Poster Reprint

ASMS 2023
6495 LC/TQ Launch

Application robustness of the 6495 triple quadrupole LC/MS system for non-stop Pesticide analysis in black tea matrix

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Introduction

System robustness is of utmost importance especially when analyzing samples for routine, in-production, type of analysis. Additionally, when evaluating samples for meaningful scientific results, analysis of a large population of samples is necessary for good population statistics.

The new **6495 triple quadrupole LC/MS system** (G6495D) is equipped with VacShield and iFunnel technology that aims to provide high sensitivity and high-performance analysis while being robust and rugged enough to withstand the effects of deposition from a complex and dirty matrix.

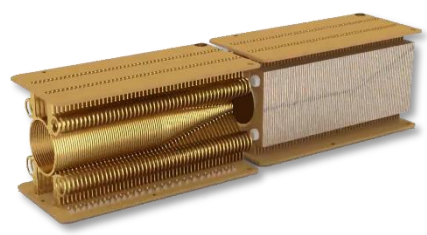
- *VacShield* –ion injector capillary removal mechanism that enables quick routine-maintenance, reduces downtime, and preserves system stability.

- *iFunnel Technology* – a dual-staged stacked ring ion funnel used to compress and concentrate the ion beam. 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 is in good operating condition.

Compared to non-iFunnel systems, the 6495 LC/TQ provides about 10x improvement in signal while providing superior injection-to-injection measurement robustness and precision at sub-millisecond dwell times.

VacShield Technology
Ease of maintenance and robustness



iFunnel Technology
For high sensitivity and precision

The Agilent 6495 triple quadrupole LC/MS system launched at ASMS 2023

Experimental

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 3000xg for 5 minutes. The black tea mixture was added into the Agilent QuEChERS Dispersive SPE for high pigment (10mL Acetonitrile per 2g 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 8g black tea of LC TOF/QTOF/QQQ Pesticide Test Mix (P/N 5190-0449) and 3 isotope labelled compounds (Atrazine-d_5, Diazinon-d_{10}, Dimethoate-d_6) for a final concentration 20pg/µL per analyte.

Sample was injected directly with no further cleanup

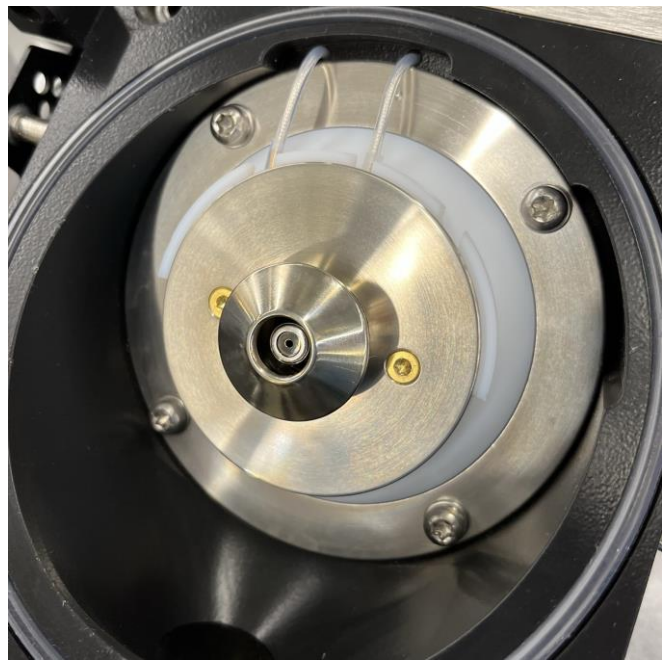
LC/MS Method

LC Parameter	Agilent 1290 Infinity LC
Guard Cartridge	Agilent Poroshell EC C18, 2.1x5 mm, 2.7 µm (P/N 821725-911)
Analytical Column	Agilent Eclipse Plus C18 RRHD, 2.1x50 mm, 1.8 µm (P/N 959757-902) @ 30°C
Injection Volume	2 µL
Mobile Phase	A) H_2O + 5mM NH_4-formate + 0.1% formic acid B) MeOH + 5mM 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

