Extraction and Analysis of Neonicotinoid Pesticides using QuEChERS Approach

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Abstract

Pesticide compounds of the neonicotinoid class have been used extensively in crop protection. The advent of the die-off of honey bees due to colony collapse disorder (CCD) has spawned investigation into pesticide exposure as the cause. Some studies have indicated that exposure of honey bees to neonicotinoids has detrimental effects on their behavior. Recently the European Union adopted a regulation restricting the use of three of these pesticides: clothianidin, thiamethoxam, and imidacloprid.

Here, a Quick Easy Cheap Effective Rugged and Safe (QuEChERS) approach was used to develop a method for extraction of neonicotinoid pesticides from matrices with which honey bees come most in contact - flower blossoms. Seven neonicotinoids were extracted from cherry, apple, and dandelion blossoms and analyzed via LC/MS/MS. A number of sorbent mixtures were tested for the cleanup method, and a mixture of PSA and C18 sorbents provided the best recoveries and matrix removal. At a spiking level of 50 ng/g, recoveries of >89% were achieved for all tested neonicotinoids. Good reproducibility was achieved with the method, as indicated by RSD values of less than 7% for spiked replicates.

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Introduction

- Recent studies have indicated neonicotinoid pesticides as having detrimental effects on the brain cells of honey bees (1).
  - Seeds that are treated with these pesticides can generate contaminated dust when using equipment that incorporates air for planting (2).
  - Some crops directly pollinated by honeybees such as cantaloupe and cucumber are treated with these pesticides (3).

Experimental

- Analyzed for seven neonicotinoid pesticides (Figure 1), spiking level of 50 ng/g.

The flower blossom species chosen were several that would be pollinated by honeybees: Dandelion, Sweet Cherry, Crab apple

- Used QuEChERS approach for extraction and cleanup (Table 1).
- Evaluated several different cleanup sorbents initially using dandelion extract:
  1. PSA/C18/ENVI-Carb™/$MgSO_4$ 50/50/50/150 mg
  2. PSA/$MgSO_4$ 50/150 mg
  3. PSA/C18/$MgSO_4$ 50/150 mg
  4. Z-Sep, 75 mg
  5. Z-Sep+, 75 mg
  6. Z-Sep/C18, 20/50 mg
- Analysis was done by LC/MS/MS using conditions in Tables 2 and 3. Matrix matched calibrations were used for quantitation, separate for each blossom type and cleanup.

