Unmatched Analytical Sensitivity in Targeted MS Detection – Pushing the analytical boundary with the Agilent 6495 Triple Quadrupole LC/MS

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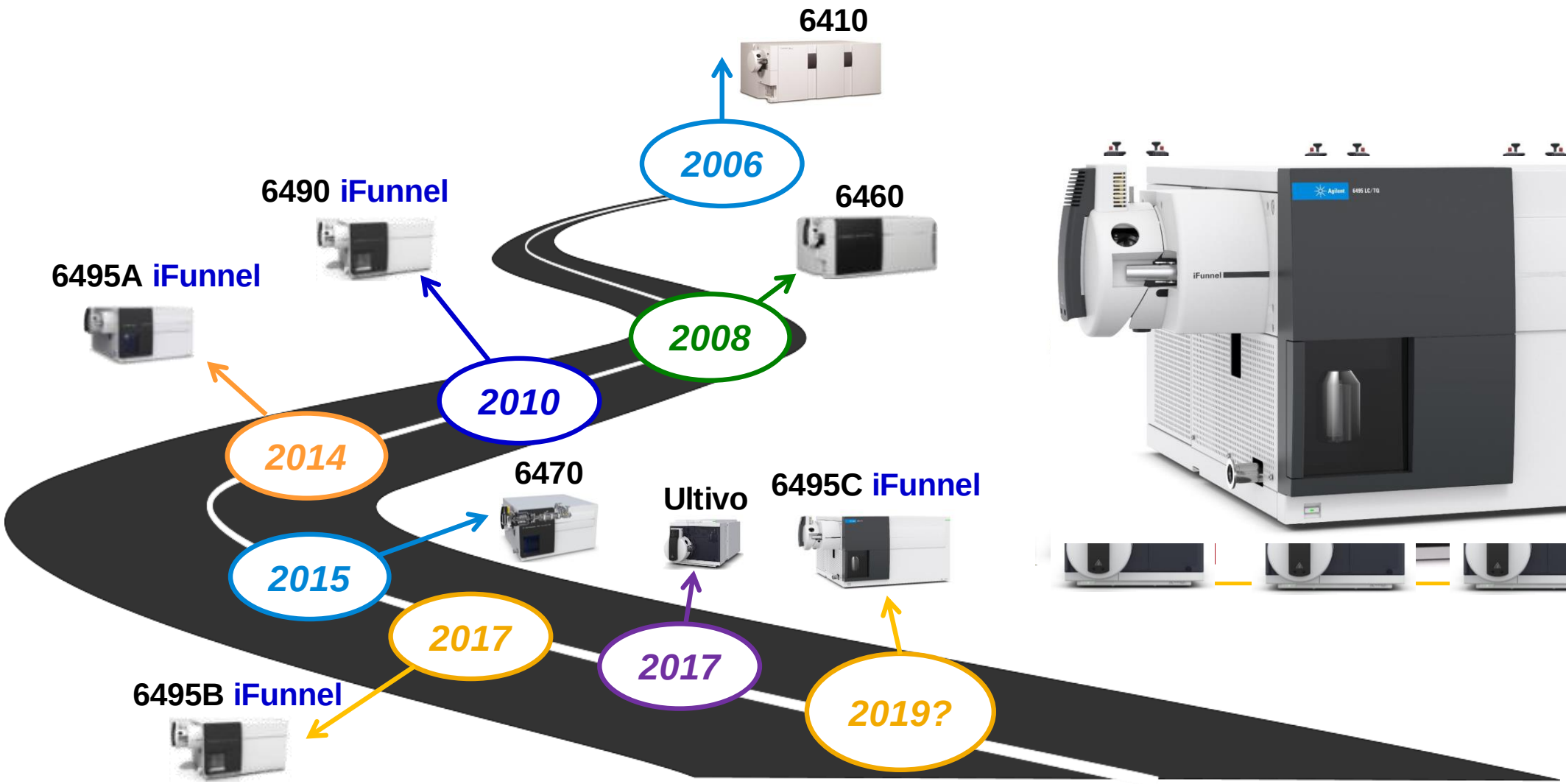
LC/MS Triple Quadrupole Product Manager

Agilent Triple Quadrupole LC/MS development



The 6495C Triple Quadrupole LC/MS with iFunnel technology

Product Feature Overview

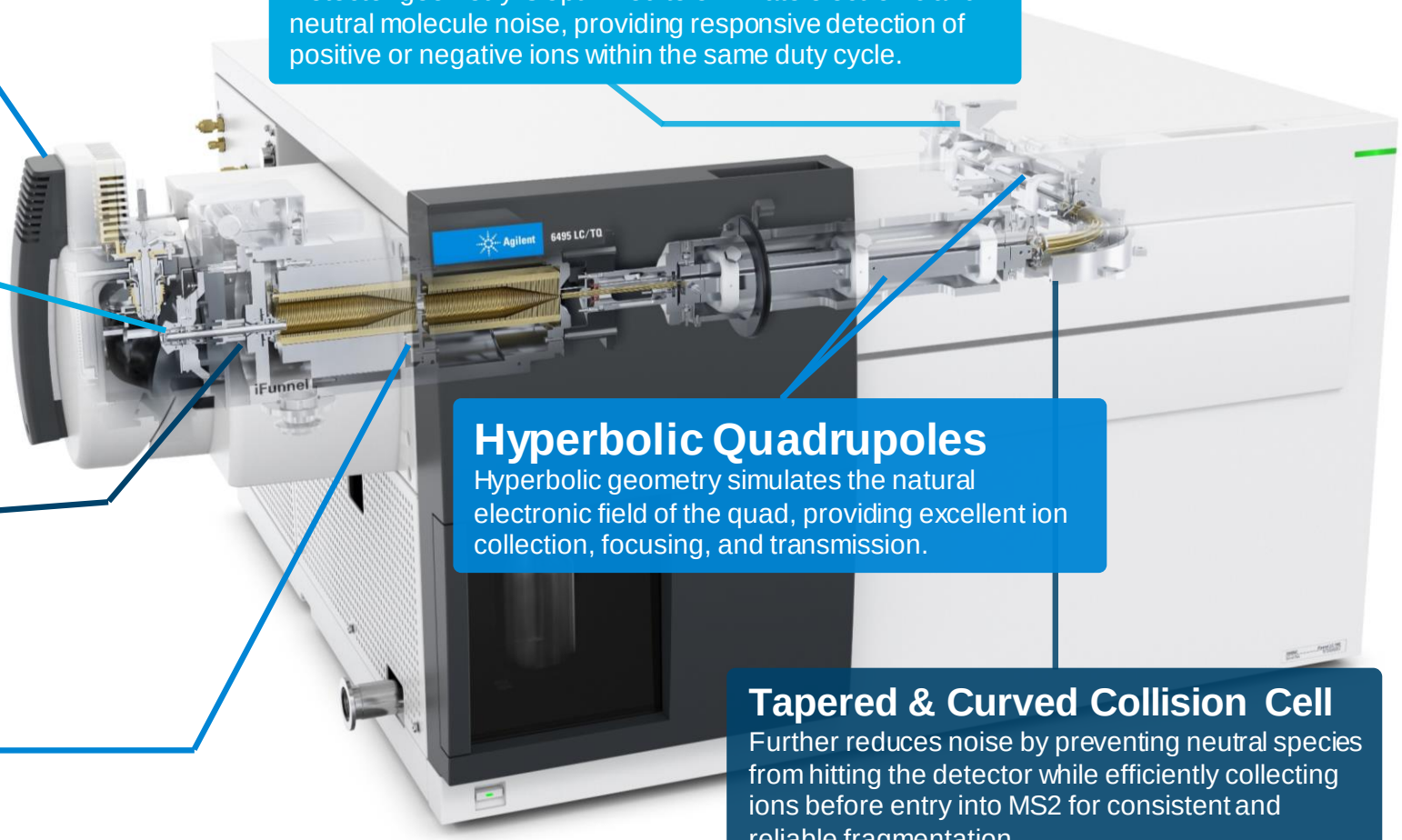
Agilent Jet Stream
Highly efficient desolvation produces 5x more ions than standard electrospray due to thermal focusing and heat transfer.

Hexabore Capillary
Six capillary inlets increases ion flux into the ion funnel by 10x for greater analytical sensitivity.

VacShield Technology
Vent free mechanism for rapid removal and maintenance of the capillary. No timing or manual switching required.

