Environmental and Food Safety Analyses by UHPLC and LC/MS Using the New Agilent 1290 Infinity LC System and Agilent Mass Spectrometers

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Applications Development
Agilent Technologies
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Topics of Discussion

UHPLC theory and advantages

Challenges in Instrument design

- Pump: flow and pressure range, mixing requirements
- Injector: injection range, principle, carryover
- Column availability, stability, variety
- Column management: temperature control, column dimensions and valve configurations
- UV/VIS detection: balancing dispersion and sensitivity
- MS Considerations: acquisition rate, optimal flow rate
Resolution Equation

Although we may be aiming for faster separations, we normally also need to maintain or increase resolution.

\[ R_S = \frac{\sqrt{N}}{4} \frac{(\alpha-1)}{\alpha} \frac{k}{(k+1)} \]

\(\alpha = \text{Selectivity} – \text{influenced by mobile and stationary phase}\)

\(N = \text{Column Efficiency} – \text{influenced by length and particle size}\)

\(k = \text{Capacity Factor} \text{ (retention)} – \text{influenced by stationary and mobile phase, gradient slope and dwell volume (gradients)}\)
Method Transitions to UHPLC sub 2 micron materials

Maintain Acceptable Flow Rates at High Linear Velocity by Progressively Decreasing Column Diameter

Starting point of 4.6mm i.d. 5um column, 1ml/min

Increase linear velocity to gain resolution. Reduce column diameter for solvent saving and maintain detection compatibility.

<table>
<thead>
<tr>
<th>Lin. Vel. (mm)</th>
<th>ml/min</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6 mm</td>
<td>0.7</td>
<td>1.4</td>
<td>2.1</td>
<td>2.8</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>3 mm</td>
<td>0.3</td>
<td>0.6</td>
<td>0.9</td>
<td>1.2</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>2.1 mm</td>
<td>0.14</td>
<td>0.29</td>
<td>0.44</td>
<td>0.58</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>1 mm</td>
<td>0.033</td>
<td>0.066</td>
<td>0.1</td>
<td>0.133</td>
<td>0.166</td>
<td></td>
</tr>
</tbody>
</table>
Increased Pressure Requirements and Enhanced Speed and Performance Associated with Reduced Particle Size

<table>
<thead>
<tr>
<th>$d_p$ ($\mu$m)</th>
<th>$\Delta P$ (bar)</th>
<th>$t_R$ (min)</th>
<th>$H_{MIN}$ ($\mu$m)</th>
<th>$N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>14.5</td>
<td>34.7</td>
<td>10.0</td>
<td>25,000</td>
</tr>
<tr>
<td>3.0</td>
<td>66.9</td>
<td>20.8</td>
<td>6.0</td>
<td>41,000</td>
</tr>
<tr>
<td>1.5</td>
<td>531</td>
<td>10.4</td>
<td>3.0</td>
<td>83,000</td>
</tr>
<tr>
<td>1.0</td>
<td>1800</td>
<td>6.9</td>
<td>2.0</td>
<td>125,000</td>
</tr>
<tr>
<td>0.75</td>
<td>4270</td>
<td>5.2</td>
<td>1.5</td>
<td>166,000</td>
</tr>
<tr>
<td>0.50</td>
<td>14400</td>
<td>3.5</td>
<td>1.0</td>
<td>250,000</td>
</tr>
</tbody>
</table>

With a 5.0 to 1.5 $\mu$m particle size reduction
• $t_R$ decreases >3-fold
• $N$ increases >3-fold

But . . . $\Delta P$ increases >35-fold
Maximum Power Range

maximum performance, flexibility, compatibility

1290 Infinity

Standard LC
Advanced UHPLC Binary Pump

- Flow path and integration of new materials

Degasser

Solvent selection valve

Mixer

High Resolution Pump drives

Multi-layer Heat exchanger

Silicon Carbide Pistons

Purge Valve

Channel A

Channel B
What to Look for in Gradient Performance

- Steep 0.5ml (25%/cv) Gradient, alkylphenones, system 38
System to System Performance
- systems 38 and 0x67, same column installed, 2ml gradient

r.t. 2.256

r.t. 2.234
Resolution – effect of increasing capacity factor $k'$ in isocratic Resolution Equation

![Graph showing R as Affected by Capacity Factor](image)
Resolution – effect of increasing capacity factor \( k^* \) in gradient peak capacity equations

\[
Pc = 1 + \frac{(t_{last} - v_0)}{pw} \quad \text{Measured}
\]

for a 1ml/min gradient, 30-90% ACN, 3x50mm 1.8um, 11ul disp., avg. analyte mc.wt. 162g/m
My Lab Environment?

