TOF-MS: a Recent Challenge in GC Residue Analysis and a Strategy for Obtaining Accurate Data

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Suggestions for implementation of NOVEL APPROACHES IN GC-BASED MRMs

- **SAMPLE PREP**: partition followed by dispersive SPE - **QuEChERS**
- **SAMPLE INTRODUCTION**: dirty matrix introduction injector - **DMI**
- **SEPARATION**: - low pressure chromatography - **LP-GC**
  - orthogonal chromatography - **GC×GC**
- **DETECTION**: time-of-the-flight mass analyzer - **TOF-MS**

- **QUALITY ASSURANCE**: **ANALYTICAL PROTECTANTS**
Strategies to examine food samples for occurrence of pesticide residues

**TARGET ANALYSIS**
- the list of analytes specified

**NON-TARGET SEARCH**
- „unknowns“ to be determined
### GC-MS systems conceivable for (multi)residue analysis

<table>
<thead>
<tr>
<th>Mass analyser</th>
<th>Mass Resolution</th>
<th>Acquisition rate</th>
<th>Detection limits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>QUADRUPOLE</strong></td>
<td>0.5 amu peak width, (R = 2m, 10%) valley</td>
<td>15–33 scans/s for mass range 300 amu</td>
<td>pg - fg (SIM mode - limited by chemical noise, further improvement in MS/MS)</td>
</tr>
<tr>
<td><strong>ION TRAP</strong></td>
<td>1 amu peak width, (R = m, 10%) valley</td>
<td>19 scans/s for mass range 300 amu</td>
<td></td>
</tr>
<tr>
<td><strong>SECTOR</strong></td>
<td>Up to 80,000 (10% valley)</td>
<td>0.15 s/decade</td>
<td>fg (SIM mode)</td>
</tr>
<tr>
<td><strong>hs-TOF</strong></td>
<td>1,400 FWHM</td>
<td>1–500 spectra/s</td>
<td>pg</td>
</tr>
<tr>
<td><strong>hr-TOF</strong></td>
<td>7,000 FWHM</td>
<td>1–20 spectra/s</td>
<td>pg</td>
</tr>
</tbody>
</table>

Complex matrix

Achieve good spectral resolution!
Improve chromatographic resolution!

Non-target search:
Full spectral information needed for identification of unknown signals
Spectral resolution of co-eluting compounds

TIC

How many compounds compose this record

12 s

deconvolution software

→ extraction of spectral information →

→ library search
What is deconvolution...?

.... extraction of signals from complex mixture

Source:: Agilent Technologies
AMDIS: Automatic Mass spectral Deconvolution Identification System

Component 1
Component 2
Component 3

TIC & SPEKTRUM

matrix

interference

ANALYTE

DECONVOLUTED PEAKS & THEIR SPECTRA
**Cause:** changing concentration of analyte in MS source during peak elution

**SCAN:** high → low m/z
Before removing skew (MSD)

Shift of ions apexes during scan of single compound

Skew removed & apexes interpolated (AMDIS)

Identical

AMDIS: de-skew function
LIMITATION when using conventional MSD

High LODs in TIC

LVI-PTV

More co-extracts introduced into GC system

MATRIX EFFECTS
Comparison of mass analyzers sensitivity - acquisition of full spectral information
UNIQUE FEATURES OF BOTH APPROACHES:

- Permanent acquisition of full mass spectra
- Absence of spectral skew (deconvolution function)
- High mass analyser efficiency up to 25% (compared to 0.05% for the quadrupole) over the mass range of a 500 amu

HR TOF
- exact mass measurement
- conventional and fast GC

fast TOF
- fast, ultra fast GC
- GC x GC

GCT, Waters
Pegasus IV, Leco
For trace levels **SIM mode** is necessary.
# GC-TOF MS instruments available at the market

