Agilent 6495 Triple Quadrupole LC/MS System

Experience A New Level of Confidence

Agilent Technologies
Overview of Topics

Markets and Applications

Technology, performance, and differentiation
• Technology innovations
• New sensitivity performance standard: Instrument Detection Limit (IDL)
• Quantitation performance
• New software capabilities

Key Applications
• Food safety – Pesticides in food matrices
• Food safety – Estrogens in milk
• Environmental – Water analysis
• Peptide quantitation

Summary: Product Values
6495 Markets and Applications

- **Biomarkers**
- **Peptide Quan**
- **High sensitivity**
- **Complex matrices**

**Protein Quant**

- **Vitamin D**
- **Endogenous steroids**

**Clinical Research**

- **Pesticides**
- **Mycotoxins**
- **Antibiotics**
- **Sample dilution**

**Food Safety**

- **Water**
- **Hormones**
- **Contaminants**
- **Direct injections**

**Environmental**

- **Research**
- **Chemical**
- **High sensitivity**

**Academia**

- **Drug discovery**
- **ADME/PK**
- **Bioanalysis**
- **Microdosing**

**Pharma**

- **Veterinary Drugs**
- **Kits & Methods**
- **tMRM**

**Veterinary**

- **Drugs of abuse**
- **Designer drugs**
- **Oral fluids**

**Forensic Toxicology**

- **Drugs of abuse**
- **Designer drugs**
- **Oral fluids**
Proven iFunnel Technology

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

- Six capillary inlets
- Samples x10 times more ion rich gas
- Removes the gas but captures the ions
- Removes neutral noise
6495 QQQ Technologies
Continued Development

1. New Enhanced Q1 Ion Optics
   - Improved ion transmission

2. New Tapered Hexapole Collision Cell
   - Effective ion collection and transmission

3. New Detector with High Energy Conversion Dynode
   - Improved NEG ion detection with low noise

Proven iFunnel Technology
- Agilent Jet Stream
- Hexabore Capillary
- Dual Ion Funnel
- Increased ion generation
- Enhanced ion sampling

Quantitative Applications
- Enhanced peak area response
- Improved peak area %RSD
- More sensitive and precise
- Lower Limits of Detection (IDL) and Quantitation (LLOQ)
- More reliable and robust
New Enhanced Q1 Ion Optics

*Improved transmission of ions and 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 and easier autotune
  - More **reliable** and **robust** performance
New Tapered Hexapole Collision Cell

*Effective collection and transmission of ions*

- Curved and Tapered Hexapole Assembly for efficient collection and transmission of product ions
- Consistent collision cell pressure for higher quality MS/MS spectra
- Designed for consistent collision energies across all QQQ platforms
- Capable of higher RF voltage on rods for higher mass transmission
New Detector with High Energy Conversion Dynode

More efficient detection of ions with low noise characteristics

• 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 m/z range of product ions

• Low noise level at 20 kV
  - Improved signal to noise

Gain in Signal with Higher HED Voltage: Anti-HER2/neu mAB Peptides

60 – 120% increase in response to 20kV vs. 10kV
6495 QQQ - Premium Performance

- **Improved sensitivity and lower LLOQs**
  - Average 3x in S/N specifications and applications

- **Improved precision and excellent accuracy** at the lowest levels
  - 3x in IDL specifications

- **Proven 6 orders of linear dynamic range**

- **Proven robustness** in complex matrix
  - Food matrix and biological matrix (plasma)

- **Improved mass range, fast scan speed and MRM acquisition rate**
Improved Sensitivity – Peak Area Response and S/N
Reserpine (+) and Chloramphenicol (-), 1 pg on-column

- The 6495 QQQ LC/MS system shows improved peak area response and S/N, in both positive and negative ion modes compared to previous designs.
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...

Variation in S/N measurements makes direct assessment difficult
**Instrument Detection Limit (IDL) is Defined by Statistics**

\[
\text{IDL}_{\text{LCMS}} = t \times \text{SD} = t \times (\%\text{RSD} / 100) \times \text{amount measured}
\]

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

%RSD vs. 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 – Peak Area %RSD
Reserpine (+) and Chloramphenicol (-) at low levels, 5 fg on-column

5 fg reserpine (+) (n=10)
3 x higher area response
Lower area %RSD

6495 QQQ
Avg. Area = 157
Area %RSD = 6.1

6490 QQQ
Avg. Area = 47
Area %RSD = 10.4

5 fg chloramphenicol (-) (n=10)
3 x higher area response
Lower area %RSD

6495 QQQ
Avg. Area = 121
Area %RSD = 2.7

6490 QQQ
Avg. Area = 40
Area %RSD = 5.6

• The improved sensitivity of the 6495 QQQ LC/MS system results in enhanced peak area response and improved area precision (%RSD), especially at the low levels
• This ultimately leads to lower instrument detection limits (IDLs) compared to previous designs
Improved Sensitivity and Precision – 6495 QQQ IDL

**IDL for the Agilent 6495 QQQ LC/MS System**

<table>
<thead>
<tr>
<th>6495 QQQ IDL</th>
<th>Amount measured</th>
<th>Replicates</th>
<th>Area %RSD</th>
<th>t (99%)</th>
<th>IDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reserpine (+)</td>
<td>1 fg</td>
<td>5 fg</td>
<td>n = 10</td>
<td>7.2</td>
<td>2.821</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.20 fg</td>
</tr>
<tr>
<td>Chloramphenicol (-)</td>
<td>1 fg</td>
<td>5 fg</td>
<td>n = 10</td>
<td>9.7</td>
<td>2.821</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.27 fg</td>
</tr>
</tbody>
</table>

**1 fg of reserpine used to measure IDL (+)***

\[
\text{IDL} = t \times \left(\frac{\%RSD}{100}\right) \times \text{Amount measured}
\]

\[
= 2.821 \times \left(\frac{7.2}{100}\right) \times 1 \text{ fg}
\]

\[
= 0.20 \text{ fg}
\]

**1 fg of chloramphenicol used to measure IDL (-)***

\[
\text{IDL} = t \times \left(\frac{\%RSD}{100}\right) \times \text{Amount measured}
\]

\[
= 2.821 \times \left(\frac{9.7}{100}\right) \times 1 \text{ fg}
\]

\[
= 0.27 \text{ fg}
\]

