Improved Detection of Food Contamination using GC/QQQ and GC/QTOF

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Agilent Technologies
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Some of the Food and Feed Contaminants that we Know About and Need to Analyze

<table>
<thead>
<tr>
<th>Acrylamide</th>
<th>Melamine</th>
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<tbody>
<tr>
<td>Aflatoxins</td>
<td>Nitrosamines</td>
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<tr>
<td>Bisphenol A &amp; F</td>
<td>Nonylphenol</td>
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<tr>
<td>Brominated Flame Retardants</td>
<td>Octylphenol</td>
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<tr>
<td>Dioxins</td>
<td>Styrene dimers &amp; Trimers</td>
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<tr>
<td>Dibenzofurans</td>
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<tr>
<td>3-Monochloropropane-1,2 Diol Esters</td>
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<tr>
<td>Synthetic Colors</td>
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<tr>
<td>Mycotoxins</td>
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<tr>
<td>Nanoparticles</td>
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<tr>
<td>PAHs</td>
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<tr>
<td>Pesticides</td>
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<tr>
<td>PCBs</td>
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<tr>
<td>Drug residues</td>
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<tr>
<td>Perfluorooctanesulfonate</td>
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<tr>
<td>Perfluorooctanoic Acid</td>
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</tr>
<tr>
<td>Phthalates</td>
<td></td>
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</tbody>
</table>
What about the ones we don’t know about?

- Acrylamide
- Aflatoxins
- Bisphenol A & F
- Brominated Flame Retardants
- Dioxins
- Dibenzofurans
- 3-Monochloropropane-1,2 Diol Esters
- Synthetic Colors
- Mycotoxins
- Nanoparticles
- PAHs
- Pesticides
- PCBs
- Drug residues
- Perfluorooctanesulfonate
- Perfluorooctanoic Acid
- Phthalates

- Melamine
- Nitrosamines
- Nonylphenol
- Octylphenol
- Styrene dimers & Trimers

Unknowns!
Presentation Outline

• Advantages of using GC/QQQ
• Sensitive analysis of pesticides and other food contaminants
• New MRM database for 1074 pesticides and environmental contaminants
• Sample prep and backflushing
• New high resolution, accurate mass GC/Q-TOF
  – Design
  – Food applications
  – Unknowns Analysis
Chemical Residue Analysis Today – GC/MS/MS is Replacing GC/MS and Selective Detectors

Agilent 7890A/7000 Series GC/QQQ
Introduced in 2008
Advantages of a QQQ as a Chromatographic Detector - Multiple Reaction Monitoring (MRM)

Spectrum with background ions (from EI)

Q1 lets only target ion 210 pass through

Collision cell breaks ion 210 apart

Q2 monitors fragments 158 and 191 for quant and qual.

No chemical background
MS/MS Eliminates Scan and SIM Interferences

**Single Quad MS**
no selectivity against ions with same m/z

**Triple Quad MS**
Selectivity by selection of product ions

*unit mass resolution*
Dieldrin EI Spectrum

Precursor ion \( m/z \) 263
GC/MS SIM vs. GC/QQQ; Dieldrin at 10 ppb

**GC/SIM**

\[ m/z \, 263, \, 265, \, 277, \, 279 \]

- **Apple**
- **Cabbage**
- **Ginseng**
- **Orange**
- **Spinach**

**GC/QQQ MRM**

\[ 263 \, & \, 191 \, & \, 263\, & \, 193 \]
Lambda-Cyhalothrin I and II in Spinach using NCI-ammonia

- Sample spiked at 5ppb I + II
- I quantified at 2.05 ppb ($R^2 = 0.9999$)
- II quantified at 1.10 ($R^2 = 1.0$)
- Summed in Mass Hunter for total of 3.15 ppb
Incurred Cypermethrin I – IV in Spinach at 0.75 ppb by NH₃ NCI GC/MS/MS
Building MRM Methods for Food Analysis
What is the hard part about developing a GC/QQQ method?