3rd Generation Ion Funnel
Collects and focuses stray ions while allowing neutrals to be efficiently pumped out for exceptionally high analytical sensitivity and reduced noise from neutrals.

Innovative HED and Detector Horn Chamber
Detector geometry is optimized to eliminate electronic and neutral molecule noise, providing responsive detection of positive or negative ions within the same duty cycle.

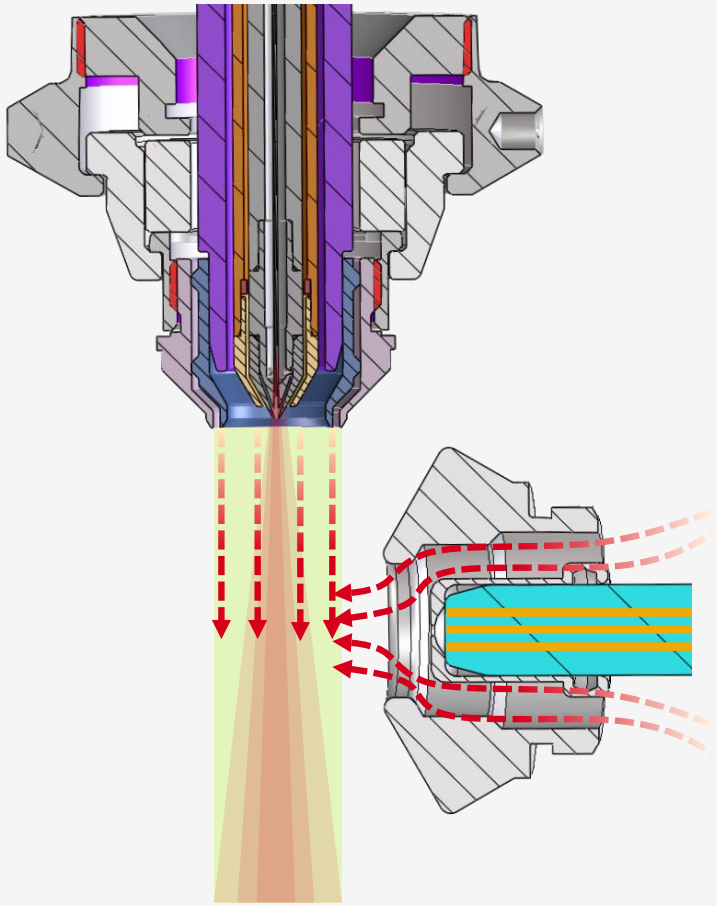


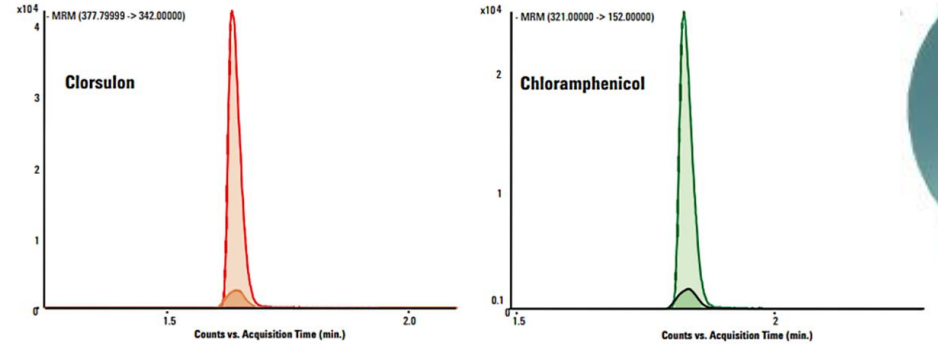
Hyperbolic Quadrupoles
Hyperbolic geometry simulates the natural electronic field of the quad, providing excellent ion collection, focusing, and transmission.

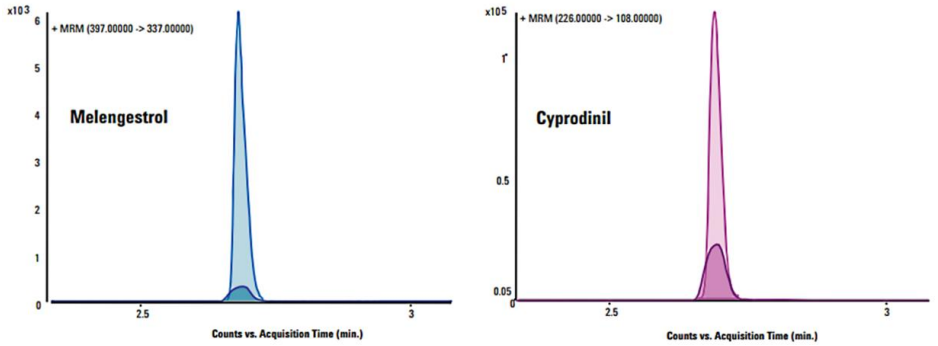
Tapered & Curved Collision Cell
Further reduces noise by preventing neutral species from hitting the detector while efficiently collecting ions before entry into MS2 for consistent and reliable fragmentation.

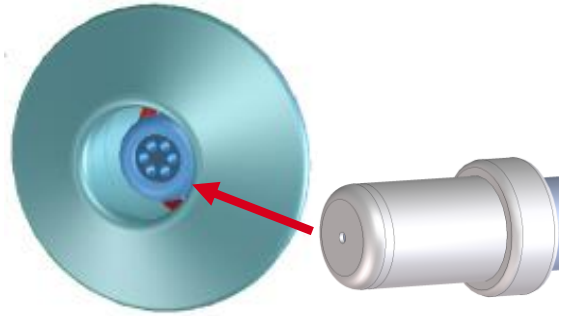
Agilent Jet Stream (AJS) source with Hexabore Capillary

Thermal focusing produces efficient heating and desolvation of droplets









Sample More Ions

The hexabore capillary has 6-inlets for greater sampling of the ion cloud. This results in more ions entering the iFunnel than a single bore capillary.

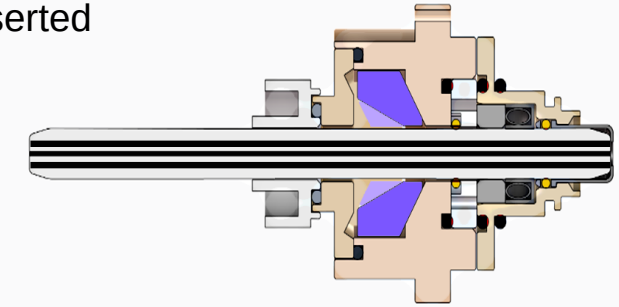
Enhanced Desolvation and Ionization with AJS

AJS features two sources contributing to greater desolvation – Sheath gas emitted from the nebulizer region confines and focuses the nebulizer spray. Counter-current drying gas flows against incoming droplets to expose bare ions for entrance into the hexabore capillary.

Capillary maintenance made simpler with VacShield

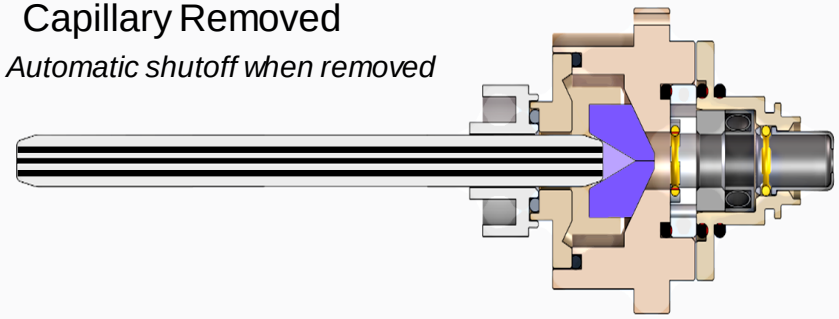
Rapid removal of capillary with automatic sealing