Improved **precision** (%RSD) and Instrument Detection Limit (IDL) achieved on the **6495 vs. the 6490**

---

*5.4 5.6 5.8 6 6.2 6.4 6.6 6.8 7 7.2 7.4 7.6 7.8 8*  

*1.45 1.55 1.65 1.75 1.85 1.95 2.05*  

Acquisition Time (min)
6495 IDL Levels: Accurate Assessment of the LLOQs
Reserpine (+) and Chloramphenicol (-)

<table>
<thead>
<tr>
<th>6495 QQQ - Reserpine (+) IDL</th>
<th>6495 QQQ – Chloramphenicol (-) IDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20 fg</td>
<td>1.02 fg</td>
</tr>
<tr>
<td>0.27 fg</td>
<td>1.25 fg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>6495 QQQ: Reserpine LLOQ = 0.2 fg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area % RSD = 18.8</td>
</tr>
<tr>
<td>n = 10 injections</td>
</tr>
<tr>
<td>%Accuracy = 108</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>6495 QQQ: Chloramphenicol LLOQ = 0.3 fg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area % RSD = 15.8</td>
</tr>
<tr>
<td>n = 10 injections</td>
</tr>
<tr>
<td>%Accuracy = 118</td>
</tr>
</tbody>
</table>

• Improved / Lower IDL levels and LLOQ levels are achieved on the new 6495 vs. the 6490
## 6495 QQQ LC/MS Performance Specifications

<table>
<thead>
<tr>
<th>Bid Specifications</th>
<th>Agilent 6490</th>
<th>Agilent 6495</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity, S/N</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive, 1 pg Reserpine on-column</td>
<td>50,000:1</td>
<td>150,000:1</td>
</tr>
<tr>
<td>Negative, 1 pg chloramphenicol on-column</td>
<td>50,000:1</td>
<td>150,000:1</td>
</tr>
<tr>
<td><strong>Sensitivity, Instrument Detection Limit (IDL)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive, Reserpine</td>
<td>2.5 fg Measured at 5 fg on-column</td>
<td>0.75 fg Measured at 1 fg on-column</td>
</tr>
<tr>
<td>Negative, chloramphenicol</td>
<td>2.5 fg Measured at 5 fg on-column</td>
<td>0.75 fg Measured at 1 fg on-column</td>
</tr>
<tr>
<td><strong>Linear dynamic range</strong></td>
<td>10⁶</td>
<td>10⁶</td>
</tr>
<tr>
<td><strong>Mass range</strong></td>
<td>5 – 1,400 Da</td>
<td>5 – 2,250 Da</td>
</tr>
<tr>
<td><strong>Maximum scan speed</strong></td>
<td>12,500 Da/sec</td>
<td>15,000 Da/sec</td>
</tr>
<tr>
<td><strong>Maximum MRM rate</strong></td>
<td>250 MMRs/sec</td>
<td>500 MMRs/sec</td>
</tr>
<tr>
<td><strong>Minimum MRM dwell time</strong></td>
<td>1 ms</td>
<td>1 ms</td>
</tr>
<tr>
<td><strong>Polarity switching</strong></td>
<td>30 ms</td>
<td>30 ms</td>
</tr>
</tbody>
</table>

- **Improved sensitivity specs:**
  - S/N
  - IDL
- Improved mass range
- Improved full scan and MRM speed
Verapamil (+) Sensitivity in Solvent
Attogram / Zeptomole Lower Limit of Quantitation

6495 QQQ: Verapamil LLOQ = 40 ag (80 zeptomoles) on-column

- Unparalleled sensitivity (LLOQ of 40 ag on-column) - better than the 6490 (100 ag)
- Excellent precision (peak area %RSD) and accuracy are observed at the lowest levels

Verapamil 100 ag on-column
Improved LLOQ, 6495 vs. 6490

6495 QQQ
Area %RSD = 11.1
6490 QQQ
Area %RSD = 19.5
Verapamil IDL – Measured at the LLOQ Level

<table>
<thead>
<tr>
<th>Amount measured</th>
<th>Replicates</th>
<th>%RSD</th>
<th>t (99%)</th>
<th>IDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 ag (LLOQ)</td>
<td>n = 10</td>
<td>15.1</td>
<td>2.182</td>
<td>17 ag</td>
</tr>
</tbody>
</table>

IDL = t x (%RSD/100) x Amount = 2.182 x (15.1/100) x 1.0 fg = 17 ag

- Excellent peak area precision (%RSD) are observed at the lowest level (LLOQ)
Bentazon (-) Sensitivity in Solvent
Sub-femtogram Limits of Detection and Quantitation

LOD = 0.2 pg/mL = 0.4 fg on-column in Solvent
LLOQ = 0.5 pg/mL = 1.0 fg on-column in Solvent

- Unparalleled sensitivity (LLOQ 0.5 pg/mL and 1.0 fg on-column) – better than the 6490
- Excellent precision (peak area %RSD) and accuracy are observed at the lowest levels
Bentazon IDL – Measured at the LLOQ Level

<table>
<thead>
<tr>
<th>Amount measured</th>
<th>Replicates</th>
<th>%RSD</th>
<th>t (99%)</th>
<th>IDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 pg/mL, 1.0 fg (LLOQ)</td>
<td>n = 7</td>
<td>12.4</td>
<td>3.143</td>
<td>0.20 pg/mL, 0.39 fg</td>
</tr>
</tbody>
</table>

IDL = t x (%RSD/100) x Amount = 3.143 x (12.4/100) x 1.0 fg = 0.39 fg

Injection volume = 2 µL

• Excellent peak area precision (%RSD) are observed at the lowest level (LLOQ)

Agilent 6495 QQQ LC/MS System
6 Orders of Linear Dynamic Range, Peptide Quantitation

**6 Orders of Linear Dynamic Range**

\[ R^2 = 0.998 \]

- Excellent assay **accuracy** and **precision** are achieved at all levels including the LLOQ level

