EURL-FV EXPERIENCES ON THE EVALUATION OF ACCURATE MASS PLATFORMS FOR PESTICIDES RESIDUES ANALYSIS IN FRUITS AND VEGETABLES

Limassol CYPRUS
26th May 2016

AMADEO R. FERNÁNDEZ-ALBA
LC-HRAMS

WHY?

BENEFITS?
Development of control programs for pesticides in Europe

Source: EFSA

No of pesticides found

1999

No of pesticides sought
Development of control programs for pesticides in Europe

Source: EFSA
## Target list: 62 pesticides

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Nº of reported results</th>
<th>Nº of NA (not sought)</th>
<th>False negatives</th>
<th>% of NA results from the total 125</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetamiprid</td>
<td>56</td>
<td>67</td>
<td>2</td>
<td>54</td>
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<tr>
<td>Carbaryl</td>
<td>101</td>
<td>24</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Cyprodinil</td>
<td>99</td>
<td>24</td>
<td>2</td>
<td>19</td>
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<tr>
<td>Diazinon</td>
<td>123</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>119</td>
<td>2</td>
<td>4</td>
<td>2</td>
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<tr>
<td>Fenhexamid</td>
<td>89</td>
<td>36</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Fludioxonil</td>
<td>85</td>
<td>36</td>
<td>4</td>
<td>29</td>
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<tr>
<td>Imidacloprid</td>
<td>64</td>
<td>60</td>
<td>1</td>
<td>48</td>
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<tr>
<td>Iprodione</td>
<td>113</td>
<td>8</td>
<td>4</td>
<td>6</td>
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<tr>
<td>Kresoxim-methyl</td>
<td>104</td>
<td>20</td>
<td>1</td>
<td>16</td>
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<td>Methomyl</td>
<td>71</td>
<td>45</td>
<td>9</td>
<td>36</td>
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<tr>
<td>Monocrotophos</td>
<td>89</td>
<td>30</td>
<td>5</td>
<td>24</td>
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<tr>
<td>Procymidone</td>
<td>121</td>
<td>4</td>
<td>0</td>
<td>3</td>
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<tr>
<td>Pyrimethanil</td>
<td>98</td>
<td>25</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Tetraconazole</td>
<td>70</td>
<td>49</td>
<td>6</td>
<td>39</td>
</tr>
<tr>
<td>Thiabendazole</td>
<td>104</td>
<td>17</td>
<td>3</td>
<td>14</td>
</tr>
</tbody>
</table>
## EUPT-FV07 (2005)

### Laboratories that reported false positives

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Lab Code</th>
<th>Sample</th>
<th>Concentration (mg/kg)</th>
<th>RL (mg/kg)</th>
<th>MPRL (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpyrifos</td>
<td>EUPT-7 116</td>
<td>Test</td>
<td>0.0016</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>Dichlofluanid</td>
<td>EUPT-7 097</td>
<td>Test</td>
<td>0.013</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Dichlofluanid</td>
<td>EUPT-7 122</td>
<td>Test</td>
<td>0.103</td>
<td>0.006</td>
<td>0.05</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>EUPT-7 125</td>
<td>Test</td>
<td>0.106</td>
<td>0.0034</td>
<td>0.05</td>
</tr>
<tr>
<td>Methidathion</td>
<td>EUPT-7 126</td>
<td>Test</td>
<td>0.176</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>EUPT-7 049</td>
<td>Test</td>
<td>0.26</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>EUPT-7 125</td>
<td>Test</td>
<td>0.005</td>
<td>0.0006</td>
<td>0.02</td>
</tr>
<tr>
<td>Vinclozolin</td>
<td>EUPT-7 097</td>
<td>Test</td>
<td>0.022</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Common Transitions

**Prometon**
- 10.54 min
- 226.3 → 142.3

**Secbumeton**
- 10.54 min
- 226.0 → 142.3

**Terbumeton**
- 10.54 min
- 226.0 → 142.3

**Counts**
- Prometon
- Secbumeton
- Terbumeton

**Acquisition Time (min)**
- 9.6 to 11.4

**LC-QqQ-MS**
Plant Origin Matrix Groups

Total number: 250 matrices
**Data base components - orange matrix compounds (1 g/mL)**