<table>
<thead>
<tr>
<th>Instrument (Manufacturer)</th>
<th>Mass Range (Da)</th>
<th>Mass Resolution</th>
<th>Acquisition Rate (spectra/s)</th>
<th>Registration of Ions</th>
<th>Ionisation Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pegasus</strong> (Leco)</td>
<td>&lt;1,000</td>
<td>Unit mass</td>
<td>1–500</td>
<td>ADC</td>
<td>EI</td>
</tr>
<tr>
<td><strong>Tempus</strong> (Thermo)</td>
<td>&lt;1,000</td>
<td>Unit mass</td>
<td>2–60</td>
<td>ADC</td>
<td>EI, CI</td>
</tr>
<tr>
<td><strong>Kronus</strong> (Scientific Analysis Instruments)</td>
<td>&lt;2,000</td>
<td>Unit mass</td>
<td>1–100</td>
<td>ADC</td>
<td>EI</td>
</tr>
<tr>
<td><strong>GCT</strong> (Waters)</td>
<td>&lt;1,500</td>
<td>7,000 FWHM</td>
<td>1–10</td>
<td>TDC</td>
<td>EI, CI, FI</td>
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<tr>
<td><strong>GCT Premier</strong> (Waters)</td>
<td>&lt;1,500</td>
<td>7,000 FWHM</td>
<td>1–20</td>
<td>TDC</td>
<td>EI, CI, FI</td>
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<tr>
<td><strong>JMS-T100GC</strong> (JEOL)</td>
<td>&lt;2,000</td>
<td>5,000 FWHM</td>
<td>1–25</td>
<td>ADC</td>
<td>EI, CI, FI</td>
</tr>
</tbody>
</table>
Principle of oa-TOF MS

Formation of fragment ions in the ion source (EI, CI)

Ejection of a part of focused ion beam into a mass analyser

Energy focusing using reflectron

Detection of “ion events”
Reflectron function

\[ E_k = \frac{1}{2}mv^2 = z e E \]

\[ t = \sqrt{\frac{m}{z}} \cdot C \]

\[ (C = d \sqrt{\frac{1}{2eE}}) \]
HR-TOF-MS

Narrow mass window setting

Example:
Matrix-matched standard of apple
Analyte: PHOSALONE, concentration: 0.013 mg/kg

Low-resolution
S/N (PtP) = 5:1
Mass window: 1 Da
m/z 182

High-resolution
S/N (PtP) = 61:1
Mass window: 0.02 Da
m/z 182.002

Significant elimination of background interferents from chromatogram

Improved detectability of analytes, low LODs
Example: analysis of 100 pesticides in baby food (buffered QUeCHERS extract)

Conventional GC–MS (SIM)

74 min

Dichlorvos $t_R = 17.6$ min
Azoxystrobin $t_R = 72.8$ min

LP-GC-HR-TOF MS

7 min

Dichlorvos $t_R = 1.7$ min
Azoxystrobin $t_R = 5.1$ min

Acquisition of full mass spectra

- Full capabilities of library reference spectra search - identification / confirmation even at very low concentrations

Library search

Pirimiphos-methyl
0.010 mg/kg
m/z 290.074
Mass window: 0.02 Da

m/z 70–700

Library search

<table>
<thead>
<tr>
<th>Hit</th>
<th>Compound Name</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>PIRIMIPHO3 METHYL</td>
</tr>
<tr>
<td>2</td>
<td>2-DIETHYLAMINO-3-[METHOXY(METHYLTHIO)PHOSPHINYL]DIETHYLAMINO</td>
</tr>
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</table>
Exact mass deconvolution, library search

0.05 mg/kg CHLORPYRIFOS in peach, acquisition rate 5 spectra/sec

potential for non-target screening
Changing acquisition speed

peak width cca 5 sec

1 point = sum (average) of 33,000 primary spectra/s

Good repeatability of signal even at lowest speed i.e. 1 spectrum s⁻¹ (contrary to scanning instruments)

↑ acq. speed ⇒
↑ points/peak ⇒
↑ LOD
## Pesticide residues in apples: LOQs (µg/kg)