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**Table:**

<table>
<thead>
<tr>
<th>Levels</th>
<th>%RSD (n = 10)</th>
<th>% Accuracy</th>
<th>RT %RSD (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 amol</td>
<td>14.0</td>
<td>109.8</td>
<td></td>
</tr>
<tr>
<td>7.5 amol</td>
<td>16.0</td>
<td>108.7</td>
<td></td>
</tr>
<tr>
<td>15 amol</td>
<td>9.4</td>
<td>105.0</td>
<td></td>
</tr>
<tr>
<td>30 amol</td>
<td>9.0</td>
<td>87.1</td>
<td></td>
</tr>
<tr>
<td>300 fmol</td>
<td>1.6</td>
<td>85.2</td>
<td>0.12</td>
</tr>
<tr>
<td>3 fmol</td>
<td>1.2</td>
<td>81.4</td>
<td></td>
</tr>
<tr>
<td>30 fmol</td>
<td>0.6</td>
<td>86.4</td>
<td></td>
</tr>
<tr>
<td>300 fmol</td>
<td>0.7</td>
<td>87.4</td>
<td></td>
</tr>
<tr>
<td>3 pmol</td>
<td>2.1</td>
<td>105.6</td>
<td></td>
</tr>
<tr>
<td>5 pmol</td>
<td>10.0</td>
<td>97.5</td>
<td></td>
</tr>
</tbody>
</table>
Proven System Robustness in Complex Food Matrix: Multi-Residue Pesticide Analysis in Black Tea

- Pesticide standards analyzed before, in the middle, and after matrix injections
- No signal decrease was observed after 3 days of operation running with heavy food matrix (black tea)
- %RSD < 6 (n = 6 data files) – Average results of > 250 pesticides analyzed
Unrivaled System Robustness in Biological Matrix: Protein Quantitation in Plasma

- Selected peptides from 42 peptides in the QC sample – normalized to Day 1 response
- **Peptide QC samples** analyzed daily after every ~25 plasma digest injections
- No significant signal degradation observed after **853 injections** of **40 µg plasma digest per injection** and **3.5 weeks of continuous operation**
- Response %RSD: 6 - 15
Ultra-Fast MRM Acquisition – 1ms Dwell Time

123 Pesticides, 1 ng/mL

***Fast MRM allows enough data points across the peak for precise and accurate multi-compound quantitation with UHPLC speed***

- **Precision** %RSD < 20
- **%Accuracy** 80 - 120

![Graph showing Calibration Curve at 1ms dwell 1 – 1000 ng/mL]

- 1,000 ng/mL
  - %Accuracy = 100.4

- 100 ng/mL
  - %Accuracy = 92.1
  - %RSD = 6.2

- 10 ng/mL
  - %RSD = 4.2

- 1 ng/mL
  - %Accuracy = 108.3
  - %RSD = 4.4
Improved Mass Range

- Improved peptide quantitation can be achieved with the 6495
  - Extended mass range up to 2,250 m/z
  - Increased detection efficiency (HED voltage up to 20kV)
6495 QQQ Key Applications

**Food Safety - Pesticides**
Analysis of >250 trace level pesticides in food matrices (e.g. black tea) with extensive sample dilutions

**Food Safety - Estrogens**
Determination of ultra-trace level estrogens in milk product

**Environmental - Water Analysis**
Quantitation of ultra-trace level hormones (EDCs) in drinking water using direct injection

**Peptide Quantitation**
Quantitation of synthetic peptide at sub-attomole level using nanoflow and standard flow chromatography

**Peptide Quantitation**
Quantitative analysis of microcystins (cyclic nonribosomal peptides) in liver extract samples using stand flow chromatography
High Sensitivity Allows Extensive Sample Dilution

1:20 dilution of > 250 pesticides spiked into black tea at 10 µg/kg (MRL), 0.1 ng/mL

1:100 dilution of pesticides in black tea at MRL, 20 pg/mL

- Multi-residue pesticide analysis in food products – most demanding food safety applications
- Improved sensitivity and precision of the 6495 allows accurate quantitation of pesticides < Maximum Residue Limits (MRLs) imposed by EU, even with extensive sample dilution
- Sample dilution leads to less matrix effects and improved method robustness
Ultimate Sensitivity for Pesticides in Negative Mode
Sub-pg/mL LLOQs and Sub-fg IDLs

Injection volume = 2 μL

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>LOD (pg/mL)</th>
<th>LLOQ (pg/mL)</th>
<th>IDL (pg/mL)</th>
<th>Neat Solvent</th>
<th>LOD (pg/mL)</th>
<th>LLOQ (pg/mL)</th>
<th>IDL (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fipronil</td>
<td>0.2</td>
<td>0.5</td>
<td>0.14</td>
<td>2.7</td>
<td>1</td>
<td>1</td>
<td>0.39</td>
</tr>
<tr>
<td>Bentazon</td>
<td>0.2</td>
<td>0.5</td>
<td>0.20</td>
<td>0.39</td>
<td>0.5</td>
<td>1</td>
<td>0.36</td>
</tr>
<tr>
<td>Teflubenzuron</td>
<td>0.5</td>
<td>1</td>
<td>0.37</td>
<td>0.73</td>
<td>5</td>
<td>5</td>
<td>2.13</td>
</tr>
<tr>
<td>Hexaflumuron</td>
<td>0.5</td>
<td>1</td>
<td>0.38</td>
<td>0.75</td>
<td>5</td>
<td>5</td>
<td>1.09</td>
</tr>
<tr>
<td>Flubendiamide</td>
<td>0.5</td>
<td>1</td>
<td>0.27</td>
<td>0.54</td>
<td>1</td>
<td>5</td>
<td>0.54</td>
</tr>
<tr>
<td>Fluazinam</td>
<td>0.5</td>
<td>1</td>
<td>1.10</td>
<td>2.20</td>
<td>5</td>
<td>5</td>
<td>2.13</td>
</tr>
</tbody>
</table>

| LOD Levels in Black Tea Matrix |

- Fipronil 1 pg/mL
- Bentazon 0.5 pg/mL
- Teflubenzuron 5 pg/mL
- Hexaflumuron 5 pg/mL
- Fluazinam 5 pg/mL
- Flubendiamide 1 pg/mL

• LLOQs < 20 pg/mL (40 fg on-column) is required to achieve MRL with 1:100 dilution.
Bentazon Sensitivity in Black Tea
Low femtogram Limits of Detection and Quantitation

**LOD** = 0.5 pg/mL = 1.0 fg in 20x Diluted Black Tea

**LLOQ** = 1 pg/mL = 2.0 fg in 20x Diluted Black Tea

- **Unparalleled sensitivity** in black tea matrix - LLOQ 1.0 pg/mL and 2.0 fg on-column
- **Excellent precision** (peak area %RSD) and **accuracy** are observed at the low levels in black tea matrix, e.g. the LLOQ level.
### Bentazon: Superb Precision & IDL in Black Tea

