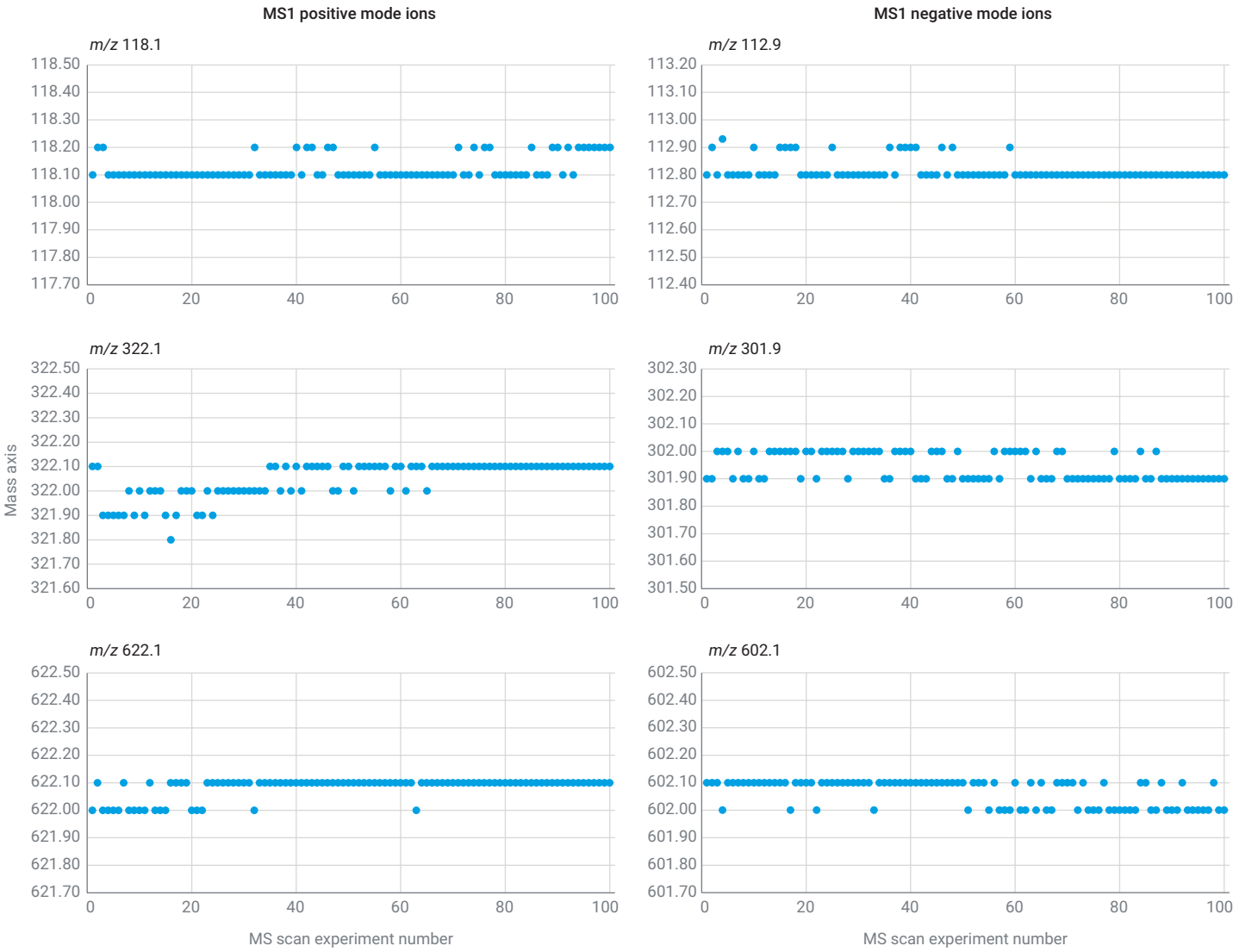


Figure 6. MS1 mass axis values of ESI-L tune ions as captured by centroided MS1 precursor ion scan.

