APPLICATION NOTE

Liquid Chromatography/
Mass Spectrometry

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Detection of Nicotinic Acetylcholine Receptor Competitive Modulator Pesticides in Honey by UPLC/TOF MS Analysis

Introduction

Neonicotinoid pesticides (Fig. 1) were introduced in the 1990s and due to their efficacy have become the most widely used insecticide class worldwide[1]. They act by binding to nicotinic acetylcholine receptors (nAChRs) in the insect nervous system and induce paralysis[2]. Sulfoximines, exemplified by Sulfoxaflor (Fig. 1), are a more recently introduced insecticide class, which also bind to the insect nAChR, but have a sufficiently different mode of action that they can be effective against neonicotinoid resistant insects[3]. The widespread use of neonicotinoids has been investigated as a possible contributing factor to honey bee colony collapse disorder[4]. Given the scale of nAChR agonist use, it is important to develop methods of detection in agricultural products. This report describes the application of UPLC®/TOF MS analysis for the detection of eight nAChR agonist pesticides in honey.

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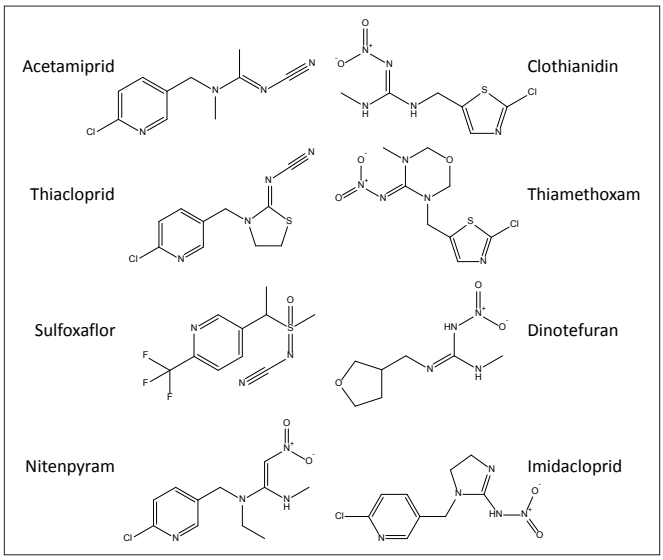

Figure 1. Structures of nAChR agonists examined in this study.

Table 1. Maximum residue limits in honey[5].

Pesticide	MoA Classification[6]	mg/kg	ppb
Acetamiprid‡	4A Neonicotinoid	0.05	50
Clothianidin	4A Neonicotinoid	0.01	10
Dinotefuran†	4A Neonicotinoid	0.01	10
Imidacloprid	4A Neonicotinoid	0.05	50
Nitenpyram†	4A Neonicotinoid	0.01	10
Sulfoxaflor§	4C Sulfoximine	0.05	50
Thiacloprid	4A Neonicotinoid	0.05	50
Thiamethoxam*	4A Neonicotinoid	0.01	10

‡Sum of acetamiprid + N-desmethyl-acetamiprid, †Default value for products for which no specific MRL is set, Article 18 (1)(b) Regulation (EC) 396/2005, §Sum of isomers, *Sum of thiamethoxam and clothianidin expressed as thiamethoxam

Experimental

Analyte standards were obtained from Sigma®, St. Louis, MO (imidacloprid) and AccuStandard®, New Haven, CT (dinotefuran, nitenpyram, thiamethoxam, clothianidin, acetamiprid, sulfoxaflor, and thiacloprid). Deuterium labeled standards (acetamiprid-D3, clothianidin-D3, thiacloprid-D4, imidacloprid-D4, and thiamethoxam-D3) were obtained from C/D/N Isotopes, Pointe-Claire, Quebec. Supported liquid extraction (SLE+) cartridges were obtained from Biotage®, Charlotte, NC. Five commercially available honeys, one organic and four conventional, were purchased locally. One conventional clover honey was used for blank and spiked samples and the four other samples were analyzed as unknowns. Internal standards were added to 50 ppb final concentration. Calibrators were prepared with analyte final concentrations of 2, 5, 10, 20, 30, 50, and 100 ppb. Thiamethoxam-D3 was used as internal standard for dinotefuran and sulfoxaflor. LC/MS analysis was performed as outlined in Tables 2 and 3. The sample preparation procedure is shown in Table 4.