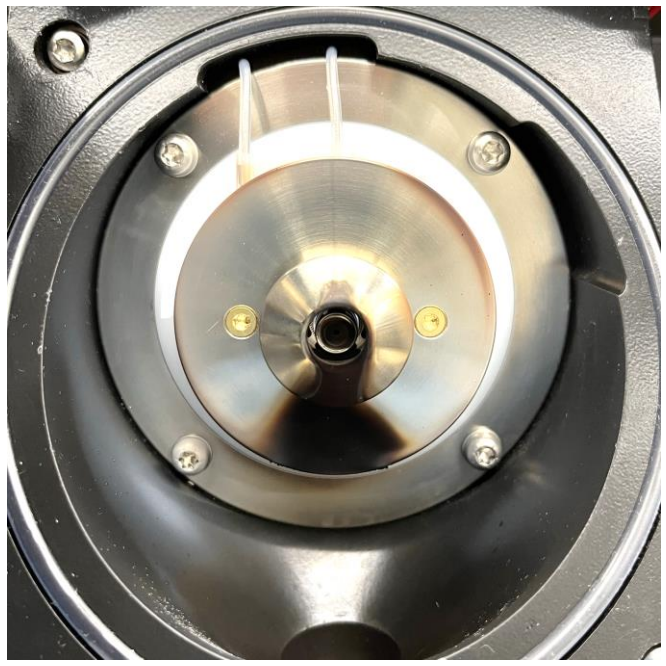
MS Source Parameters	Agilent Jet Stream Source
Drying Gas	15 L/min @ 290°C
Sheath Gas	12 L/min @ 325°C
Nebulizer	40 psi
Capillary Voltage	4000 V (+), 3000 (-)
Nozzle Voltage	1500 V (+/-)

