Mass Spectrometry for food analysis

Food Industry Segment Team
November 2009
Agilent’s 37 Years in Mass Spectrometry

Nearly 40,000 systems – Applied to Chromatographic Analysis

5930A 1971

5992A 1976

5970B MSD 1986

5971 - 5975 1988 - present

6100 Single Quad 2006 - Present

7000 GC-QQQ 2008 - Present

LC/MSD Series 1997-2005

6400 Triple Quad 2006 - Present

ESI-TOF Series 2003-Present

6500 Q-TOF 2006 - Present

4500, 7500, 7700 Series ICP/MS 1994-Present
Agilent’s NEW Analyzers and Application Kits help you reduce your number of steps between startup and results, so you can:

1. Implement new, more advanced technologies quickly
2. Lower method development costs
3. Produce consistent, high-quality data from day one

Learn more @ www.agilent.com/chem/applicationkit
Food Analysis using ICP-MS
New levels of efficiency to remove interference

Collision cell evolution with 7700

- 18% longer rods
- 15% smaller ID
- 16% higher pressure
- 20% higher frequency

All means that a much larger energy discrimination step to remove interferences.
At cell entrance, analyte and polyatomic ion energies overlap. Energy spread of both groups of ions is narrow, due to ShieldTorch System.

Energy loss from each collision with a He atom is the same for analyte and polyatomic ion, but polyatomics are bigger and so collide more often.

By cell exit, ion energies no longer overlap; polyatomics are rejected using a bias voltage “step”. Analyte ions have enough residual energy to get over step; polyatomics don’t (energy discrimination).

*KED = Kinetic Energy Discrimination
Blank Acid Matrices and IPA in No Gas Mode

Color of spectrum indicates which matrix gave each interfering peak

Unspiked 5% HNO₃ + 5% HCl + 1% H₂SO₄ + 1% IPA Matrix

Unspiked Matrix – ALL peaks are due to polyatomic interferences

Multiple polyatomic interferences affect almost every mass – Interferences are matrix-dependent

No Gas Mode
Many polyatomic interferences remain (or new ones are created) in H₂ (reaction) Mode. Interferences are different in each Matrix! How does He mode (default on the 7700) perform?

Unspiked 5% HNO₃ + 5% HCl + 1% H₂SO₄ + 1% IPA Matrix

Many polyatomic interferences can still be seen in H₂ Mode.
Blank Acid Matrices and IPA in He Mode

Color of spectrum indicates which matrix gave each interfering peak

Unspiked 5% HNO₃ + 5% HCl + 1% H₂SO₄ + 1% IPA Matrix

ALL polyatomic interferences are removed in He Mode (same cell conditions)

ALL polyatomic interferences are removed in He Mode
Is sensitivity still OK?
Matrix Mix with Spike (10ppb) in He Mode

Consistent sensitivity and perfect template match for all elements

10ppb Spike in 5% HNO₃ + 5% HCl + 1% H₂SO₄ + 1% IPA Matrix

Consistent high sensitivity for all isotopes of all elements in He Mode

Good signal for all spike elements at 10ppb Spike. Perfect template fit for all elements – no residual interferences and no loss of analyte signal by reaction

He Mode
ICP-MS Interfacing Options

- LC-ICP-MS
- GC-ICP-MS
- CE-ICP-MS

Optional Conventional (Organic compound) Detector(s)
Speciation with **LC-ICP-MS** and **GC-ICP-MS**

ICP-MS is a sensitive, selective and specific detector for most compounds containing an element that can be measured by ICP-MS (any except H, He, N, O, F, Ne or Ar)

**As Speciation using LC-ICP-MS**

*Chromatogram of mixed organo-tin standard*

- containing 20ng/mL (ppb) each compound.
- Elution order is: Sn, MBT, TPrT, DBT, MPhT, TBT, TeBT, TPeT, DPhT TPhT.
- DL’s typically fg

**Sn Speciation using GC-ICP-MS**

Conventional “organo-metallic” compounds:
- Inorganic vs organic arsenic
- Organo-tin
- Methyl-mercury, etc

More unusual elements/comounds:
- Pesticide and OP nerve agent residues
- PBDEs
- Nanoparticles
LCMS and GCMS

Routine Analysis
Identify Unknowns

**(semi)-volatile or non-polar** (moderate)-polar or thermally labile

GAS phase

LIQUID phase

GC/QQQ MRM
GC/Q DRS

LC/QQQ MRM
LC/Q

TOF
QTOF

Exact Mass Database Search

Agilent Technologies
Target, Unknown or Suspect?