Capillary Inserted

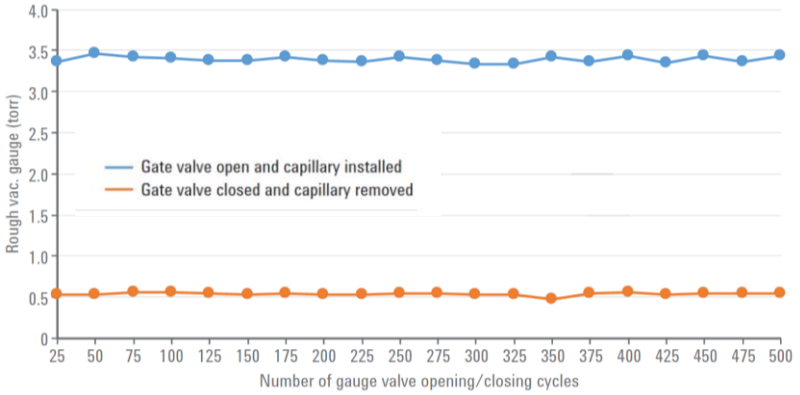


Capillary Removed

Automatic shutoff when removed

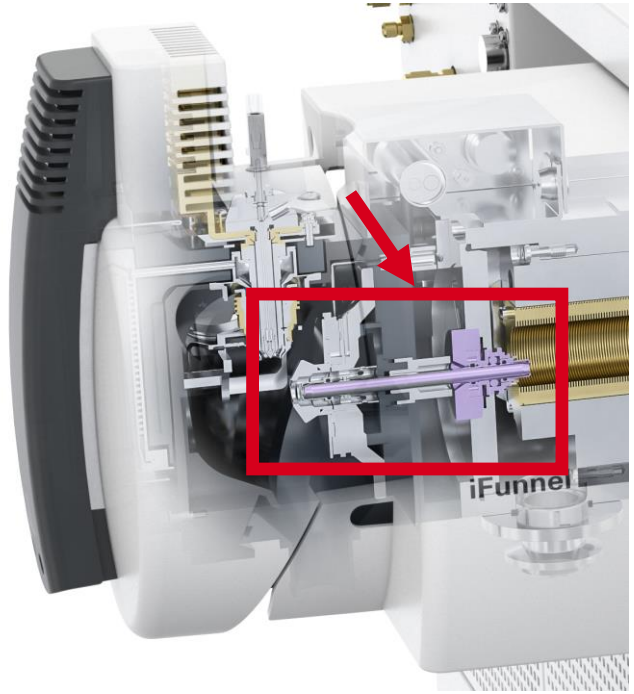


Demonstration of capillary insertion and removal cycles



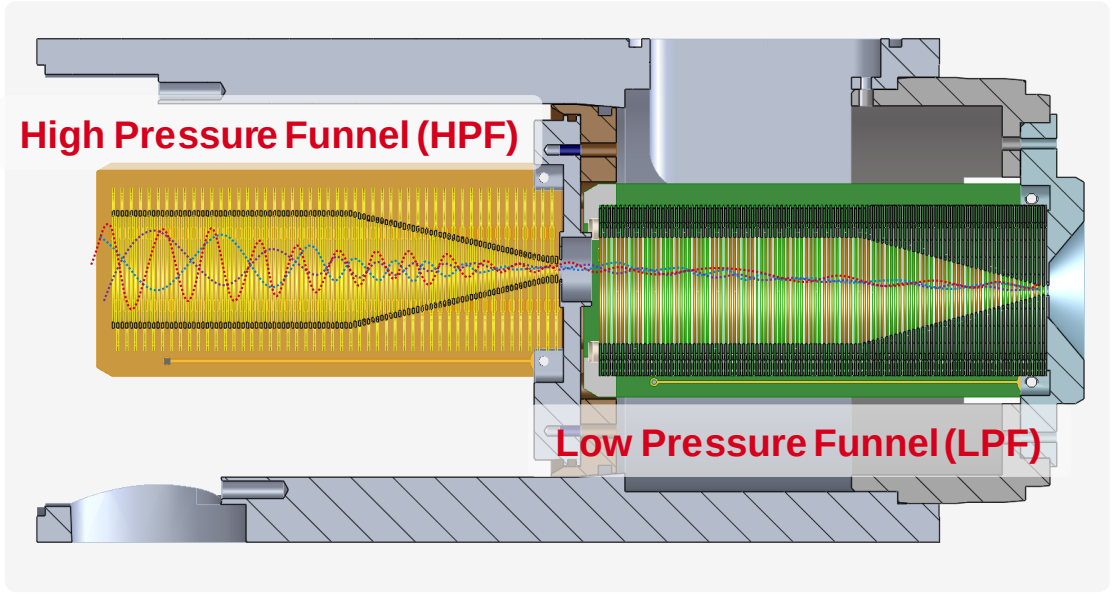
VacShield on the 6495C

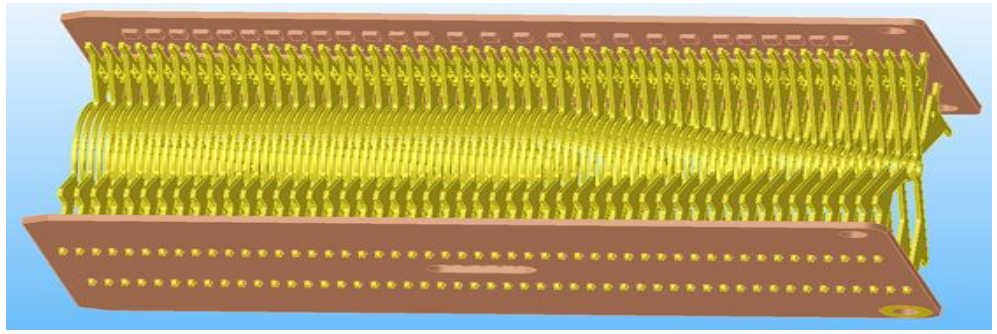
With VacShield, capillary maintenance is made easy; no manual valves, no venting, no downtime.