Black Tea Samples Injected

Inlet Before Experiment Run

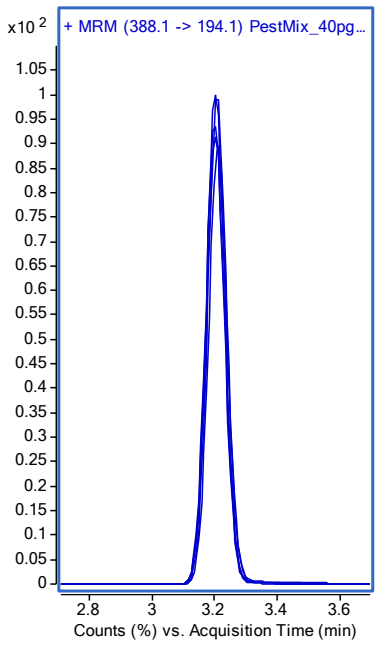


Inlet After Experiment Run

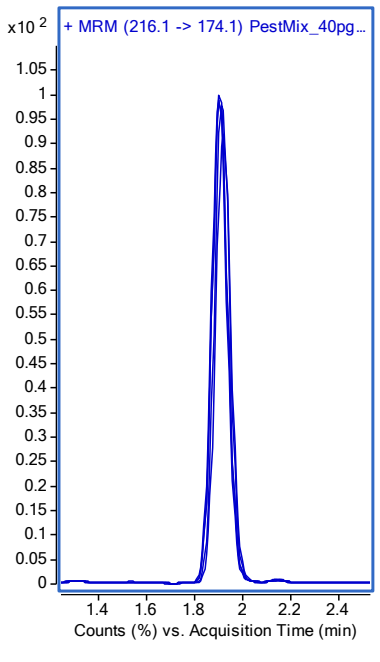


Replicate MRM chromatograms every 500 Injections

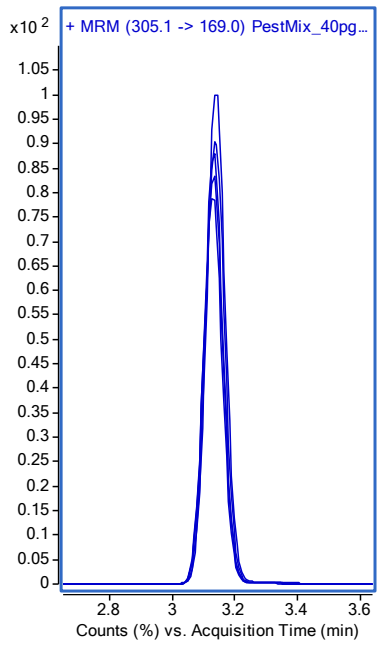
Pyraclostrobin



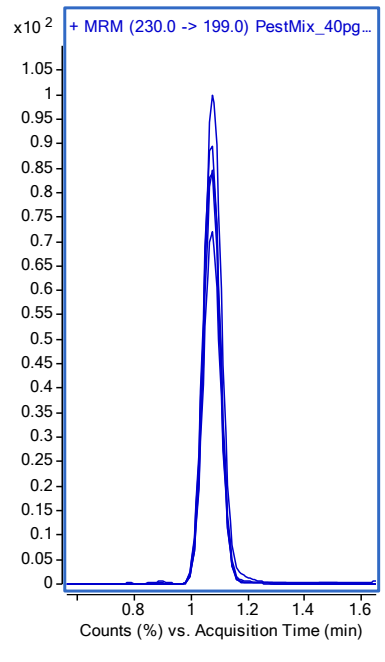
Atrazine



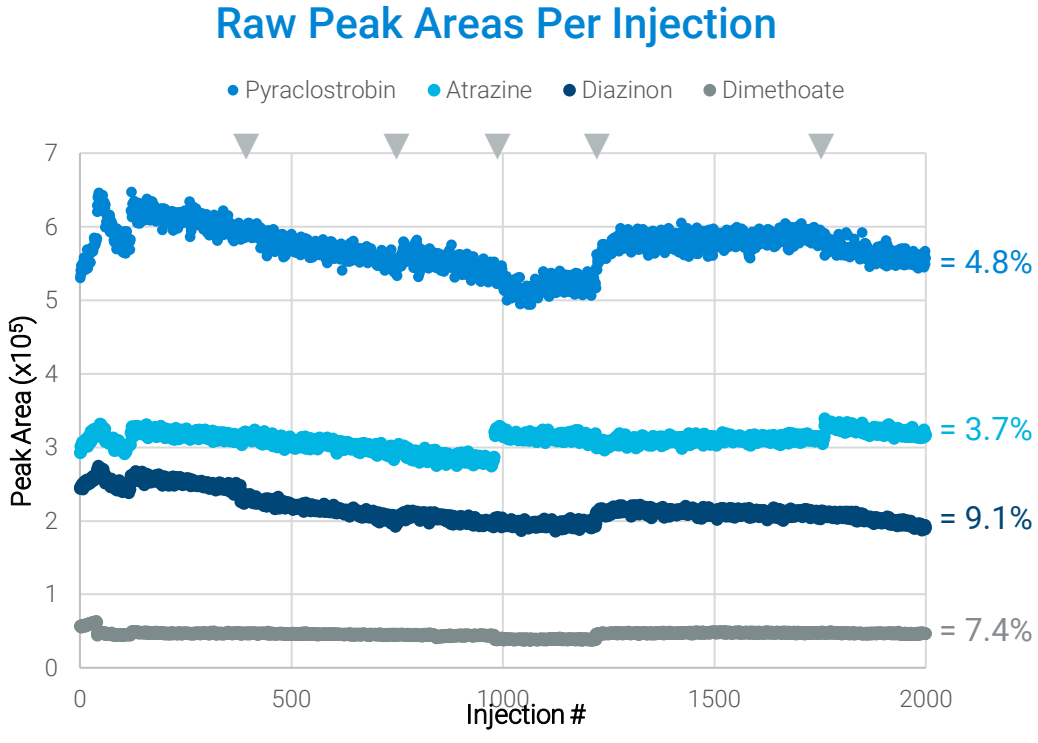
Diazinon



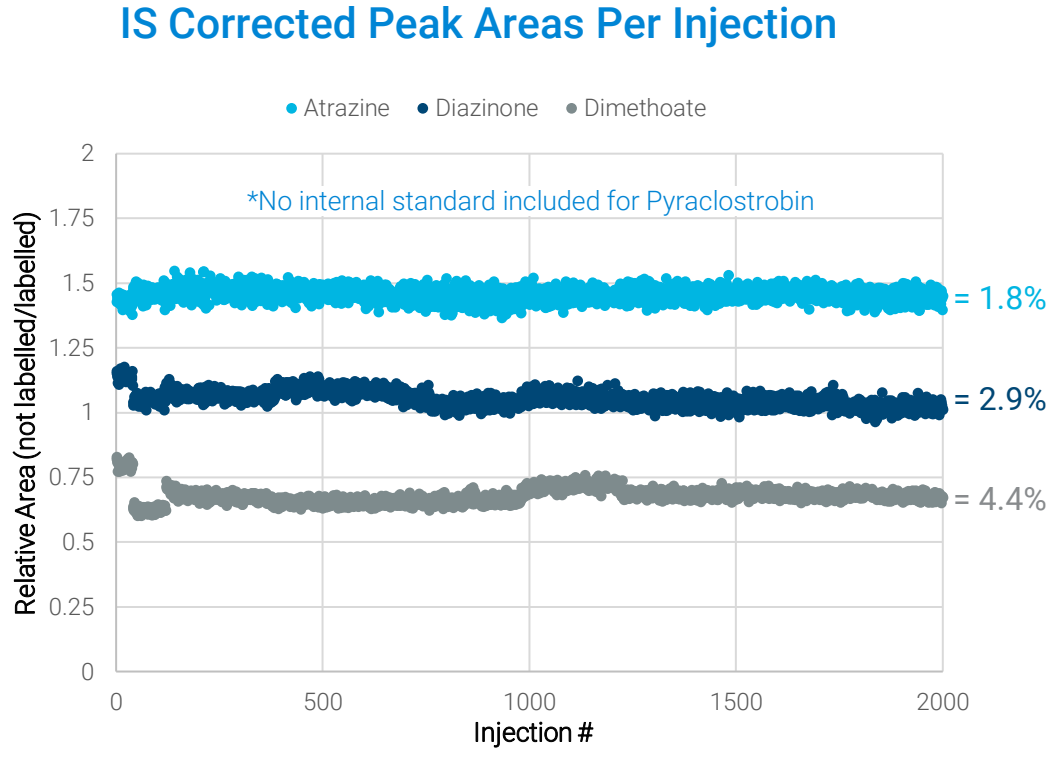
Dimethoate



Raw Peak Areas Per Injection



IS Corrected Peak Areas Per Injection



Results and Discussion

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

- 2000 MRM acquisitions of black tea sample injections
- 400 blank injections
- 100 precursor scans (to check MS1 calibration)
- 100 Full scans (to check MS2 calibration)

The instrument was operated for 16 days uninterrupted. A total black tea matrix volume of 4mL was injected. LC flow was directed to the ion source at all time during the chromatography run. During this time, no MS maintenance events such as cleaning, re-tuning, or mass calibration was carried out.

Raw Peak Areas for each analyte were plotted as a function of injection #. Raw Area abundance breakpoints were observed and coincided with LC-maintenance events such as solvent top-off and clogged injector needle change (marked by ▼). The first 40 injections had varied abundance due to needle wash not flowing correctly.