Figure 1. Neonicotinoid Pesticides

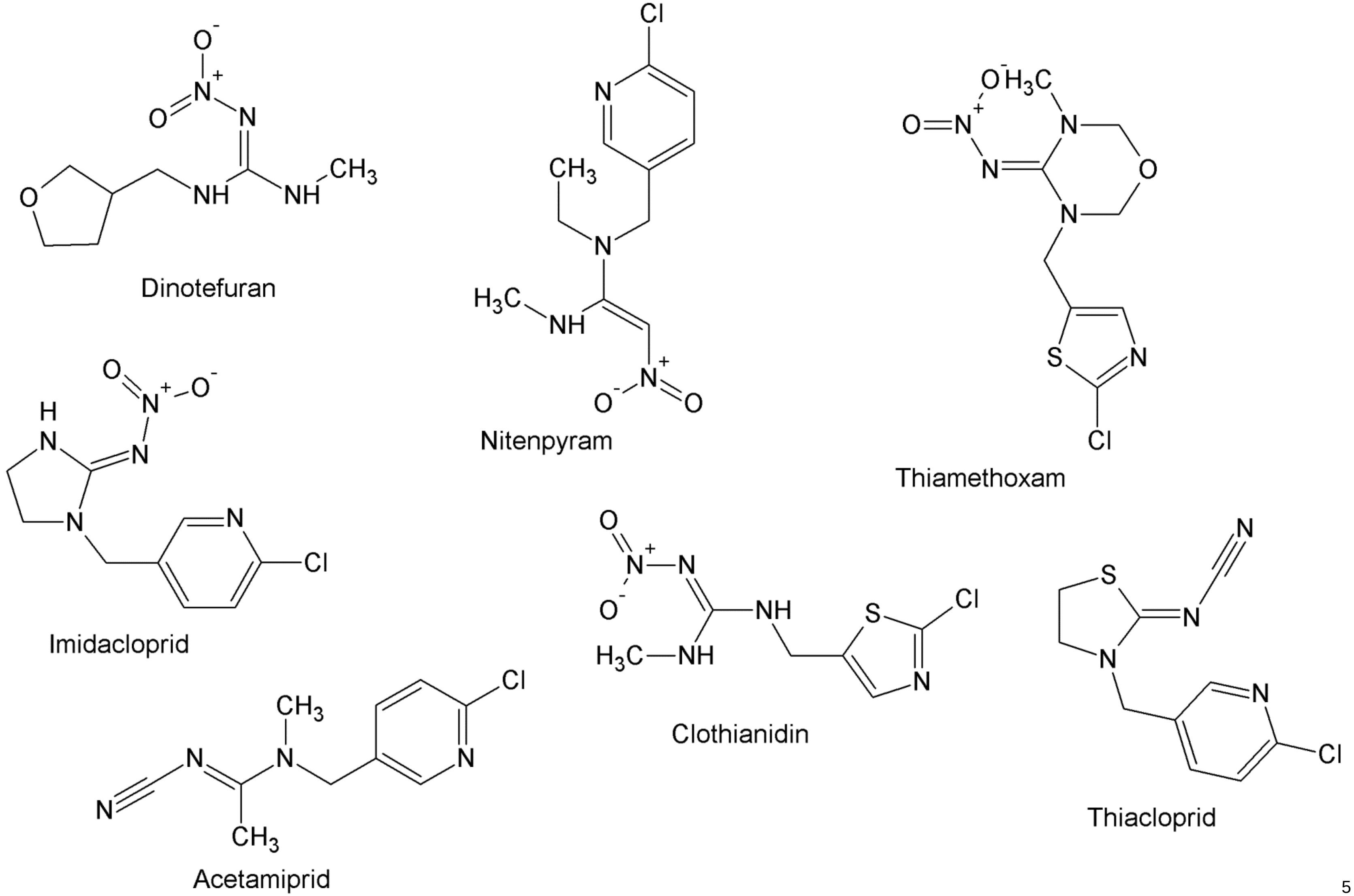

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Table 1. QuEChERS Extraction and Cleanup Method for Flower Blossoms

Place 3 g pulverized flower blossoms into a 50 mL centrifuge tube. Add 10 mL dei. water and homogenize sample. Add 15 mL ACN.

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Add Supel™ Que Acetate tube, mix for 1 min.

↓

Centrifuge samples for 5 min at 3400 rpm.

↓

Separate ACN supernatant.

↓

Add 1 mL supernatant to 2 mL tube containing cleanup sorbent.

↓

Shake for 1 min and centrifuge for 3 min at 3500 rpm.

↓

Draw off 700 µL of supernatant, and evaporate to dryness at 50 °C under nitrogen..

↓

Reconstitute in 200 µL of 50:50 0.1% formic acid:0.1% formic acid in MeOH. → Analyze by LC/MS/MS.

Table 2. LC/MS/MS Analysis Conditions

instrument:	Agilent® 1100 / AB-Sciex 3200 Q-Trap
column:	Ascentis® Express C18, 10 cm x 3.0 mm, 2.7 µm
mobile phase:	(A) 0.1% formic acid in water; (B) 0.1% formic acid in methanol
gradient:	30% B from 0 to 5 min; to 100% B in 0.2 min; held at 100% B for 5.3 min; to 30% B in 0.5 min; held at 30% B for 5 min
flow rate:	0.5 mL/min
temp.:	ambient
det.:	MS, ESI(+), MRM, see Table 3
injection:	3 µL

Table 3. MRMs Used

Name	MRM
Acetamiprid	223.2/126.0
Clothianidin	250.0/132.0
Dinotefuran	203.2/129.2
Imidacloprid	256.0/175.2
Nitenpyram	271.2/225.0
Thiacloprid	253.0/125.8
Thiamethoxam	292.1/211.0