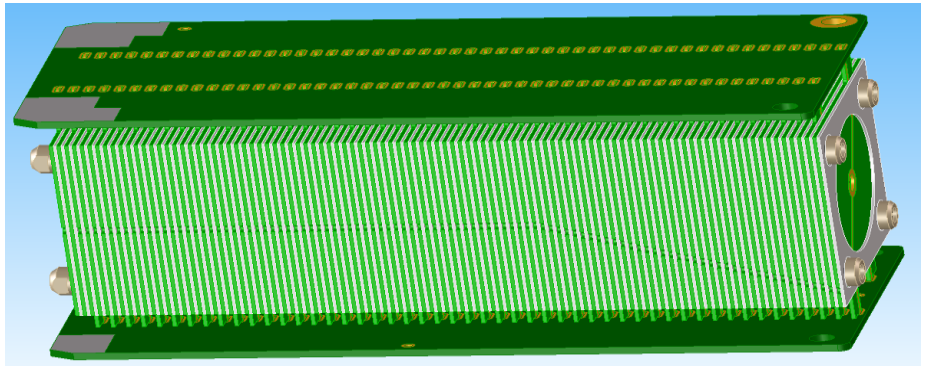


Third-Generation iFunnel

Next-generation ion funnel provides high precision & analytical sensitivity

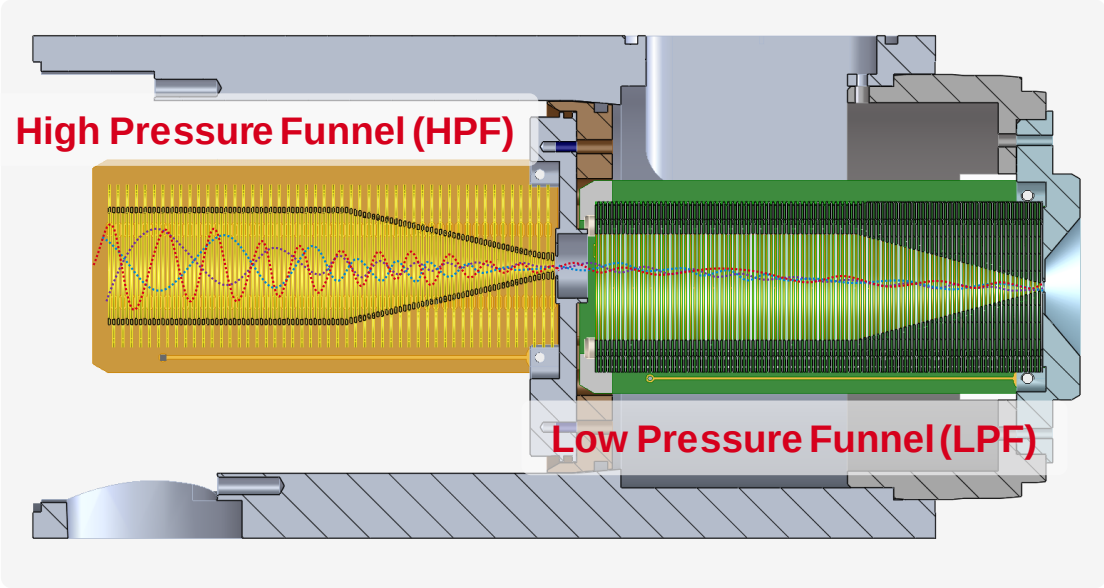






Third-Generation iFunnel

Next-generation ion funnel provides high precision & analytical sensitivity

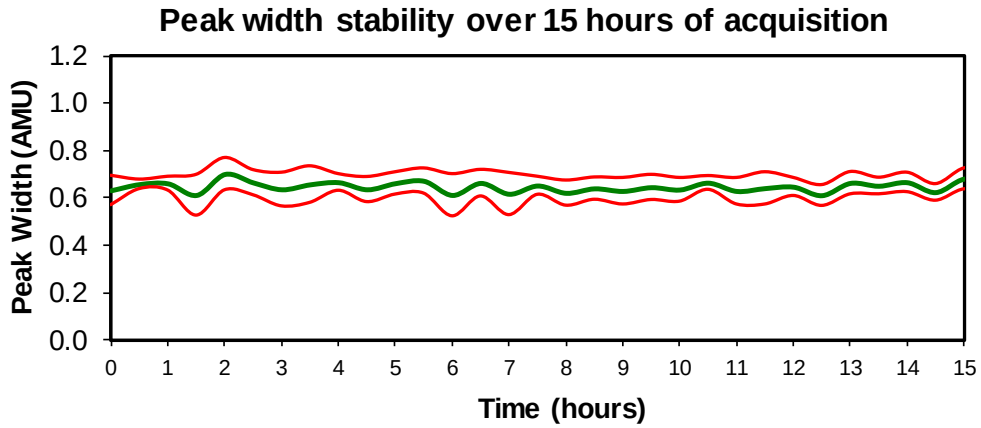


MS120 Vacuum Pump

The inclusion of the MS120 pump is closely tied with the high performance of the third-generation iFunnel.

Agilent's iFunnel technology

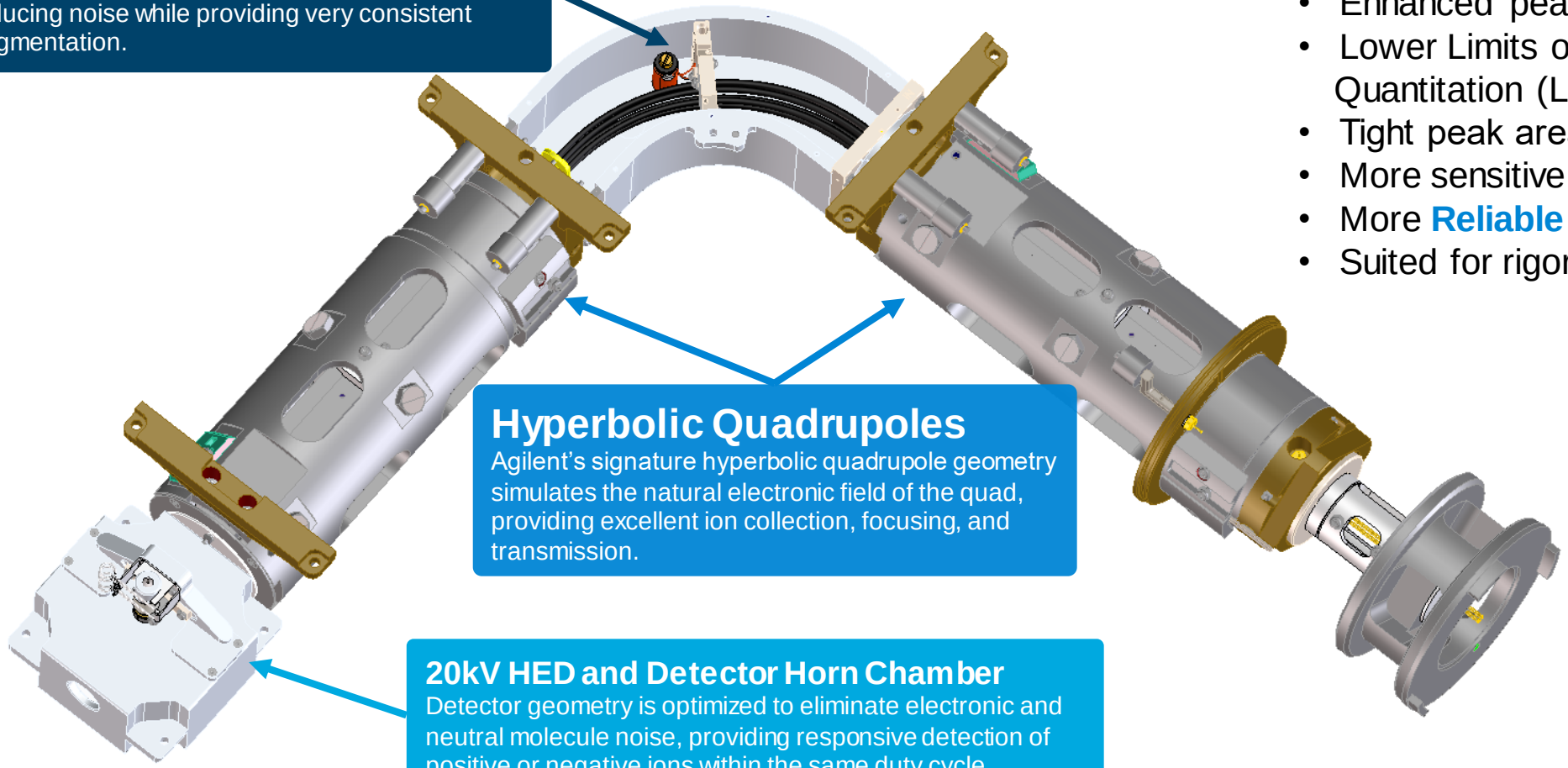
With a carefully engineered design, Agilent's iFunnel technology provides the 6495C with unprecedented analytical sensitivity, signal stability, and the industry's lowest IDLs.



Curved Ion Optics Rail

Continual development & improvement for the highest sensitivities

Tapered & Curved Collision Cell
Prevents neutral species from hitting the detector, reducing noise while providing very consistent fragmentation.



Hyperbolic Quadrupoles
Agilent's signature hyperbolic quadrupole geometry simulates the natural electronic field of the quad, providing excellent ion collection, focusing, and transmission.

20kV HED and Detector Horn Chamber
Detector geometry is optimized to eliminate electronic and neutral molecule noise, providing responsive detection of positive or negative ions within the same duty cycle.

Customer Application Outcomes:

- Enhanced peak area response
- Lower Limits of Detection (IDL) and Quantitation (LLOQ)
- Tight peak area %RSD's
- More sensitive and precise
- More **Reliable** and **Robust**
- Suited for rigorous **Routine** analysis

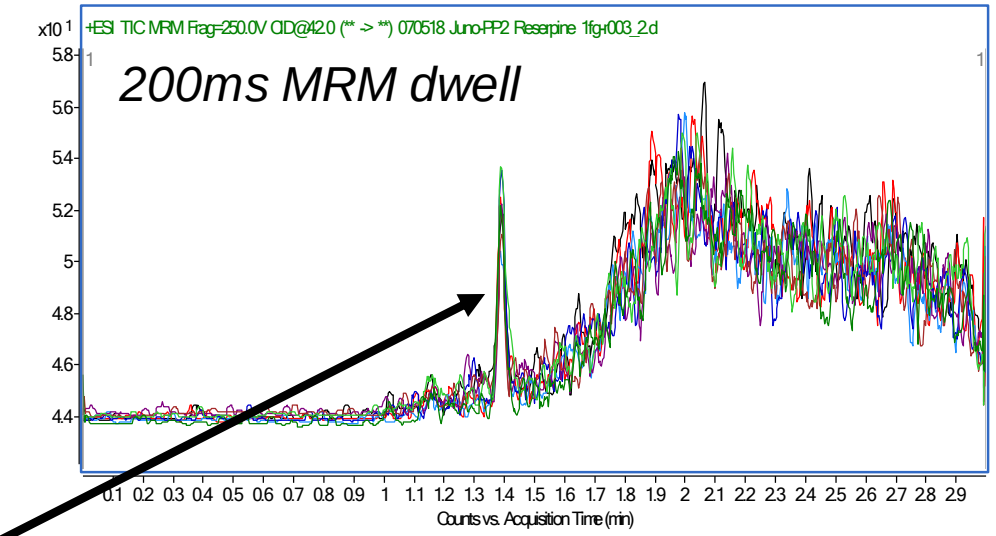
Instrument Detection Limit (IDL) of the 6495C

