LC-MS/MS multiresidue method used for pesticides analysis in fruits and vegetables

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Pesticides

- Approximately 2000 known pesticides and ~300 widely used

- Very different physico-chemical properties

- Agricultural practices are different from one country to another

- Large variety of commodities to control
Multiresidue method

« Simultaneous analysis of a large number of substances in various matrices »

Goals

The method must include as many substances as possible and must be accurate, precise, sensitive, fast, widely applicable, selective, easy and robust.
Gas chromatography

For many years, gas chromatographic methods have been used for pesticides monitoring

GC-ECD, GC-NPD, GC-MS

- Limitation to volatile substances
- Poorly detectable substances
- Poor chromatography (tailing problem etc…)

=> Complementary analytical method are required for some substances.
Pesticides monitoring

Volatile substances:
- mainly organochlorinated and organophosphorous compounds

GC-ECD, NPD analysis
GC-MS confirmation

Polar substances:
- Various chemical families (carbamates, imidazoles, …)

LC-ESI-MS/MS analysis and confirmation
<table>
<thead>
<tr>
<th>Acetamiprid</th>
<th>Cyproconazole</th>
<th>Furathiocarb</th>
<th>Oxamyl</th>
<th>Tebuconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldicarb</td>
<td>Cyprodinil</td>
<td>Hexythiazox</td>
<td>Oxine</td>
<td>Tebufenpyrad</td>
</tr>
<tr>
<td>Azametiphos</td>
<td>Diethofencarb</td>
<td>Imazalil</td>
<td>Phenthoate</td>
<td>Terbufos</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>Difenconazole</td>
<td>Imidacloprid</td>
<td>Phosalone</td>
<td>Thiabendazole</td>
</tr>
<tr>
<td>Bendiocarb</td>
<td>Dimethoate</td>
<td>Indoxacarbd</td>
<td>Pirimicarb</td>
<td>Thiacloprid</td>
</tr>
<tr>
<td>Benfuracarb</td>
<td>Dimethomorph</td>
<td>Iprovalicarb</td>
<td>Prochloraz</td>
<td>Thiobencarb</td>
</tr>
<tr>
<td>Benodanil</td>
<td>Dimetilan</td>
<td>Isazofos</td>
<td>Propamocarb</td>
<td>Thiodicarb</td>
</tr>
<tr>
<td>Benzoximate</td>
<td>Dinocap</td>
<td>Mepanipyrim</td>
<td>Propetamphos</td>
<td>Thiofanox</td>
</tr>
<tr>
<td>Buprofezin</td>
<td>Diphenylamine</td>
<td>Metalaxyl</td>
<td>Propoxur</td>
<td>Thiometon</td>
</tr>
<tr>
<td>Butocarboxym</td>
<td>Ethiophencarb</td>
<td>Methiocarb</td>
<td>Pymetrozine</td>
<td>Thiophanate-ethyl</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>Fenazaquin</td>
<td>Methomyl</td>
<td>Pyridaben</td>
<td>Thiophanate-methyl</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>Fenoxycarb</td>
<td>Monocrotophos</td>
<td>Pyrifenvox</td>
<td>Tocolphos-methyl</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>Fenproparthrin</td>
<td>Myclobutanil</td>
<td>Pyrimethanil</td>
<td>Triforine</td>
</tr>
<tr>
<td>Clofentezine</td>
<td>Fludioxonil</td>
<td>Omethoate</td>
<td>Spinosyn A</td>
<td>Vamidothion</td>
</tr>
<tr>
<td>Cymoxanil</td>
<td>Fluquinconazole</td>
<td>Oxadixyl</td>
<td>Spinosyn D</td>
<td></td>
</tr>
</tbody>
</table>
Pesticides

Thiodicarb

Oxamyl

Omethoate

Carbaryl

Thiabendazole

Imazalil

Diphenylamine

Indoxacarb

Spinosyn A
The goal of sample preparation is to obtain a sample compatible with the analytical system.
Sample preparation

- Homogenisation
- Weighing 20g
- Add 10 ml of water
- pH adjustment between 6-7
- Add 40 ml of ethyl acetate
- Shake 15 min
- Centrifugation
- Evaporation of 5 ml of supernatant
- Reconstitution in 1 ml of methanol
- Filtration on nylon filter 0.45 µm