<table>
<thead>
<tr>
<th>Amount measured</th>
<th>Replicates</th>
<th>%RSD</th>
<th>t (99%)</th>
<th>IDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 pg/mL, 2.0 fg (LLOQ)</td>
<td>n = 7 injections</td>
<td>11.4</td>
<td>3.143</td>
<td>0.36 pg/mL, 0.72 fg</td>
</tr>
</tbody>
</table>

IDL = \( t \times \left(\frac{\%RSD}{100}\right) \times \text{Amount} = 3.143 \times \left(\frac{11.4}{100}\right) \times 2.0 \text{ fg} = 0.72 \text{ fg} \)

**Injection volume = 2 \mu L**

**Excellent peak area precision** (%RSD) are observed at the lowest level in black tea matrix.
Five Orders of Linear Dynamic Range

Fipronil (-) in 1:20 diluted black tea
1 pg/mL – 100 ng/mL (2 fg - 200 pg)
5 Orders
$R^2 = 0.995$

<table>
<thead>
<tr>
<th>Levels</th>
<th>%RSD (n = 7)</th>
<th>%Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 pg/mL</td>
<td>10.41</td>
<td>82.4</td>
</tr>
<tr>
<td>5 pg/mL</td>
<td>5.84</td>
<td>87.2</td>
</tr>
<tr>
<td>10 pg/mL</td>
<td>6.41</td>
<td>82.6</td>
</tr>
<tr>
<td>50 pg/mL</td>
<td>6.10</td>
<td>97.4</td>
</tr>
<tr>
<td>100 pg/mL</td>
<td>3.86</td>
<td>100.8</td>
</tr>
<tr>
<td>500 pg/mL</td>
<td>1.73</td>
<td>108.8</td>
</tr>
<tr>
<td>1 ng/mL</td>
<td>2.93</td>
<td>112.5</td>
</tr>
<tr>
<td>5 ng/mL</td>
<td>2.25</td>
<td>119.7</td>
</tr>
<tr>
<td>10 ng/mL</td>
<td>2.54</td>
<td>116.8</td>
</tr>
<tr>
<td>50 ng/mL</td>
<td>2.62</td>
<td>102.3</td>
</tr>
<tr>
<td>100 ng/mL</td>
<td>2.51</td>
<td>95.8</td>
</tr>
</tbody>
</table>

Spinosyn A (+) in 1:20 diluted black tea
1 pg/mL – 100 ng/mL (2 fg - 200 pg)
5 Orders
$R^2 = 0.997$

<table>
<thead>
<tr>
<th>Levels</th>
<th>%RSD (n = 7)</th>
<th>%Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 pg/mL</td>
<td>15.0</td>
<td>110.7</td>
</tr>
<tr>
<td>5 pg/mL</td>
<td>10.9</td>
<td>85.7</td>
</tr>
<tr>
<td>10 pg/mL</td>
<td>6.8</td>
<td>97.2</td>
</tr>
<tr>
<td>50 pg/mL</td>
<td>3.6</td>
<td>86.0</td>
</tr>
<tr>
<td>100 pg/mL</td>
<td>5.4</td>
<td>100.5</td>
</tr>
<tr>
<td>500 pg/mL</td>
<td>7.3</td>
<td>96.3</td>
</tr>
<tr>
<td>1 ng/mL</td>
<td>6.5</td>
<td>105.0</td>
</tr>
<tr>
<td>5 ng/mL</td>
<td>2.1</td>
<td>105.8</td>
</tr>
<tr>
<td>10 ng/mL</td>
<td>3.6</td>
<td>111.3</td>
</tr>
<tr>
<td>100 ng/mL</td>
<td>1.2</td>
<td>96.2</td>
</tr>
</tbody>
</table>

• Wide (5 orders) **linear dynamic range**, excellent assay **accuracy** and **precision** at all levels
Signal Improvements for High Relevance Pesticides

- Improved sensitivity (peak area gain of 3x) are observed on the new 6495 vs. the 6490.
Improved Precision & Lower LLOQs in Black Tea

Improved precision (%RSD) are observed, particularly at the lowest levels (LLOQs).

The enhanced peak area response and improved precision (%RSD) means pesticides achieve lower LLOQs (≤ MRL level) using the new 6495.

- 67% of pesticides were easily detected with a %RSD < 20 with 1:100 dilution.
- This means LLOQs < 20 pg/mL (ppt).
- %RSD < 20 meets SANCO guidelines.

Comparison of Area %RSD for 50 Pesticides at MRL

# of pesticides detected at MRL

6495

- 67% of pesticides were easily detected with a %RSD < 20 with 1:100 dilution.
- This means LLOQs < 20 pg/mL (ppt).
- %RSD < 20 meets SANCO guidelines.
Dilution allows More Efficient Ionization of Pesticides

**Alanyacarb, 10 µg/kg in Black Tea**

- **No Dilution**
- 1:5
- 1:10
- 1:20
- 1:50
- 1:100

**Oxamyl, 10 µg/kg in Black Tea**

- **No Dilution**
- 1:5
- 1:10
- 1:20
- 1:50
- 1:100
Recovery (%) in Black Tea with Different Dilutions

