

Analysis of Neonicotinoid Pesticides in Bee Pollen by LC/MS/MS



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What is Bee Pollen?

- Flower pollen collected by bees combined with enzymes and nectar
- Pollen collected at flowers and packed into sacs on the bees back legs forming little pellets
- Composed of protein (20%), lipids (6%), carotenoids, flavonoids, and phenolics
 - Composition dependant on plant bee pollen collected from

Bee Collecting Pollen



Bee Pollen Collection



Pollen Samples

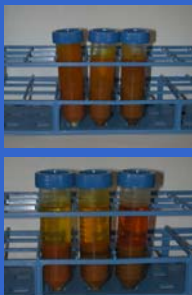


Why Study Neonicotinoids in Pollen?

- Honeybees are important pollinators of many crops
- Colony Collapse Disorder (CCD) is a new threat to honeybee populations
- The cause of CCD is unknown but some people suspect pesticides play a role
- Imidacloprid, a neonicotinoid pesticide, has been shown to be harmful to honeybees at low concentrations (crucial level in the 1-10 PPB range; Bonmatin *et al.* 2005)

Extract with a Modified QuEChERS Procedure

- 10 g of pollen plus:
 - 6 g magnesium sulfate
 - 1.5 g sodium acetate
 - 10 mL water
 - 20 ml acetonitrile
 - C-13 Alachor I.S.
- Shake / Vortex
- Sonicate
- Centrifuge



Extraction (cont.)

- Decant liquid
- Combine with
 - 3 g magnesium sulfate
 - 1 g PSA
 - 3 mL toluene
- Shake
- Centrifuge
- Decant liquid
- Concentrate to <1 mL with nitrogen for LC/MS analysis



LC Conditions

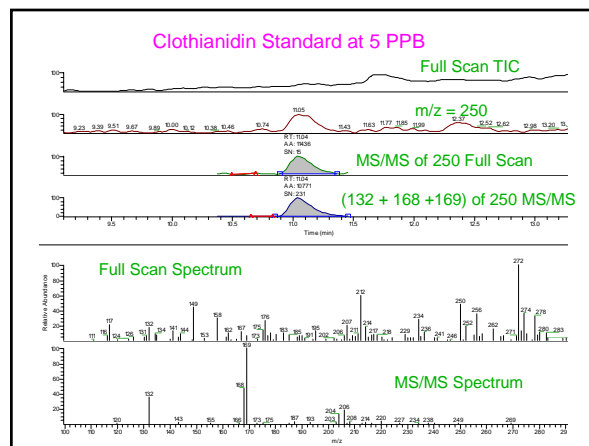
- Column: Zorbax SB-C18, 2.1 x 150 mm, 5 micron
- 10 Microliter injection
- 0.25 mL/min
- Mobile phase solvents
 - A: 0.02% Formic acid in water
 - B: 0.02% Formic acid in methanol
- Mobile phase gradient (time-%A)
 - 0-75, 2-75, 12-30, 14.5-30, 16.5-15, 18-15, 22-0, 28-0, 29-75, 33-75, post run 3 min

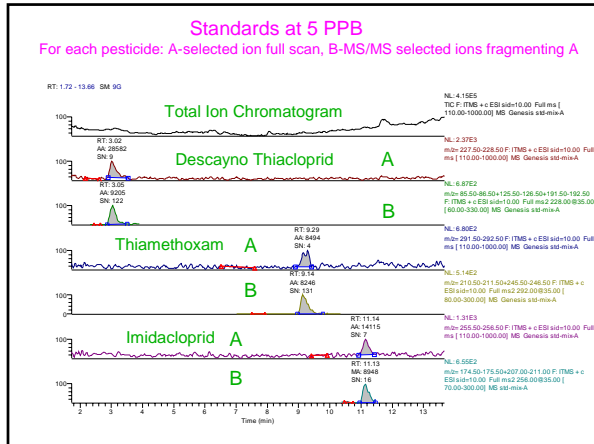
Mass Spectrometric Conditions

- Thermo – LTQ (linear ion trap)
- Positive ion electrospray at 3.5 Kev
- Multiple segments/scans
 - First scan of each segment is full scan (100-1000 amu)
 - Subsequent scans monitor MS/MS transitions for targeted compounds
 - Quantification on selected ions of MS/MS data

