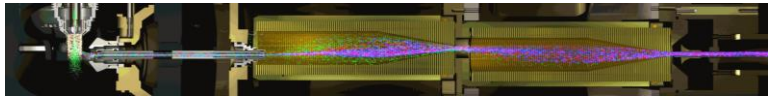
Ultrasensitive LC MS/MS: Agilent 6470 and 6495 LC-QQQ

ROCK SOLID Performance for Trace-Level Quantitation

Agilent Technologies

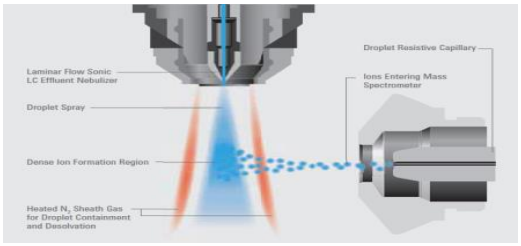
Brief History of Agilent 6400 QQQ LC/MS



6410

2006

6460 Agilent Jet Stream



2008

6430

2009

6490 iFunnel

2010

6420

2011

6460 ESI

2012

6495 iFunnel

2014

6470 AJS

2015

Agilent 6470 and 6495 Triple Quadrupole LC/MS

- **It all starts with the source!**
- **Improved Robustness**
- **Improved performance at Intrument Detection Limit (IDL Specification)**
- **Up to 4000 MRM transitions per method with DynamicMRM**
- **Simultaneous Quantitation and ID Confirmation with TriggeredMRM**

Higher Throughput

+

Higher Confidence

=

Higher Productivity

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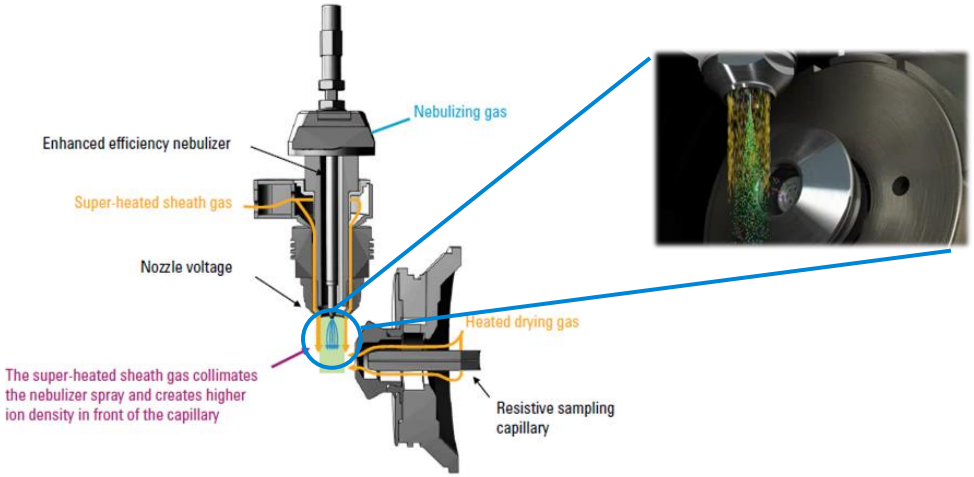
6470 - 6495 QQQ Technologies

Enhanced Performance in a Smaller Space



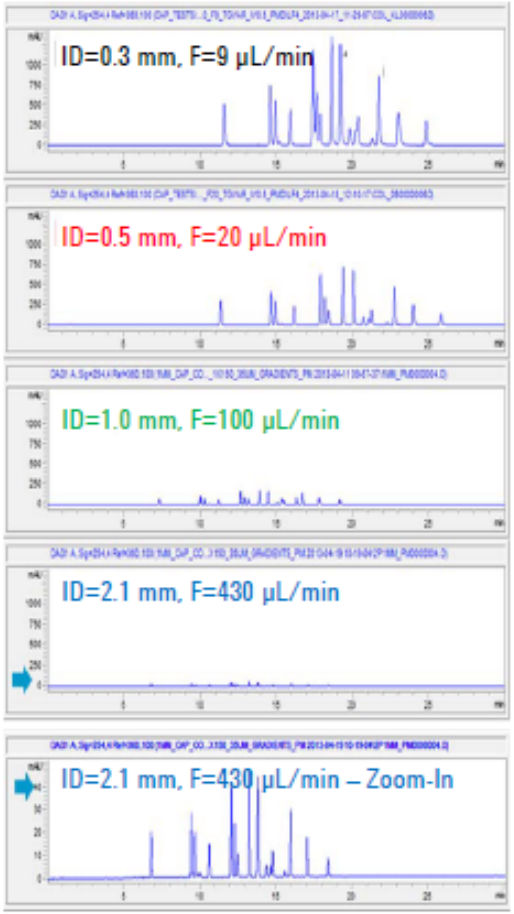
Agilent Jet Stream Technology

- **Thermal gradient focusing**
- **Efficient desolvation**
- **Creates an ion rich zone**
- **Up to 10x gains in sensitivity**

Agilent Jet Stream Technology

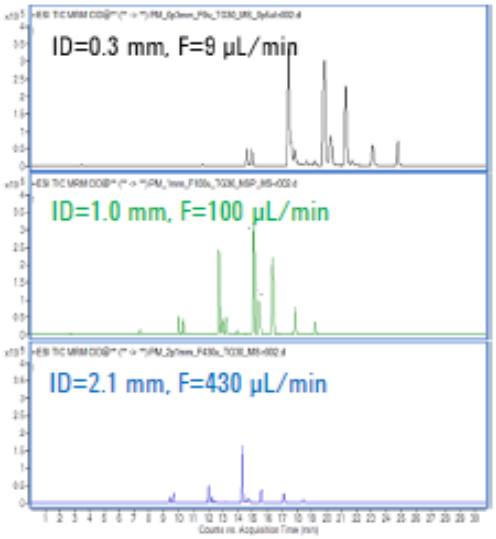
UV (254 nm)