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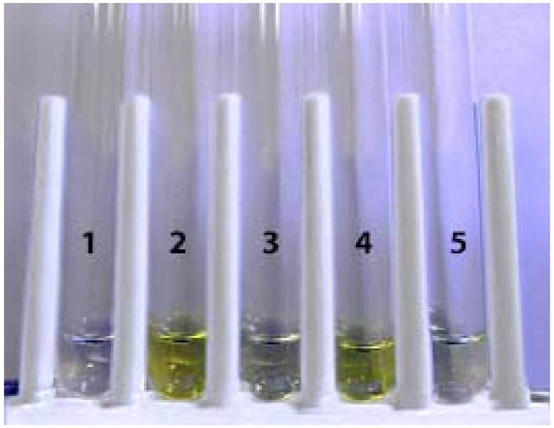
Results

Initial evaluation of cleanup sorbents

An initial screening of several cleanup sorbents was done using single samples of 50 ng/g spiked dandelion blossom extract, quantified against a single point standard in solvent. After cleanup, the color remaining in the extracts was as indicated in Figure 2. PSA/C18/ENVI-Carb resulted in the cleanest extract. The darkest color remained in the PSA and Z-Sep cleaned extracts.

Figure 2. Color Remaining in Dandelion Extract after Cleanup



1. PSA/C18/ENVI-Carb
2. PSA
3. PSA/C18
4. Z-Sep
5. Z-Sep+

Intensity of color in extracts: 1<5<3<2=4

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Table 4. Results of Cleanup Evaluation

Sorbents	Recovery	Result
PSA/C18/ENVI-Carb	0-10%	Clear extract, poor recovery
PSA	0-10%	Not adequate cleanup
PSA/C18	50-120%	Best recoveries, not the cleanest extract
Z-Sep	40-80%	Not adequate cleanup
Z-Sep+	0-10%	No to poor recovery

Determination of final cleanup method, and pesticide recoveries

C18 was added to the Z-Sep to determine if recoveries would be similar to PSA/C18, but with a cleaner extract than Z-Sep alone. Comparisons were made to an uncleaned extract (Table 5).

Table 5. Pesticide Recoveries from Spiked Replicates of Dandelion

n=3	Z-Sep/C18	PSA/C18	No Clean*
Acetamiprid	94 (13)	99 (1)	102
Clothianidin	102 (8)	99 (6)	87
Dinotefuran	104 (7)	101 (2)	75
Imidacloprid	96 (12)	93 (4)	120
Nitenpyram	77 (5)	105 (1)	36
Thiacloprid	98 (13)	94 (2)	107
Thiamethoxam	106 (10)	98 (1)	81

*quant. against single point standard in solvent

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- Recoveries were comparable using Z-Sep/C18 and PSA/C18.
- %RSDs were higher using Z-Sep/C18 cleanup, indicating an incompatibility between the target compounds and the Z-Sep.
- Sorbents removed ion-supression causing matrix resulting in better recoveries.
- PSA/C18 was chosen for the final cleanup.
- The method was then used to extract cherry and crab apple blossoms spiked at 50 ng/g (Table 6).