When plotting each analyte against their isotopically labeled internal standard, LC-maintenance events were corrected for, except for carryover, which remained until the next sample vial. It is important to note that this robustness test was completed after a set number of sample injections and physical time constraint; i.e. not due to instrument failure.

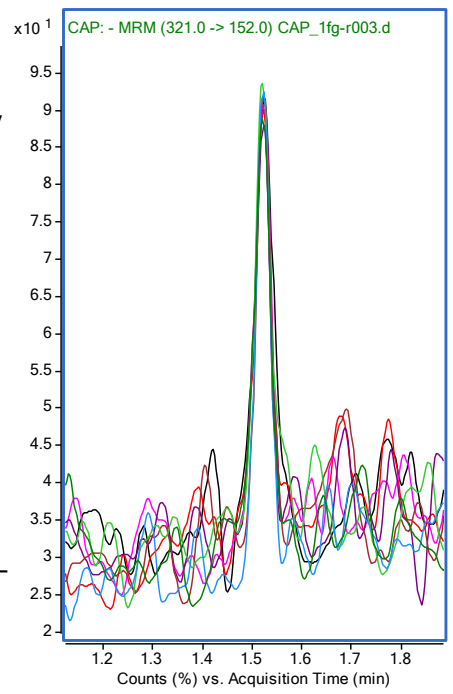
Peak area statistics for each monitored analyte

Analyte	Raw Peak Area %RSD	IS Corrected Peak Area Ratio %RSD
Pyraclostrobin	4.8%	No ISI correction
Atrazine	3.7%	1.8%
Diazinon	9.1%	2.9%
Dimethoate	7.4%	4.4%

Negative Mode Sensitivity Check (1fg Chloramphenicol)

Once the robustness test was concluded, a Negative Mode Sensitivity Check was immediately executed on the system to test for negative mode instrument detection limit of 1 fg Chloramphenicol on-column.

Using *n*=8 injections, the response reproducibility of Chloramphenicol was determined to be RSD=13.9% indicating good sensitivity and reproducibility even without follow-up cleaning or maintenance.

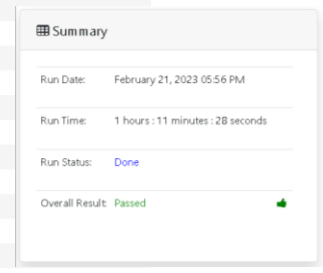


The system was found to be within tolerance and no issues with the instrument were flagged at the conclusion of the robustness test.

Tune Results Before Experiment (Autotune)

Results Summary

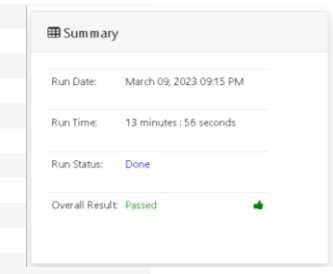
Procedure	Result
Initialize System Tune	Passed
Start tune Quad_Standard mode	Passed
Sure Dip Quad RF	Passed
Run Detector Noise	Done
Wait for Ion Source Settle	Passed
Sure Evaluate Signal Stability	Passed
Coarse Mass Calibration	Passed
Lag Factors Calibration	Passed
Normalize Detector Gain	Passed
Mass 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



Tune Verification After Experiment (Checktune)

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



*Checktune results list was shorter than Autotune since no tuning or calibration was carried out

Conclusions

- Peak area statistics revealed excellent response reproducibility, spanning over 2,000 injections and 16 days of non-stop operation. (Analytes RSD<10%)
- Examination of the ionization region shows severe matrix deposition, but did not interfere with the analysis
- Immediately after robustness testing, Negative Mode Sensitivity Check demonstrated excellent performance for detection of 1fg Chloramphenicol on-column.
- The system was able to pass the Checktune procedure without flagging any issues affecting system performance.

References

5991-6357EN – Routine Multiresidue Pesticide Analysis using the Agilent 6470 Triple Quadrupole Mass Spectrometer

https://www.agilent.com/en/promotions/asms

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