- **m/z:** 100-700
  - Rt: 4-8 min

- **m/z:** 100-400
  - Rt: 7-13 min

**8017 Matrix compounds**

**Miner 3D Enterprise**

- DB: 750 components
- Orange: 2743 matrix compounds
Haloxypf at 0.01 mg/kg in Aubergine. LC-MS/MS

Std in solvent at 0.01 mg/kg

Ethyl Acetate method

NL method

Citrate QuEChERS without clean-up method

Citrate QuEChERS method

Ion Ratio: 0.38

Ion Ratio: 0.34 Diff.: 11%

Ion Ratio: 0.27 Diff.: 29%

Ion Ratio: 0.21 Diff.: 45%

Ion Ratio: 0.39 Diff.: 3%
Quantitation and Accurate Mass Analysis of Pesticides in Vegetables by LC/TOF-MS

Imma Ferrer,* E. Michael Thurman, and Amadeo R. Fernández-Alba

Pesticide Residue Research Group, University of Almería, 04120 Almería, Spain

Figure 1. Chemical structures of imidacloprid, acetamiprid, and thiacloprid.

Table 2. LC/TOF-MS Accurate Mass Measurements for the Chloronicotinyl Pesticides and Their Fragments in a Tomato-Matched Matrix

<table>
<thead>
<tr>
<th>compound</th>
<th>elemental composition</th>
<th>theoretical mass (0.05 mg/kg)</th>
<th>measured mass</th>
<th>error (ppm)</th>
<th>concentration (0.5 mg/kg)</th>
<th>measured mass</th>
<th>error (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>imidacloprid</td>
<td>C_{9}H_{11}N_{5}O_{2}Cl</td>
<td>256.0596</td>
<td>256.0596</td>
<td>0.1</td>
<td>256.0597</td>
<td>210.0664</td>
<td>-1.3</td>
</tr>
<tr>
<td></td>
<td>C_{9}H_{11}N_{5}Cl</td>
<td>210.0667</td>
<td>210.0663</td>
<td>-1.8</td>
<td>210.0664</td>
<td>209.0587</td>
<td>-0.7</td>
</tr>
<tr>
<td></td>
<td>C_{9}H_{11}N_{5}Cl</td>
<td>209.0589</td>
<td>209.0587</td>
<td>-0.7</td>
<td>209.0587</td>
<td>175.0977</td>
<td>-0.7</td>
</tr>
<tr>
<td></td>
<td>C_{9}H_{11}N_{5}</td>
<td>175.0978</td>
<td>175.0983</td>
<td>2.7</td>
<td>175.0977</td>
<td>126.0105</td>
<td>0.0</td>
</tr>
<tr>
<td>acetamiprid</td>
<td>C_{10}H_{12}N_{5}Cl</td>
<td>223.0745</td>
<td>223.0746</td>
<td>0.5</td>
<td>223.0749</td>
<td>126.0105</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>C_{10}H_{12}N_{5}Cl</td>
<td>126.0105</td>
<td>126.0106</td>
<td>0.8</td>
<td>126.0105</td>
<td>126.0106</td>
<td>0.8</td>
</tr>
<tr>
<td>thiacloprid</td>
<td>C_{10}H_{12}N_{5}SCl</td>
<td>253.0309</td>
<td>253.0311</td>
<td>0.7</td>
<td>253.0313</td>
<td>126.0103</td>
<td>-1.6</td>
</tr>
<tr>
<td></td>
<td>C_{10}H_{12}N_{5}Cl</td>
<td>126.0105</td>
<td>126.0107</td>
<td>1.6</td>
<td>126.0105</td>
<td>126.0106</td>
<td>1.6</td>
</tr>
</tbody>
</table>
**Pyrimethanil at 0.01 mg/kg**