- **Known Targets**
  - QQQ or GC-Quad SIM or LC-TOF

- **Unknown Contaminants**
  - LC-TOF with deconvolution or GC-scan Quad MS

- **Suspects analysis**
  - Large QQQ panels or LC-TOF or GC-scan Quad MS with Database searches
Screening: Deconvolution/Pattern recognition

A way of condensing ions together from the same compound

Advantage: Resulting pattern can be used to id compound.

Advantage: Resulting pattern can be found even when other interfering ions are in excess. In effect sensitivity to low level compounds can be increased.

What is required for reliable pattern recognition?

Something characteristic:

Rich spectral pattern generation

Accurate Mass
GCMS
DRS: Deconvolution Reporting Software.
Separate co-eluting compounds

TIC & Spectrum

Deconvoluted peaks and spectra

- Sum of ions in Component 1
- Sum of ions in Component 2
- Sum of ions in Component 3
Scan at 5.615 min

The power of deconvolution is appreciated while comparing the top two spectra (raw scan and the compound spectrum hidden in the raw scan).

Deconvoluted/extracted spectrum

A component in the scan above.

Library spectrum Carbaryl

Application Note 5989-7670: Replacing Multiple 50-Minute GC and GC-MS/SIM Analyses with One 15-Minute Full-Scan GC-MS Analysis for Non-targeted Pesticides Screening and >10x Productivity Gain
Grape Extract
DRS found
Flusilazole

Flusilazole EICs
Advance pneumatics allow precise RT control.

Hence with *Retention Time Locking* permanent retention times can be achieved even with column maintenance.

Precision of 0.001 psi control
Agilent DRS Databases with retention times

• Pesticides and Endocrine Disruptors (including Japan “positive list”) 926 compounds
• Hazardous Chemicals 731 compounds

Conditions

– **Locked** retention time method on HP5-MS column
– Mass spectra from **autotuned** GC/MSD
If we used RTL, DRS, & CFT, we could get…

![Graph showing data]

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**Screen for 926 pesticides in one 21-minute GC/MS run**

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**Data File**
- **Date/Time:** 2:45:59 PM Thursday, February 19, 2009

**MSD Deconvolution**
- **Sample Name:** carrot1

**The NIST library was searched for the components that**

<table>
<thead>
<tr>
<th>R.T.</th>
<th>Cas #</th>
<th>Compound Name</th>
<th>AMDIS</th>
<th>AMDIS Match</th>
<th>R.T. Diff sec.</th>
<th>Reverse Match</th>
<th>Hit Num.</th>
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<tbody>
<tr>
<td>4.4196</td>
<td>33704619</td>
<td>Cashmeran</td>
<td>Chem</td>
<td>13.59</td>
<td>60</td>
<td>67</td>
<td>35</td>
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<tr>
<td>6.9401</td>
<td>1517222</td>
<td>Phenanthrene-d10</td>
<td>35.96</td>
<td>24.68</td>
<td>92</td>
<td>9.2</td>
<td>79</td>
</tr>
<tr>
<td>8.0245</td>
<td>84695</td>
<td>Diisobutyl phthalate</td>
<td>16.19</td>
<td>9.88</td>
<td>83</td>
<td>9.2</td>
<td>81</td>
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<tr>
<td>9.27</td>
<td>84742</td>
<td>Di-n-butylphthalate</td>
<td>61.5</td>
<td>23.38</td>
<td>95</td>
<td>7.5</td>
<td>92</td>
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<tr>
<td>12.136</td>
<td>72559</td>
<td>p,p'-DDE</td>
<td>6.15</td>
<td>3.52</td>
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<td>15</td>
<td>82</td>
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<td>72548</td>
<td>p,p'-DDD</td>
<td>2.6</td>
<td>1.55</td>
<td>69</td>
<td>12.5</td>
<td>77</td>
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<tr>
<td>12.954</td>
<td>789026</td>
<td>o,p'-DDT</td>
<td>2.6</td>
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<td>13.5916</td>
<td>50293</td>
<td>p,p'-DDT</td>
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<td>1.33</td>
<td>70</td>
<td>12</td>
<td>71</td>
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<tr>
<td>13.9963</td>
<td>115866</td>
<td>Triphenyl phosphate</td>
<td>15.11</td>
<td>9.18</td>
<td>95</td>
<td>16.1</td>
<td>84</td>
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<tr>
<td>14.9048</td>
<td>117817</td>
<td>Bis(2-ethylhexyl)phthalate</td>
<td>4.46</td>
<td>56</td>
<td>9.6</td>
<td>64</td>
<td>62</td>
</tr>
<tr>
<td>14.99</td>
<td>2385855</td>
<td>Mirex</td>
<td>1.38</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>6.943</td>
<td></td>
<td>Phenanthrene-d10</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
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</table>
Combination of ChemStation, AMDIS, and NIST gives complete info for both unknowns and target compounds