1290 Infinity LC
- Fluorescence
- Diode Array
- Outboard 12 position solvent select valves
- Column Compartment with 2 position 6 port valve
- Autosampler
- Binary pump with integral vacuum degasser and solvent select valves
What Kind of Lab Environment is Needed?
Design Considerations for UHPLC Sample Introduction

- Injection principle (variable loop, fixed loop designs)
- Usable injection volume range
- Speed of injection cycle
- Minimal dispersion
- Minimal carryover
- Minimal sample waste
Design Considerations for UHPLC Samplers

• Injection principle (variable loop, fixed loop designs)

Fixed loop designs leave flush solution in loop, which can distort peak shape or alter selectivity in scouting separations.
Tips for minimizing dispersion in LC systems

- Keep injection volumes small, ensure weak diluent when possible
- Ensure autosampler flush solvent is matched with starting conditions

**Figure 2. Effect of Strong Diluent Injections – Bandbroadening**

Methyl, propyl and butyl paraben in methanol diluent. 250nm UV, Direct injection to column. Inj. volume 1, 2, 5 and 10ul

Methanol diluted sample, gradient from 30% ACN initial condition, 3x50mm 1.8um column
Flexibility in injection options – more freedom of choice - extending the capabilities

- Syringe Pump
- Low pressure Valve
- Valve option 1
- Valve option 2
- Rail for external Valve

Applications:
- a) Automated needle seat back-flushing for lower carry over
- b) Fixed loop injection mode for fastest cycle times
- c) By-pass metering device for lower delay volume
- d) ...and more ...........

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Carry-over comparison
- two system designs

0.021% on popular UHPLC system (PLUNO mode)

0.002% to 0.004% on another UHPLC without needle seat backflushing

Column: 2.1x50mm BEH C 18
Design Considerations for Column Compartments

- Column management: use any analytical column size or hardware
- Use fully porous, superficially porous or monolithic materials
- Temperature control: sub-ambient to 90C for all application types with independent temperature zones for each column compartment
- Valve configurations: 6-port, 10-port, two position valves for 2 column selection, including alternating column regeneration (10 port only)
- With 2 column compartment modules, 9-port eight position column selection valves, you may expand column selection to 4 columns with independent thermal zones, or up to 8 columns total
- Change valve type in minutes using universal drive valves and RFID identified liquid ends
UHPLC Therm. Column Compartment
- look for flexibility:

New 2PS/6PT, 2PT/10PT and 9PT/8PS high pressure valves

RFID-tag, stores type, pressure range, valve switches
Design Considerations for UHPLC Optical Detection

• Maximizing sensitivity without excessive cell volume
• Flow rate and gradient compositional stability
• Refractive index correction approaches
  • direct correction through optical design,
  • acquisition with reference wavelength,
  • baseline subtraction from reference chromatogram
Long Path Cartridge Cell
- *Optofluidic waveguides*

High Light Transmission due to Total-Internal Reflection (TIR) principle (~100 % Light efficiency)
supports longer path length cell designs

**Benefits:**
- High sensitivity (S/N) with small cell volumes (dispersion effects)
- Coating-free fused silica (no special care instructions or smiling baseline effects)
- Easy cell selection (one cell for all major applications)
- Cartridge design for ease of use
Effect of Data Acquisition Rate (time constant)
Peak Width, Resolution and Peak Capacity in Ultra-Fast LC

80Hz versus 20Hz
- 30% Peak Width
+ 30% Resolution
+ 40% Peak Capacity
+ 70% Apparent Column Efficiency

80Hz versus 10Hz
- 55% Peak Width
+ 90% Resolution
+ 120% Peak Capacity
+ 260% Apparent Column Efficiency
Conclusion – UHPLC Design Requirements

• Look for best overall performance and feature set according to your application requirements (routine vs. method development, etc.)