✓ Optimizing the GC method
✓ Identifying the best MRM transitions for each compound
  ✓ Purchase the standards - $$$$$$
  ✓ Run each one in scan mode
  ✓ Choose possible precursor ions
  ✓ Run Product Ion Scans
  ✓ Choose the best transitions
  ✓ Optimize collision energy
  ✓ Create the method
  ✓ Test the method

Method development can take days or weeks
Agilent has built methods for you.

New Comprehensive MRM Database for Pesticides and Other Food/Environmental Contaminants

- 8000+ optimized MRM for >1000 Pesticides & Pollutants
  -- based on >3500 injections on $70,000 worth of chemical standards

- Extensive flexibility allows method optimization
  - average of 8 MRM transitions with relative intensity for each compound
    -- provides alternatives to avoid matrix interference
  - compound classification, CAS number etc. in Excel format
    -- allows easy searching and sorting for method customization
  - three GC methods with Retention Times (RTs) and Retention Indexes (RIs)
    -- allows maximum freedom to follow user’s workflow

- Tools and macros in Database
  -- build a MRM acquisition method, based on your compound list, in 5 mins

- Available Now!

We bought standards and made >3500 runs, so you don’t have to!
**Average and exact Molecular Weight**

Each compound is classified in two categories.

Database has RTs (and RIs) to be used with three GC methods (CF-40min, CP-40min, and CF-20min).
### Flexibility: Excel Format, Relative and Absolute Transition Intensity

#### MassHunter Format

<table>
<thead>
<tr>
<th>Common Name</th>
<th>ISTD?</th>
<th>Precursor</th>
<th>M51 Resolution</th>
<th>Product</th>
<th>M52 Resolution</th>
<th>Dwell Time (ms)</th>
<th>CE (V)</th>
<th>Intensity</th>
<th>(Q0 / Qu)</th>
<th>Chinese Name</th>
<th>Group</th>
<th>Japanese Name</th>
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**Color Scales:** Red denotes strong intensity and blue denotes weak intensity among ALL transitions.

**Compound names in Chinese and Japanese**

- **Flexibility**: Excel Format, Relative and Absolute Transition Intensity
- **MassHunter Format**: The absolute and relative intensities of transitions
- **One Quant and several Qualification ions for each compound**

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**December 14, 2011**
Sample Prep and Method Ruggedness
QuEChERS Sample Preparation

Quick
Easy
Cheap
Effective
Rugged
Safe

- Very popular in labs all over the world
- “Just enough” sample preparation means that extracts can be dirty

Agilent supplies QuEChERS Kits with pre-weighed reagents in sealed packets.
The QuEChERS Method for Pesticide Residues

1) Shake sample with solvent and salts
2) Centrifuge for 1 min
3) Mix a portion with a sorbent
4) Centrifuge for 1 min
5) Analyze Pesticides

Slide courtesy of Dr. Stephen Lehotay, USDA
Heavy Compounds May Be Left in Head of Column After Each Injection

These heavy materials build up and travel further into the column with each injection.

This buildup of heavy materials causes retention time shifts, peak distortion, higher bleed, ghost peaks, and loss of sensitivity.
Backflushing After Each Injection

Backflushing removes heavy materials after each injection.
What Happens when you Don’t Backflush

Dandelion root powder full scan (m/z 45-650) analysis without backflushing

Run time = 20 min

Acetonitrile blank analysis after the dandelion root powder analysis without backflushing and run time of 20 min

Matrix peaks observed in two subsequent blank injections!

Run time = 35 min (additional 15 min at 290°C)

At least additional 10 min at 290°C needed to elute the less volatile matrix components (e.g. sterols).