Concentration sensitive



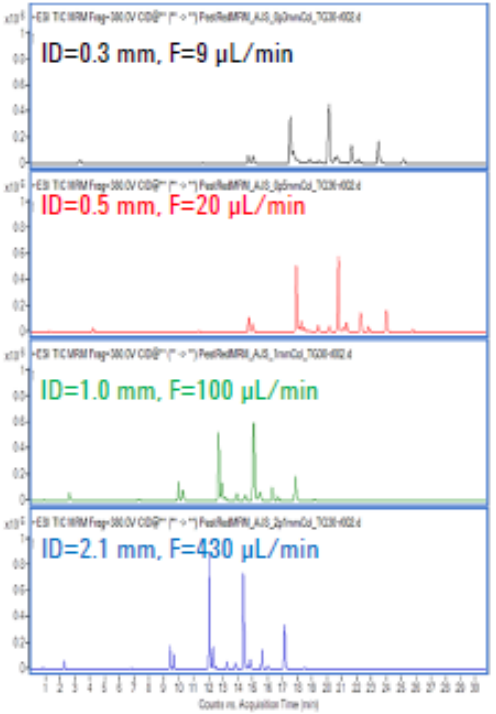
Conventional ESI

Mixed response

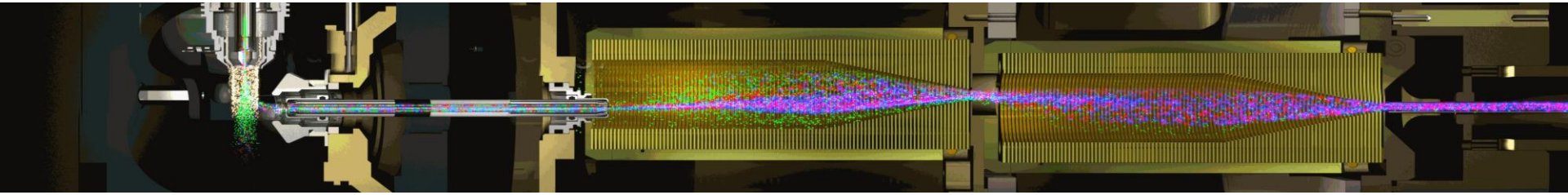


JetStream Technology

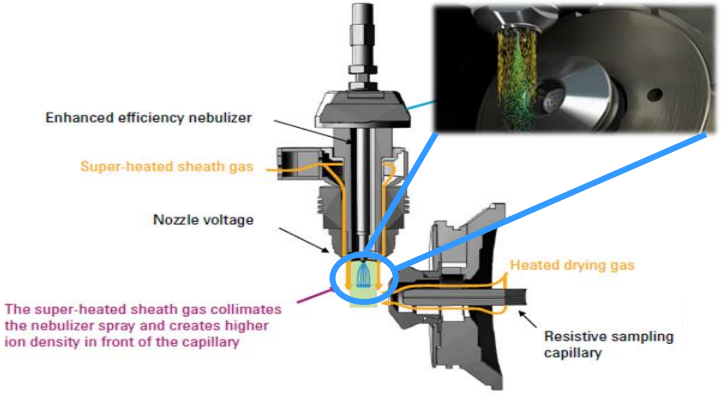
Mass sensitive



Unique to 6495: Proven iFunnel Technology

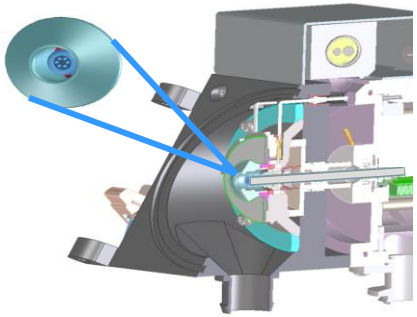


Agilent Jet Stream



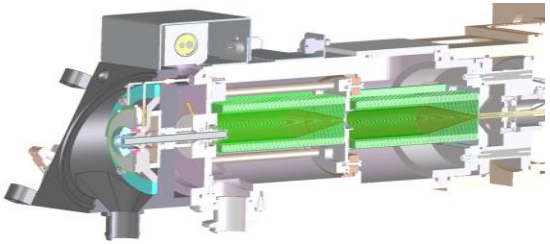
- Thermal gradient focusing
- Efficient desolvation
- Creates an ion rich zone

Hexabore Capillary



- Six capillary inlets
- Samples x10 times more ion rich gas

Dual Ion Funnel



- Removes the gas but captures the ions
- Removes neutral noise

Agilent 6470 and 6495 Triple Quadrupole LC/MS

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6470 - 6495 QQQ Technologies

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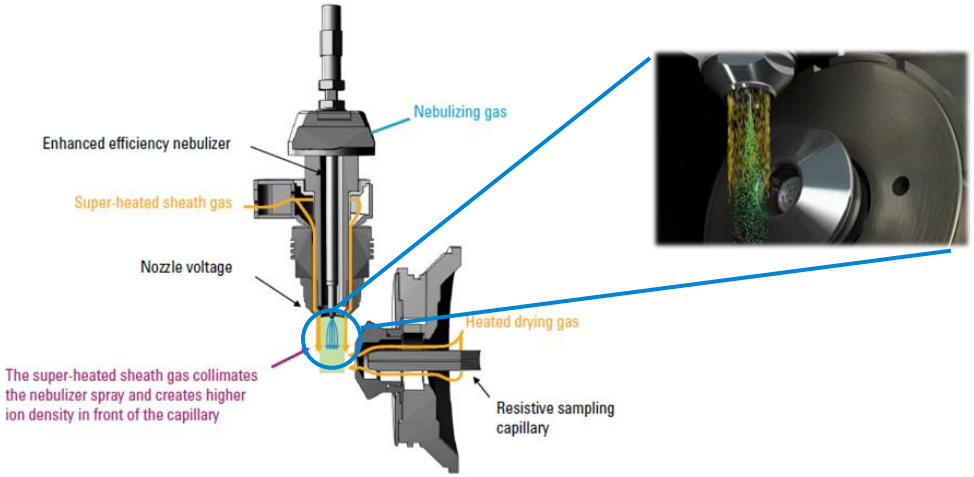

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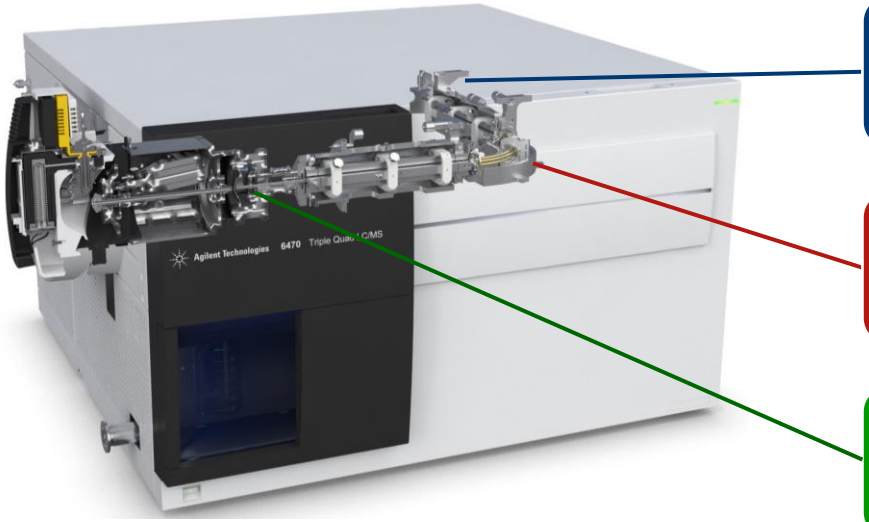

3

- An Ion Detector with High Energy Conversion Dynode and Low Noise
- **Improved ion detection**

2

- A Curved and Tapered Hexapole Collision Cell
- **Effective ion collection**

1

- Enhanced Q1 Ion Optics
- **Improved ion transmission**

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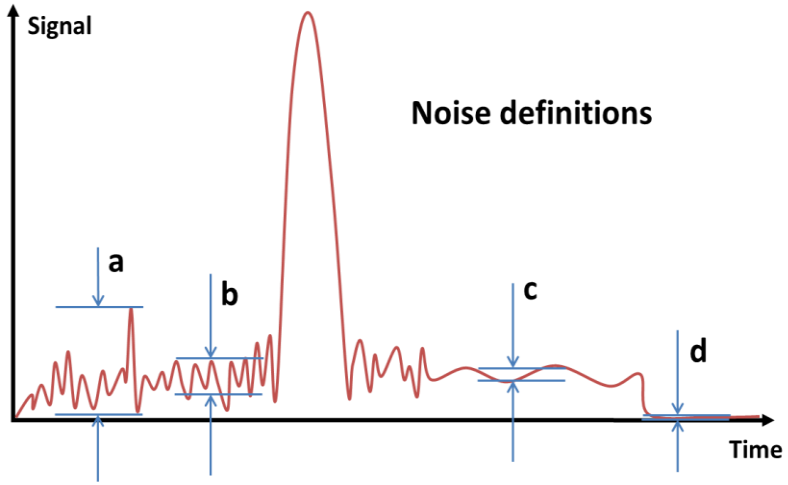
Many Ways to Manipulate S/N

Increase signal

- Increase the gain
- Narrow chromatographic peak width
- Increase scan averaging

Lower noise

- Select noise region
- Narrow the width of noise region
- Adjust baseline
- Apply peak smoothing & noise filtering
- Vary noise calculation algorithms: Peak-to-Peak, RMS, and ...



a. Peak-to-peak noise
b. RMS noise
c. Smoothing
d. Baseline noise filtering

Variation in S/N measurements makes direct assessment difficult

Instrument **D**etection **L**imit (IDL) is Defined by Statistics

$$IDL_{LCMS} = t \times SD = t \times (\%RSD / 100) \times \text{amount measured}$$

Based on a well-established statistical formula, follows regulatory guidelines

IDL

- The minimum amount of analyte that is detectable and distinguishable from background noise with a confidence level

t

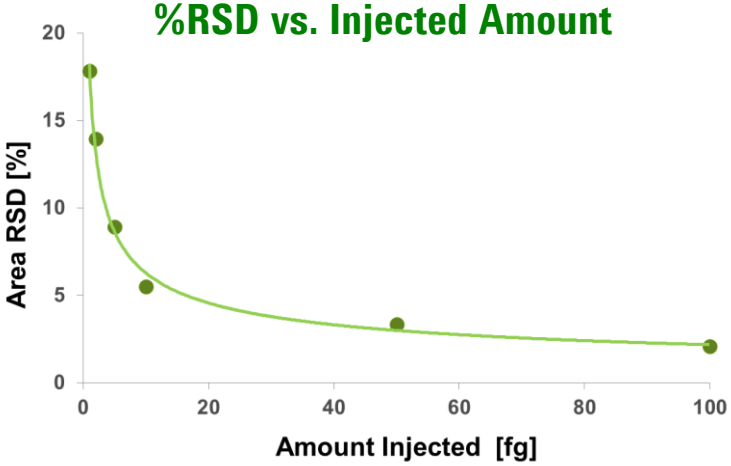
- Student "t" value, for
  - **99%** confidence level
  - **n – 1** degree of freedom

%RSD

- Relative standard deviation / **precision** of peak area at the amount measured
- From **n** replicate injections

Amount measured

- Limited to **2 – 5 x** times higher than the **Detection Limit (DL)**



- Theoretical fitting of %RSD is based on ion statistics
- %RSD increases at lower injected amount

Why Add IDL Specs for QQQ LC/MS?