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>No dilution (n = 5)</th>
<th>Dilution 1:5 (n = 5)</th>
<th>Dilution 1:10 (n = 5)</th>
<th>Dilution 1:20 (n = 5)</th>
<th>Dilution 1:50 (n = 5)</th>
<th>Dilution 1:100 (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetamiprid</td>
<td>29.4 ± 0.8</td>
<td>57.3 ± 1.4</td>
<td>67.5 ± 3.7</td>
<td>79.9 ± 2.9</td>
<td>91.8 ± 5.2</td>
<td>109.5 ± 3.4</td>
</tr>
<tr>
<td>Alanycarb</td>
<td>10.4 ± 1.3</td>
<td>73.9 ± 2.2</td>
<td>81.5 ± 14.3</td>
<td>85.7 ± 11.1</td>
<td>87.6 ± 4.7</td>
<td>121.7 ± 10.8</td>
</tr>
<tr>
<td>Aldicarb</td>
<td>36.9 ± 1.0</td>
<td>69.9 ± 1.4</td>
<td>78.0 ± 3.5</td>
<td>91.0 ± 4.2</td>
<td>95.2 ± 8.8</td>
<td>104.9 ± 14.1</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>56.9 ± 1.8</td>
<td>80.1 ± 3.8</td>
<td>80.8 ± 4.1</td>
<td>96.1 ± 7.2</td>
<td>102.6 ± 6.6</td>
<td>116.4 ± 9.6</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>33.9 ± 1.7</td>
<td>68.6 ± 2.4</td>
<td>84.1 ± 5.4</td>
<td>89.0 ± 7.9</td>
<td>88.2 ± 8.8</td>
<td>84.7 ± 7.5</td>
</tr>
<tr>
<td>Diuron</td>
<td>79.7 ± 4.0</td>
<td>90.4 ± 7.0</td>
<td>91.7 ± 4.9</td>
<td>94.9 ± 7.2</td>
<td>89.2 ± 7.3</td>
<td>100.9 ± 13.5</td>
</tr>
<tr>
<td>Flufenoxuron</td>
<td>95.4 ± 1.1</td>
<td>88.8 ± 1.6</td>
<td>89.4 ± 3.8</td>
<td>93.3 ± 5.8</td>
<td>100.0 ± 6.1</td>
<td>119.2 ± 13.9</td>
</tr>
<tr>
<td>Monocrotophos</td>
<td>4.6 ± 0.3</td>
<td>13.9 ± 0.3</td>
<td>21.8 ± 0.8</td>
<td>33.8 ± 1.1</td>
<td>58.5 ± 2.0</td>
<td>95.1 ± 5.7</td>
</tr>
<tr>
<td>Oxamyl</td>
<td>20.8 ± 0.7</td>
<td>52.6 ± 1.9</td>
<td>65.0 ± 2.0</td>
<td>79.7 ± 3.0</td>
<td>91.2 ± 4.6</td>
<td>110.6 ± 5.2</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>40.0 ± 1.4</td>
<td>45.9 ± 0.9</td>
<td>46.6 ± 3.8</td>
<td>52.2 ± 1.7</td>
<td>70.9 ± 2.9</td>
<td>97.3 ± 2.0</td>
</tr>
</tbody>
</table>

- Cells shaded in green show %recovery of 80 – 120, which are in compliance with SANCO requirements.
- Pesticides show full recovery and no signal suppression with 1:100 dilution.
- Neat solvent calibration curve can be used for pesticides quantitation with 1:100 dilution.
6495 QQQ Key Applications

**Food Safety - Pesticides**
Analysis of >250 trace level pesticides in food matrices (e.g. black tea) with extensive sample dilutions

**Food Safety - Estrogens**
Determination of ultra-trace level estrogens in milk product

**Environmental - Water Analysis**
Quantitation of ultra-trace level hormones (EDCs) in drinking water using direct injection

**Peptide Quantitation**
Quantitation of synthetic peptide at sub-attomole level using nanoflow and standard flow chromatography

**Peptide Quantitation**
Quantitative analysis of microcystins (cyclic nonribosomal peptides) in liver extract samples using standard flow chromatography
Sensitive Quantitation of Estrogens in Cow Milk

Eight estrogens spiked in cow milk at 0.1 µg/kg, corresponding to 0.1 ng/mL

<table>
<thead>
<tr>
<th>ID</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Estriol (-)</td>
</tr>
<tr>
<td>2</td>
<td>17-α-Estradiol (-)</td>
</tr>
<tr>
<td>3</td>
<td>17-β-Estradiol (-)</td>
</tr>
<tr>
<td>4</td>
<td>Ethynyl estradiol (-)</td>
</tr>
<tr>
<td>5</td>
<td>Estrone (-)</td>
</tr>
<tr>
<td>6</td>
<td>Diethylstilbestrol (-)</td>
</tr>
<tr>
<td>7</td>
<td>Hexestrol (-)</td>
</tr>
<tr>
<td>8</td>
<td>Dienestrol (-)</td>
</tr>
</tbody>
</table>

- Estrogen levels in milk products are regulated by government agencies down to 1 µg/kg
- Highly **sensitive** and **precise** quantitation of estrogens was achieved using the new 6495 with a simple QuEChERS procedure without derivatization or enrichment.
- **LLOQs** for all estrogens (<< 0.1 µg/kg) are far below the regulatory requirement (1 µg/kg)
Highest Sensitivity and Excellent Precision
LLOQs and IDLs at pg/mL (fg) level

<table>
<thead>
<tr>
<th>Compounds</th>
<th>LLOQ (pg/mL)</th>
<th>%RSD n = 7</th>
<th>IDL pg/mL</th>
<th>IDL fg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estriol</td>
<td>5</td>
<td>11.3</td>
<td>1.8</td>
<td>8.9</td>
</tr>
<tr>
<td>17-α-Estradiol</td>
<td>10</td>
<td>8.4</td>
<td>2.7</td>
<td>13.3</td>
</tr>
<tr>
<td>17-β-Estradiol</td>
<td>10</td>
<td>11.4</td>
<td>3.6</td>
<td>18.0</td>
</tr>
<tr>
<td>Ethynyl estradiol</td>
<td>10</td>
<td>11.7</td>
<td>3.7</td>
<td>18.5</td>
</tr>
<tr>
<td>Estrone</td>
<td>5</td>
<td>9.6</td>
<td>1.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
<td>10</td>
<td>12.8</td>
<td>2.0</td>
<td>10.1</td>
</tr>
<tr>
<td>Hexestrol</td>
<td>5</td>
<td>11.1</td>
<td>1.7</td>
<td>8.7</td>
</tr>
<tr>
<td>Dienestrol</td>
<td>5</td>
<td>7.6</td>
<td>1.2</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Injection volume = 5 µL
Estriol: Superb Precision and IDL at Low-fg Level

<table>
<thead>
<tr>
<th>Estriol Amount measured</th>
<th>Replicates</th>
<th>%RSD</th>
<th>t (99%)</th>
<th>Estriol IDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 pg/mL, 25 fg (LLOQ)</td>
<td>n = 7 injections</td>
<td>11.3</td>
<td>3.143</td>
<td>1.8 pg/mL, 8.9 fg</td>
</tr>
</tbody>
</table>