• Choose flexible injection designs that allow variable or fixed loop capability, should usage priorities change

• Versatile column compartments support a wide range of applications on one common platform

• Understand available column technologies to ensure a good mix of performance and chemical selectivity
Pharmaceuticals in Water

- Rising concern about pharmaceuticals in surface water
- Wide range of analytes at low levels
- High speed MS sampling and mass resolution needed
Choose the Appropriate MS Technology

<table>
<thead>
<tr>
<th><strong>MS/MS</strong></th>
<th><strong>TOF</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Target compound analysis</td>
<td>• Identification of unknowns</td>
</tr>
<tr>
<td>• MRM</td>
<td>• High mass accuracy</td>
</tr>
<tr>
<td>• Quantitation in complex matrices</td>
<td>• Fast acquisition</td>
</tr>
<tr>
<td></td>
<td>• Full scan</td>
</tr>
</tbody>
</table>

Note: MRM with two transitions is normally required
UHPLC is Important for Environmental Chemistry

Many analytes in complex matrices

Maximum peak capacity and resolution

1.8 micron columns and high pressure

Agilent ZORBAX
RR-HT columns – 600 bar
RR-HD columns – 1200 bar
Improve Group 1 Analysis with 1.8 Micron

EPA method
2.1 x 100 mm
3.5 micron
22 minutes

Modified method
2.1 x 100 mm
1.8 micron
Eclipse Plus C-18
750 bar
11 minutes

Higher pressure with reduced particle size – more power
Improve Further with Gradient Change

<table>
<thead>
<tr>
<th>Time</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>10% ACN</td>
</tr>
<tr>
<td>3.4</td>
<td>10% ACN</td>
</tr>
<tr>
<td>20.0</td>
<td>100% ACN</td>
</tr>
<tr>
<td>20.3</td>
<td>100% ACN</td>
</tr>
</tbody>
</table>

2.1 x 100 mm
1.8 micron
Eclipse Plus C-18
750 bar
11 minutes

<table>
<thead>
<tr>
<th>Time</th>
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</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>10% ACN</td>
</tr>
<tr>
<td>1.7</td>
<td>10% ACN</td>
</tr>
<tr>
<td>10.0</td>
<td>100% ACN</td>
</tr>
<tr>
<td>10.3</td>
<td>100% ACN</td>
</tr>
</tbody>
</table>

2.1x 50 mm
1.8 micron
Eclipse Plus C-18
375 bar
6 minutes

shorter column further increases throughput
Fast Resolution of 12 Tetracyclines

Good resolution with shallow gradient on short column
Peak Capacity for 90 Pharmaceuticals in 20 min

Cpd 1: 0.764: +ESI EIC(177.1019, 178.1058, 179.1105) Scan Frag=190.0V Groups1-4+Extras_resolution_100mm.d

2.1 x 50 mm, 1.8 micron Eclipse Plus C-18,
750 bar observed operating pressure at 0.6 ml/min
1290 Infinity LC

“Infinity” resolution for complex mixtures

<table>
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</tr>
<tr>
<td>20.0</td>
<td>100% ACN</td>
</tr>
<tr>
<td>20.3</td>
<td>100% ACN</td>
</tr>
</tbody>
</table>
Ultrafast Chromatography for 15 Analyte Subset

2.1x 50 mm
1.8 micron
Eclipse Plus C-18
750 bar
1 minute

Peak Width 0.7 sec

<table>
<thead>
<tr>
<th>Time</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>10% ACN</td>
</tr>
<tr>
<td>1.5</td>
<td>100% ACN</td>
</tr>
</tbody>
</table>

Ultimate speed on a short column with ballistic gradient
Rapid Analysis of Boulder Wastewater Effluent

6 compounds identified
1 ng/L to 10 ng/L

2.1x 50 mm, 1.8 micron Eclipse Plus C-18, running at 750 bar

It works on real samples, too!!
Pesticides

- Number of Compounds limited with Fast Target Analyses
- Rapid screening of many pesticides needed
- Complex matrices make identification difficult
### Find These Compounds in a Sample