S/N

- Many factors impact S/N:
  - Lower noise
  - Increase signal
- Significant variation in S/N measurements
- A relatively high level is used for S/N measurement
- Fails to estimate the true limits of detection and quantitation (LLOQ)
- Not a good metric of sensitivity performance

IDL (%RSD)

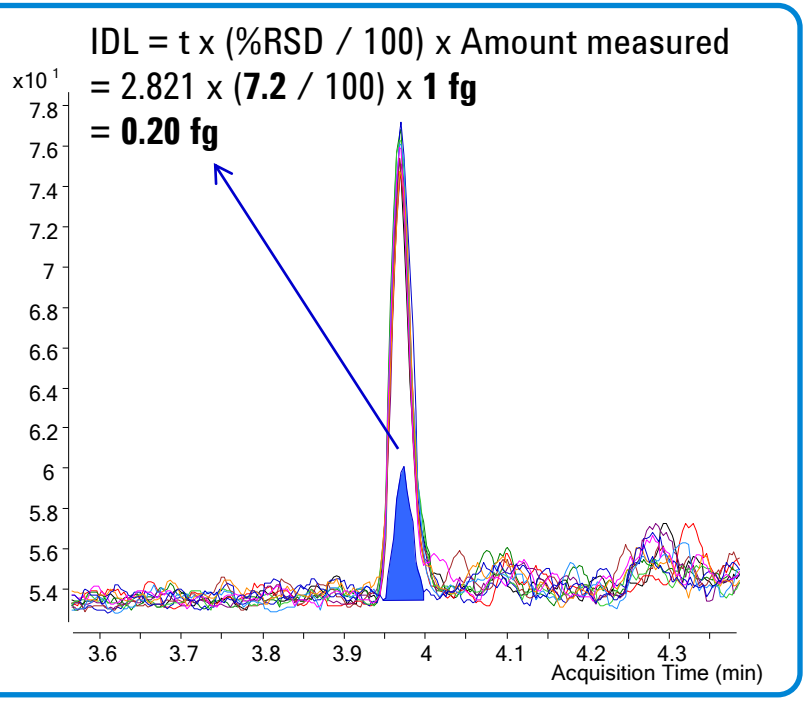
- Access sensitivity performance from area %RSD (precision) of replicate injections
- Based on a well established statistical formula – follows IUPAC / EPA guidelines
- An analytical low level is used for IDL measurement – determined using calibration curve
- Accurate assessment of the true limits of detection and quantitation (LLOQ)
- A better and more rigorous sensitivity performance metric

Improved Sensitivity and Precision – 6495 QQQ **IDL**

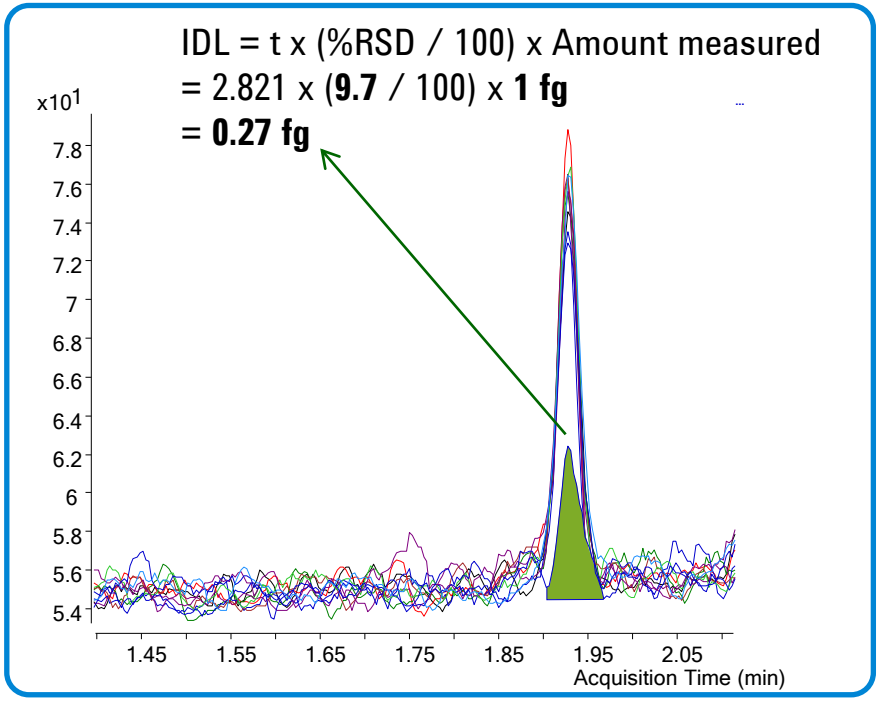
IDL for the Agilent 6495 QQQ LC/MS System

6495 QQQ IDL	Amount measured	Replicates	Area %RSD	t (99%)	IDL
Reserpine (+)	**1 fg**	n = 10	**7.2**	2.821	**0.20 fg**
Chloramphenicol (-)	**1 fg**	n = 10	**9.7**	2.821	**0.27 fg**

1 fg of reserpine used to measure IDL (+)



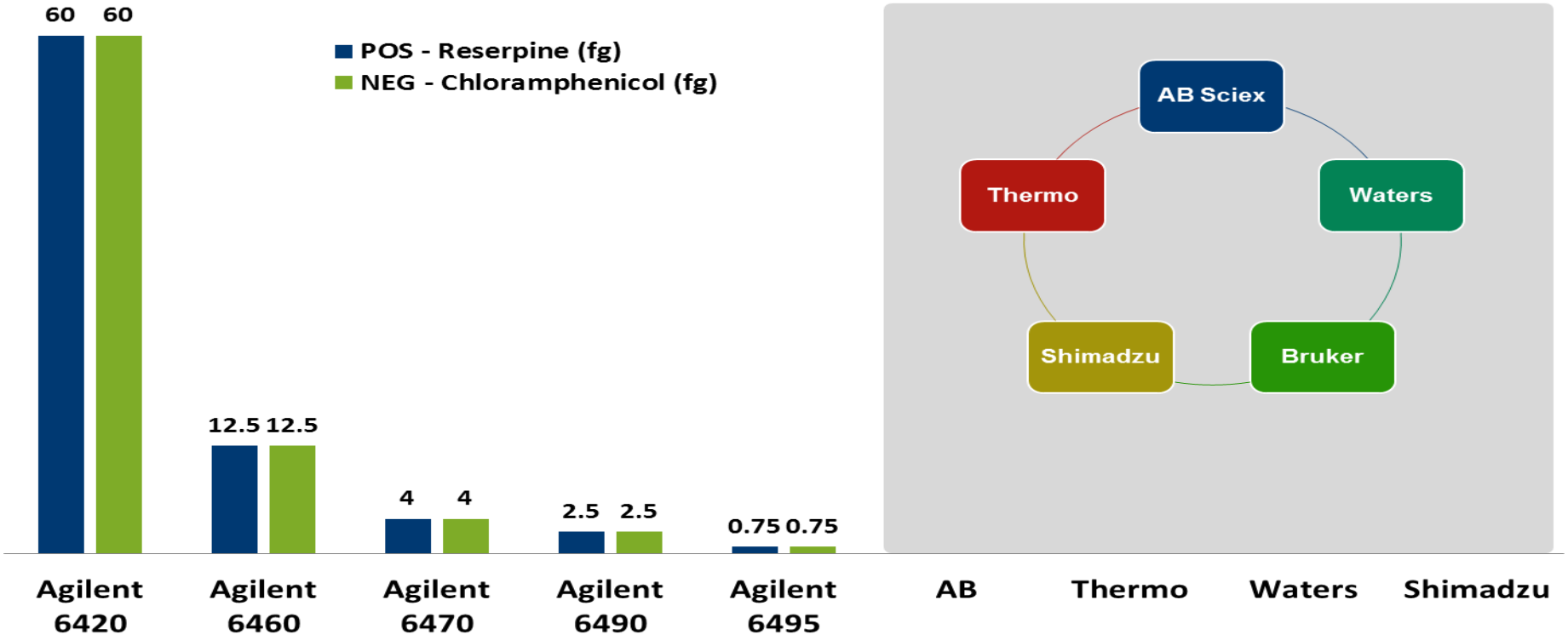
1 fg of chloramphenicol used to measure IDL (-)



$$IDL_{LCMS} = t \times SD = t \times (\%RSD / 100) \times \text{amount measured}$$

IDL Specifications for 6400 QQQ LC/MS

Agilent Differentiator!