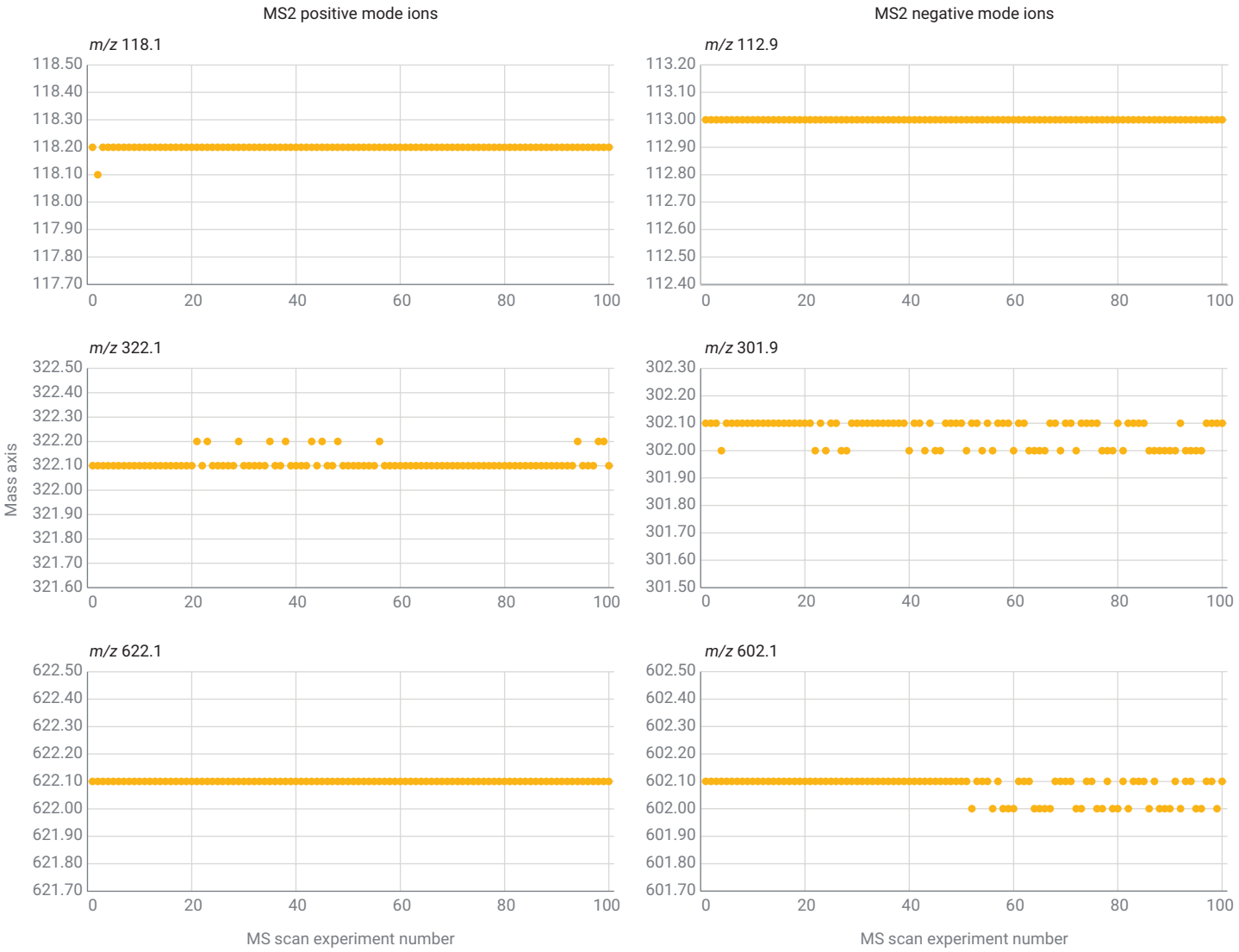


Figure 7. MS2 mass axis values of ESI-L tune ions as captured by centroided MS2 full scan.