<table>
<thead>
<tr>
<th>Compound</th>
<th>Compound</th>
<th>Compound</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methamidophos</td>
<td>Carbofuran</td>
<td>Forchlorfenuron</td>
<td>Mefenacet</td>
</tr>
<tr>
<td>1-Naphthol, Methomyl</td>
<td>Formetanate</td>
<td>-Linuron</td>
<td>-Diflufenuron</td>
</tr>
<tr>
<td>Fenuron</td>
<td>Acetamiprid</td>
<td>-Fludioxonil</td>
<td>-Dithianthox</td>
</tr>
<tr>
<td>Acephate</td>
<td>Butoxycarboxim</td>
<td>-Clothianidin</td>
<td>-Sulfathiazole</td>
</tr>
<tr>
<td>Fuberidazole</td>
<td>Methomyl</td>
<td>-Triacloprid</td>
<td>-Fenamid</td>
</tr>
<tr>
<td>Propomocarb</td>
<td>Dicalphos</td>
<td>-Icompatra</td>
<td>-Flutyl</td>
</tr>
<tr>
<td>Tricyclazole</td>
<td>Bendiocarb</td>
<td>-Piroximox</td>
<td>-Fenoxycarb</td>
</tr>
<tr>
<td>Butocarboxim</td>
<td>Dioxyacar</td>
<td>-Demeton-S Phorate</td>
<td>-Furalaxy</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>Mepanipyrim</td>
<td>-Diethofencarb</td>
<td>-Methodidathox</td>
</tr>
<tr>
<td>Isoprocarb</td>
<td>Mevinphos</td>
<td>-Mepronil</td>
<td>-Clofentez</td>
</tr>
<tr>
<td>Cyromixan</td>
<td>Ethiofencarb</td>
<td>-Nitenpyramid</td>
<td>-Fenamiphos</td>
</tr>
<tr>
<td>Cycuron</td>
<td>Methiocarb</td>
<td>-Metropothyne</td>
<td>-Fenpropiromorph</td>
</tr>
<tr>
<td>Pyrimethanil</td>
<td>Cyprodinil</td>
<td>-Neburon</td>
<td>-Diazinon</td>
</tr>
<tr>
<td>Diamidafo</td>
<td>Prometom</td>
<td>-Fenthion</td>
<td>-Buprofex</td>
</tr>
<tr>
<td>Thiabendazole</td>
<td>Secubetone</td>
<td>-Oxadixyl</td>
<td>-Fenaquin</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>Terbumetone</td>
<td>-Metalaxyl</td>
<td>-Quinoxycarb</td>
</tr>
<tr>
<td>Dinofuran</td>
<td>Ametryn</td>
<td>-Propetamphos</td>
<td>-Iponoxazol</td>
</tr>
<tr>
<td>Aldicarbol-sulfoxide</td>
<td>Tebuthiuron</td>
<td>-Penconazole</td>
<td>-Tebuconazole</td>
</tr>
<tr>
<td>Isoproturon</td>
<td>Dimethoate</td>
<td>-Ethofumesate</td>
<td>-Fensulfothiophor</td>
</tr>
<tr>
<td>Promecarb</td>
<td>Flonicamid</td>
<td>-Vamidothion</td>
<td>-Epidiphene</td>
</tr>
<tr>
<td>Aminoacid</td>
<td>Diuron</td>
<td>-Terbufos</td>
<td>-Diffbenzuron</td>
</tr>
<tr>
<td>Propoxur</td>
<td>Fluometuron</td>
<td>-Iprobenfos</td>
<td>-Fenamidone</td>
</tr>
<tr>
<td>Chlortoluron</td>
<td>Sidoron</td>
<td>-Myclobutanil</td>
<td>-Triazophos</td>
</tr>
<tr>
<td>Omeoxtane</td>
<td>Carbethamide</td>
<td>-Chloroxuron</td>
<td>-Hexaconazole</td>
</tr>
<tr>
<td>Simetryn</td>
<td>Dicrotophos</td>
<td>-Thiamethoxam</td>
<td>-Kresoxim methyl</td>
</tr>
<tr>
<td>Monolinuron</td>
<td>Carbofuran,</td>
<td>-Parathion</td>
<td>-Nuamrul</td>
</tr>
<tr>
<td>Metribuzin</td>
<td>-3 hydroxy</td>
<td>-Cyproconazole</td>
<td>-Trifurso</td>
</tr>
<tr>
<td>Pyrectrozone</td>
<td>Pirimicarb</td>
<td>-Uniconazole</td>
<td>-Flusilazol</td>
</tr>
<tr>
<td>Pyracarbolid</td>
<td>Prometryn</td>
<td>-Amitraz</td>
<td>-Bupirimate</td>
</tr>
<tr>
<td>Thiofanox</td>
<td>-Terbutryn</td>
<td>-Triadimenol</td>
<td>-Azinphos-methyl</td>
</tr>
<tr>
<td>Oxamyl</td>
<td>-Ethoprop</td>
<td>-Imazilil</td>
<td>-Triticconazole</td>
</tr>
<tr>
<td>Thidiazuron</td>
<td>-Cyanothphos</td>
<td>-Spiroxamise</td>
<td>-Tebupirimfos</td>
</tr>
<tr>
<td>Methabenzthiazuron-Fonophos</td>
<td></td>
<td>-Quinalphos</td>
<td>-Metconazole</td>
</tr>
</tbody>
</table>