Agilent 6470 and 6495 Triple Quadrupole LC/MS

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- **Up to 4000 MRM transitions per method with DynamicMRM**
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Higher Throughput

+

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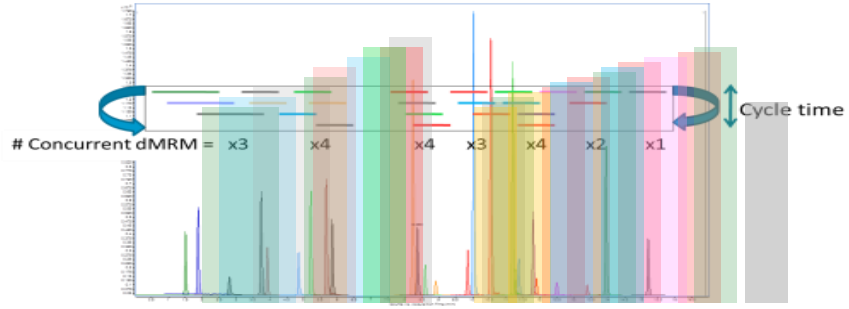
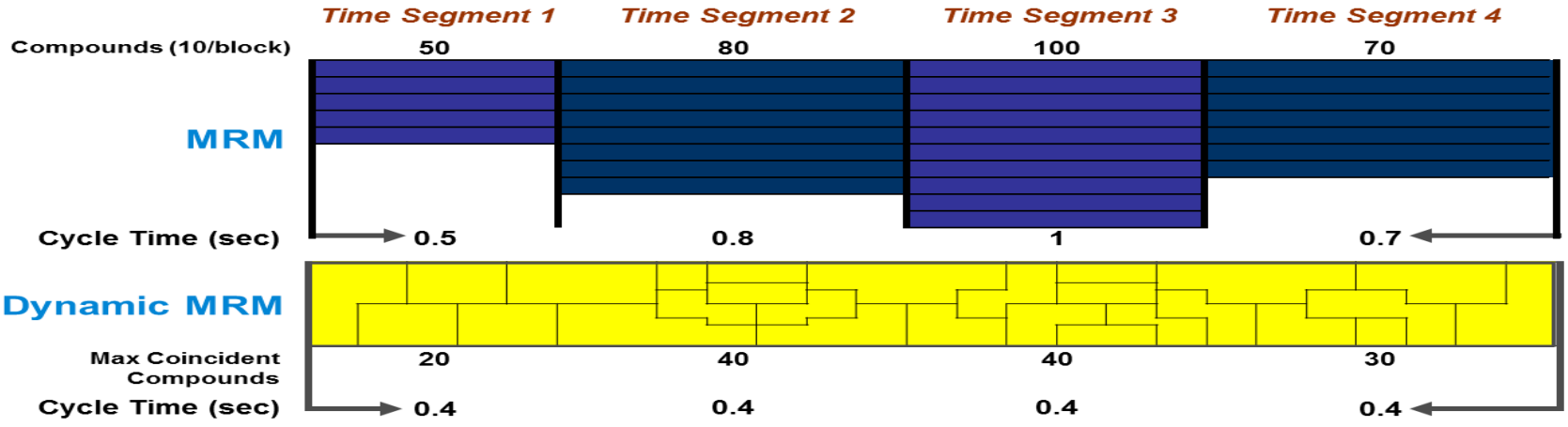
Higher Productivity

Dynamic MRM (dMRM)

Dynamic MRM, Fast MRM Speed and Polarity Switch

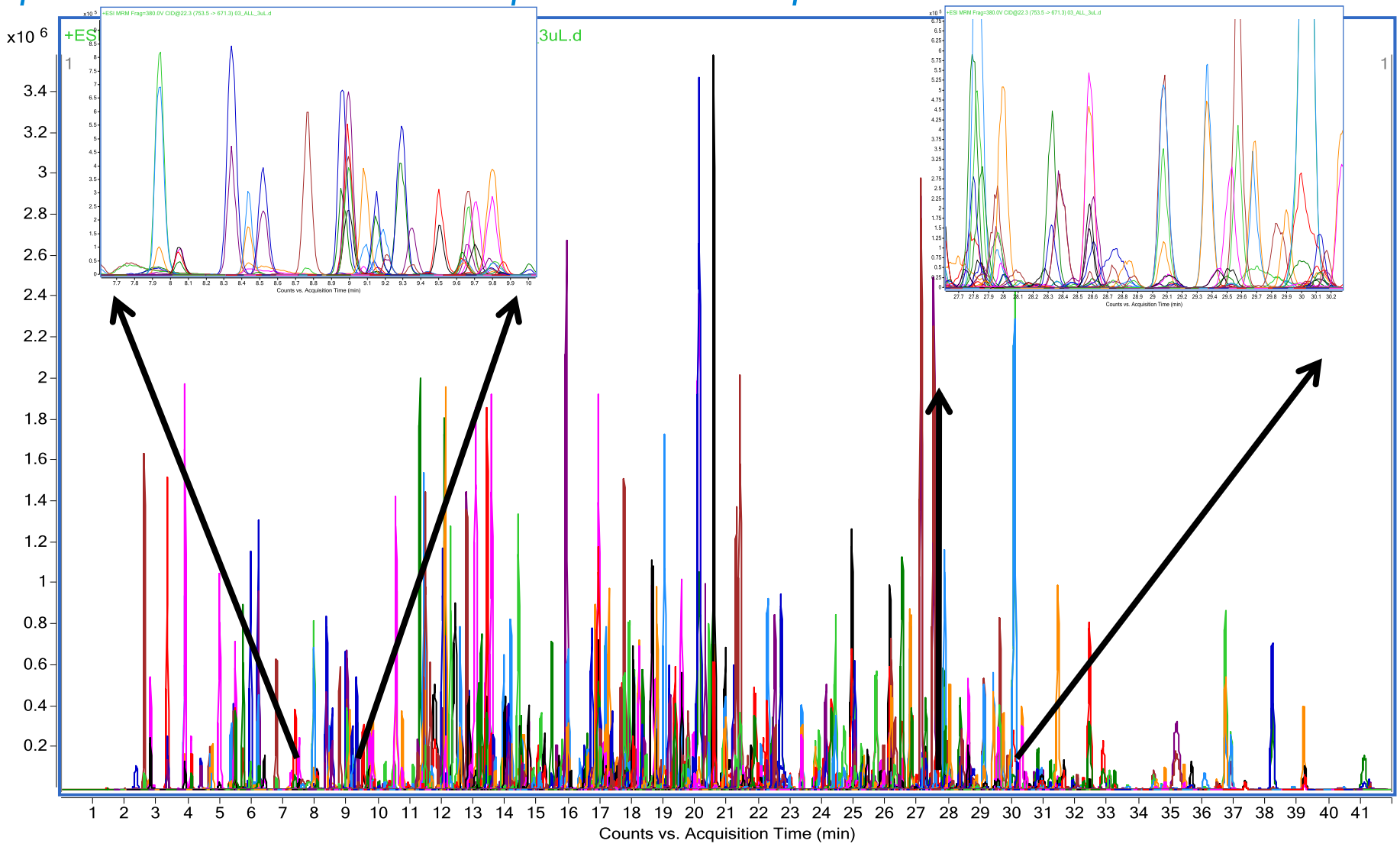


- Group MRMs in RT windows instead of time segments
- 2x shorter cycle time supports narrow UHPLC peaks
- Supported by very fast MRM speed (1 ms dwell) and polarity switch (20 ms)



Dynamic MRM (dMRM):

Up to 4000 MRM Transitions per Run in Complex Matrix!