Results provided by Dr. Katarina Mastovska, Covance Laboratories
Typical Backflush Configuration: Analysis Mode

- **7890A GC**
- **Aux EPC**
- **Y ml/min**
- **Z ml/min**
- **Z mL/min = very low flow**
- **2-m x 0.25 mm
deactivated fused silica
(Optional Retention Gap)**
- **15-m HP-5ms UI
(0.25mm id x0.25um)**
- **0.65-m fused silica
(0.15 mm id)
Or Second column**
- **Purged Ultimate Union**
- **Purged Union**
- **700B GC MS/MS
Or 5975C MSD**

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*December 14, 2011*
Purged Union: Backflush Mode

Split/Splitless Injection Port

7890A GC

Vent

Decrease inlet pressure during backflush

2-m x 0.25 mm deactivated fused silica
(Optional Retention Gap)

15-m HP-5ms UI
(0.25mm id x0.25um)

Purged Ultimate Union

0.65-m fused silica
(0.15 mm id)
Or Second column

Aux EPC

7000B GC MS/MS
Or
5975C MSD

December 14, 2011
The Purged Ultimate Union (PUU) configurations

Vocabulary

Post-column
- inlet
- Capillary column
- Purged Ultimate Union
- Restrictor
- Mass Spectrometer (Turbo Only)

Mid-column
- inlet
- Capillary column
- Purged Ultimate Union
- Mass Spectrometer (Turbo or Diff)

Mid-column
- inlet
- Short capillary column
- Purged Ultimate Union
- Longer capillary column
- Mass Spectrometer (Turbo or Diff)
Backflushing Eliminates Less Volatile Matrix Components (Using Mid-Column Backflush)

Dandelion root powder full scan (m/z 45-650) analysis with backflushing

Last analyte RT = 18.45 min
Deltamethrin (m/z 253>172)

Run time = 20 min

Backflushing starts

Acetonitrile blank analysis with extended run

No matrix peaks from the previous injection observed!

Results provided by Dr. Katarina Mastovska, Covance Laboratories
Long-term System Performance

Overlays of GC-MS/MS chromatograms for selected analytes in spiked samples obtained within the sequence of 125 matrix injections

Dichlorvos
m/z 185>93

Malathion
m/z 173>99

Ethion
m/z 231>129

Phosalone
m/z 367>182

Deltamethrin
m/z 253>174

Ginseng
Root
Powder

Saw
Palmetto
Berry
Powder

Scutellaria
Powdered
Extract

Results provided by Dr. Katarina Mastovska, Covance Laboratories
Backflush: Many Advantages for GC/MS(/MS) Analysis of Complex Samples (‘Dirty Matrices’) 

- Provides **more consistent GC retention times**
- Provides better, **more consistent mass spectra** throughout a sequence
  - **Reduces chemical noise** - reduced carryover of matrix
  - **Higher quality quantitation** without increase in interfering ions
- **Reduces contamination** of the source
- **Reduces analysis time**
- **Increases lifetime of analytical column**
- **Change columns, liners, septa without venting!**
Evolution of Benchtop GC-MS at HP / Agilent

1982
5970A MSD

1988
5971A MSD

1991
5972A MSD

1996
5973A MSD

2005
5975A MSD…

2008
7000 QQQ

2010
240 ITD
Evolution of Benchtop GC-MS at HP / Agilent

- **1982**
  - 5970A MSD

- **2005**
  - 5975A

- **2008**
  - 7000 QQQ

- **2010**
  - 240 ITD

- **2011**
  - 73A MSD
  - ITD
7200 Series Q-TOF for GC/MS
High Resolution and Accurate Mass

7000 GC/MS  QQQ based
6500 LC/MS  QTOF based
7200 Analyzer
Four stages of pumping
Removable Ion Source

30 min to change source and make new run
Internal Reference Mass (IRM) Compound

CAS 858-46-8, 1,3,5-Triazine, 2,4,6-tris(pentafluoroethyl)-, C$_9$F$_{15}$N$_3$, 434.9847

Accurate mass EI spectrum

\[ [M]^+ = 434.9847 \]

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<thead>
<tr>
<th>Measured mass</th>
<th>Accurate Mass</th>
<th>Mass Accuracy ppm</th>
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Accurate mass EI spectrum (December 14, 2011)
What Will the 7200 Q-TOF Do for You?