<table>
<thead>
<tr>
<th>R.T.</th>
<th>Cas #</th>
<th>Compound Name</th>
<th>Agilent ChemStation Amount (ng)</th>
<th>AMDIS Match</th>
<th>R.T. Diff sec.</th>
<th>Reverse Match</th>
<th>Hit Num.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1437</td>
<td>3228033</td>
<td>Promecarb artifact [5-isopropyl-3-methylphenol]</td>
<td>68</td>
<td>6.1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2.1437</td>
<td>1450722</td>
<td>Ethanone, 1-(2-hydroxy-5-methylphenyl)-</td>
<td></td>
<td></td>
<td>89</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2.2759</td>
<td>97530</td>
<td>Eugenol</td>
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<td>2.7</td>
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<tr>
<td>3.5107</td>
<td>877096</td>
<td>2,4,5,6-Tetrachloro-m-xylene</td>
<td>2.02</td>
<td>93</td>
<td>3.5</td>
<td>60</td>
<td>2</td>
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<td>3.6664</td>
<td>126738</td>
<td>Tributyl phosphate</td>
<td>81</td>
<td>5.2</td>
<td>86</td>
<td>2</td>
<td></td>
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<tr>
<td>4.6126</td>
<td>120127</td>
<td>Anthracene</td>
<td>81</td>
<td>-12.2</td>
<td>91</td>
<td>8</td>
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<tr>
<td>4.8290</td>
<td>333415</td>
<td>Diazinon</td>
<td>69</td>
<td>1.3</td>
<td>72</td>
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</tbody>
</table>

With SIM/Scan mode, target data can be acquired with optimum sensitivity.
Seventeen (17) surface water samples

<table>
<thead>
<tr>
<th></th>
<th>*CDFA</th>
<th>Agilent DRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targets found:</td>
<td>37</td>
<td>same 36 + 34 additional</td>
</tr>
<tr>
<td>(not counting ISTD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>False positive:</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Processing time:</td>
<td>~8 hours Chemstation only</td>
<td>20 minutes</td>
</tr>
</tbody>
</table>

What’s the point? **Save time and Money**

*CDFA is the California Department of Food and Agriculture
Data files courtesy of Dr. Mark Lee and Steve Siegel
LC-TOF: Molecular feature extraction

- **$^{35}\text{Cl}$**: 297.0564, +1.997
- **$^{37}\text{Cl}$**: 299.0533, +1.997

Molecular structure with peaks at m/z values:
- 297.0564
- 300.0555
- 301.0503

Chemical formula and structural details provided for molecular feature extraction.
LC-TOF: Molecular Feature Extraction

Before
## Molecular Feature Extraction: The results

<table>
<thead>
<tr>
<th>charge state</th>
<th>RT</th>
<th>m/z</th>
<th>m0</th>
<th>vol</th>
<th>width</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M</td>
<td>6.761</td>
<td>317.1384</td>
<td>4.22</td>
<td>0.047</td>
<td></td>
</tr>
<tr>
<td>2 M+H</td>
<td>6.763</td>
<td>318.1457</td>
<td>3.53</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>3 M+H+1</td>
<td>6.762</td>
<td>319.1499</td>
<td>0.63</td>
<td>0.051</td>
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</tr>
<tr>
<td>4 M+H+2</td>
<td>6.759</td>
<td>320.1531</td>
<td>0.06</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td>6 M</td>
<td>6.761</td>
<td>300.1125</td>
<td>4.19</td>
<td>0.039</td>
<td></td>
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<tr>
<td>7 M+H</td>
<td>6.762</td>
<td>301.1192</td>
<td>300.1119</td>
<td>1.72</td>
<td>0.049</td>
</tr>
<tr>
<td>8 M+H+1</td>
<td>6.762</td>
<td>302.1225</td>
<td>0.30</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>10 M+Na</td>
<td>6.762</td>
<td>323.1016</td>
<td>300.1131</td>
<td>1.65</td>
<td>0.048</td>
</tr>
<tr>
<td>11 M+Na+1</td>
<td>6.764</td>
<td>324.1052</td>
<td>0.29</td>
<td>0.050</td>
<td></td>
</tr>
<tr>
<td>12 M+Na+2</td>
<td>6.752</td>
<td>325.1059</td>
<td>0.02</td>
<td>0.015</td>
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<tr>
<td>13 M+K</td>
<td>6.765</td>
<td>339.0768</td>
<td>300.1136</td>
<td>0.06</td>
<td>0.037</td>
</tr>
<tr>
<td>16 2M+Na</td>
<td>6.759</td>
<td>623.2137</td>
<td>300.1127</td>
<td>0.11</td>
<td>0.032</td>
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<tr>
<td>17 2M+Na+1</td>
<td>6.761</td>
<td>624.2161</td>
<td>0.05</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td>19 M</td>
<td>6.762</td>
<td>420.1554</td>
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<td>0.053</td>
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<tr>
<td>20 M+H</td>
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<td>421.1627</td>
<td>420.1554</td>
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<tr>
<td>21 M+H+1</td>
<td>6.764</td>
<td>422.1664</td>
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<tr>
<td>22 M+H+2</td>
<td>6.761</td>
<td>423.1744</td>
<td>0.11</td>
<td>0.054</td>
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</tr>
</tbody>
</table>
Screening Using Accurate Mass – LC/QTOF & TOF

Molecular Feature Extractor Algorithm

Database Search: Diazepam detected
Easily ID 400 Compounds in Honey Sample in 5 min!