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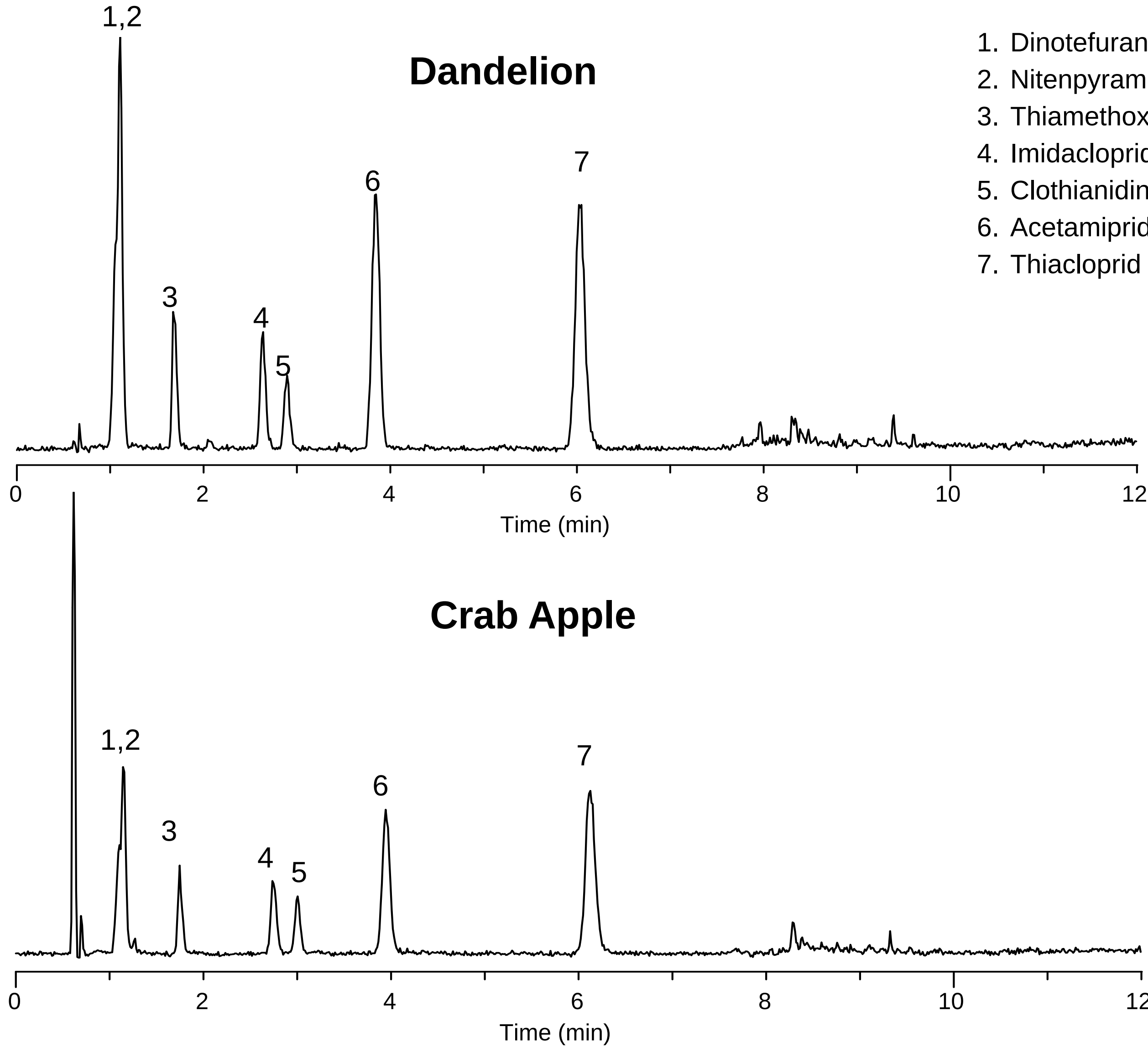
Table 6. Pesticides Recoveries and Reproducibility from Dandelion, Cherry, and Crab Apple Blossoms

n=3	Dandelion	Cherry	Crab apple
Acetamiprid	99 (1)	96 (2)	105 (4)
Clothianidin	99 (6)	106 (2)	106 (7)
Dinotefuran	101 (2)	103 (4)	89 (4)
Imadicloprid	93 (4)	97 (4)	90 (3)
Nitenpyram	105 (1)	107 (2)	97 (6)
Thiacloprid	94 (2)	99 (1)	104 (2)
Thiamethoxam	98 (1)	99 (3)	93 (8)

- Good recoveries and reproducibility obtained for all three blossom types.
- PSA/C18 cleanup reduced color of both cherry and crab apple blossom extracts (not shown).
- Cherry extract had the most LC/MS/MS background; but it did not interfere with quantitation (Figure 3).