IDL = (RSD) × (t-statistic) × (conc on-column)

Instrument Detection Limit

IDL is a statistical estimation of the minimal amount on column that can be detected. It considers multiple factors:

- Peak area of a sample
- Injection-injection reproducibility
- T-tests at 99% confidence (noise ≠ signal)

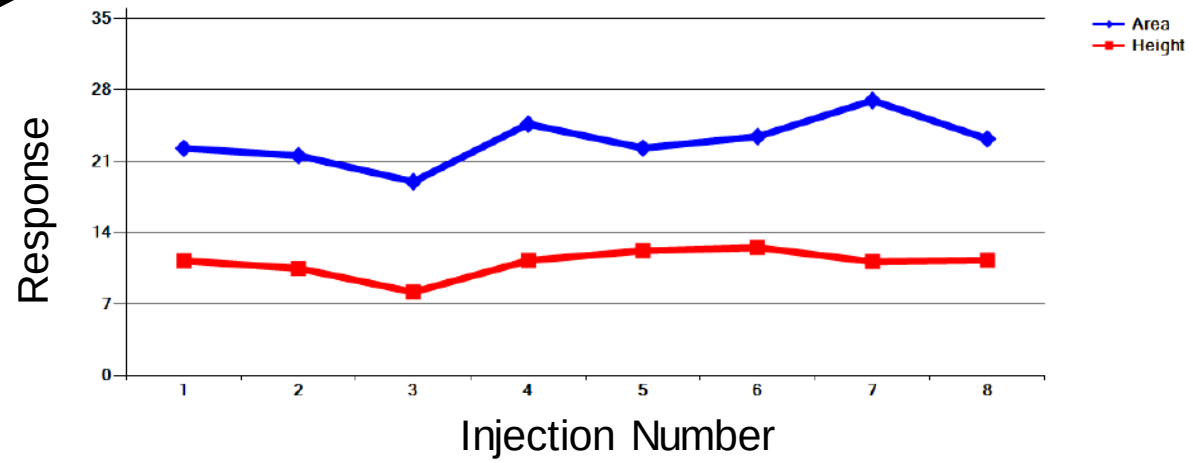


Based on 1fg of Reserpine on-Column

IDL = (0.101) × (2.998) × (1 fg)

IDL = 0.30 fg on-Column

Lots of margin to confidently detect closer to the baseline!



6495C Technical Brief – IDL Measurements at 0.5 ms and 1 ms

Instrument conditions change at reduced dwell times, IDL characterizes its performance limit

Not your typical IDL measurement

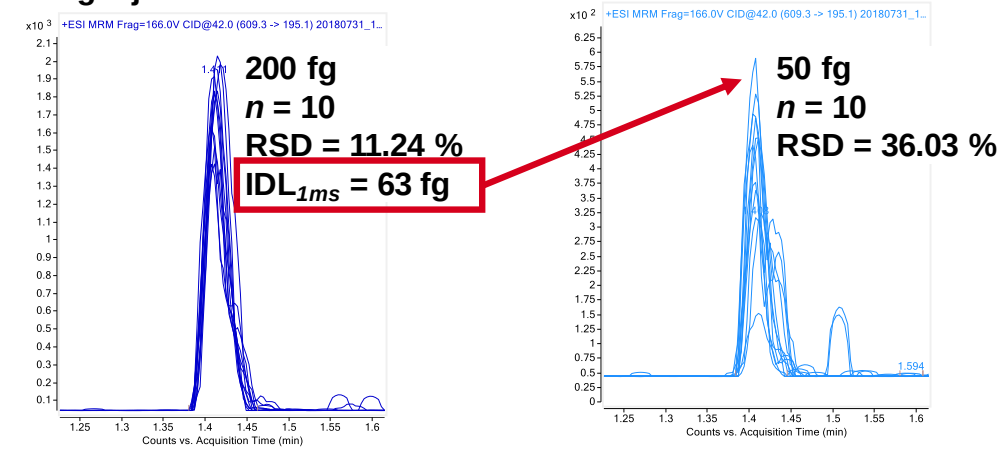
Simulating a busy MRM method, reserpine IDL was characterized using 0.5 ms and 1 ms dwell times. The study demonstrates the ability to confidently detect analytes at these settings while still able to confidently acquire near the instrument's detection limit.

Acquisition Dwell Times

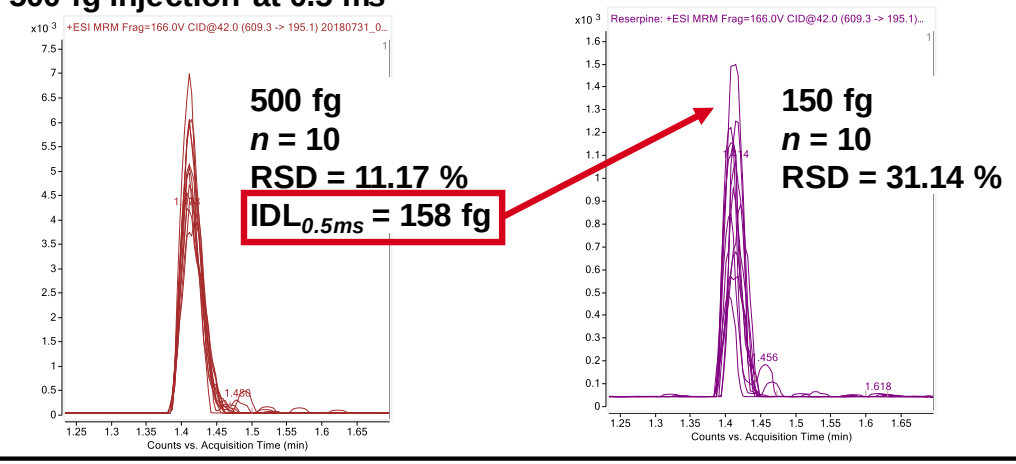
Dwell Time	on-Column Amt.	Calculated IDL
200 ms	1 fg	0.30 fg
1 ms	200 fg	63 fg
0.5 ms	500 fg	158 fg