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>LOQs</th>
<th>Pesticide</th>
<th>LOQs</th>
<th>Pesticide</th>
<th>LOQs</th>
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<tbody>
<tr>
<td>endosulfan-SO4</td>
<td>1.1</td>
<td>o,p-DDD</td>
<td>2.8</td>
<td>endrin</td>
<td>5.3</td>
<td>methamidophos</td>
<td>9.6</td>
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<td></td>
</tr>
<tr>
<td>penceonazole</td>
<td>1.3</td>
<td>p,p-DDT</td>
<td>3.0</td>
<td>chlorpyrifos-methyl</td>
<td>5.4</td>
<td>mevinphos</td>
<td>9.8</td>
<td></td>
<td></td>
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<tr>
<td>vinclozolin</td>
<td>1.4</td>
<td>procymidone</td>
<td>3.1</td>
<td>cypermethrin I</td>
<td>5.4</td>
<td>acephate</td>
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<tr>
<td>chlorothalonil</td>
<td>1.7</td>
<td>cyprodinyl</td>
<td>3.5</td>
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<td>5.4</td>
<td>fenthion</td>
<td>10.0</td>
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<tr>
<td>tetracoxime methyl</td>
<td>2.0</td>
<td>ethion</td>
<td>3.5</td>
<td>cypermethrin III</td>
<td>5.4</td>
<td>folpet</td>
<td>11.0</td>
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<tr>
<td>triadimefon</td>
<td>2.0</td>
<td>endosulfan-beta</td>
<td>3.6</td>
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<td>pyridaben</td>
<td>2.0</td>
<td>fenitrothion</td>
<td>4.0</td>
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<td>phosphamidone I</td>
<td>12.0</td>
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<td>beta-cyfluthrin II</td>
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<td>captan</td>
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<tr>
<td>p,p-DDE</td>
<td>2.3</td>
<td>fenarimol</td>
<td>4.3</td>
<td>tebuconazole</td>
<td>6.1</td>
<td>iprodione</td>
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<tr>
<td>bifenthrin</td>
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<td>dichlofluanid</td>
<td>4.4</td>
<td>azinfos-Et</td>
<td>6.1</td>
<td>hexythiazox</td>
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<tr>
<td>HCHγ (lindane)</td>
<td>2.4</td>
<td>dichlorvos</td>
<td>4.5</td>
<td>phosalone</td>
<td>6.4</td>
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<tr>
<td>p,p-DDE</td>
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<td>bromopropylate</td>
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<td>malathion</td>
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<td>imazalil</td>
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<tr>
<td>HCHα</td>
<td>2.5</td>
<td>pirimiphos-methyl</td>
<td>4.8</td>
<td>heptenophos</td>
<td>7.2</td>
<td>fenoxy carb</td>
<td>15.0</td>
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<td></td>
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<tr>
<td>heptachlor</td>
<td>2.5</td>
<td>difenylamin</td>
<td>4.9</td>
<td>monocrotophos</td>
<td>7.2</td>
<td>propham</td>
<td>24.0</td>
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<tr>
<td>aldrin</td>
<td>2.5</td>
<td>parathion</td>
<td>5.0</td>
<td>trifloxystrobin</td>
<td>7.2</td>
<td>metalaxyl</td>
<td>30.0</td>
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<td></td>
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<tr>
<td>o,p-DDT</td>
<td>2.6</td>
<td>triazamate</td>
<td>5.0</td>
<td>cyhalothrin</td>
<td>7.4</td>
<td>chlorfenvinphos I</td>
<td>30.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCHβ</td>
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<td>dieldrin</td>
<td>5.0</td>
<td>methacrifos</td>
<td>7.5</td>
<td>chlorpropham</td>
<td>32.0</td>
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<tr>
<td>HCHδ</td>
<td>2.7</td>
<td>permethrin I</td>
<td>5.0</td>
<td>dimethoate</td>
<td>7.8</td>
<td>difenoconazole-I</td>
<td>32.0</td>
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<tr>
<td>HCB</td>
<td>2.8</td>
<td>permethrin II</td>
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<td>bupirimate</td>
<td>7.8</td>
<td>difenoconazole-II</td>
<td>32.0</td>
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<tr>
<td>quinalphos</td>
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<td>parathion-methyl</td>
<td>5.2</td>
<td>pirimicarb</td>
<td>9.1</td>
<td>fenvalerate I</td>
<td>48.0</td>
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</tr>
<tr>
<td>o,p-DDE</td>
<td>2.8</td>
<td>tolclofos-methyl</td>
<td>5.2</td>
<td>thiabendazole</td>
<td>9.3</td>
<td>fenvalerate II</td>
<td>48.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*LOQs expressed as Lowest Calibration Levels (LCL), RSD 8 – 12%*
Limitations of hr-TOF MS