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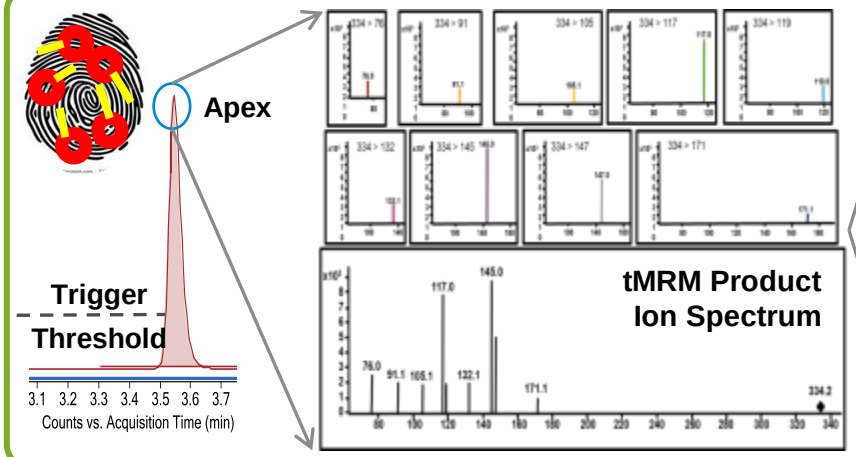
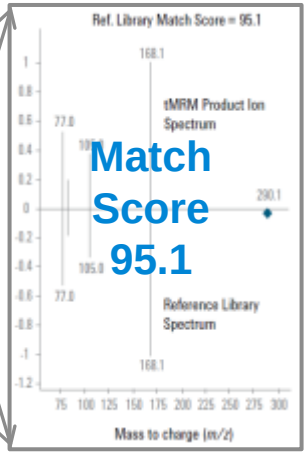
Triggered MRM (tMRM)

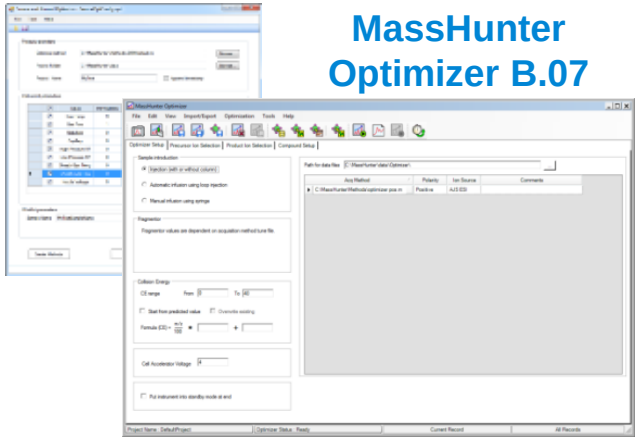
Triggered MRM, tMRM Database and Library





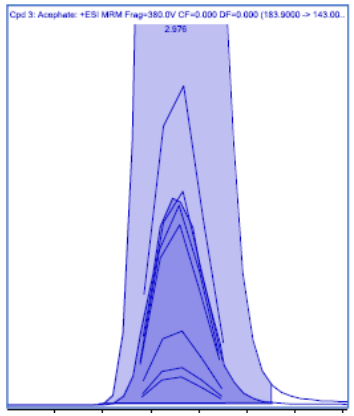
- tMRM database accelerates acquisition method setup
- tMRM library – Match Score for confirmation
- tMRM is Fast and Sensitive
- UHPLC compatible
- Simultaneous Quantitation, Screening and Confirmation

Enhanced Optimizer Workflow in MassHunter B.07 (to Better Support tMRM)



MassHunter Optimizer B.07

- Find <u>10 MRM</u> transitions in Optimizer
- Injection volume flexibility
- Improved method editing



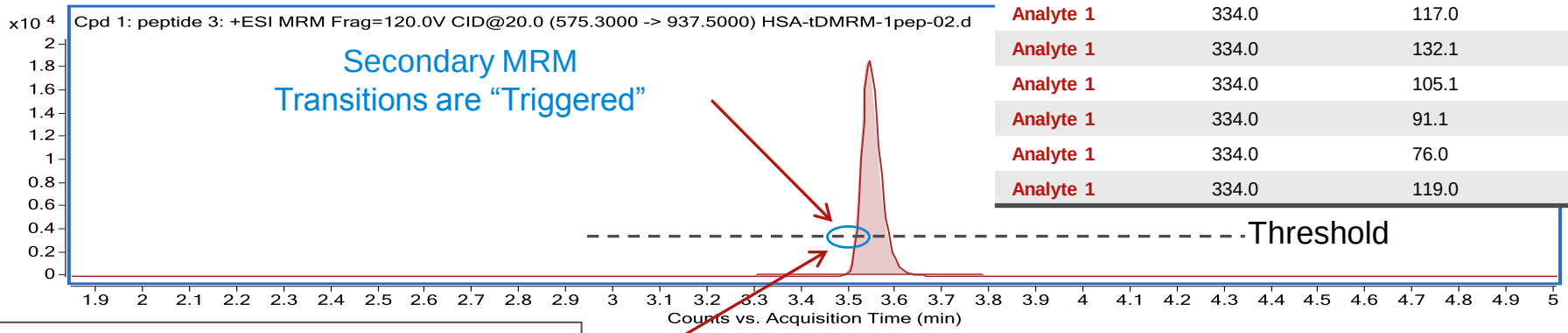
Triggered MRM (tMRM)

QTrap Approach

- Scan the entire finger print
- Not very fast and sensitive

QQQ tMRM Approach

- Focus on ten fingerprint features (10 MRMs)
- Maintains good MDL
- Fast – UHPLC compatible



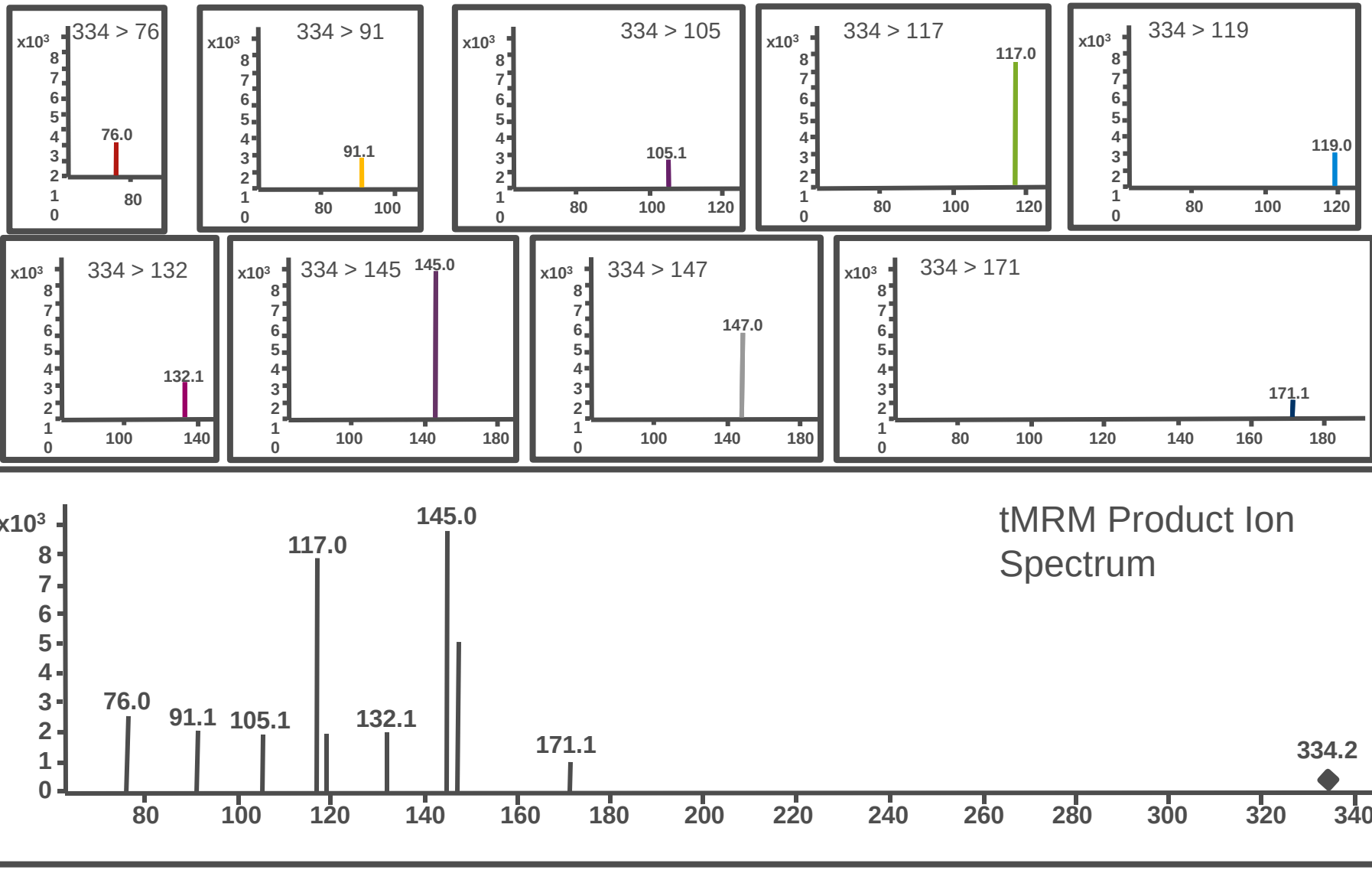
Triggered cycle (above threshold)