Determination of IDL **Confirmation of IDL**

200 fg injection at 1 ms

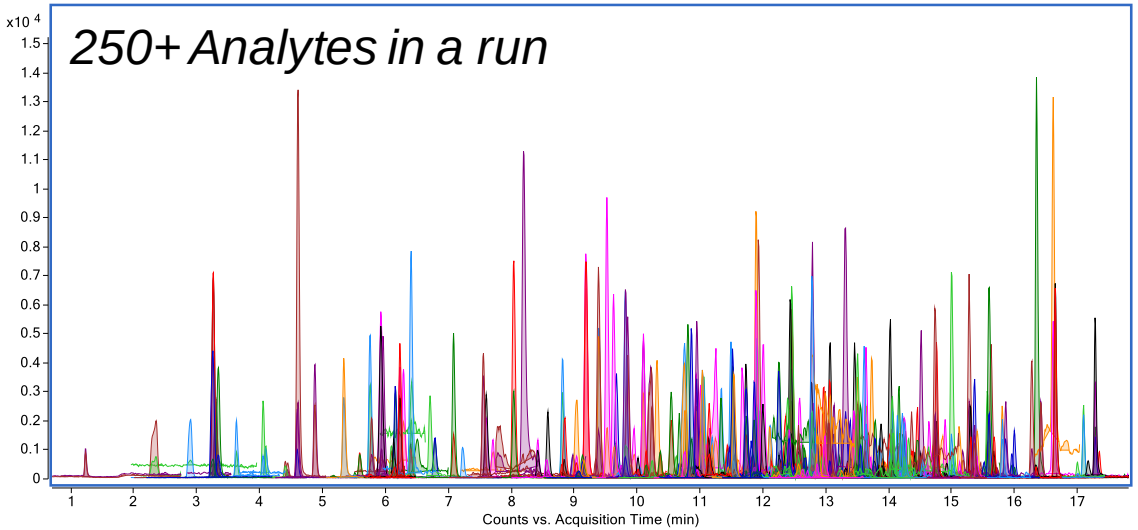


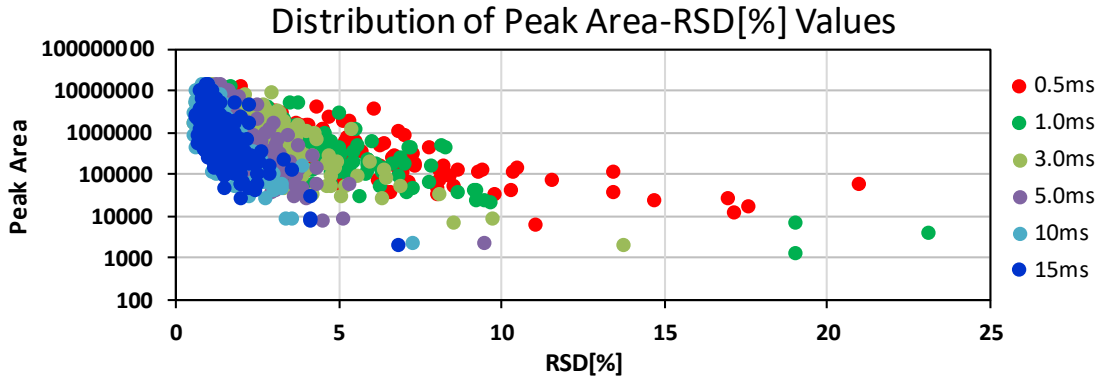
500 fg injection at 0.5 ms



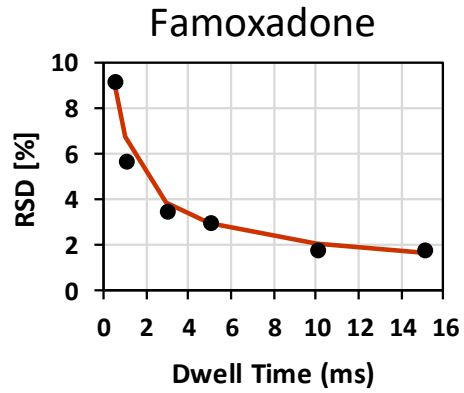
Impact of Dwell Time and Ion Flux on MRM Measurement Precision

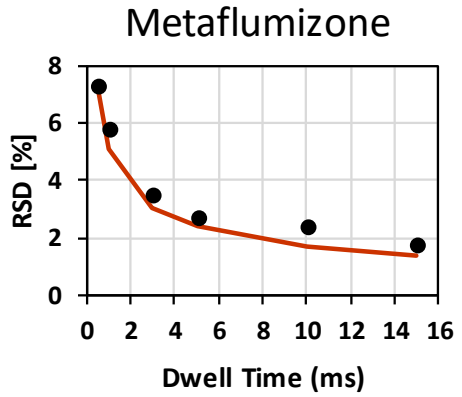
Check out this poster by Behrooz Zekavat (WP-444)

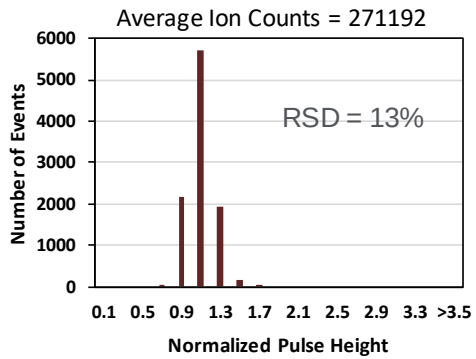


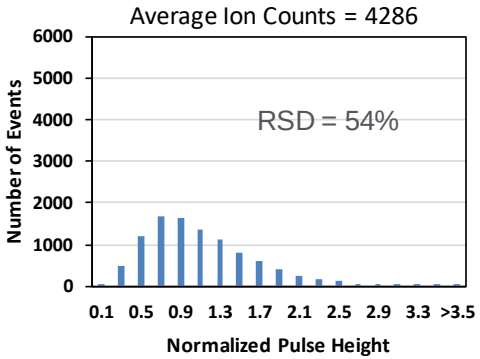


Injection-injection variability at various dwell times









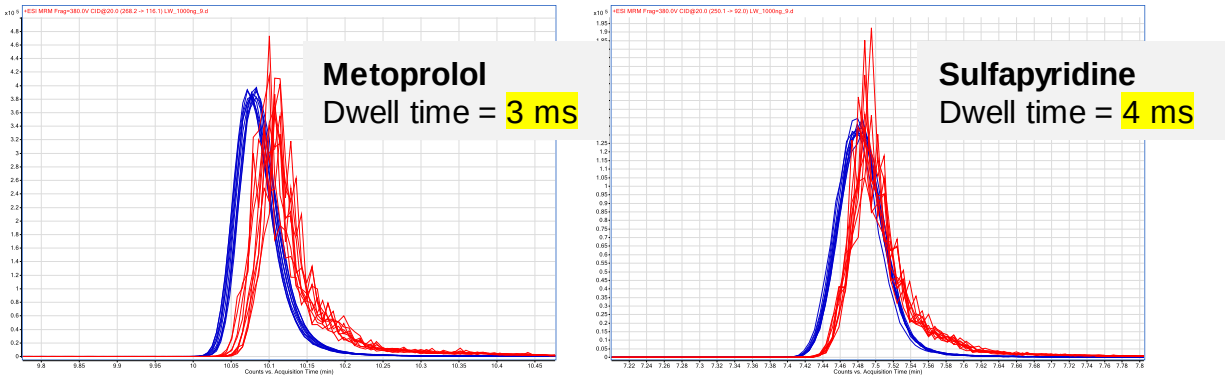
6495C Technical Brief – LC Peak Shape at Low Dwell Times

Superior ion sampling stability produces improved LC peak shape

Ideal chromatographic peak shape

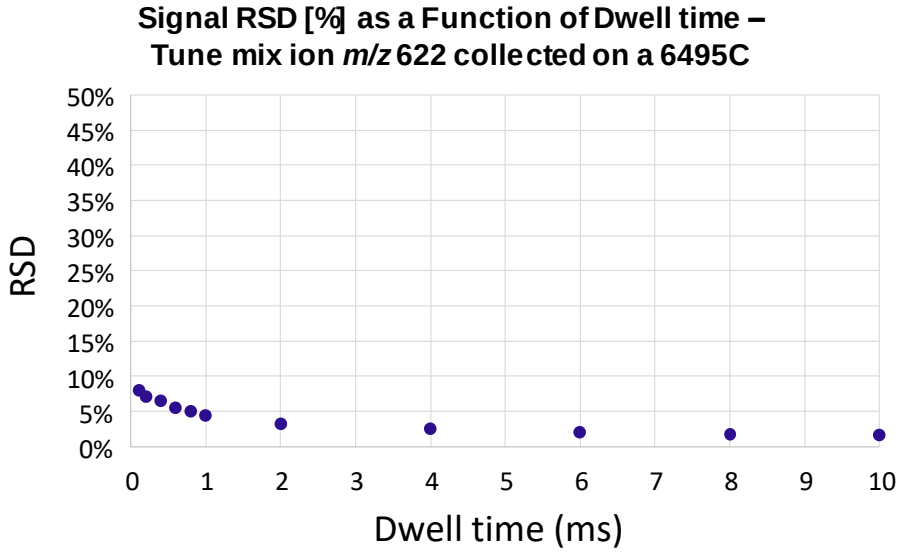
technological innovations in 6495C result in highly synchronous hardware, providing effortless chromatographic peak shape during low dwell time MRM analysis.