TOF mode

• High resolution full spectral acquisition
• Accurate mass measurements
• Fast acquisition of full spectra

MS/MS mode

• Full product ion spectral acquisition
  – With high resolution and accurate mass

Ideal tool for solving complex analytical problems
Resolving power & Mass accuracy

Resolving Power:
\[ R = \frac{m_z}{\text{FWHM}} \]

Mass Accuracy:
\[ \Delta m_z = \frac{dm}{m_z} \times 10^6, \text{ parts per million (ppm)} \]

PFTBA mass 614
C12F24N=613.964203

\[ \begin{align*}
R &= \frac{614}{0.68} = 903 \\
\Delta m_z &= \frac{0.1}{614} = 160 \text{ ppm}
\end{align*} \]

\[ \begin{align*}
P_w &= 0.68 \\
\end{align*} \]

\[ \begin{align*}
R &= \frac{614}{0.0423} = 14522 \\
\Delta m_z &= \frac{0.0004}{613.96} = 0.7 \text{ ppm}
\end{align*} \]
Ethion Spectrum

\[ C_5H_{12}O_2PS_3^+ = 230.9737 \]

\[ C_9H_{22}O_4P_2S_4 \text{ M}^+ = 383.9871 \]
Enhanced Selectivity using Accurate Mass

Ethion @ 5 ppb in Ginseng Extract
Extracted Ions = 230.9737 and 383.9871 +/- 10 ppm
Fludioxonil in Frozen Blueberry Extract – 38 ppb in extract

Signal/Noise = 83

Signal/Noise = 831
Many Possible Formulas with Quadrupole or Ion Trap - *But only a few with Accurate Mass TOF*

Accurate mass reduces risk of investing effort on the wrong molecule.

---

### Possible Number of Chemical Formulas at m/z 272

#### Formulas made of:
C, H, N, O, F, & Cl

<table>
<thead>
<tr>
<th>mass uncertainty, ppm</th>
<th>mass uncertainty, amu</th>
<th># of Possible Formulas</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>0.3</td>
<td>7657</td>
</tr>
<tr>
<td>368</td>
<td>0.1</td>
<td>4050</td>
</tr>
<tr>
<td>100</td>
<td>0.03</td>
<td>1223</td>
</tr>
<tr>
<td>37</td>
<td>0.01</td>
<td>466</td>
</tr>
<tr>
<td>10</td>
<td>0.003</td>
<td>120</td>
</tr>
<tr>
<td>4</td>
<td>0.001</td>
<td>43</td>
</tr>
<tr>
<td>1</td>
<td>0.0003</td>
<td>11</td>
</tr>
<tr>
<td>0.4</td>
<td>0.0001</td>
<td>5</td>
</tr>
<tr>
<td>0.1</td>
<td>0.00003</td>
<td>2</td>
</tr>
</tbody>
</table>

Octafluoronaphthalene (CAS 313-72-4)

\[ C_{10}F_8 = 271.98667 \]
The Problem – Confirm Most Likely Structure
Kava Extract - Compound “B”, \( \text{C}_{16}\text{H}_{14}\text{O}_{4} \)
(Rings + Double Bonds = 10)

For the 5 candidate structures, only one fit the losses identified by CID experiments on multiple precursor ions.

<table>
<thead>
<tr>
<th>( m/z ) (experimental)</th>
<th>Formula</th>
<th>Error (ppm)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>269.0802</td>
<td>( \text{C}<em>{16}\text{H}</em>{13}\text{O}_{4} )</td>
<td>2.2</td>
<td>80.7</td>
</tr>
<tr>
<td>193.0494</td>
<td>( \text{C}<em>{10}\text{H}</em>{9}\text{O}_{4} )</td>
<td>0.6</td>
<td>96.7</td>
</tr>
<tr>
<td>167.0334</td>
<td>( \text{C}<em>{8}\text{H}</em>{7}\text{O}_{4} )</td>
<td>3.0</td>
<td>N/A</td>
</tr>
<tr>
<td>166.0259</td>
<td>( \text{C}<em>{8}\text{H}</em>{6}\text{O}_{4} )</td>
<td>0.6</td>
<td>N/A</td>
</tr>
<tr>
<td>138.0310</td>
<td>( \text{C}<em>{7}\text{H}</em>{6}\text{O}_{3} )</td>
<td>1.1</td>
<td>98.1</td>
</tr>
<tr>
<td>110.0359</td>
<td>( \text{C}<em>{6}\text{H}</em>{6}\text{O}_{2} )</td>
<td>3.0</td>
<td>N/A</td>
</tr>
<tr>
<td>95.0127</td>
<td>( \text{C}<em>{5}\text{H}</em>{3}\text{O}_{2} )</td>
<td>0.9</td>
<td>99.5</td>
</tr>
</tbody>
</table>
Agilent 7200 Series GC-QTOF
Identifying and quantification of S- and N-containing compounds in beverages
Problem – Compounds affecting taste and flavor