Using n = 8 injections of 1 fg on-column (df = 7), the response reproducibility of chloramphenicol was determined to be RSD = 13.9%, resulting in an IDL = 0.42 fg chloramphenicol. The IDL result implies that 0.42 fg of chloramphenicol can be detected on-column and differentiated from random noise with 99% statistical confidence. The results, shown in Figure 8, indicate good sensitivity and reproducibility of the 6495D LC/TQ without follow-up cleaning, preparation, or maintenance immediately after the robustness test.

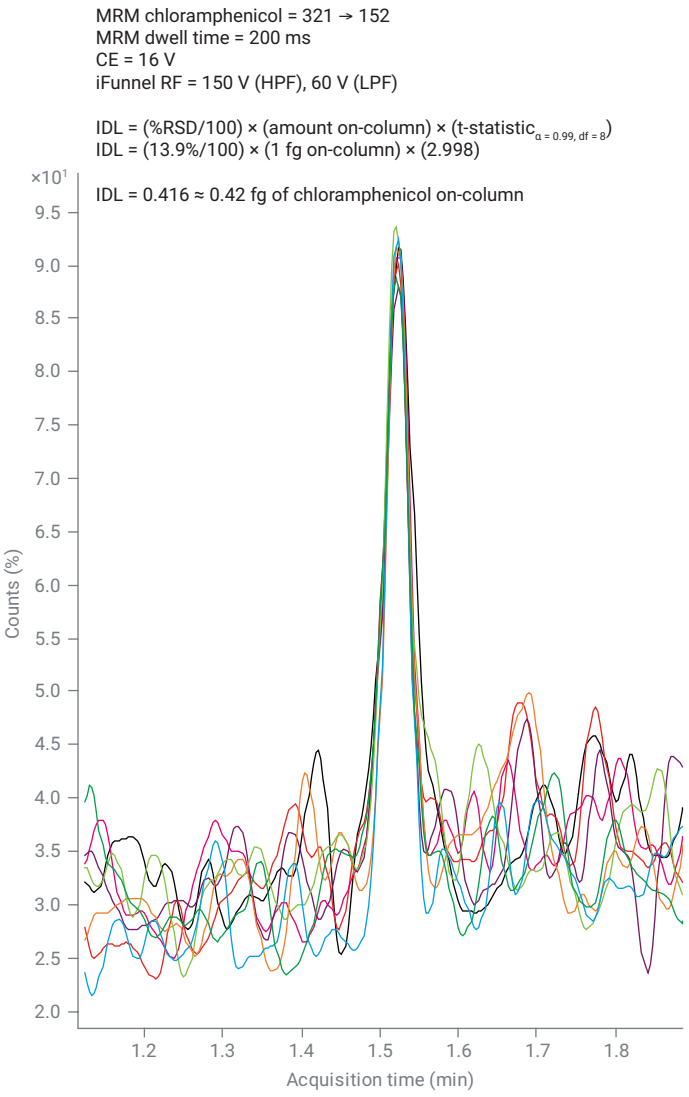


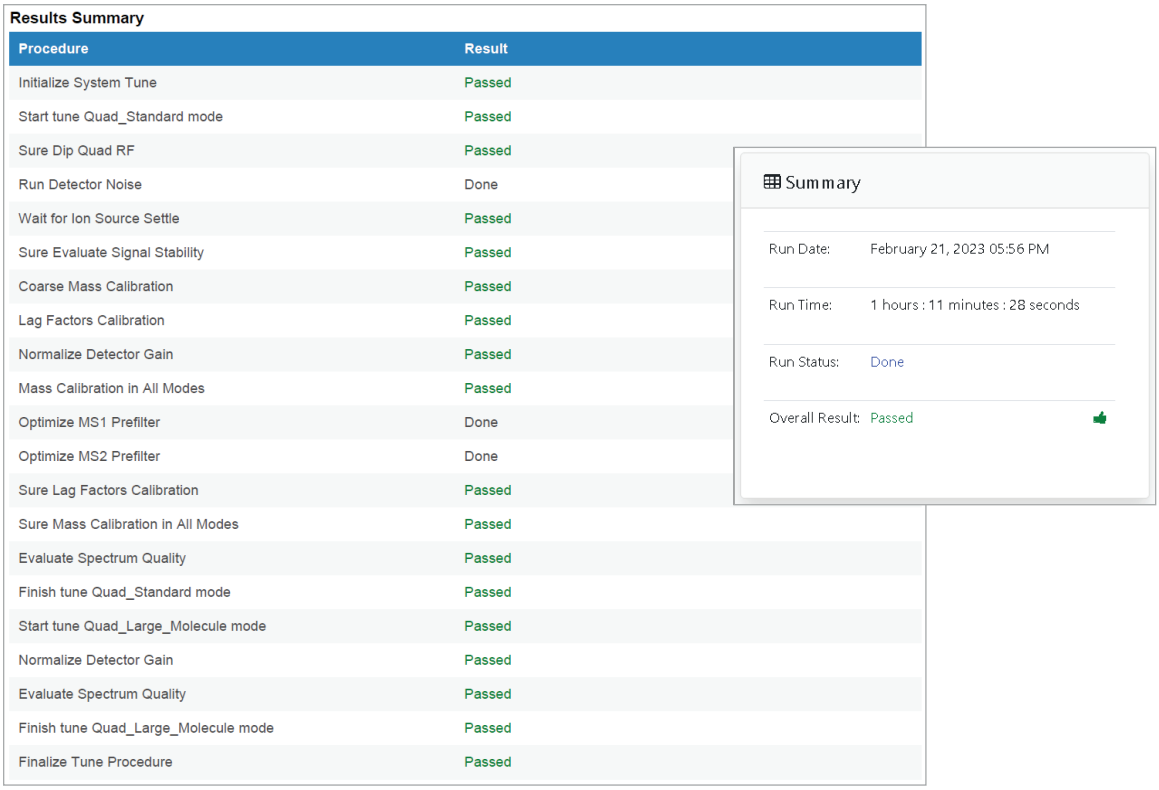
Figure 8. Summary of instrument detection limit results calculated from 1 fg of chloramphenicol on-column, using a 99% confidence threshold for n = 8 injections.

Final instrument status report using Checktune