IDL = t x (%RSD/100) x Amount = 3.143 x (10.4/100) x 25 fg = 8.9 fg

Inj # | Peak Area | S/N (Peak to peak) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>11.1</td>
</tr>
<tr>
<td>2</td>
<td>54</td>
<td>11.1</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>9.8</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>9.8</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>10.5</td>
</tr>
<tr>
<td>6</td>
<td>54</td>
<td>8.6</td>
</tr>
<tr>
<td>7</td>
<td>52</td>
<td>9.9</td>
</tr>
</tbody>
</table>

%RSD 11.3 Ave. 10.1

Injection volume = 5 µL

- Excellent peak area precision (%RSD) are observed at the lowest levels (LLOQs)
Confident Estrogen Quantitation in Milk

- Excellent linearity ($R^2 \geq 0.998$), accuracy and precision are achieved at all levels.
## Summary of Estrogen Quantitation in Milk

Excellent linearity, precision, accuracy and matrix recovery

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$R^2$</th>
<th>%RSD, n = 7</th>
<th>%Accuracy</th>
<th>%Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.2 µg/kg</td>
<td>1 µg/kg</td>
</tr>
<tr>
<td>Estriol</td>
<td>0.9988</td>
<td>0.94 – 5.33</td>
<td>94.6 – 106.0</td>
<td>92.9</td>
</tr>
<tr>
<td>17-α-Estradiol</td>
<td>0.9973</td>
<td>1.32 – 5.31</td>
<td>90.6 – 112.0</td>
<td>92.2</td>
</tr>
<tr>
<td>17-β-Estradiol</td>
<td>0.9985</td>
<td>1.45 – 3.86</td>
<td>91.1 – 113.8</td>
<td>92.9</td>
</tr>
<tr>
<td>Ethynyl estradiol</td>
<td>0.9984</td>
<td>1.59 – 6.61</td>
<td>90.1 – 114.5</td>
<td>90.2</td>
</tr>
<tr>
<td>Estrone</td>
<td>0.9987</td>
<td>1.45 – 3.76</td>
<td>92.4 – 110.7</td>
<td>94.0</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
<td>0.9985</td>
<td>2.27 – 5.04</td>
<td>94.9 – 104.6</td>
<td>83.5</td>
</tr>
<tr>
<td>Hexestrol</td>
<td>0.9981</td>
<td>0.88 – 4.58</td>
<td>90.1 – 112.8</td>
<td>86.1</td>
</tr>
<tr>
<td>Dienestrol</td>
<td>0.9995</td>
<td>1.24 – 4.34</td>
<td>95.6 – 102.7</td>
<td>82.9</td>
</tr>
</tbody>
</table>
Food Safety - Pesticides
Analysis of >250 trace level pesticides in food matrices (e.g. black tea) with extensive sample dilutions

Food Safety - Estrogens
Determination of ultra-trace level estrogens in milk product

Environmental - Water Analysis
Quantitation of ultra-trace level hormones (EDCs) in drinking water using direct injection

Peptide Quantitation
Quantitation of synthetic peptide at sub-attomole level using nanoflow and standard flow chromatography

Peptide Quantitation
Quantitative analysis of microcystins (cyclic nonribosomal peptides) in liver extract samples using standard flow chromatography
Endocrine Disrupting Chemical (EDCs) levels in municipal water supplies are regulated by government agencies down to ng/L (ppt) level.

The highly sensitive 6495 allows the quantitation of EDCs at sub ng/L using direct injection.

No need for time consuming offline SPE or enrichment.
Highest Sensitivity and Excellent Precision
LLOQs and IDLs at sub-ng/L Level

<table>
<thead>
<tr>
<th>Compounds</th>
<th>LLOQ (ng/L)</th>
<th>%RSD n = 8</th>
<th>IDL (ng/L)</th>
<th>% Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estriol</td>
<td>1</td>
<td>5.6</td>
<td>0.168</td>
<td>94.8</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>&lt; 0.5</td>
<td>13.5</td>
<td>0.202</td>
<td>102.2</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.1</td>
<td>10.3</td>
<td>0.031</td>
<td>114.5</td>
</tr>
<tr>
<td>17α-Ethynylestradiol</td>
<td>1.75</td>
<td>14.8</td>
<td>0.78</td>
<td>98.4</td>
</tr>
<tr>
<td>Equilin</td>
<td>&lt; 0.2</td>
<td>3.7</td>
<td>0.022</td>
<td>115.1</td>
</tr>
<tr>
<td>Estrone</td>
<td>&lt; 0.2</td>
<td>7.2</td>
<td>0.043</td>
<td>115.1</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>&lt; 0.2</td>
<td>4.3</td>
<td>0.026</td>
<td>107.2</td>
</tr>
</tbody>
</table>
17β-Estradiol: Superb Precision and Ultra-Low IDL

<table>
<thead>
<tr>
<th>17β-Estradiol Measured</th>
<th>Replicates</th>
<th>%RSD</th>
<th>t (99%)</th>
<th>IDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 ng/L (LLOQ)</td>
<td>n = 8 injections</td>
<td>13.5</td>
<td>2.998</td>
<td>0.202 ng/L</td>
</tr>
</tbody>
</table>

MDL = t x (%RSD/100) x Amount = 2.998 x (13.5/100) x 0.5 ng/L = 0.202 ng/L

• Excellent peak area precision (%RSD) are observed at the lowest levels (LLOQs)
Confident Quantitation of EDCs in Drinking Water

• Excellent **linearity** \((R^2 \geq 0.995)\), assay **accuracy** and precision are achieved at all levels.
Summary of Hormone Quantitation in Drinking Water