- Detection / identification / quantification of very narrow peaks difficult – not enough data points per peak

10 spectra/s

250 spectra/s

Peak 150 ms wide at the base “INVISIBLE“

Fast TOF
Example:
TOF-MS detection of co-eluted chorpyrifos + parathion peaks

Peak width: 2 s
30 spectra per peak needed for deconvolution
Example:
Separation of target analyte (dichlorvos, 0.01 mg/kg) from co-extract

1D-GC

INTERFERENCE:

- $m/z$ 109 (quantification)
- $m/z$ 79 (identification)

no spectral match with NIST library

COMPREHENSIVE CHROMATOGRAPHY: dramatic increase of chromatographic resolution
Orthogonal GC (GC×GC) set-up

→ two columns with differing selectivity connected via modulator

1st dimension
Typically narrow bore non polar column
(30 m × 0,25 mm I.D. × 0,25 μm film)

2nd dimension
Typically microbore polar column
(1 m × 0,1 mm I.D. × 0,1 μm film)
Two-dimensional chromatogram
**Example:**
Separation of target analyte (dichlorvos, 0.01 mg/kg) from co-extract

Dichlorvos reliably identified and quantified → result of increased GC peak capacity

<table>
<thead>
<tr>
<th>Hit</th>
<th>Name</th>
<th>Reverse</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phosphoric acid, 2,2-dichlorovinyl dimethyl ester</td>
<td>940</td>
<td>62-73-7</td>
</tr>
<tr>
<td>2</td>
<td>Phosphoric acid, 2,2-dichlorovinyl dimethyl ester</td>
<td>729</td>
<td>62-73-7</td>
</tr>
<tr>
<td>3</td>
<td>Phosphoric acid, 2,2-dichlorovinyl dimethyl ester</td>
<td>707</td>
<td>62-73-7</td>
</tr>
<tr>
<td>4</td>
<td>Phosphoric acid, 2,2-dichlorovinyl dimethyl ester</td>
<td>704</td>
<td>62-73-7</td>
</tr>
<tr>
<td>5</td>
<td>Phosphoric acid, 2,2-dichlorovinyl dimethyl ester</td>
<td>700</td>
<td>62-73-7</td>
</tr>
</tbody>
</table>

Separation of target analyte (dichlorvos, 0.01 mg/kg) from co-extract

HMF

Peak True - sample "mstd"

Library Hit - similarity 727, "Phosphoric acid, 2,2-dichlorovinyl dimethyl ester"

<table>
<thead>
<tr>
<th>Peak True</th>
<th>Library Hit</th>
</tr>
</thead>
<tbody>
<tr>
<td>79</td>
<td>727</td>
</tr>
</tbody>
</table>