Figure 3. TICs from LC/MS/MS Analysis of Flower Blossom Extracts after PSA/C18 Cleanup



1. Dinotefuran
2. Nitenpyram
3. Thiamethoxam
4. Imidacloprid
5. Clothianidin
6. Acetamiprid
7. Thiacloprid

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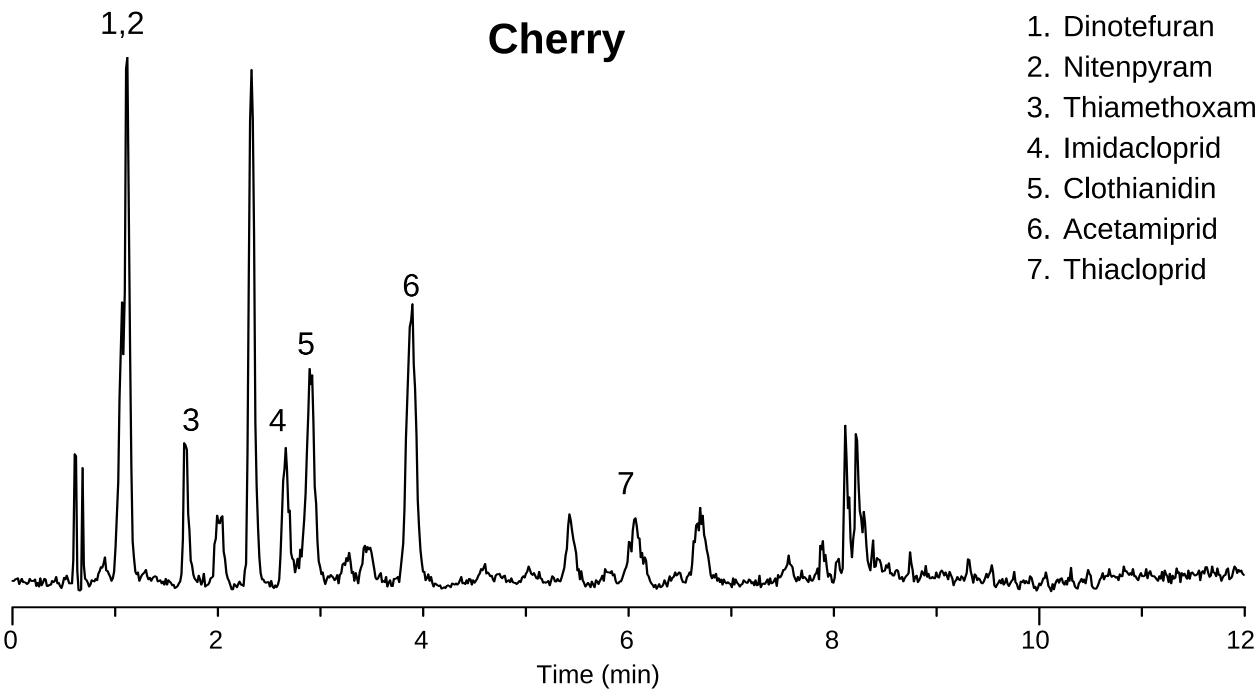
Figure 3. TICs from LC/MS/MS Analysis of Flower Blossom Extracts after PSA/C18 Cleanup (contd.)



1. Dinotefuran
2. Nitenpyram
3. Thiamethoxam
4. Imidacloprid
5. Clothianidin
6. Acetamiprid
7. Thiacloprid

Conclusions

- A QuEChERS method for the analysis of neonicotinoid pesticides from flower blossoms was developed.
- For cleanup, addition of ENVI-Carb to PSA/C18 yielded the cleanest extracts but poor pesticide recoveries.
- PSA/C18 cleanup yielded the best recoveries for the neonicotinoid pesticides, and extract cleanliness was adequate for LC/MS/MS analysis.
- The zirconia-based Z-Sep and Z-Sep+ sorbents were not compatible with these pesticides, as evidenced by the poor recoveries when using these for cleanup.

References

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