Pyrimethanil

[M+H⁺] m/z 200.1182

0.01 mg/kg in onion

RT = 7.05 min

Dilution 5x
EUPT Sample: coriander

Linuron
Standard in solvent
Ion ratio: 1.8

Linuron (0.125 mg/kg)
Real sample of coriander
Ion ratio: 2.4

FALSE NEGATIVE
EUPT Sample of coriander

Linuron

Matrix interference
BUT.....
Fenazaquin in tomato extract, calibration with monoisotopic ion, with ion containing one $^{13}$C atom and with ion containing two $^{13}$C atoms. Quantitation with monoisotopic ion possible up to 0.100 mg/kg. Higher concentration levels have to be quantified with ion containing two $^{13}$C atoms.
Influence of resolution on detection

Exact mass of bupirimate: 317.1642

Resolution 17500

- Two non resolved matrix ions.
  Mass error 4.7 ppm
  False detect of bupirimate

Resolution 70000

- Matrix ion.
  Mass error -30.1 ppm

Matrix ion. Mass error 27.1 ppm
2005
LOOKING FOR
PERFORMANCE

2016
ACQUIRED
LOQ

Tomato
- Target Analysis
- Non Target MS² (DIA-VMW)
- Non Target MS² (DIA)

Apple
- Target Analysis
- Non Target MS² (DIA-VMW)
- Non Target MS² (DIA)

Orange
- Target Analysis
- Non Target MS² (DIA-VMW)
- Non Target MS² (DIA)

Onion
- Target Analysis
- Non Target MS² (DIA-VMW)
- Non Target MS² (DIA)
Linearity LC-HRAMS

Spinach

- Pirimiphos-methyl
- Ethoprophos
- Flusilazole
- Azoxystrobin
- Imidacloprid
- Zoxamide
Azoxystrobin 0.01 mg/kg in tomato
Dilution 5x (mass errors in ppm)
Ethirimol 0.1 mg/kg in tomato, MS² spectrum
Thiabendazole
$C_{10}H_7N_3S \ (m/z \ 202.0433)$

- $C_{0.25}H_{0.50}N_{0.5}O_{0.5}Cl_{0.5}Br_{0.5}S$

**Number of possible molecular formulae**

- 80
- 60
- 40
- 20
- 0

**Resolution**

- ~ 25 000 FWHM
- ~ 70 000 FWHM

**Absolute mass error relative to Thiabendazole**

- ~ 2 000
- ~ 20 000
- ~ 40 000
- ~ 200 000
- ~ 400 000 (FWHM)

- 5 ppm
- 500 ppm
- 2 000 ppm
- 4 000 ppm
- 8 000 ppm

- Cl, Br
- Cl, Br
Resolution dependence on m/z
Experimental data
Number of Pesticides with fragments using Orbitrap in Full Scan mode

Solvent 0.10 mg/kg
Analysis of real samples by LC-HRAMS MS²

Metalaxyl (XIC m/z 280.1543 ± 5 ppm). **Full scan MS.** Resolution 70000

- Pepper spiked at 0.01 mg/kg metalaxyl
- Grapefruit Real sample 1
- Grapefruit Real sample 2
Metalaxyl (XIC m/z 280.1543 ± 5 ppm).

**Full scan MS. Resolution 70000**

**Expected fragments:**
- 148.1119
- 160.1119
- 192.1380
- 220.1380

**Standard of Metalaxyl**

**Grapefruit Real sample**

**Library MS/MS spectrum**

- 192.1382
- 160.1119
- 192.1380
- 220.1380

**Experimental MS/MS spectrum**

**FALSE POSITIVE**
Combining Full Scan MS and MS$^2$ Mode
Pesticide residue method MS²

- **Full Scan**
  - Detection/Identification (mass and retention time)
  - Quantitation

- **MS/MS (Target and Non target MS/MS)**
  - Identification (at least one fragment)
**Tomato 0.01 mg/kg**