Productivity: ~20 sample / person and day
LC-ESI-MS/MS system

Liquid chromatography coupled to a triple quadrupole mass spectrometer Quattro \textit{micro}^\text{TM} (Waters-Micromass) equipped with an electrospray source operated in positive ionization mode.
Two MS/MS transitions per substances:
  MH⁺ as mother ions (if present)  
  and two specific daughter ions
Cyproconazole

MH+ 292

Daughter scan of 292

MS scan

Cyproconazole, 10 µg/ml, 10 µl/min, cone 25 V; Collision 30 V

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Acquisition is carried out in 11 acquisition groups

1 MS/MS transition by substances

Dwell time of 50ms for each MS/MS transitions

More compounds could be added to screening
Chromatography

Column: Nucleosil 100-5 C18 HD, 70 x 2 mm I.D.
Gradient: [A] 0.1% of formic acid in water
          [B] 0.1% of formic acid in MeOH
Flow rate: 300 µl/min
Injection: 10 µL

Spiked sample at 100 µg/kg
Difficult to validate for all combination of substances and matrices.

Validation was carried out for:

1. All substances
2. Representative matrices
Method validation

1. **Response fonction**
   Linearity verified in the analytical domain 10-1000 µg/kg

2. **Detection and quantification limits**
   LOD <10 µg/kg for almost all compounds

3. **Extraction recovery**
   Majority of compounds with recovery >80%

4. **Accuracy and repeatability**
   80% < accuracy < 120% and repeatability < 20%

5. **Matrix effects**
Matrix effects

- Test the response of sample with or without the presence of matrices

Blank sample → Extraction → Blank extract → Spike → Spiked Blank extract → Aqueous standard
Matrix effects

- 13 matrices have been tested
  lemon, grape, strawberry, apple, nectarine, tomato, carrot
  green salad, cucumber, eggplant, spinach, potato, pepper

  Mean matrix effect for all substances of 92%
  ranging from 63 to 133%!
Specificity

- Specificity means the ability of a method to distinguish between the analyte being measured and other substances » European guidelines EC/657/2002

Authorised substances: minimum 3 identification points
Forbidden substances: minimum 4 identification points

<table>
<thead>
<tr>
<th>Technique(s)</th>
<th>Number of ions</th>
<th>Identification Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-MS (EI or CI) or LC-MS</td>
<td>n ions</td>
<td>n</td>
</tr>
<tr>
<td>GC-MS/MS or LC-MS/MS</td>
<td>1 precursor + 1 daughters ions</td>
<td>2.5</td>
</tr>
<tr>
<td>GC-MS/MS or LC-MS/MS</td>
<td>1 precursor + 2 daughters ions</td>
<td>4</td>
</tr>
<tr>
<td>GC-MS/MS or LC-MS/MS</td>
<td>2 precursor, each with 1 daughter</td>
<td>5</td>
</tr>
</tbody>
</table>
Specificité

Standard Isazofos
Retention time: 13.58 min

Sample
Retention time: 13.52 min

MS/MS transition
314 -> 162
314 -> 120

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Non-compliant sample

- Confirmatory method is needed to identify without ambiguity the incriminated substance and to quantify without error at the level of interest.

  - External matrix matched calibration for quantification

  - Test non-compliant sample using a second MS/MS transition for the incriminated substance
Pesticides monitoring program

Analytical strategy in our laboratory today:

- **~2000 samples/year**
  - Acetonitrile-Hexane extract
    - GC-ECD, NPD, FPD analysis
    - GC-MS confirmation
  - Ethyl acetate extract
    - LC-MS/MS analysis and confirmation

Volatile substances:
- mainly organochlorinated and organophosphorous compounds

Polar substances:
- Various chemical families:
  - carbamates, imidazoles, conazoles…
**Pesticides monitoring program**

