Chemical residues in food and water; challenges for a future sustainable agriculture

Liquid Chromatography-Tandem Mass Spectrometric Ion-Switching Determination of Chlorantraniliprole and Flubendiamide in Fruits and Vegetables

Pierluigi Caboni, Paolo Cabras

INTRODUCTION

The anthranilic and phthalic diamides, chlorantraniliprole (CAP) and flubendiamide (FLU), represent a new class of very effective insecticides.

Allosteric activators of the ryanodine-sensitive intracellular calcium release channel (ryanodine receptor).

Extremely potent against lepidopterous pests including those resistant to neonicotinoid and pyrethroid insecticides.
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**Action sites of commercially available pesticides**

**Insect Neuromuscular Signaling Pathway**

- **Excitatory Neuron**
  - OPs
  - Carbamates
  - AChE
  - Neonicotinoids
  - Spinosad

- **Motor Neuron**
  - Glut
  - GABA

- **Muscle**
  - Mitochondria
  - Insecticides

**Pyrethroids**
- Indoxacarb

**Na+/K+ channels**

**Ryanodine receptors RyRs**

**Ryanodine receptors** (*RyRs*) form a class of intracellular calcium channels in various forms of excitable animal tissue like muscles and neurons.

**Ryanodine receptors** mediate the release of calcium ions from the sarcoplasmic reticulum, an essential step in muscle contraction.
Ryanodine is an alkaloid found in the South American plant (Flacourtiaceae) originally used as an insecticide. The compound has extremely high affinity to the ryanodine receptor. It binds with such high affinity to the receptor that it was used as a label for the first purification of that class of ion channels and gave its name to it. At nanomolar concentrations, ryanodine locks the receptor in a half-open state, whereas it fully closes them at micromolar concentration. The effect of the nanomolar-level binding is that ryanodine causes release of calcium from calcium stores in the sarcoplasmic reticulum leading to massive muscular contractions.

Uses

Pending target crops for these insecticides are cotton, corn, grape, pome fruits, potatoes, and strawberry.
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- CAP is being developed by DuPont Crop Protection and Syngenta Crop Protection,
- FLU is being codeveloped by Nihon Nohyaku and Bayer CropScience.

AIM OF THIS WORK

To develop and validate an analytical method for the simultaneous determination of the two insecticides on fruits and vegetables by liquid chromatography electrospray tandem mass spectrometry (triple quad)
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**LC/MSMS ESI**

*electrospray ionization*

Simultaneous Positive and Negative electrospray ionization for CAP and FLU quantitation

*ion-switching ESI*

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**LC/MS ESI(+)**: Chloroantraniliprole (CAP)

Full scan spectrum for CAP showed the characteristic isotopic pattern \([A+2^{37}\text{Cl}]\) of compounds with 2 chlorines and 1 bromine.

Very intense \([\text{M+H}]^+\) and \([\text{M+Na}]^+\) adduct ions.

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**LC/MSESI (-) : Flubendiamide (FLU)**

*Full scan* spectrum for FLU showed the following adducts:

- \([\text{M-H}]^- \text{ (m/z 681.3)}\)
- \([\text{M+35Cl}]^- \text{ (m/z 717.3)}\)
- \([\text{M+37Cl}]^- \text{ (m/z 719.4)}\)

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**Q-TOF experiments for Chlorantraniliprole**

\[ \text{C}_{16}\text{H}_{14}\text{BrCl}_2\text{N}_5\text{O}_2 \]

483.15

480.970792
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Collision induced fragmentation experiment

Proposed chlorantraniliprole fragmentation pathway
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Q-TOF experiments for Flubendiamide

\[
\text{C}_{23}\text{H}_{22}\text{F}_{4}\text{N}_{2}\text{O}_{4}\text{S} \\
682.39 \\
682.023329
\]

Collision induced fragmentation experiment
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ESI-MS/MS (+/-): MRM

<table>
<thead>
<tr>
<th>compound</th>
<th>MW</th>
<th>Precursor ion mass (m/z)</th>
<th>First transition mass (m/z)</th>
<th>CE (V)</th>
<th>Second transition mass (m/z)</th>
<th>CE (V)</th>
<th>Third transition mass (m/z)</th>
<th>CE (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAP</td>
<td>481</td>
<td>484 [M+2]+</td>
<td>484.3 → 285</td>
<td>-14</td>
<td>484.3 → 453</td>
<td>-16</td>
<td>484.3 → 287</td>
<td>-14</td>
</tr>
<tr>
<td>FLU</td>
<td>682</td>
<td>681 [M-H]-</td>
<td>681.4 → 253</td>
<td>+28</td>
<td>681.4 → 274</td>
<td>+16</td>
<td>681.4 → 271</td>
<td>+16</td>
</tr>
<tr>
<td>IS</td>
<td>444</td>
<td>445 [M+H]+</td>
<td>445.5 → 169</td>
<td>+20</td>
<td>445.5 → 171</td>
<td>-28</td>
<td>445.5 → 258</td>
<td>-14</td>
</tr>
</tbody>
</table>