Example of complex matrix – Coffee extract

- **2-formyl thiophene** and **2-acetyl thiazole** are common contaminants

- Low sensory threshold and can have negative effect on product flavor or aroma
- Easy to separate from each other
- Often requires sophisticated extraction/enrichment procedures and/or powerful 2D GC techniques for separation from matrix for quantitation
- GC-Q-TOF Method highlights:
  - Simple Liq / Liq extraction in Dichloromethane (10:1 enrichment)
  - 1:10 split injection with S/SL inlet
  - DB-5MS column 30 m x 0.25 mm x 0.25 μm
Standards at 100 pg On Column

2-formyl thiophene
Formula $\text{C}_5\text{H}_4\text{OS}$
MW: 112

2-acetyl thiazole
Formula $\text{C}_5\text{H}_5\text{NOS}$
MW: 127
TIC and EICs of Coffee Extract

2-formyl thiophene
$m/z$ 111.9983 ± 100 ppm

2-acetyl thiazole
$m/z$ 127.0092 ± 100 ppm
2-Acetyl Thiazole calibration

STD addition calibration curve for 2-acetyl thiazole in spiked coffee (STD amount: 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 pg)

Average mass error = 1.1 ppm (max 1.6) over entire concentration range

m/z 127.0092
Mass window: ±20 ppm
Photodegradation Products of Beer

- Completely untargeted (initially) study of beer photodegradation
- Method highlights
  - 30 min extraction at 30 °C using manual SPME holder and conditioned 50/30 µm DVB/Carboxen/PDMS StableFlex SPME fiber (Supelco), no agitation
  - Desorption at 300 °C for 2 min in the SSL injector; 1:10 split
  - Agilent J&W column DB-5MS 30 m x 0.25 mm x 0.25 µm
Changes in the Chromatogram

Appears following the exposure of the sample to direct sunlight. Peak height is dependent on the duration of exposure to the sun.

No exposure to direct sunlight
3 hours
6 hours
Summary of MS/MS Experiments

[Diagram showing mass spectra and molecular formulas]
Unknowns Analysis – A feature in MassHunter Quant

- Run after finishing Quant Analysis
- Deconvolutes chromatogram
- Library search deconvoluted “components”
  - Can use retention times to filter hits
- Sort into target compounds and non-target compounds
- Easy review of results
- Export spectra to mass spec library
  - Easy way to build an accurate mass library from acquired data
Unknowns Analysis – A feature in MassHunter Quant

- TIC
- Deconvoluted accurate mass spectrum
- Library match - Ditalimfos
- Component EICs
Fundamental Benefits (Agilent 7200 Q-TOF)

• High resolution (> 10K, typically > 13K FWHM)
  – Increased detector selectivity (few interferences)

Accurate mass measurements (low to sub-ppm)

  – TOF mode - Typically < 2 ppm
  – Q-TOF mode (MS/MS) Typically < 5 ppm
  – Valuable qualitative information about each ion

Structural elucidation: Accurate Mass MS/MS

  – High sensitivity tool to complement NMR

Hyper-selective, Hi-Res MS/MS

“Fast” (up to 50 spectra/sec), full spectra acquisition with excellent sensitivity
The Agilent Portfolio of Benchtop GC-MS Systems

Thank You