**Note:** Some compounds are noted with parentheses (E) and (Z), indicating their optical isomers (E = e, Z = Z).
How Did We Do It?

1290 Infinity LC  
High pressure

Zorbax Eclipse Plus C18 column  
High resolution

6230 TOF with JetStream  
High sensitivity

Pesticide database  
Screening
Is 3 Minutes Fast Enough?

5pg on-column

Remarkable specificity, speed and sensitivity
TOF Can Identify More Compounds in Less Time

224 pesticides at 50 pg each
217 ionized & detected in positive mode (97%, Find by Formula)

2.1 x 50 mm x 1.8 micron
Eclipse Plus C-18
900 bar
1.5 mL/min
1290 Infinity

TOF fast acquisition rates ensure maximum throughput
High Accuracy Confirmed by Database

### 3-minute 1290/6230 TOF Screening (224 +ve Pesticide suite)

<table>
<thead>
<tr>
<th>Analyte amount (on-column)</th>
<th># of compounds identified (%)</th>
<th>Average Mass Accuracy (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 fg</td>
<td>45 (20%)</td>
<td>0.24</td>
</tr>
<tr>
<td>1.25 pg</td>
<td>96 (43%)</td>
<td>0.51</td>
</tr>
<tr>
<td>2.5 pg</td>
<td>124 (55%)</td>
<td>0.49</td>
</tr>
<tr>
<td>5 pg</td>
<td>163 (73%)</td>
<td>0.12</td>
</tr>
<tr>
<td>25 pg</td>
<td>202 (90%)</td>
<td>0.70</td>
</tr>
<tr>
<td>50 pg</td>
<td>217 (97%)</td>
<td>0.06</td>
</tr>
<tr>
<td>125 pg</td>
<td>224 (100%)</td>
<td>0.60</td>
</tr>
</tbody>
</table>

More than 70% confirmed with only 5pg on column
Capabilities

• Full-Spectral Acquisition
• Accurate mass
• Fast acquisitions
• High Spectral Resolution
New 1290 Infinity LC with 6540 QTOF LC/MS
ECC of 100 Pesticides in 3 min using new Agilent 1290 Infinity LC with the new Agilent 6540 QTOF

With FULL SPECTRA Benefit from FAST RUN TIMES
Example mass spectrum from data on 3 min run with 1290 Infinity LC and 6540 QTOF.