Excellent linearity, precision and accuracy at all levels

<table>
<thead>
<tr>
<th>Compounds</th>
<th>LLOQ (ng/L)</th>
<th>IDL (ng/L)</th>
<th>$R^2$</th>
<th>Precision (%RSD, n = 8)</th>
<th>%Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estriol</td>
<td>0.4</td>
<td>0.168</td>
<td>0.997</td>
<td>0.81 – 10.6</td>
<td>93.6 – 113.6</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>&lt; 0.5</td>
<td>0.202</td>
<td>0.996</td>
<td>0.73 – 13.7</td>
<td>87.5 – 120.3</td>
</tr>
<tr>
<td>Testosterone</td>
<td>&lt; 0.1</td>
<td>0.031</td>
<td>0.994</td>
<td>2.09 – 10.3</td>
<td>88.8 – 114.5</td>
</tr>
<tr>
<td>17α-Ethynylestradiol</td>
<td>1.75</td>
<td>0.78</td>
<td>0.996</td>
<td>2.50 – 16.5</td>
<td>95.3 – 107.2</td>
</tr>
<tr>
<td>Equilin</td>
<td>&lt; 0.2</td>
<td>0.022</td>
<td>0.997</td>
<td>3.00 – 5.21</td>
<td>88.9 – 115.1</td>
</tr>
<tr>
<td>Estrone</td>
<td>&lt; 0.2</td>
<td>0.043</td>
<td>0.996</td>
<td>2.57 – 9.41</td>
<td>91.2 – 115.1</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>&lt; 0.1</td>
<td>0.026</td>
<td>0.995</td>
<td>1.48 – 9.07</td>
<td>89.2 – 107.2</td>
</tr>
</tbody>
</table>
**6495 QQQ Key Applications**

- **Food Safety - Pesticides**
  Analysis of >250 trace level pesticides in food matrices (e.g. black tea) with extensive sample dilutions

- **Food Safety - Estrogens**
  Determination of ultra-trace level estrogens in milk product

- **Environmental - Water Analysis**
  Quantitation of ultra-trace level hormones (EDCs) in drinking water using direct injection

- **Peptide Quantitation**
  Quantitation of synthetic peptide at sub-attomole level using nanoflow and standard flow chromatography

- **Peptide Quantitation**
  Quantitative analysis of microcystins (cyclic nonribosomal peptides) in liver extract samples using stand flow chromatography
Outstanding Sensitivity with Standard Flow LC

- Low attomole **sensitivity** for synthetic peptide spiked in tryptic digest using the 6495 with **standard flow chromatography**
- Six orders of **linear dynamic range**
- Excellent **precision** and **accuracy** are observed at all levels including the LLOQ level

### Levels

<table>
<thead>
<tr>
<th>Levels</th>
<th>%RSD (n = 10)</th>
<th>% Accuracy</th>
<th>RT %RSD (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 amol</td>
<td>14.0</td>
<td>109.8</td>
<td></td>
</tr>
<tr>
<td>7.5 amol</td>
<td>16.0</td>
<td>108.7</td>
<td></td>
</tr>
<tr>
<td>15 amol</td>
<td>9.4</td>
<td>105.0</td>
<td></td>
</tr>
<tr>
<td>30 amol</td>
<td>9.0</td>
<td>87.1</td>
<td></td>
</tr>
<tr>
<td>300 fmol</td>
<td>1.6</td>
<td>85.2</td>
<td>0.12</td>
</tr>
<tr>
<td>3 fmol</td>
<td>1.2</td>
<td>81.4</td>
<td></td>
</tr>
<tr>
<td>30 fmol</td>
<td>0.6</td>
<td>86.4</td>
<td></td>
</tr>
<tr>
<td>300 fmol</td>
<td>0.7</td>
<td>87.4</td>
<td></td>
</tr>
<tr>
<td>3 pmol</td>
<td>2.1</td>
<td>105.6</td>
<td></td>
</tr>
<tr>
<td>5 pmol</td>
<td>10.0</td>
<td>97.5</td>
<td></td>
</tr>
</tbody>
</table>
Excellent Precision and Low Attomole Level IDL

<table>
<thead>
<tr>
<th>Amount measured</th>
<th>Replicates</th>
<th>%RSD</th>
<th>t (99%)</th>
<th>IDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0 amol (LLOQ)</td>
<td>n = 10 injections</td>
<td>14.0</td>
<td>2.821</td>
<td>2.0 amol</td>
</tr>
</tbody>
</table>

MDL = t x (%RSD/100) x Amount = 2.821 x (14.0/100) x 5.0 amol = 2.0 amol

- Excellent peak area **precision** (%RSD) are observed at the lowest levels (LLOQs).

---

**Injection #** | **Peak Area**
---|---
1 | 30.5
2 | 28.7
3 | 30.4
4 | 33.91
5 | 35.1
6 | 31.5
7 | 34.5
8 | 20.7
9 | 26.7
10 | 26.6

%RSD = 14.0
Ultimate Sensitivity with Nanoflow LC

- Ultimate sub-attomole sensitivity for synthetic peptide spiked in tryptic digest using the 6495 with nanoflow chromatography
- Wide (over 5 orders) linear dynamic range
- Excellent precision and accuracy are observed at all levels including the LLOQ level

<table>
<thead>
<tr>
<th>Levels (amol)</th>
<th>%RSD</th>
<th>%Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>4.52</td>
<td>103.2</td>
</tr>
<tr>
<td>1</td>
<td>8.50</td>
<td>80.2</td>
</tr>
<tr>
<td>10</td>
<td>5.75</td>
<td>84.6</td>
</tr>
<tr>
<td>100</td>
<td>4.71</td>
<td>88.9</td>
</tr>
<tr>
<td>1000</td>
<td>1.23</td>
<td>85.6</td>
</tr>
<tr>
<td>10,000</td>
<td>1.09</td>
<td>97.6</td>
</tr>
<tr>
<td>100,000</td>
<td>1.69</td>
<td>100.4</td>
</tr>
</tbody>
</table>
6495 QQQ Key Applications

Food Safety - Pesticides
Analysis of >250 trace level pesticides in food matrices (e.g. black tea) with extensive sample dilutions

Food Safety - Estrogens
Determination of ultra-trace level estrogens in milk product

Environmental - Water Analysis
Quantitation of ultra-trace level hormones (EDCs) in drinking water using direct injection

Peptide Quantitation
Quantitation of synthetic peptide at sub-attomole level using nanoflow and standard flow chromatography

Peptide Quantitation
Quantitative analysis of microcystins (cyclic nonribosomal peptides) in liver extract samples using stand flow chromatography
Microcystins are cyanotoxins that may cause serious damage to the liver of mammals.

Microcystins are challenging compounds to analyze due to the low levels in biological matrices.

MRM chromatograms of microcystins Using the 6495 with 1290 UHPLC

- RR
- YR
- LR
- LA
- LY
- LW
- LF
Outstanding Sensitivity and Linear Dynamic Range
Excellent Peak Area Precision %RSD at the LLOQ Levels (pg/mL)

- **Excellent linearity** ($R^2 \geq 0.99$), assay **accuracy** and **precision** are achieved for all seven microcystins and at all levels.