Result of increased GC peak capacity

HMF

Separated coextract
**Difficult pesticides**

**METHAMIDOPHOS**
in GPC purified peach extract (10 pg injected)

<table>
<thead>
<tr>
<th>Hit</th>
<th>Name</th>
<th>Reversed Similarity</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Metamidophos</td>
<td>841</td>
<td>10265-92-6</td>
</tr>
<tr>
<td>2</td>
<td>Phosphoramidothioic acid, O,S-dimethyl ester (mainlib)</td>
<td>829</td>
<td>0-00-0</td>
</tr>
<tr>
<td>3</td>
<td>Phosphoramidothioic acid, O,S-dimethyl ester</td>
<td>829</td>
<td>10265-92-6</td>
</tr>
<tr>
<td>4</td>
<td>1H-Pyrrole, 2,3-dimethyl-</td>
<td>818</td>
<td>600-28-2</td>
</tr>
</tbody>
</table>

**1D:** spectral match 611 (only)

**2D:** very good spectral match
# ACEPHATE in GPC purified peach extract (10 pg injected)

<table>
<thead>
<tr>
<th>Hit</th>
<th>Name</th>
<th>Reverse Similarity</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>Acephate</strong></td>
<td>810</td>
<td>30560-19-1</td>
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<tr>
<td>2</td>
<td>Acephate</td>
<td>821</td>
<td>30560-19-1</td>
</tr>
<tr>
<td>3</td>
<td>Acephate (mainlib)</td>
<td>804</td>
<td>0-00-0</td>
</tr>
<tr>
<td>4</td>
<td>Acephate</td>
<td>804</td>
<td>30560-19-1</td>
</tr>
<tr>
<td>5</td>
<td>Disulfide, ethyl 1-methylethyl</td>
<td>697</td>
<td>53966-36-2</td>
</tr>
</tbody>
</table>
RESULTS:
Analysis of 91 pesticides by high speed GCxGC/TOF-MS

LODs < 0.01 mg/kg, RSD 5 – 24% at 0.02 mg/kg level

The latest eluting deltamethrin at 10.3 min

TOTAL ANALYSIS TIME: 11 MIN
## Problem:
Late eluting broad peaks of pyrethroids

\[ \rightarrow 6 - 7 \text{ segments} / \text{peak} \]

\[ \rightarrow \text{higher LODs} \]

### LODs (ug/kg) in 2D \( \rightarrow \) lower by factor 2 - 50

<table>
<thead>
<tr>
<th>pesticide</th>
<th>m/z</th>
<th>1D noise</th>
<th>1D S/N</th>
<th>1D LOD</th>
<th>2D noise</th>
<th>2D S/N</th>
<th>2D LOD</th>
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<tr>
<td>methamidophos</td>
<td>94</td>
<td>400</td>
<td>43.7</td>
<td>6</td>
<td>60</td>
<td>28</td>
<td>2</td>
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<tr>
<td>dichlorvos</td>
<td>109</td>
<td>3000</td>
<td>24</td>
<td>10</td>
<td>50</td>
<td>261</td>
<td>0.2</td>
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<tr>
<td>acephate</td>
<td>136</td>
<td>400</td>
<td>25</td>
<td>10</td>
<td>40</td>
<td>30</td>
<td>2</td>
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<tr>
<td>dimethoate</td>
<td>125</td>
<td>1000</td>
<td>62</td>
<td>4</td>
<td>60</td>
<td>40</td>
<td>1.3</td>
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<tr>
<td>lindane</td>
<td>181</td>
<td>1000</td>
<td>16.4</td>
<td>15</td>
<td>30</td>
<td>66</td>
<td>0.8</td>
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<tr>
<td>carbaryl</td>
<td>144</td>
<td>1200</td>
<td>37.2</td>
<td>6.7</td>
<td>50</td>
<td>78</td>
<td>0.6</td>
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<tr>
<td>methiocarb</td>
<td>168</td>
<td>600</td>
<td>35.2</td>
<td>7.1</td>
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<td>34</td>
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<td>272</td>
<td>210</td>
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</tbody>
</table>

* estimated from the analysis of peach matrix-matched standard at 50 mg/ml for both techniques

** estimated from the analysis of peach matrix-matched standard at 100 mg/ml for both techniques
GCxGC/HS-TOF-MS: A powerful tool for non-target analysis