Comparison of two iFunnel designs – Blue trace on 6495C



Metoprolol
Dwell time = 3 ms

Sulfapyridine
Dwell time = 4 ms

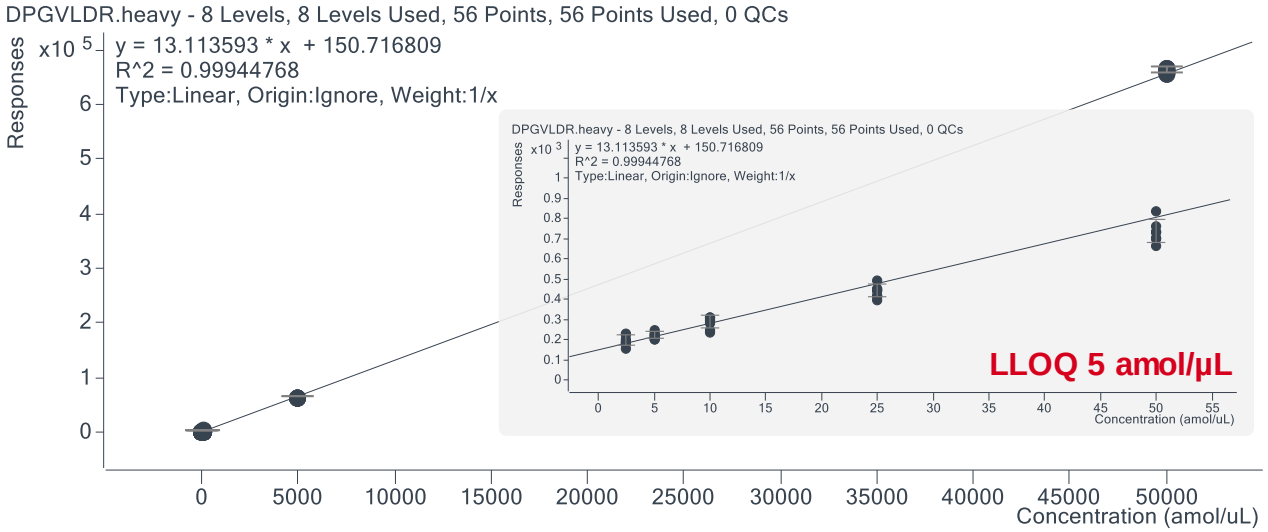


Exceptional signal stability

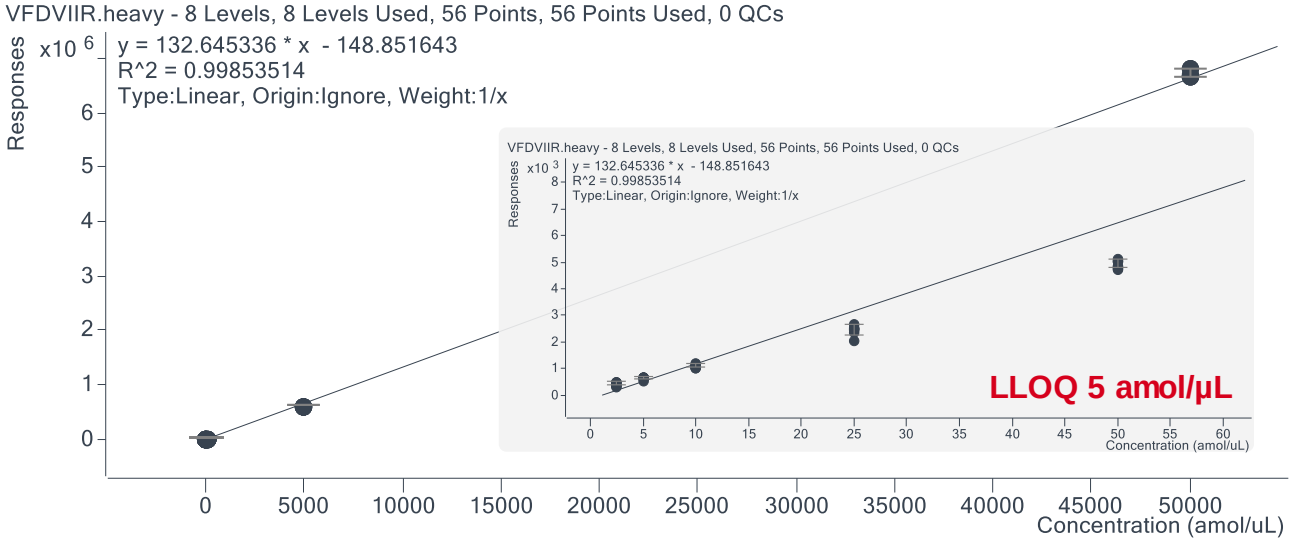
Presented is true signal stability of tune mix at various dwell times, demonstrating the exceptional stability of your signal for every analysis.

Application Brief – Peptide Quantitation

Peptide quant for host cell proteins in a purified mAb protein matrix using stable isotope-labeled standard peptides



Protein name/ molecular weight	Protein S100-A11 (G3HUU6) / 11,241Da	
Peptide sequences	DPGVLDR	
Original SIL peptide concentration (amol/µL)	%RSD (n=7)	accuracy
2.5	16.10%	152.80%
5	**11.60%**	**118.20%**
10	10.50%	89.70%
25	20.90%	76.20%
50	18.10%	82.00%
100	16.40%	86.40%
5000	0.70%	94.00%
50000	0.70%	100.70%



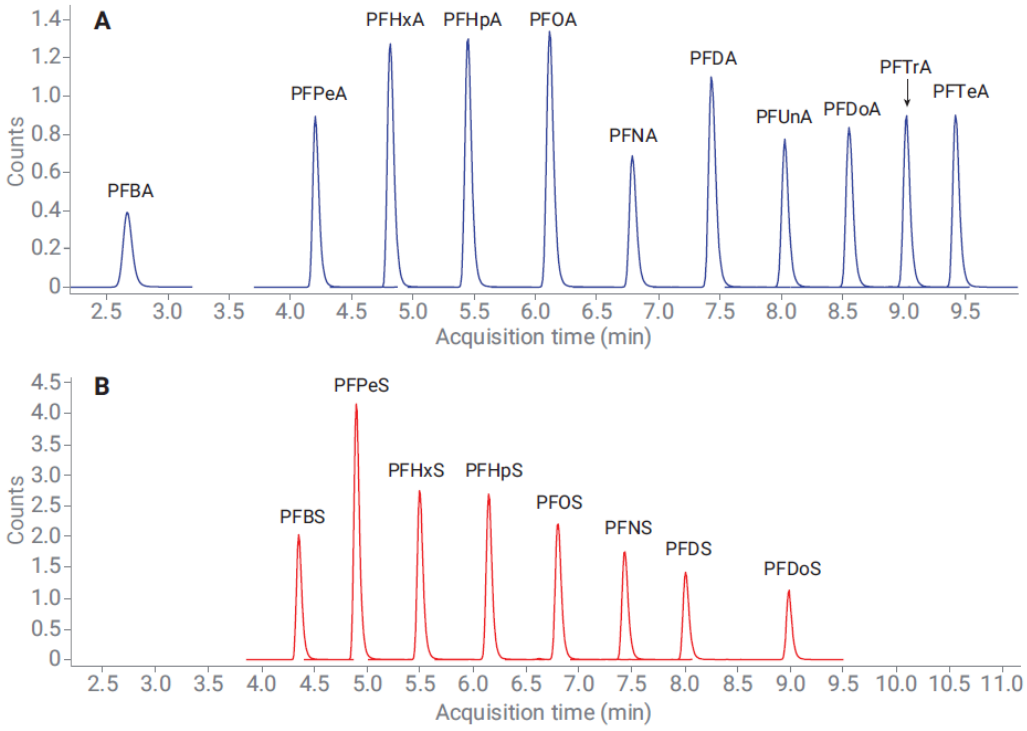
Protein name/ molecular weight	Histidyl-tRNA synthetase (P12081) / 58233 Da	
Peptide sequences	VFDVIIR	
Original SIL peptide concentration (amol/µL)	%RSD (n=7)	accuracy
2.5	11.60%	168.00%
5	**8.70%**	**116.70%**
10	7.40%	92.10%
25	9.50%	77.00%
50	4.10%	77.60%
100	3.70%	77.80%
5000	1.40%	89.70%
50000	0.80%	101.10%