**RR, LLOQ**: 2.5 pg/mL
%RSD = 2.97
n = 3

**LY, LLOQ**: 50 pg/mL
%RSD = 10.3
n = 3

**RR, LLOQ**: 2.5-50,000 ng/mL
> 4 Orders of Linear Dynamic Range
$R^2 = 0.995$

**LY, LLOQ**: 50-50,000 ng/mL
3 Orders of Linear Dynamic Range
$R^2 = 0.998$
Analysis of Microcystins in Liver Extract

- The highly sensitive 6495 allows confident quantitation of microcystins at pg/mL levels in liver extract samples
Summary of Microcystins Quantitation
Excellent linearity, precision and accuracy at all levels

<table>
<thead>
<tr>
<th>Compounds</th>
<th>LLOQ (pg/mL)</th>
<th>Range (pg/mL)</th>
<th>$R^2$</th>
<th>Precision (%RSD, $n = 3$)</th>
<th>%Accuracy</th>
<th>Liver Extract (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR</td>
<td>2.5</td>
<td>2.5 - 50,000</td>
<td>0.9949</td>
<td>0.13 - 6.24</td>
<td>93.9 - 111.7</td>
<td>930</td>
</tr>
<tr>
<td>YR</td>
<td>2.5</td>
<td>2.5 - 50,000</td>
<td>0.9906</td>
<td>1.30 - 13.51</td>
<td>91.6 - 111.9</td>
<td>107</td>
</tr>
<tr>
<td>LR</td>
<td>2.5</td>
<td>2.5 - 50,000</td>
<td>0.9921</td>
<td>0.41 - 11.35</td>
<td>92.2 - 107.5</td>
<td>155</td>
</tr>
<tr>
<td>LA</td>
<td>25</td>
<td>25 - 50,000</td>
<td>0.9984</td>
<td>0.57 - 5.40</td>
<td>85.8 - 101.9</td>
<td>179</td>
</tr>
<tr>
<td>LY</td>
<td>50</td>
<td>50 - 50,000</td>
<td>0.9975</td>
<td>2.80 – 1034</td>
<td>88.8 - 119.4</td>
<td>292</td>
</tr>
<tr>
<td>LW</td>
<td>25</td>
<td>25 - 50,000</td>
<td>0.9987</td>
<td>0.66 - 14.3</td>
<td>85.6 - 105.1</td>
<td>176</td>
</tr>
<tr>
<td>LF</td>
<td>25</td>
<td>25 - 50,000</td>
<td>0.9975</td>
<td>1.62 - 6.35</td>
<td>85.5 - 115.0</td>
<td>195</td>
</tr>
</tbody>
</table>
MassHunter Acquisition B.07

- Support for 6495 QQQ LC/MS
- Faster and accurate autotune
- Improved workflows
- Optimizer to better support tMRM
- Dynamic MRM (dMRM) and triggered MRM (tMRM)
- iFunnel express, Study manager, Skyline automation, application kits and solutions
• New autotune reduces the time by **40 min** (40%) when both POS & NEG polarities are tuned
• New checktune reduces the time by **2 min** (25%) when both polarities are checktuned
• Enhanced quadrupole optics tuning algorithms results in higher ion transmission
Dynamic MRM (dMRM)

**Dynamic MRM, Fast MRM Speed and Polarity Switch**

- Group MRM s 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)

### Time Segment

<table>
<thead>
<tr>
<th>Compounds (10/block)</th>
<th>Time Segment 1</th>
<th>Time Segment 2</th>
<th>Time Segment 3</th>
<th>Time Segment 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>80</td>
<td>100</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>

**MRM**

- Cycle Time (sec) 0.5 0.8 1.0 0.7

**Dynamic MRM**

- Max Coincident Compounds 20 40 40 30
- Cycle Time (sec) 0.4 0.4 0.4 0.4

**Image Details:**
- Cycle time chart with concurrent dMRMs indicated.
- Comparison of cycle times and compounds in different time segments.
Triggered MRM (tMRM)

**Triggered MRM, tMRM Database and Library**

- Triggered MRM, tMRM Database and Library
- Apex
- tMRM Product Ion Spectrum
- Match Score 95.1
- 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)**

- Find 10 MRM transitions in Optimizer
- Injection volume flexibility
- Improved method editing

![Enhanced Optimizer Workflow in MassHunter B.07](image-url)
iFunnel Express

iFunnel Express – Source and iFunnel Optimization

Method Generation

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

MH Source and Funnel Optimizer

Acquire Data for Each Parameter

MassHunter Quant

Excel File for Optimized Parameter
Study Manager and Skyline Automation

Study Manager – Fully Automated Quantitation Workflow

1. Specify input File
2. Specify Quant Method
3. Specify Report
4. Submit and go!

Skyline Automation

- Automated workflows for peptide quantitation
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.
Summary: Value of the New 6495 QQQ LC/MS

- **Increased sensitivity**
  - Lowest Limits of Quantitation

- **Excellent precision & accuracy**
  - at the Lowest Levels

- **Wide linear dynamic range**

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**Streamlined Analytical Workflow**
- More Productive Lab

**Reproducible, High Quality Data**
- More Confident Results

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**Proven robustness and stability in complex matrices**

**Fast MRM speed, dynamic and triggered MRM**

**Screen, Confirm and Quantify with UHPLC Speed**

**Ease to use, workflow driven software, kits and solutions**

**Ease of Operation & Fast Set-up**
- Get Answers Quickly!

Confident quantitation and confirmation for the most demanding applications
6495 Brochure: 5991-4541EN

6495 App Note: 5991-4687EN

6495 App Note: 5991-4686EN

6495 App Note: 5991-4685EN

6495 Video
ASMS 2014, Baltimore
Celebrating Achievement

CELEBRATING ACHIEVEMENT IN
MASS SPECTROMETRY

AGILENT BREAKFAST MEETINGS AT ASMS | June 16th – June 18th, 2014 | 7:00 – 8:00 am

AGILENT’S HOSPITALITY SUITE

DATES: June 16 – 18, 2014
8:00 am – 11:00 pm
LOCATION: Key Baltimore 5
Agilent will host a hospitality suite on Monday, Tuesday, and Wednesday evenings. Come celebrate with us and recharge our booth mass spectrometry technology.

Agilent Technologies