Prior to any robustness testing and experimentation, the instrument was tuned and calibrated using SWARM Autotune, ensuring optimal voltage settings for good transmission of ions, proper mass calibration, and proper detector response level. The results of the Autotune function are shown in Figure 9.

At the end of robustness testing and negative-mode IDL checkout, the final evaluation of instrument fitness was determined through standard instrument Checktune. The built-in Checktune procedure evaluated several parameters to determine if the instrument is within calibration, output mass spectra are of acceptable quality, and if certain components are within good operational function.

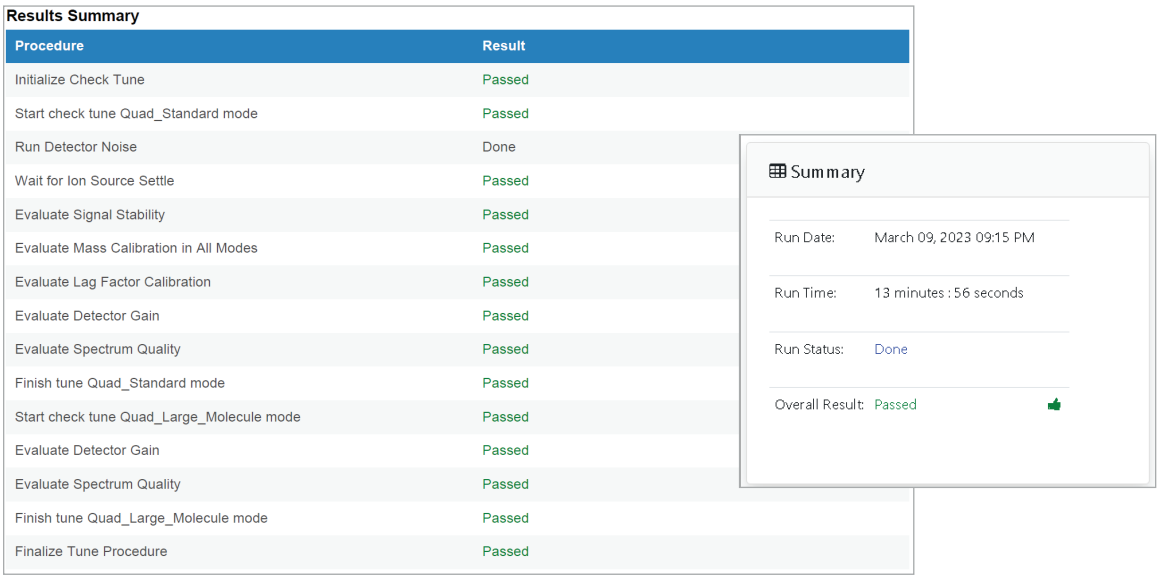
The Checktune results shown in Figure 10 indicate that the instrument was within tolerance, and no issues were flagged at the conclusion of this 16-day robustness experiment.