Compound	Precursor	Product
Analyte 1	334.0	145.0
Analyte 1	334.0	117.0
Analyte 1	334.0	132.1
Analyte 1	334.0	105.1
Analyte 1	334.0	91.1
Analyte 1	334.0	76.0
Analyte 1	334.0	119.0

Primary cycle (below threshold)

Compound	Precursor	Product
Analyte 1	334.0	145.0
Analyte 1	334.0	117.0

tMRM Composite Product Ion Spectrum



tMRM Library Searching – Match Score for Confirmation

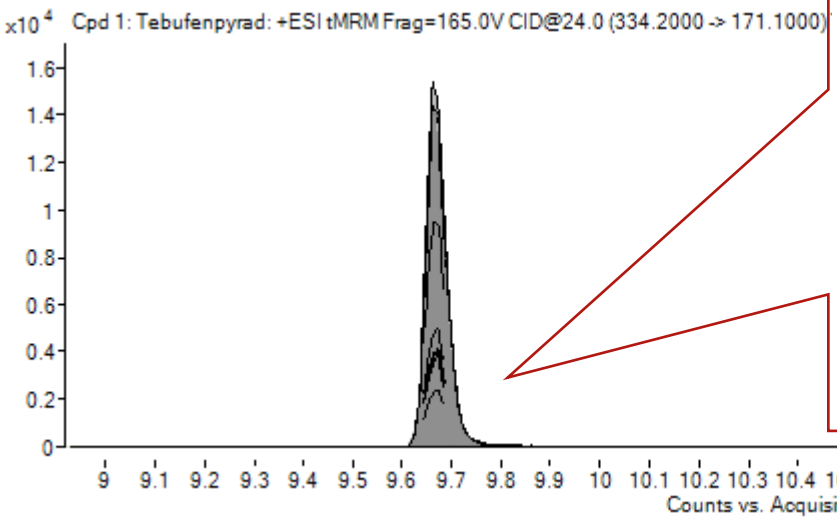
Show/Hide	Label	Polarity	Cpd	Name	Score	Start	RT	End	Width	m/z	Mass (DB)
☑	Cpd 1: Tebufenpyrad	Positive	1	Tebufenpyrad	96.75	9.602	9.664	9.761	0.046	334.2	333.8557

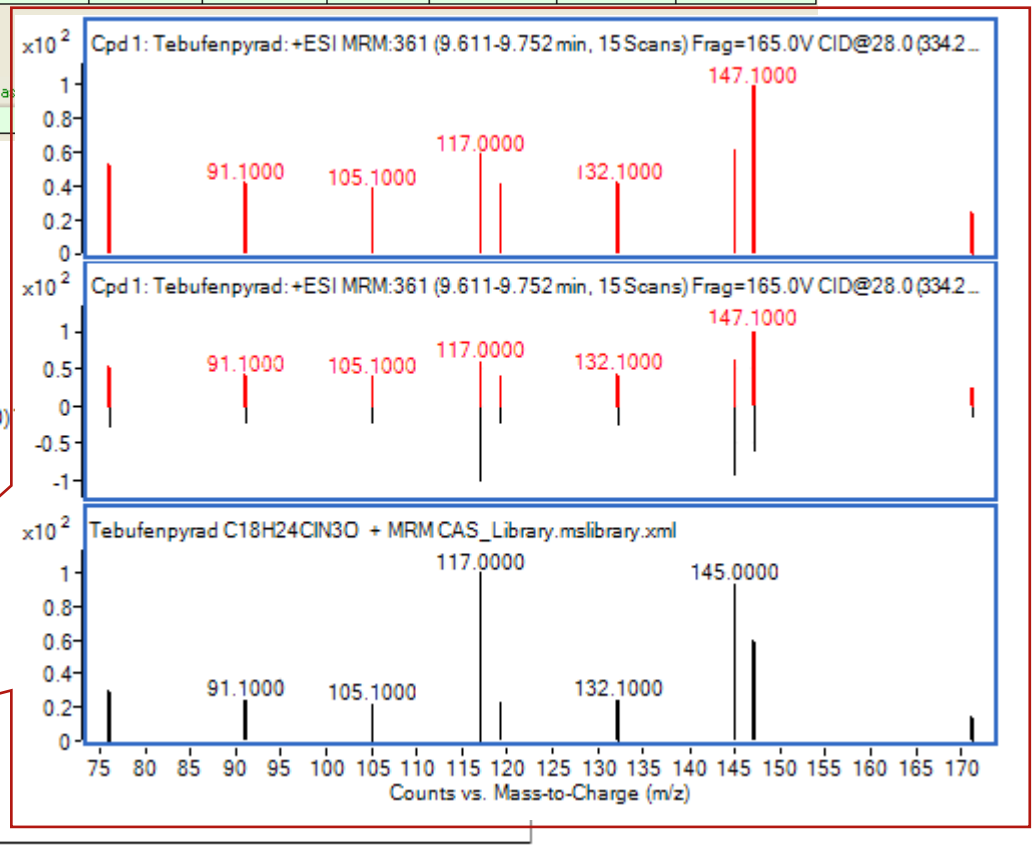
Best	Name	Formula	CAS	Score	Mass (DB)	RT	Score (Lib)	Precursor	Find by MRM	Score (Acq)	ID Source
◉	Tebufenpyrad	C18H24ClN3O	119168-77-3	96.75	333.8557	9.664	96.75				LibSearch