N,N’-bis-(5-chloro-2-methoxyphenyl)phthalamide was used as internal standard

FRUITS & VEGETABLES

- Apples
- Grapes
- Pears
- Eggplants
- Peppers
- Tomatoes
Extraction procedure:
- Samples were analyzed, unwashed, and in a raw state.
- Samples were extracted with acetonitrile after salting out with NaCl and MgSO₄.
- 1 mL of the mixture was evaporated.
- Residue was taken up with the mobile phase and submitted to chromatographic analysis in the MRM mode.

Chromatographic separation:
- Performed on a Zorbax Column Eclipse XDB C8 (4.6 mm × 150 mm i.d., 3 μm).
- Mobile phase consisted of (A) acetonitrile 80% and (B) water 20% containing 0.1% formic acid.
- Flow rate of 0.4 mL/min, and the injection volume 10 μL.
- ESI was operated in the positive and negative ion mode simultaneously.
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**ESI-MS/MS (+/-): chromatogram**

- Highly selective method
- No interfering peaks

Chromatogram of (1) chlorantraniliprole, (2) flubendiamide, (3) internal standard. A) tomatoes blank; B) matrix fortified at 1.6 µg/kg

**Limit of Detection and Quantification (µg/kg) of Chlorantraniliprole and Flubendiamide**

<table>
<thead>
<tr>
<th>Matrix</th>
<th>LOD</th>
<th>LOQ</th>
<th>slope</th>
<th>slope ratio</th>
<th>LOD</th>
<th>LOQ</th>
<th>slope</th>
<th>slope ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>solvent*</td>
<td>0.1</td>
<td>0.5</td>
<td>0.202</td>
<td></td>
<td>0.1</td>
<td>0.5</td>
<td>0.200</td>
<td></td>
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<tr>
<td>eggplant</td>
<td>0.8</td>
<td>1.6</td>
<td>0.011</td>
<td>0.05</td>
<td>0.4</td>
<td>0.8</td>
<td>0.334</td>
<td>1.67</td>
</tr>
<tr>
<td>tomato</td>
<td>0.8</td>
<td>1.8</td>
<td>0.022</td>
<td>0.11</td>
<td>0.4</td>
<td>0.8</td>
<td>0.318</td>
<td>1.59</td>
</tr>
<tr>
<td>pepper</td>
<td>0.8</td>
<td>1.6</td>
<td>0.022</td>
<td>0.11</td>
<td>0.4</td>
<td>0.8</td>
<td>0.045</td>
<td>0.23</td>
</tr>
<tr>
<td>apple</td>
<td>0.8</td>
<td>1.6</td>
<td>0.012</td>
<td>0.06</td>
<td>0.4</td>
<td>0.8</td>
<td>0.217</td>
<td>1.09</td>
</tr>
<tr>
<td>pear</td>
<td>0.8</td>
<td>1.6</td>
<td>0.021</td>
<td>0.10</td>
<td>0.4</td>
<td>0.8</td>
<td>0.256</td>
<td>1.29</td>
</tr>
<tr>
<td>grape</td>
<td>0.8</td>
<td>1.6</td>
<td>0.016</td>
<td>0.08</td>
<td>0.4</td>
<td>0.8</td>
<td>0.041</td>
<td>0.21</td>
</tr>
</tbody>
</table>

*LOD and LOQ of solvent are expressed as µg/L.
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Matrix Effect & Recovery Experiments

For CAP we observed a reduction of the analytical response for all fruits and vegetables.

For FLU we observed an enhanced response for pears, tomatoes, apples,

Recovery ranged from 82 to 117% with a standard deviation between 1 and 14%.

LC/MSMS: Linearity and efficiency experiments

Calibration range was linear:
• from 2 to 1000 mg/L for CAP with $r > 0.992$
• from 1 to 1000 mg/L for FLU with $r > 0.996$

For the precision experiments the highest and lowest variation coefficients were 7.5 and 2.9%, respectively, under the conditions of repeatability, and 1.1 and 0.6%, respectively, for intraday comparisons.
CONCLUSIONS

The proposed LC-MS/MS analytical method for the determination of diamides in different food matrices was

- sensitive
- fast
- precise
- accurate
- robust

and can be used to monitor chlorantraniliprole and flubendiamide residues in fruits and vegetables.

Acknowledgements

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Thank you for your attention!