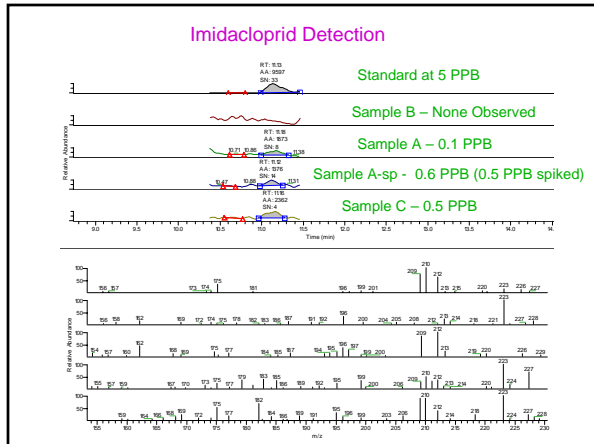
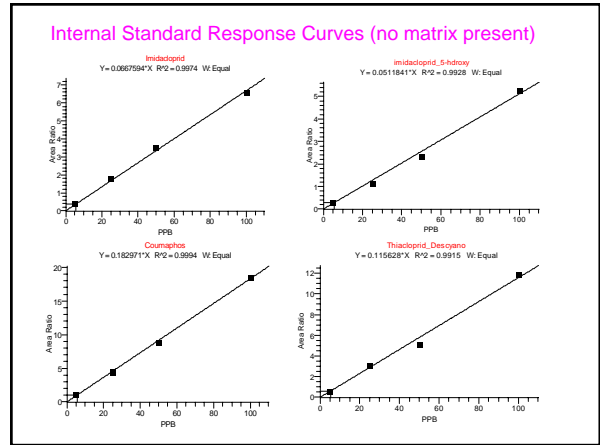
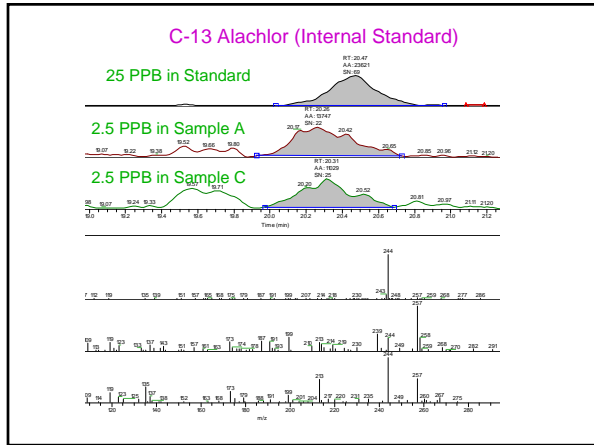
Compounds Monitored

- Neonicotinoids
 - (# metabolites monitored in addition to parent)
 - Imidacloprid (5 metabolites)
 - Acetamaprid (3 metabolites)
 - Dinotofuran (2 metabolites)
 - Thiacloprid (1 metabolite)
 - Thiamethoxam (4 metabolites)
 - Clothianadin (thiamethoxam metabolite CGA322704)
- Pesticides used to treat beehives
 - Coumaphos
 - Fluvalinate
 - Amitraz





Linearity and Limits of Detection

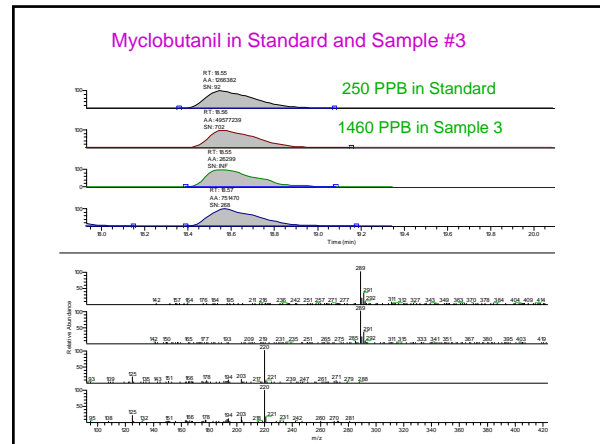


Orange – not found spiked or unspiked
 Yellow – found in spiked sample not in unspiked
 Blue – found in spiked and unspiked

Internal Standard	Y	R ²	W	Y	R ²	W	Y	R ²	W	Y	R ²	W
Thiamethoxam Metabolite CGA35198	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Thiamethoxam Metabolite CGA35199	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Thiamethoxam Metabolite CGA35200	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Thiamethoxam Metabolite CGA35201	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Thiamethoxam Metabolite CGA35202	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Thiamethoxam Metabolite CGA35203	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Thiamethoxam Metabolite CGA35204	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
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Thiamethoxam Metabolite CGA35286	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Thiamethoxam Metabolite CGA35287	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Thiamethoxam Metabolite CGA35288	NF	NF	N									

Other Pesticides Detectable?

- QuEChERS extractions can typically be used for LC/MS or GC/MS
- GC/MS test run had too many interfering compounds to reliably determine pesticides
- Other LC/MS detectable pesticides can be observed, some at high concentrations
 - Observed compounds include: carbaryl, atrazine and myclobutanil



Conclusions

- Method will be useful for parent neonicotinoids at the required detection limits
- Alternate extraction technique required for many neonicotinoid metabolites
- Other pesticides can be detected in pollen by these procedures

We would like to thank the
Connecticut Department of Health for
purchasing the LTQ-MS