1265 fruits samples from the Swiss market

Results from January 2002 to September 2003

<table>
<thead>
<tr>
<th>FRUITS</th>
<th>Nb of sample</th>
<th>Without residue</th>
<th>With residue &lt;MRL</th>
<th>With residue &gt;MRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pome fruits</td>
<td>246</td>
<td>80 (32%)</td>
<td>164 (67%)</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>Stone fruits</td>
<td>217</td>
<td>84 (39%)</td>
<td>130 (60%)</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>Berries and small fruits</td>
<td>480</td>
<td>143 (30%)</td>
<td>292 (61%)</td>
<td>45 (9%)</td>
</tr>
<tr>
<td>Citrus fruits</td>
<td>172</td>
<td>31 (18%)</td>
<td>119 (69%)</td>
<td>22 (13%)</td>
</tr>
<tr>
<td>Exotic fruits</td>
<td>85</td>
<td>57 (67%)</td>
<td>25 (29%)</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Miscellaneous fruits</td>
<td>65</td>
<td>56 (86%)</td>
<td>9 (14%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>1265</strong></td>
<td><strong>451 (35.6%)</strong></td>
<td><strong>739 (58.4%)</strong></td>
<td><strong>75 (5.9%)</strong></td>
</tr>
</tbody>
</table>
# Pesticides Monitoring Program

## 1306 Vegetables Samples from the Swiss Market

Results from January 2002 to September 2003

<table>
<thead>
<tr>
<th>VEGETABLES</th>
<th>Nb of sample</th>
<th>Without residue</th>
<th>With residue &lt;MRL</th>
<th>With residue &gt;MRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root vegetables</td>
<td>40</td>
<td>38 (95 %)</td>
<td>2 (5 %)</td>
<td>0 (0 %)</td>
</tr>
<tr>
<td>Leafy vegetables</td>
<td>445</td>
<td>182 (41 %)</td>
<td>205 (46 %)</td>
<td>58 (13 %)</td>
</tr>
<tr>
<td>Fruiting vegetables</td>
<td>616</td>
<td>350 (57 %)</td>
<td>245 (40 %)</td>
<td>21 (3 %)</td>
</tr>
<tr>
<td>Legume vegetables</td>
<td>42</td>
<td>34 (81 %)</td>
<td>8 (19 %)</td>
<td>0 (0 %)</td>
</tr>
<tr>
<td>Miscellaneous vegetables</td>
<td>163</td>
<td>116 (71 %)</td>
<td>34 (21 %)</td>
<td>13 (8 %)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>1306</strong></td>
<td><strong>720 (55.2%)</strong></td>
<td><strong>494 (37.8%)</strong></td>
<td><strong>92 (7.0%)</strong></td>
</tr>
</tbody>
</table>
Pesticides monitoring program

Majority of samples contained more than one residues!

Statistical data from January to September 2003
## Multiresidue sample!

### Grape sample

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Level (mg/kg)</th>
<th>Swiss MRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpyrifos (I)</td>
<td>1.08</td>
<td>0.5</td>
</tr>
<tr>
<td>Folpet (F)</td>
<td>0.78</td>
<td>3</td>
</tr>
<tr>
<td>Fenitrothion (I)</td>
<td>0.65</td>
<td>0.5</td>
</tr>
<tr>
<td>Bromopropylate (A)</td>
<td>0.62</td>
<td>2</td>
</tr>
<tr>
<td>Fludioxonil (F)</td>
<td>0.52</td>
<td>3</td>
</tr>
<tr>
<td>Dicofol (A)</td>
<td>0.32</td>
<td>2</td>
</tr>
<tr>
<td>Cyprodinil (F)</td>
<td>0.30</td>
<td>3</td>
</tr>
<tr>
<td>Fenazaquin (A)</td>
<td>0.21</td>
<td>0.20</td>
</tr>
<tr>
<td>Fenpropathrin (I)</td>
<td>0.09</td>
<td>0.02</td>
</tr>
<tr>
<td>Cymoxanil (F)</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Dimethomorph (F)</td>
<td>0.04</td>
<td>2</td>
</tr>
<tr>
<td>Tebuconazole (F)</td>
<td>0.03</td>
<td>1</td>
</tr>
</tbody>
</table>
Acknowledgement

- Dr Patrick EDDER, Dr Claude CORVI
- Maria BLANCO, Corinne BERGER, Eric PITTET
- Waters-Micromass