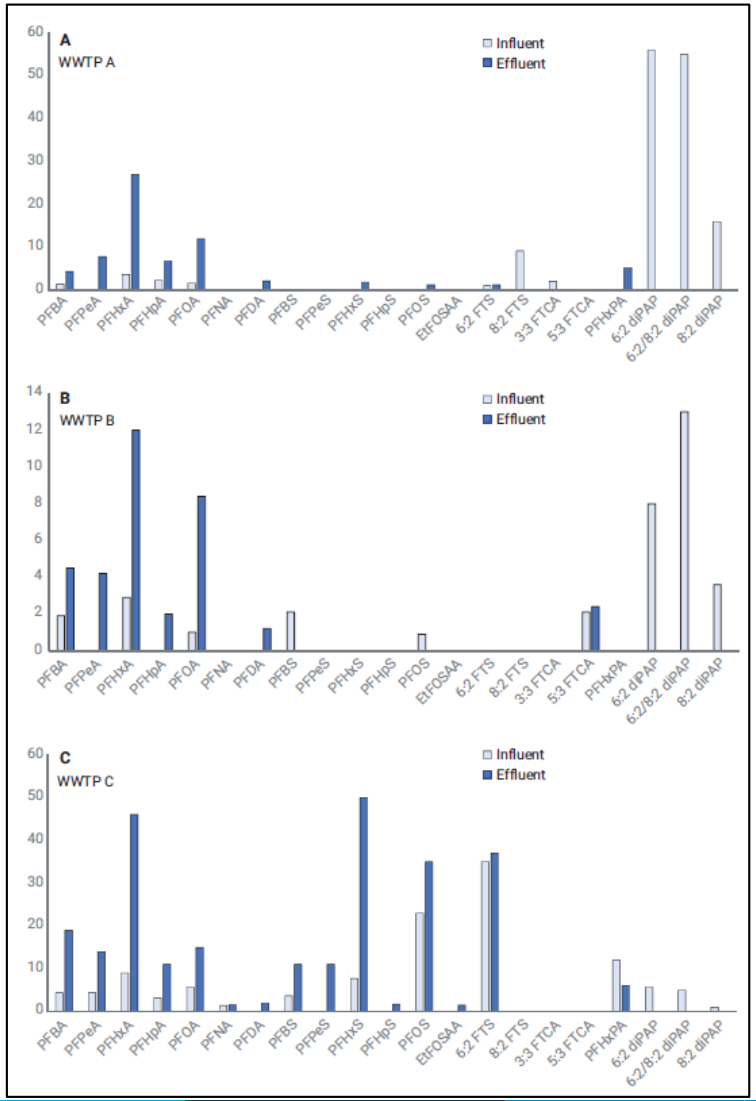
Application – Known and Emerging PFAS in Water

Minimal sample prepration and concentration

Compound	MDL (ng/L)	MQL (ng/L)
PFBA	0.59	0.75
PFPeA	0.71	0.89
PFHxA	0.87	1.1
PFHpA	0.84	1.1
PFOA	0.28	0.35
PFNA	0.61	0.77
PFDA	0.71	0.89
PFUnA	0.80	1.0
PFDoA	1.2	1.5
PFTrA	1.4	1.8
PFTeA	0.67	0.84
PFBS	0.49	0.62
PFPeS	1.2	1.5
PFHxS	0.69	0.88
PFHpS	0.79	1.0
PFOS	0.78	1.0
PFNS	1.0	1.3

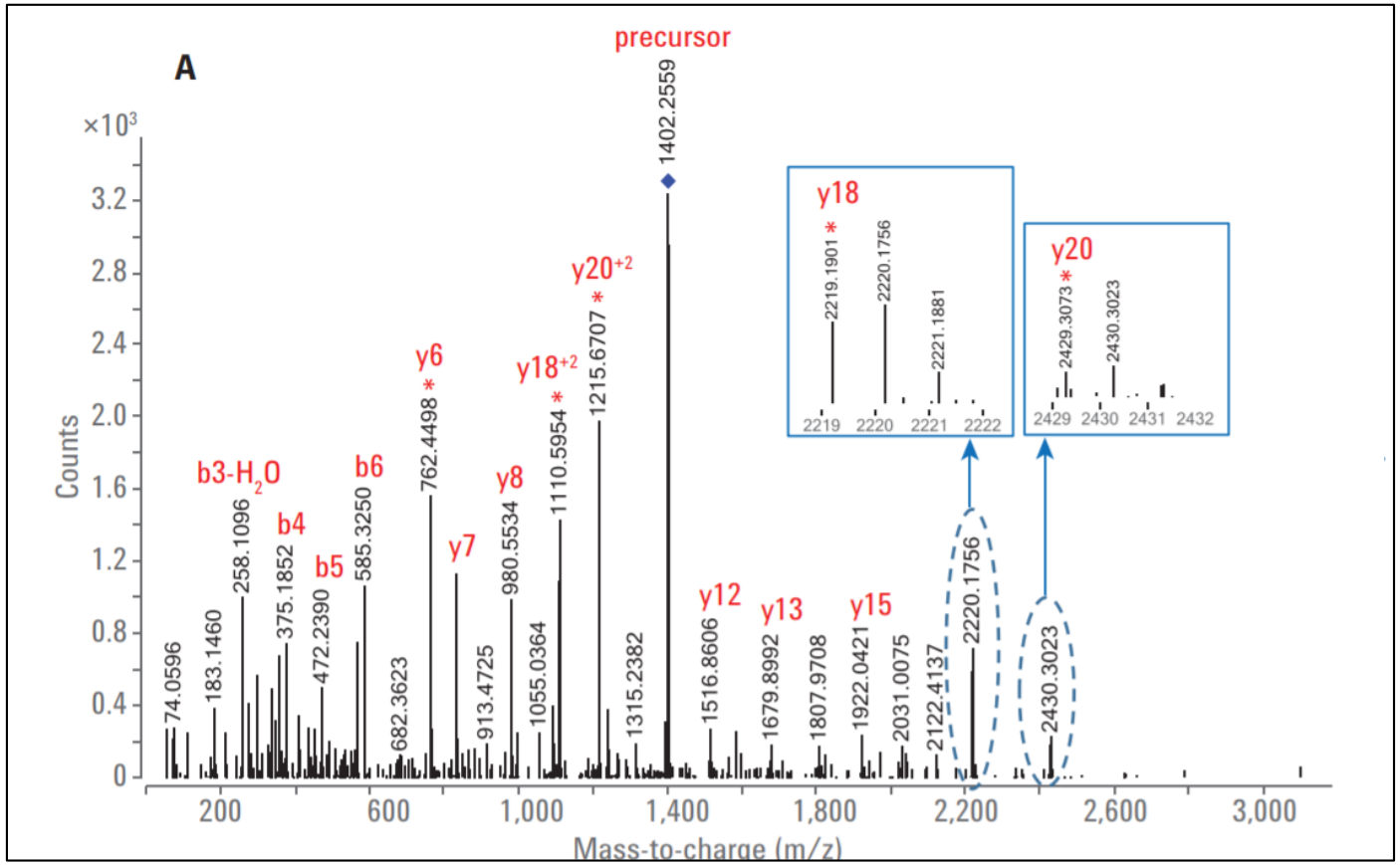
"The method was applied to influent and effluent samples from three Australian wastewater treatment plants (WWTPs). Twenty-one PFAS were detected in wastewater samples at concentrations ranging from <MDL to 56 ng/L"





Extended mass range for high m/z peptide ions

Analysis of tryptic digests of mAb antibodies and HeLa cell membranes



High m/z Precursor & Fragment Ions

For life science research, information rich peptides and other analytes of interested can be in the range of 2000 Da or higher. The 6495C is equipped with quadrupole & electronics to enable broader MRM coverage.

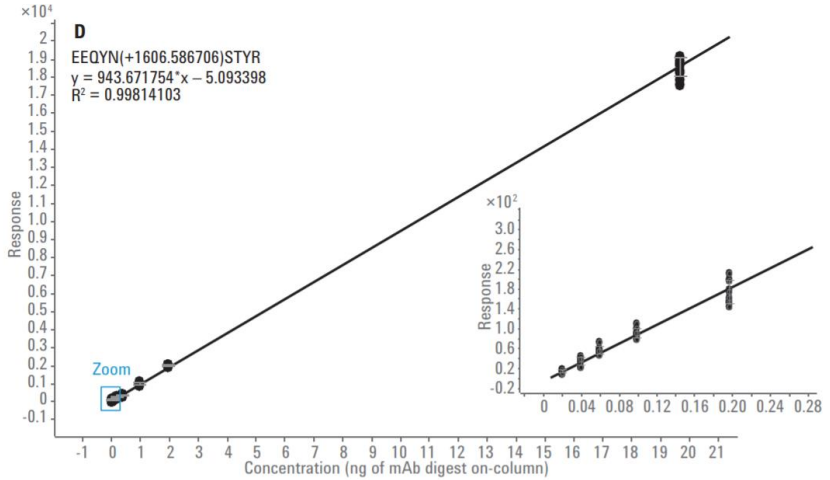


Figure 3. Quantification for G1F glycopeptide EEQYN[+1606.6]STYR.

The 6495C Triple Quadrupole LC/MS with iFunnel technology

Reliability & Robustness for demanding applications.

Born out of our drive for innovation and improvement, Agilent's 6495C Triple Quadrupole LC/MS is an innovative, powerful, and reliable mass spectrometer for customer applications demanding the lowest limits of detection, sampling speed, and signal reproducibility.

Experience the highest level of confidence with the 6495C Triple Quadrupole LC/MS.