Results Summary

Procedure	Result
Initialize System Tune	Passed
Start tune Quad_Standard mode	Passed
Sure Dip Quad RF	Passed
Run Detector Noise	Done
Wait for Ion Source Settle	Passed
Sure Evaluate Signal Stability	Passed
Coarse Mass Calibration	Passed
Lag Factors Calibration	Passed
Normalize Detector Gain	Passed
Mass Calibration in All Modes	Passed
Optimize MS1 Prefilter	Done
Optimize MS2 Prefilter	Done
Sure Lag Factors Calibration	Passed
Sure Mass Calibration in All Modes	Passed
Evaluate Spectrum Quality	Passed
Finish tune Quad_Standard mode	Passed
Start tune Quad_Large_Molecule mode	Passed
Normalize Detector Gain	Passed
Evaluate Spectrum Quality	Passed
Finish tune Quad_Large_Molecule mode	Passed
Finalize Tune Procedure	Passed

Figure 9. Autotune results report carried out prior to robustness experimentation.



Results Summary

Procedure	Result
Initialize Check Tune	Passed
Start check tune Quad_Standard mode	Passed
Run Detector Noise	Done
Wait for Ion Source Settle	Passed
Evaluate Signal Stability	Passed
Evaluate Mass Calibration in All Modes	Passed
Evaluate Lag Factor Calibration	Passed
Evaluate Detector Gain	Passed
Evaluate Spectrum Quality	Passed
Finish tune Quad_Standard mode	Passed
Start check tune Quad_Large_Molecule mode	Passed
Evaluate Detector Gain	Passed
Evaluate Spectrum Quality	Passed
Finish tune Quad_Large_Molecule mode	Passed
Finalize Tune Procedure	Passed

Figure 10. Checktune results report carried out after robustness experimentation. The Checktune results list was shorter than Autotune since no tuning or calibration were carried out.

Conclusion

The 6495D triple quadrupole LC/MS system was designed to provide superior robustness and ultimate sensitivity for the most challenging routine analysis. Through this robustness test for analyzing pesticides in black tea matrix, the following was concluded:

- Peak area statistics revealed excellent response reproducibility, spanning over 2,000 injections and 16 days of nonstop operation. All monitored analytes produced a raw peak area abundance RSD < 10% or an IS-corrected peak area abundance ratio RSD < 5%.
- Examination of the ionization/desolvation region showed severe matrix deposition and charring of sample components, which did not interfere with the analysis or instrument performance.
- Immediately after robustness testing, a negative mode sensitivity checkout demonstrated excellent performance for detection of 0.4 fg of chloramphenicol on-column with 99% confidence.
- Immediately after testing, the system successfully passed the Checktune procedure without flagging any issues affecting system performance while demonstrating that system calibration and settings were still within acceptable tolerance.
- These results indicate that the instrument is highly robust and suitable for high-throughput analysis of complex samples.

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