Name	ID	Num Peaks	m/z (prec.)	Score (Lib)
Tebufenpyrad	1	9	334.2	96.75

Best	Name	Formula	CAS	Score
○	Tebufenpyrad			100

Library match score: 96.75





tMRM Application Kits For LC/MS

Targeted Screening & Confirmation with QQQ

Pesticides

Test Mix: 254 compounds

DB: 700+ compounds

Library: 200+ compounds

Veterinary Drugs

Test Mix: 146 compounds

DB: 500+ compounds

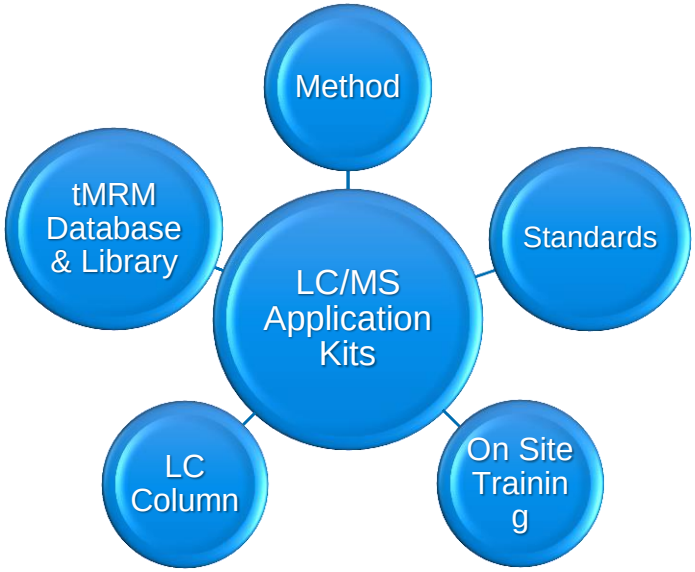
Library: 100+ compounds

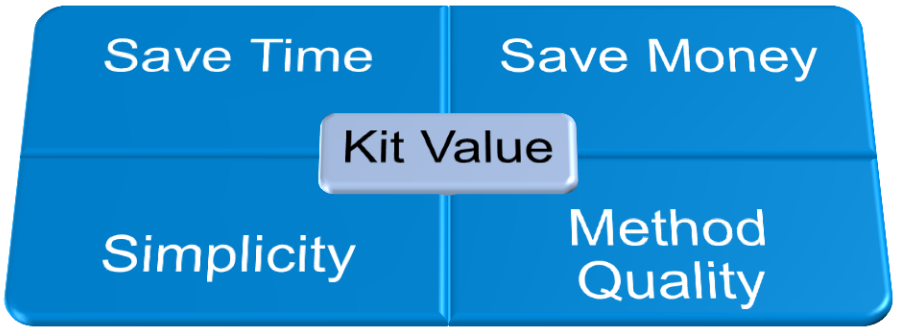
Forensic Toxicology

Test Mix: 139 compounds

DB: 2500+ compounds

Library: 100+ compounds





- ***Agilent unique data dependent acquisition for fast and sensitive compound screening, quantitation and confirmation.***

Applications for 6470 and 6495 QQQ LC/MS

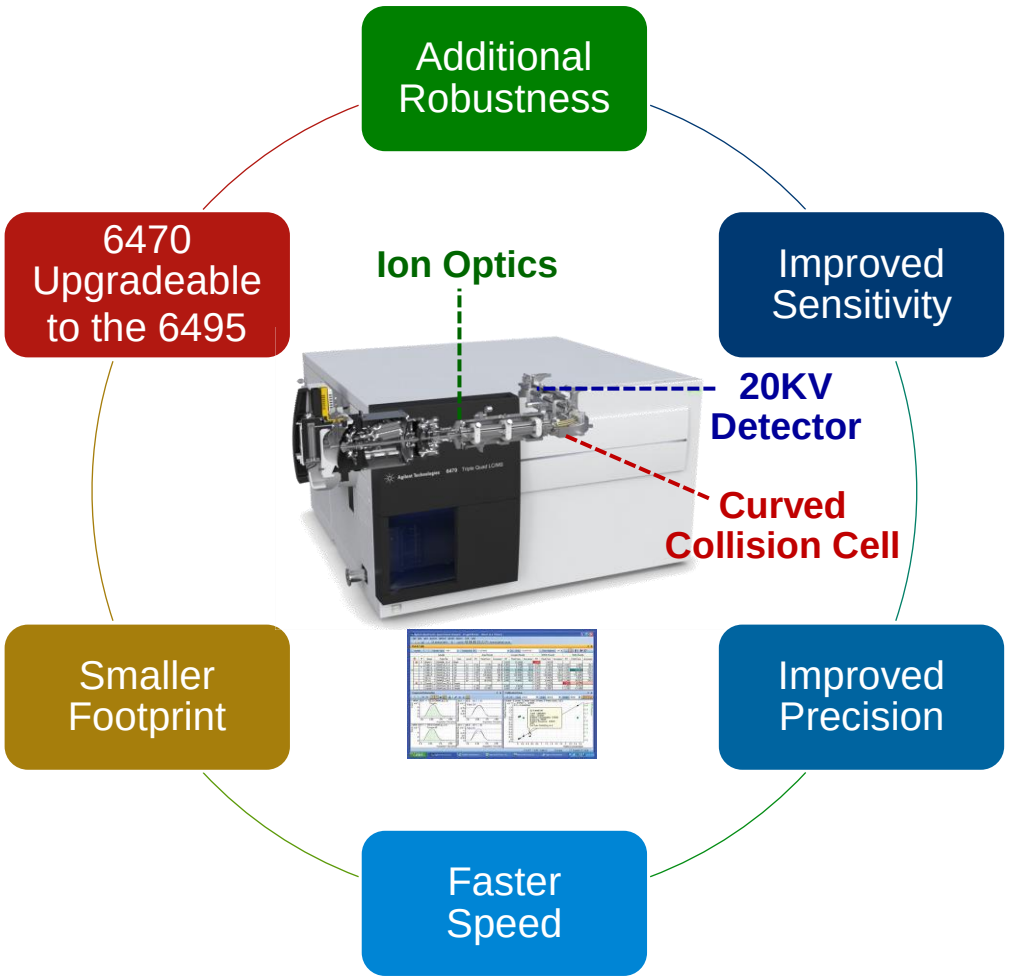
Which one is needed?

	6470	6495
	**Food Safety - Pestcides** Large Panels, High Throughput	**Food Safety - Pesticides** Tough matrices, ultra-trace levels
	**Environmental – Water Analysis** PPCPs in surface water using direct injection	**Environmental - Water Analysis** Ultra-trace level hormones (EDCs) in drinking water using direct injection
	**Pharmaceutical –** High-throughput analysis of drugs & metabolites in plasma	**Clinical - Peptide Quantitation** Quantitation of peptides at sub-attomole level using nanoflow and standard flow chromatography

0

Summary: 6470 and 6495 QQQ LC/MS

Rock Solid Performance for **Confident** Quantitation and Highest Lab **Productivity**



Less Maintenance Cleaning Reliable Consistent Results over Longer Time (24/7)

Streamlined Analytical Workflow

Reproducible High Quality Data

Higher Throughput at UHPLC Speed

Bench space can be optimized

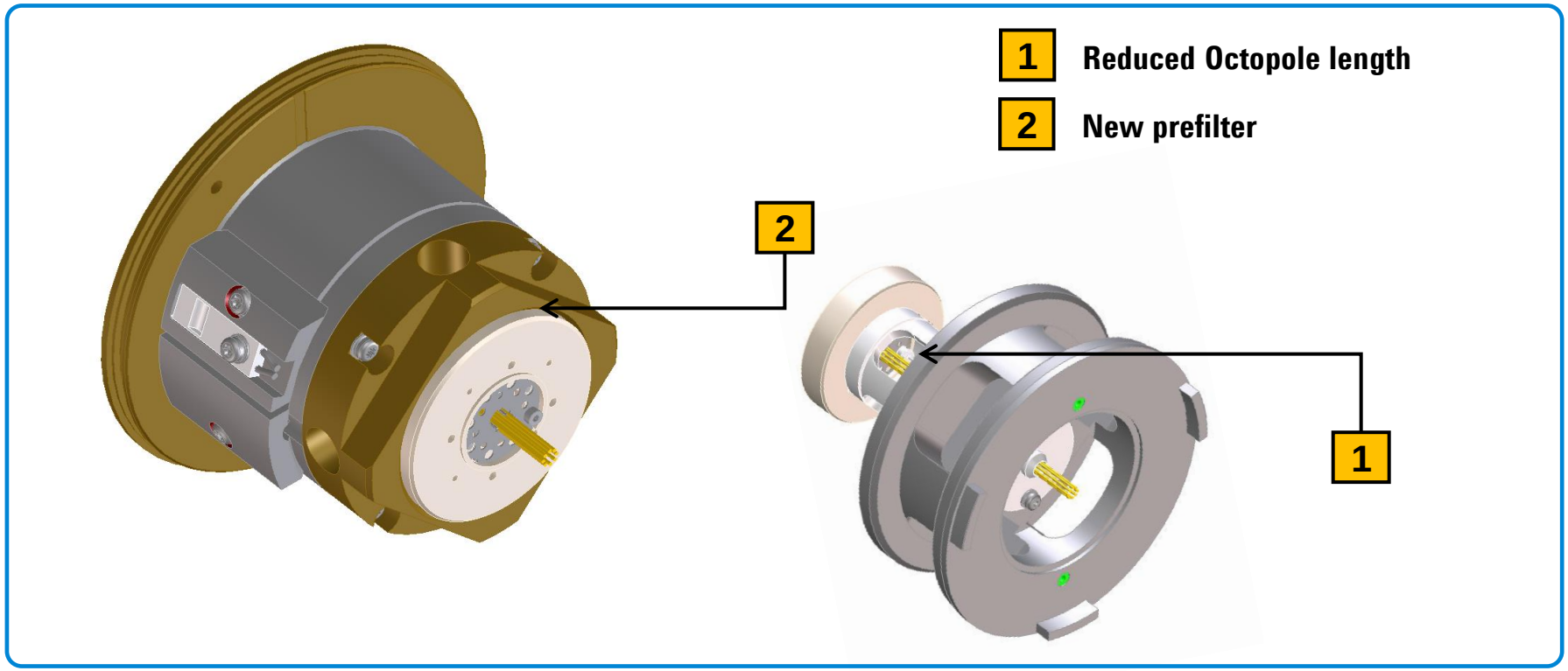
Protection of Your Investment

Merci pour votre attention!