Note the mass resolution at 10 spectra per second.
How Do We Do It with a Triple Quad
Dynamic MRM Required for Overlapping Peaks

Necessary to have at least 20 points across the peak

TIC in Black

once again, true UHPLC demands fast data acquisition
Dynamic MRM Acquisition Parameters – Customized Methods from Database

*Note that, with Dynamic MRM, the user sets the cycle time and dwell times are automatically managed.*
Dynamic MRM 11 Compounds (22 MRMs) eluting in about 1 min window

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Precursor Ion</th>
<th>Product Ion</th>
<th>Retention Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinosulfuron</td>
<td>414.1</td>
<td>183</td>
<td>5.579</td>
</tr>
<tr>
<td>Cinosulfuron (Q)</td>
<td>414.1</td>
<td>157</td>
<td>5.579</td>
</tr>
<tr>
<td>Chlorotoluron</td>
<td>213.1</td>
<td>72</td>
<td>5.642</td>
</tr>
<tr>
<td>Chlorotoluron (Q)</td>
<td>213.1</td>
<td>140</td>
<td>5.642</td>
</tr>
<tr>
<td>Atrazine</td>
<td>216.1</td>
<td>174</td>
<td>5.682</td>
</tr>
<tr>
<td>Atrazine (Q)</td>
<td>216.1</td>
<td>132</td>
<td>5.682</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>202.1</td>
<td>145</td>
<td>5.736</td>
</tr>
<tr>
<td>Carbaryl (Q)</td>
<td>202.1</td>
<td>117</td>
<td>5.734</td>
</tr>
<tr>
<td>Carboxin</td>
<td>236.1</td>
<td>143</td>
<td>5.836</td>
</tr>
<tr>
<td>Carboxin (Q)</td>
<td>236.1</td>
<td>87</td>
<td>5.836</td>
</tr>
<tr>
<td>Chlorsulfuron</td>
<td>358.0</td>
<td>167</td>
<td>5.896</td>
</tr>
<tr>
<td>Chlorsulfuron (Q)</td>
<td>358.0</td>
<td>141</td>
<td>5.896</td>
</tr>
<tr>
<td>Ethiofencarb</td>
<td>226.1</td>
<td>107</td>
<td>5.937</td>
</tr>
<tr>
<td>Ethiofencarb (Q)</td>
<td>226.1</td>
<td>164</td>
<td>5.936</td>
</tr>
<tr>
<td>Dodemorph</td>
<td>282.3</td>
<td>116</td>
<td>6.073</td>
</tr>
<tr>
<td>Dodemorph (Q)</td>
<td>282.3</td>
<td>98</td>
<td>6.074</td>
</tr>
<tr>
<td>Diuron</td>
<td>233.0</td>
<td>72</td>
<td>6.101</td>
</tr>
<tr>
<td>Diuron (Q)</td>
<td>233.0</td>
<td>160</td>
<td>6.101</td>
</tr>
<tr>
<td>Cyprodinil</td>
<td>226.1</td>
<td>108</td>
<td>6.245</td>
</tr>
<tr>
<td>Cyprodinil (Q)</td>
<td>226.1</td>
<td>93</td>
<td>6.246</td>
</tr>
<tr>
<td>Difenoxuron</td>
<td>287.1</td>
<td>123</td>
<td>6.509</td>
</tr>
<tr>
<td>Difenoxuron (Q)</td>
<td>287.1</td>
<td>72</td>
<td>6.509</td>
</tr>
</tbody>
</table>
Typical results: 10 pg Atrazine on-column using Dynamic MRM 6460A QQQ with Jet Stream Technology

- Avg. Signal Height: 15,650
- Avg. Signal Area: 50,966
- RSD: 3.2 %
- Estimated LOQ: 100 fg or less
- 6-7 data points above FWHM
- 3 sec FWHM, 6 sec @10% valley
- 20 data points baseline-to-baseline
Dynamic MRM of 300 Pesticides with Two Transitions Each

Run with 1200-SL LC 2.1 x 100 C18 Eclipse PLUS 1.8 u
Pesticide Mix run with Agilent 1290 Infinity LC: Benefit of \textit{HIGHER PEAK CAPACITY}

LC 2.1 x 150 C18 Eclipse PLUS HD 1.8 $\mu$, 0.6 mL/min, 800 bar
Conclusions

• For maximum peak capacity, choose high pressure rated columns and optimize effective gradient slope ($k^*$) vs. available system pressure and preferred analysis time. Use the Agilent Method Translator for modeling.

• Use MS conditions that allow 20 cycles/second for sharp peaks of 1-3 seconds, either LC/MS/MS by triple quadrupole with fast dwell times of 5 msec*, or LC/TOF-MS or Q/TOF-MS and 20 cycles per second (i.e.~500 transients per second).