<table>
<thead>
<tr>
<th>m/z</th>
<th>Ion</th>
<th>Formula</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>358.20123</td>
<td>(M+H)+</td>
<td>C21H28NO4</td>
<td>100%</td>
</tr>
</tbody>
</table>

Detailed Formula Generation

Results

List of compounds with calculated Molecular Formulas

| Compound mass spectrum with theoretical isotope pattern overlaid |

Detailed Molecular Formula Calculation Results

Overlaid compound chromatograms show each individual compound found.
The advantage of 2ppm Accurate Mass in screening

Reserpine \((C_{33}H_{40}N_{2}O_{9})\) has a protonated ion at 609.28066

Single quad reports mass to +/- 0.1 = 165 ppm

Number of possible formulas using only C, H, O & N

- 165 ppm: 209
- 10 ppm: 13
- 5 ppm: 7
- 3 ppm: 4
- 2 ppm: 2
1. Tune jet stream fragmenter to retain sugar moieties or to create sugar ‘series’.

2. Select intact ion or fragment ions for MSMS e.g. 287 is base molecule

3. MS/MS spectrum of selected ion (287) helps confirm identity of base molecule through MSMS fragment prediction and neutral loss prediction
Profiling: Comparing samples
Common features present in both samples and all replicates

646 Differential Features

Agilent Technologies
Looking for Differences
Show me those compounds that changed by 2 or more

- Look for changes in expression
- Look for reasons for a change in product quality
- Remove compounds present in your blank
- Create inclusion list for msms

84 Differential Features
LCMS and GCMS data: Further statistical analysis

- Collect/compare RT and mass from many MS systems
- Abundance Tests
- Class Prediction
- Clustering
- Hierarchy Trees
- Pathway Analysis
- Function Analysis
Further statistical analysis possible with Mass profiler Professional (son of Genespring)

Multiple experiment types combined

Guided workflow for ease-of-use
Wizard-Driven Data Analysis

Guided Workflow - Find Differential Expression (Step 3 of 8)

Steps
1. Summary Report
2. Experiment Grouping
3. QC on samples
4. Filter Abundance
5. Significance Analysis
6. Fold Change
7. GO Analysis
8. Find Significant Path...

QC on samples
Sample quality can be assessed by examining the values in the PCA plot and other experiment specific quality plots. To remove a sample from your experiment, select the sample from any of the views and click on the Add/Remove button. If a sample is removed, re-summation of the remaining samples will be performed.

Experiment Grouping

<table>
<thead>
<tr>
<th>Samples</th>
<th>Treatment</th>
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</thead>
<tbody>
<tr>
<td>01040B-002</td>
<td>Untreated</td>
</tr>
<tr>
<td>01040B-004</td>
<td>Untreated</td>
</tr>
<tr>
<td>01040B-005</td>
<td>Untreated</td>
</tr>
<tr>
<td>01040B-006</td>
<td>Untreated</td>
</tr>
<tr>
<td>01040B-007</td>
<td>Untreated</td>
</tr>
<tr>
<td>01040B-008</td>
<td>Untreated</td>
</tr>
<tr>
<td>01040B-011</td>
<td>Untreated</td>
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<tr>
<td>01040B-013</td>
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<tr>
<td>01040B-014</td>
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<td>01040B-016</td>
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<td>Treated</td>
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<tr>
<td>01040B-020</td>
<td>Treated</td>
</tr>
<tr>
<td>01040B-021</td>
<td>Treated</td>
</tr>
</tbody>
</table>

3D PCA Scores

Legend - 3D PCA Scores

- Treated
- Untreated

Description
- Algorithm: Principal Components Analysis
- Parameters: Column Indices = [1:14]
- Printing option = [mainPrincipalComponents, [14]]
- Per center off = false
- Scale = true
- 3D Scores = true
- PCA on = Columns

Agilent Technologies
Pathways with Up/Down regulation data
Portrait with Vegetables (*The Greengrocer*)
Artist: Giuseppe Arcimboldo
b. 1527  d. 1593, Milan, Italy

Thank You for your attention