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Full scan</th>
<th>QTOF</th>
<th>Orbitrap</th>
<th>MS²</th>
<th>QTOF</th>
<th>Orbitrap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoxystrobin</td>
<td>404.1241</td>
<td>-1.5</td>
<td>0.0</td>
<td>372.0979</td>
<td>0.6</td>
<td>-0.3</td>
</tr>
<tr>
<td>Diazinon</td>
<td>305.1083</td>
<td>-1.5</td>
<td>0.3</td>
<td>169.0794</td>
<td>0.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>220.9532</td>
<td>0.5</td>
<td>0.5</td>
<td>109.0047</td>
<td>-1.9</td>
<td>1.6</td>
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<tr>
<td>Fenamiphos - sulfone</td>
<td>336.1029</td>
<td>-0.2</td>
<td>0.6</td>
<td>266.0246</td>
<td>-1.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Profenofos</td>
<td>372.9424</td>
<td>1.9</td>
<td>0.5</td>
<td>302.8642</td>
<td>1.0</td>
<td>-0.3</td>
</tr>
</tbody>
</table>

**Orange 0.01 mg/kg**

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Full scan</th>
<th>QTOF</th>
<th>Orbitrap</th>
<th>MS²</th>
<th>QTOF</th>
<th>Orbitrap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoxystrobin</td>
<td>404.1241</td>
<td>0.4</td>
<td>0.0</td>
<td>372.0979</td>
<td>1.4</td>
<td>0.3</td>
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<td>0.0</td>
</tr>
<tr>
<td>Profenofos</td>
<td>372.9424</td>
<td>-0.4</td>
<td>0.0</td>
<td>302.8642</td>
<td>1.1</td>
<td>-1.0</td>
</tr>
</tbody>
</table>
TARGET ANALYSIS (DATA DEPENDENT ACQUISITION)

- TOF
- MS² (DATA INDEPENDENT ACQUISITION)  FS/AIF-MS²

- TOF
- MS²  DIA-VMW (FS/SWATH)

- TOF
- 0.1 – 0.5 s

- TOF
- 0.1 - 0.5 s
**LC-QTOF-MS**

**Auto MS/MS mode**

Full scan detection

**All ions mode**

Formetanate Identified with two fragments ions

**Formetanate Tomato 0.02 mg/kg**

No MS/MS spectrum

Counts vs. Mass-to-Charge (m/z)

Counts vs. Acquisition Time (min)

Counts vs. Mass-to-Charge (m/z)
**IDA mode**

Full scan detection

![IDA mode spectrum](image)

No MS/MS spectrum

**SWATH mode**

Propoxur Identified by fragments ions

![SWATH mode spectrum](image)
TARGET ANALYSIS (DATA DEPENDENT ACQUISITION) FS/dd-MS²

Orbitrap

**FS/AIF-MS²**
- 70k
- 70k

**FS/vDIA-MS²**
- 70k
- 35k
- 35k
- 35k
- 35k
- 35k
- 0.27 s
- 1.28 s
Fenazaquin
0.02 mg/L in solvent
Repeatability
(Five Analyses)

Tomato
0.01 mg/kg

% of compounds

RSD 0-5%
RSD 0-10%
RSD 0-20%

3 segments/ R=35000
5 segments/ R=35000
3 segments/ R=17500
5 segments/ R=17500
AIF/dd MS2
11 Matrices

- Lettuce
- Cucumber
- Orange
- Green bean
- Onion
- Apple
- Parsley
- Leek
- Tomato
- Garlic
- Spinach
Identification

