Identification of Imidacloprid Metabolites in Onions Using High Resolution Mass Spectrometry and Accurate Mass Tools

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Objectives

• Objectives – Dissipation Study
  – Study the chemical fate and distribution of an insecticide, imidacloprid, in plants and soil in a greenhouse environment.
  – Use of LC/Q-TOF-MS to measure the various degradation products and metabolites of imidacloprid in plant, soil, and leachate water.

• Why Imidacloprid?
  – Imidacloprid is a widely used insecticide to control aphids and flies in many vegetable and greenhouse crops.
  – Known to be toxic to honey bees and other beneficial insects.
  – Given its chlorinated structure it is amenable to LC/Q-TOF-MS analysis for parent and metabolite studies.
Introduction

• What was done — Onions and Lettuce
  – Onions (allium cepa) and Lettuce (Paris Island romaine) were chosen as two representative crops both a root and leaf crop that may receive imidacloprid as a systemic application (i.e. application to soil) — Valuable comparison of vegetable results.

• Pesticide application — Imidacloprid
  – Our study involved a single application of imidacloprid to soil and its subsequent fate over a two-month period of greenhouse life.
Fate Pathways in Plants

1. Systemic application to soil

2. Sorption and Degradation in Soil

3. Leaching

4. Rhizosphere Degradation and Uptake

5. Translocation into Plant
Set-up Procedure

- Purchased seed for both lettuce and onion from a local greenhouse.
- Obtained potting soil at CU Greenhouse of known composition, which was free of weed seeds.
- Wetted soil and mixed with hands to form a soft, lofty consistency.
- Planted 3 seeds per 2 inch square pot (March).
- Placed in a well sunlit area of greenhouse with temps that varied from 68 F at night to 90 F during the day.
- Watered daily or as needed for 4 weeks until plants reached a height of approximately 6 inches.
- Re-potted into 4 inch pots for the remainder of the study (April).
- Application of pesticides began after 55 days from planting (end of April). Sampling began at 28 days after application (May).
From seed to plant...
Almost there…
Pesticide Application:

- Concentration: 2.5 µg/mL
- 200 mL of water (with pesticide) applied to each pot/plant.
- Triplicate samples.

Sampling Schedule:

- Water Samples (leachate) collected 3 times (1 per week).
- Soil samples collected after 1 month, 10 and 15 days later (total of 36 samples).
- Plant samples collected after 1 month, 10 and 15 days later (total of 36 samples).
Water Analysis

Filter sample if needed

Spike water with deuterated imidacloprid 100 µL

Analyze by LC/Q-TOF-MS
Soil and Plant Extractions

1 gram of soil
3 grams of plant (onion/lettuce)

Add 6 mL of MeOH/H₂O (80%/20%)

Rotate sample for 30 minutes

Spike with deuterated Std and vortex

Centrifuge sample at 3500 rpm for 15 min.

Decant MeOH/H₂O extract

Evaporate MeOH to 0.5 mL. Filter

Analyze by LC/Q-TOF-MS
Plant Extraction
Accurate Mass, Time-of-Flight

- Full-spectrum data available, even for metabolites or degradation products.
- Retrospective analyses are possible (use of databases).
- Valuable information from isotopic pattern.
- Formula generation is useful for elemental elucidation.

LC/Q-TOF-MS
Agilent Model 6540
Specifications:
- 4 GHz detector rate
- 40,000 resolving power
- < 2 ppm accuracy
Accurate Mass Tools

- Database search of known metabolites from literature.
- Chlorine filter for new metabolites. Structure elucidation.
- Mass Profiler for differences between non-treated soil/plants (controls) and treated soil/plants.
- Diagnostic ion searches and MS-MS experiments.
Typical Chromatograms of Plants
Database Search for known Metabolites

Reported plant metabolites of Imidacloprid

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Elemental Composition</th>
<th>Ret. Time (min.)</th>
<th>Exact Mass [M+H]^+</th>
<th>Chemical Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolite-1</td>
<td>C₉H₆ClN₄</td>
<td>6.6</td>
<td>211.0745</td>
<td><img src="Imidacloprid_Guanidine_Analogue.png" alt="Chemical Structure" /></td>
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<td>Guanidine Analogue</td>
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<td>4-Hydroxyimidacloprid</td>
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<tr>
<td>Urea Analogue</td>
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</tbody>
</table>

Major Imidacloprid Metabolite from Database

Imidacloprid Parent from Database

m/z 211
m/z 256

C₉H₁₂ClN₄⁺ Exact Mass: 256.0596
All Ion MS/MS vs High Fragmentor of Diagnostic ion m/z 126.0105