**Example**

- Tomato sample from the EU Proficiency Test containing a wide range of pesticide residues
- The aim - testing the ability to detect pesticides in complex matrix

*No information about target analytes provided in advance*
RESULTS OF NON-TARGET SCREENING

IDENTIFIED PESTICIDES (NIST library) – deconvoluted:

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Similarity</th>
<th>Pesticide</th>
<th>Similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethoate</td>
<td>895</td>
<td>Endosulphan I</td>
<td>883</td>
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<tr>
<td>Diazinon</td>
<td>929</td>
<td>Imazalil</td>
<td>568</td>
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<td>Chlorothalonil</td>
<td>855</td>
<td>Endosulphan II</td>
<td>882</td>
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<tr>
<td>Metalaxyl</td>
<td>837</td>
<td>Bromopropylate</td>
<td>854</td>
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<tr>
<td>Procymidine</td>
<td>808</td>
<td>Azoxystrobin</td>
<td>758</td>
</tr>
<tr>
<td>Thiabendazole</td>
<td>834</td>
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</tr>
</tbody>
</table>

Oxydemethon-Me imidacioprid Acrinatrin

These pesticides were not present in the used library version nor in standard
WHAT ARE THE CHALLENGES OFFERED BY TOF MS?

hsTOF
- Unit resolution
- High sensitivity
- Very fast data acquisition

hrTOF
- High mass accuracy
- Very high sensitivity
- Fast data acquisition

Full spectral information available even at low levels

- UNKNOWNS IDENTIFICATION / ON-LINE CONFIRMATION
- RELIABLE, UNBIASED QUANTIFICATION

Main requirements and expectations in analysis of pesticide residues

- Low limits of detection
- **GOOD ACCURACY**
- High degree of confirmation
- Robustness

QUALITY CONTROL PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS

Document Nº SANCO/10232/2006

24/March/2006

- Short analysis time
- Broad MRM scope
- Affordable cost
MATRİX EFFECTS: trueness issue both in LC and GC

Change of a chromatographic peak intensity and shape of affected compounds when they are injected in the presence of a complex matrix

Response enhancement

Response diminishment

**Example:**

GC/MS analysis (splitless injection) of pesticide residues (0.02 mg/kg) in apple extract (A) and net solvent (B)

**Real life sample**

**standard in net solvent**
**Example:**

GC/MS (SIM, m/z 87) splitless injection 5 pg dimethoate in crude plant extract (1.5 µL, 2.5g matrix /ml)
Elimination vs. compensation for matrix effects

- **ELIMINATION**
  - No active sites in GC system
  - No matrix compounds

- **COMPENSATION**
  - Standard addition
  - **Matrix-matched standards**
  - Isotopically labeled internal standards

- **Analyte protectants**

**CHALLENGE!**

**Impossible in practice**

**Impractical for routine**
GENERAL CONCEPT: Replacement of laborious matrix-matched standards by addition of compounds simulating (mimicking) matrix to standard in pure solvent

Compounds strongly interacting with active sites in the GC system

USE OF ANALYTE PROTECTANTS

→ obtaining more accurate data

PHOSALON

0.022 mg/kg

0.005 mg/kg, apple

0.0045 mg/kg

solvent

matrix

solvent + APs

matrix + APs

apple

0.005 mg/kg,
Comparison of Generated Data

Accurate data over a broad analyte spectrum!

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Relative Intensity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-ethoxy-1,2-propandiol (4 mg/mL)</td>
<td>1340%</td>
</tr>
<tr>
<td>L-gulonic acid γ-lactone (1 mg/mL)</td>
<td>1450%</td>
</tr>
<tr>
<td>D-glucitol (1 mg/mL)</td>
<td></td>
</tr>
</tbody>
</table>

Baby food: 0.015 mg/kg

Without analyte protectants

With analyte protectants
Enjoy moving ahead.....