QUESTIONS??

Enhanced Q1 Ion Optics with Optimized Prefilter

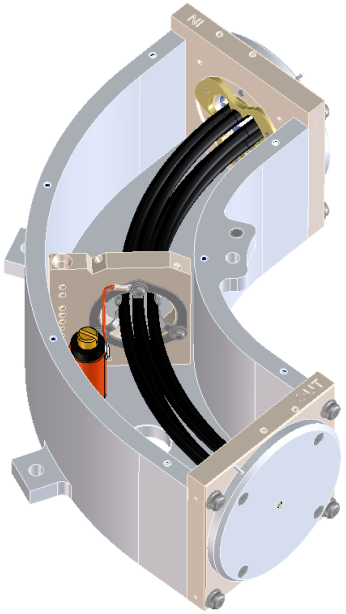
Improved transmission of precursor ions and additional system robustness



- New optimized MS 1 prefilter geometry for improved precursor **ion transmission**
  - Improved peak area response and peak area %RSD, more **sensitive** and **precise**
- New optical lens elements for reduced the probability of contamination
  - More **reliable** and **robust** performance

Curved and Tapered Hexapole Collision Cell

Effective collection and transmission of product ions and smaller footprint



6460 QQQ LC/MS
- Linear Collision Cell

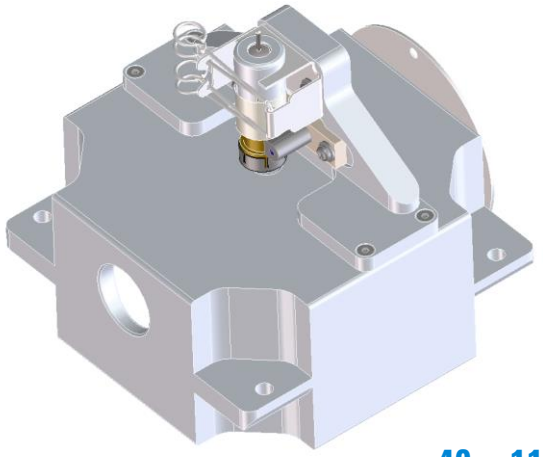
30% smaller

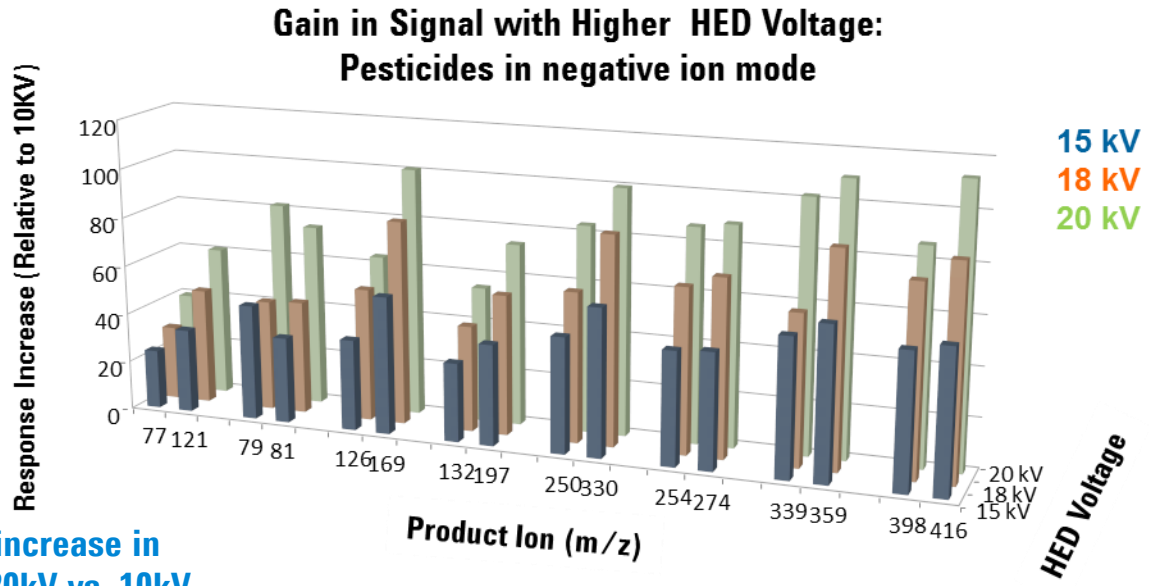
6470 QQQ LC/MS
- Curved Collision Cell

- Curved and Tapered Hexapole Assembly for efficient collection and transmission of product ions
- Designed for consistent collision energies across all QQQ platforms
- A **compact**, smaller benchtop footprint

New Detector with High Energy Conversion Dynode

More efficient detection and quantitation of ions with low noise characteristics





40 – 113% increase in response to 20kV vs. 10kV

- Improved **ion detection** efficiency with High Energy Dynode (HED) voltage up to 20 kV
  - Improved peak area response and peak area %RSD in positive & negative ion mode
  - Improved **sensitivity** and **precision** for a wide mass range
- Low noise level at 20 kV
  - Improved signal to noise

iFunnel Express

iFunnel Express – Source and iFunnel Optimization

Method Generation → **Acquisition** → **Optimization**

MH Source and Funnel Optimizer

Create Methods & Worklist

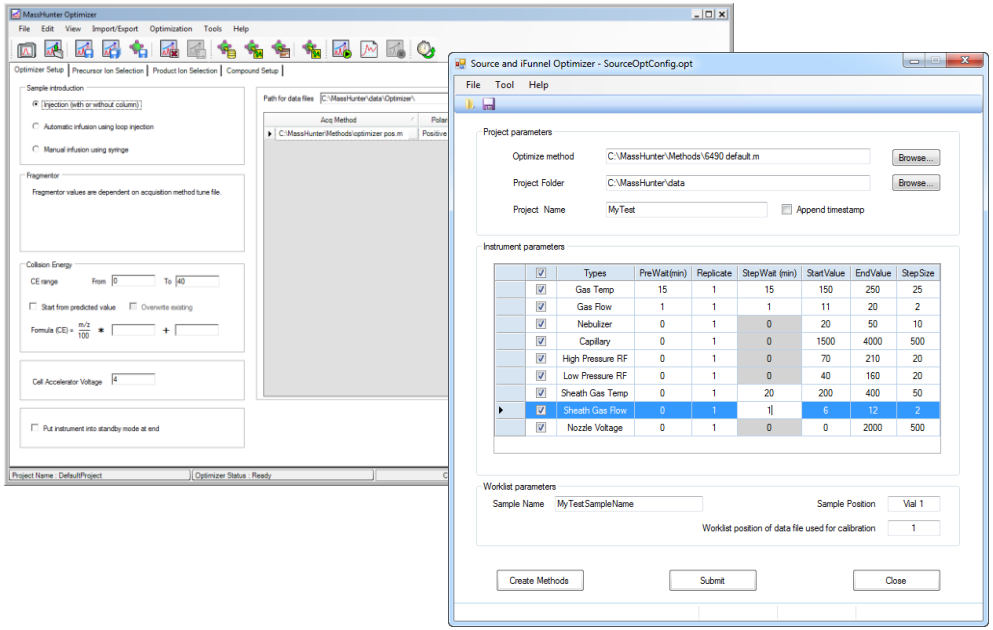
Acquire Data for Each Parameter

Data Files

MassHunter Quant

Integrate for Peak Data

Excel File for Optimized Parameter



- **Automate the source/iFunnel optimization process**