Counts vs. Acquisition Time (min)

+ESI EIC(126.0105) Scan Frag=220.0V CID@30.0 Onion Plant 512 all ions at 30V.d

+ESI EIC(126.0105) Scan Frag=190.0V Onion Plant 5-12-12.d
All Ion MS/MS of Onion with Imadlcloprid and Blank Onion of Diagnostic Extracted Ion $m/z$ 126.0105

Onion with Imadlcloprid day 12 CID 30eV

Onion no Imadlcloprid applied
MS-MS of Guanidine Metabolite

C$_9$H$_{12}$ClN$_4^+$
Exact Mass: 211.0745

C$_6$H$_5$ClN$_2^+$
Exact Mass: 126.0105

C$_3$H$_6$N$_3^+$
Exact Mass: 84.0556

C$_9$H$_{11}$N$_4^+$
Exact Mass: 175.0978

Graphical representation of the mass spectrometry data and metabolite structures.
Distribution of Imidacloprid

m/z 211, imidacloprid metabolite

m/z 256, imidacloprid

Onion Plant

m/z 211, imidacloprid metabolite

m/z 256, imidacloprid

Onion Soil
Chlorine Filter Approach

Molecular Feature Extraction (MFE)

C\textsubscript{9}H\textsubscript{10}ClN\textsubscript{5}O\textsubscript{2} \rightarrow C\textsubscript{14}H\textsubscript{16}ClN\textsubscript{5}O\textsubscript{4}
MS-MS of m/z 354 Metabolite

$C_9H_{10}ClN_5O_2$ ? $C_{14}H_{16}ClN_5O_4$

Glutamic acid

$C_6H_9NO_4$
Exact Mass: 147.0532

$C_{14}H_{17}ClN_4O_4^+$
Exact Mass: 354.0964

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6/11/2013
MS-MS of m/z 226 Metabolite

+ESI Scan (5.974-6.046 min, 7 Scans) Frag=190.0V

C₆H₅ClN⁺
Exact Mass: 126.0105

C₉H₁₀ClN₄⁺
Exact Mass: 209.0589

C₉H₉N₄⁺
Exact Mass: 173.0816

C₉H₁₃ClN₅⁺
Exact Mass: 226.0854

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

Imidacloprid-Amine Metabolite
Mass Profiler Approach

Comparison:
- 3 “blanks”: non-treated onion.
- 3 samples: treated onion with imidacloprid.
## Additional Metabolites

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<th>Ret. Time (min.)</th>
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<td>Metabolite-4</td>
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<td>Metabolite-6</td>
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<td><img src="image2" alt="Chemical Structure" /></td>
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<td>Olefin of Guanidine Analogue</td>
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<td>Metabolite-7</td>
<td>C_{9}H_{12}ClN_{5}</td>
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<td>Imidacloprid-Amine Analogue</td>
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<td>Metabolite-8</td>
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<td>Olefin-imidacloprid-Amine Analogue</td>
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<tr>
<td>Metabolite-10</td>
<td>C_{12}H_{16}ClN_{5}O_{4}</td>
<td>10.7</td>
<td>354.0964</td>
<td><img src="image5" alt="Chemical Structure" /></td>
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<tr>
<td>Glutamic acid Conjugate of Imidacloprid olefin Guanidine</td>
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</tr>
</tbody>
</table>
Metabolite distribution

Pie diagram of plant metabolites in onion and lettuce as a percentage of the total.
Distribution of Imidacloprid

Water → Less than 1% parent compound, no metabolite present.

Soil → Mostly parent compound, small percentage of major metabolite (m/z 211).

Plant → Mostly metabolite, small percentage of parent.
Mass Balance

Mass Balance of Compound Added in Total Watering (µmoles of compound)
500 µg ~ 2 µmoles

2. Total Mass in Soil for parent and metabolites (µmoles of compounds)

3. Total Mass in Plant for parent and metabolites (µmoles of compounds)

3. Total Mass in leachate water for parent and metabolites (µmoles of compounds)
Mass Balance

![Graph showing mass balance of imidacloprid and metabolite m/z 211 in water, plant, and soil relative to Onion-1 to Onion-9.](image)
Conclusions

LC/Q-TOF-MS was applied successfully to the identification of new metabolites of imidacloprid in onions and lettuce.

Minor leaching of parent compound or metabolites was observed during the dissipation experiment.

Mass balance studies show that metabolites are found mainly in the plant.

Parent compound (imidacloprid) was mainly found in the soil.

Identification of unknown compounds using accurate mass data can be endless…
Acknowledgments

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