166 pesticides x 11 matrices = 1826 results

% of compounds

0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%

dd MS2 vDIA AIF

0.01 mg/kg
0.1 mg/kg
## EUPT-FV-17 Broccoli 2015

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Assigned value (mg/kg)</th>
<th>Obtained value (Difference)</th>
<th>AIF</th>
<th>dd MS²</th>
<th>vDIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupirimate</td>
<td>0,165 (±50%)</td>
<td></td>
<td>0,157 (-5%)</td>
<td>0,152 (-8%)</td>
<td>0,160 (-3%)</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>0,512 (±50%)</td>
<td></td>
<td>0,415 (-19%)</td>
<td>0,404 (-21%)</td>
<td>0,404 (-21%)</td>
</tr>
<tr>
<td>Diazinon</td>
<td>0,071 (±50%)</td>
<td></td>
<td>0,051 (-28%)</td>
<td>0,051 (-28%)</td>
<td>0,051 (-28%)</td>
</tr>
<tr>
<td>Difenoconazol</td>
<td>0,53 (±50%)</td>
<td></td>
<td>0,625 (18%)</td>
<td>0,631 (19%)</td>
<td>0,610 (15%)</td>
</tr>
<tr>
<td>Diflubenzuron</td>
<td>0,319 (±50%)</td>
<td></td>
<td>0,367 (15%)</td>
<td>0,332 (4%)</td>
<td>0,354 (11%)</td>
</tr>
<tr>
<td>Metoxyfenozide</td>
<td>0,349 (±50%)</td>
<td></td>
<td>0,300 (-14%)</td>
<td>0,304 (-13%)</td>
<td>0,293 (-16%)</td>
</tr>
<tr>
<td>Spinosaad</td>
<td>0,051 (±50%)</td>
<td></td>
<td>0,044 (-14%)</td>
<td>0,044 (-14%)</td>
<td>0,044 (-14%)</td>
</tr>
<tr>
<td>Thiabendazole</td>
<td>1,90 (±50%)</td>
<td></td>
<td>2,07 (9%)</td>
<td>2,01 (6%)</td>
<td>2,00 (5%)</td>
</tr>
<tr>
<td>Trifloxystrobin</td>
<td>0,466 (±50%)</td>
<td></td>
<td>0,592 (27%)</td>
<td>0,592 (27%)</td>
<td>0,559 (20%)</td>
</tr>
<tr>
<td>Pendimethalin</td>
<td>0,062 (±50%)</td>
<td></td>
<td>0,070 (13%)</td>
<td>0,059 (-5%)</td>
<td>0,065 (5%)</td>
</tr>
</tbody>
</table>
• “Old” or rarely detected compounds

• **Complex residue definition**
  Cycloxydim including degradation and reaction products which can be determined as 3-(3-thianyl)glutaric acid S-dioxide (BH 517-TGSO$_2$) and/or 3-hydroxy-3-(3-thianyl)glutaric acid S-dioxide (BH 517-5-OH-TGSO$_2$) or methyl esters thereof, calculated in total as cycloxydim

• “Very expensive” analytical standards

• Compounds “produced” during the analysis
The graph shows the area (AREA) of Diafenthiuron over time (min) in different solvents and samples:

- **solvent AcN**
- **AcN/H2O**
- **Broccoli**

As the time increases, the area for each solvent and sample also increases, with the broccoli sample showing a distinct pattern compared to the others.
Diethiuron in Broccoli

LC-QTOF-MS
LC-QTOF-MS

Diafenthion in Orange

AREA

- St1
- St2
- St3
- R1_t1
- R1_t2
- R1_t3
- R2_t1
- R2_t2
- R2_t3

- Difenthiuron
- Sulfomonoxide
- Urea
Number of Laboratories analysing by HRAMS in the last three EUPTs-FV-SM

<table>
<thead>
<tr>
<th></th>
<th>Using HRMs</th>
<th>Not Using HRMs</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM06</td>
<td>18</td>
<td>49</td>
</tr>
<tr>
<td>SM07</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>SM08</td>
<td>29</td>
<td>53</td>
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</tbody>
</table>
% of Laboratories analysing by HRAMS in the last three EUPTs-FV-SM

- SM06: 27% Using HRMs, 73% Not Using HRMs
- SM07: 29% Using HRMs, 71% Not Using HRMs
- SM08: 35% Using HRMs, 65% Not Using HRMs
Participation EUPT-FV-SM08

24 EU/ EFTA Countries

Austria
Belgium
Bulgary
Croatia
Cyprus
Czech Republic
Denmark
Estonia
Finland
France
Germany
Greece
Hungary
Italy
Ireland
Latvia
Netherlands
Norway
Romania
Serbia
Slovenia
Spain
Sweden
Switzerland
United Kingdom

HRAMS

Czech Republic
Denmark
France
Germany
Greece
Italy
Netherlands
Norway
Romania
Spain
Sweden
United Kingdom
Thank You for Your Attention