

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

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**Agilent Technologies** 

# **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 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.



Of

Imidacloprid



 $C_{9}H_{11}CIN_{5}O_{2}^{+}$ Exact Mass: 256.0596

# 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.



# **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...



ASMS 61st Conference on Mass Spectrometry & Allied Topics 6/11/2013











# **Pesticide Application**

#### **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







Analyze by LC/Q-TOF-MS



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## **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 <u>Specifications</u>: 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**



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# All Ion MS/MS vs High Fragmentor of Diagnostic ion m/z 126.0105



#### All Ion MS/MS of Onion with Imadicloprid and Blank Onion of Diagnostic Extracted Ion *m/z* 126.0105



## **MS-MS of Guanidine Metabolite**



## **Distribution of Imidacloprid**



#### **Onion Soil**



## **Chlorine Filter Approach**

Molecular Feature Extraction (MFE)

Q102 209: 30.696: +ESI EIC(435.3677, 436.3715, 457.3495, 458.3531 ...) Scan Frag=190.0V Onion Plant 5-12-12.d

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0	pd ⊽₽	<mark>RT</mark> マ ₱	Label	Υ.	Show/Hide	+ Formula	⊽∀₽	m/z ⊽+¤	Height ⊽ +Þ	Mass (Tgt) ⊽+¤	Diff (Tgt, ppm) 🗸	'₽ S
<b>+</b>	1387	14.094	Cpd 1387: C9 H	110 CI N5 O	2	C9 H10 CI	N5 O2	256.0578	601240			
÷	336	6.544	Cpd 336: C	:9 H11 CI N	4	C9 H11	CI N4	211.0738	3575138			
÷	314	6.011	Cpd 314:	C9 H9 CI N	4	C9 H9	CI N4	209.0582	145349			
÷	337	6.544	Cpd 337	: C6 H4 CI	V	C6 H	4 CI N	126.0104	393210			
÷	773	10.964	Cpd 773: C14 H	116 CI N5 O	4 👿	C14 H16 CI I	N5 O4	354.0955	254645			
	1413	14.257	Cpd	1413: 14.25	7			297.0552	99487			
÷	625	10.192	Cpd 625: C12 H	114 CI N5 O	2	C12 H14 CI I	N5 O2	296.0891	101477			
÷	785	11.045	Cpd 785: C12 H	14 CI N5 O	2	C12 H14 CI I	N5 O2	296.0903	87287			
	1135	12.814	Cpd	1135: 12.81	4			257.0241	390498			
	1700	17.054	Cpd	1700: 17.05	4			258.0189	218712			
	1	2.019		Cpd 1: 2.01	9 🔽			146.1649	225109			
	2	2.022		Cpd 2: 2.02	2 📝			129.1386	371703			
ind	3	2.023		Cpd 3: 2.02	3			112.1123	329900			
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## **MS-MS of m/z 226 Metabolite**



## **Mass Profiler Approach**

#### Comparison:

- 3 "blanks": non-treated onion.
- 3 samples: treated onion with imidacloprid.



		Feature summary										Exp	periment(3)	
		ID	RT	SD	Mass	SD	Abundance	RSD	Freq.	Mark	RT	Mass	Abundance	RSD
	19	19	17. <mark>55</mark> 7	0.001	298.0186	0.0003	22940380	1.10	3				0	
Þ	20	20	6.378	0.007	210.0667	0.0001	22010370	1.10	3		6.378	210.0667	44020740	0.04
	21	21	18.157	0.002	675.3111	0.0004	21588940	0.36	6		18.156	675.3113	28663160	0.03
	22	22	18.220	0.002	666.2873	0.0007	21056570	0.20	6		18.219	666.2878	24956140	0.01
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## **Additional Metabolites**

Metabolites	Elemental Composition	Ret. Time (min.)	Exact Mass [M+H] <sup>+</sup>	Chemical Structure
Metabolite-4 Methylated Imidacloprid	C <sub>10</sub> H <sub>14</sub> ClN <sub>5</sub>	8.3	240.1010	CH <sub>3</sub>   HN/NH
Metabolite-6 Olefin of Guanidine Analogue	C9H9ClN₄	6.4	209.0589	
Metabolite-7 Imidacloprid-Amine Analogue	C <sub>9</sub> H <sub>12</sub> ClN <sub>5</sub>	6.0	226.0854	CI N NH2
Metabolite-8 Olefin-imidacloprid-Amine Analogue	C <sub>9</sub> H <sub>10</sub> ClN <sub>5</sub>	4.8	224.0697	CI N NH2
Metabolite-10 Glutamic acid Conjugate of Imidacloprid olefin Guanidine	C <sub>14</sub> H <sub>16</sub> ClN <sub>5</sub> O <sub>4</sub>	10.7	354.0964	

## **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  $\rightarrow$  Mostly parent compound, small percentage of major metabolite (m/z 211).
- Plant  $\rightarrow$  Mostly metabolite, small percentage of parent.

## **Mass Balance**

Mass Balance of Compound Added in Total Watering (µmoles of compound)

500 ua ~ 2 umoles -

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**



# 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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