Table 2. Mass spectrometry.

MassIQ™ and TOF MS Driver Software
Data analysis: AxION® Solo 1.2 Software
Ultraspray™ 2 Dual Probe electrospray source
Positive pulse-trap mode, D7: 12 µs, D8: 27 µs
2 spectra per second acquisition rate
m/z range 100 to 600
Drying gas: 16 L/min. at 350 °C
Endplate heater: medium
Calibrant solvent: methanol + 0.1% formic acid
Low calibrant: 2 µg/mL caffeine
High calibrant: 1 µg/mL hexamethoxyphosphazine
Left ESI probe (calibrant): 20 psi
Right ESI probe (column): 80 psi
Capillary exit: 80V, Skimmer: 25V

Table 3. Liquid chromatography.

Altus® A30 UPLC® Solvent Delivery Module
Altus A30 UPLC® Sampling Module with Active Preheater
Brownlee™ SPP Phenyl-Hexyl column: 2.1 x 100 mm, 2.7 µm, p/n N9308485
0.5 µm porosity stainless steel pre-column filter
Column temperature: 45 °C, Sample temperature: 20 °C
Mobile phase A: water + 5 mM ammonium formate
Mobile phase B: methanol + 5 mM ammonium formate
Autosampler needle wash: 10% acetonitrile in water
Flow rate: 0.4 mL/min., Equilibration: 3 min.
Sample injection: 10 µL, 5 replicates

Table 4. Sample preparation.

Add 8.35 mL water to 5 g honey, shake 5 min.
Add internal standards in 0.5 mL water
Add analyte stock and/or water (1 mL total volume)
Shake 30 min.
Add 0.15 mL ammonium hydroxide (28%)
Shake 5 min.
Add 10 ml of mixture to SLE+ cartridge
Apply a brief pulse of vacuum and incubate 5 min.
Add 20 mL dichloromethane, incubate 5 min.
Apply vacuum 2 min. to collect eluate
Repeat with 20 mL additional dichloromethane
Collect second eluate to same tube
Dry under a stream of nitrogen in a 40 °C water bath
Reconstitute with 1 mL of sample solution (below)
5% methanol + 5 mM ammonium formate in water

Results

Figure 2 shows an extracted ion chromatogram (EIC) of the eight analytes in neat solution at 250 ng/mL. Sulfoxaflor is a mixture of isomers and produced two chromatographic peaks. The earlier eluting sulfoxaflor peak co-eluted with acetamiprid (peak 6) on the phenyl-hexyl column. Both sulfoxaflor peaks could be resolved from acetamiprid on a C18 column (data not shown), however, an isobaric interferent in honey extracts co-eluted with acetamiprid on the C18 column, therefore the phenyl-hexyl column was utilized for this study. Honey samples spiked at three levels were prepared as outlined in Table 4 and analyzed by LC/TOF MS. Data files were processed using AxION® Solo software and analytes were identified based on mass accuracy, isotope abundance, retention time, and EIC peak intensity. Figure 3 shows an example mass spectrum (left panel) and extracted ion chromatogram (right panel) for thiacloprid in a 50 ppb level honey extract. The analyte signal (left panel, blue) shows the expected isotopic pattern as does the deuterated internal standard (red). The thiacloprid EIC (right panel, blue) shows good signal to noise and peak shape at the expected retention time. A summary view of the dataset results is shown in Figure 4. A green color indicates positive analyte identification while red indicates the analyte was not detected. These results show a positive identification of all analytes at 5, 10, and 50 ppb levels and no identification of analytes in the unspiked blank. Table 5 lists the calculated EIC signal-to-noise ratios (average of five replicates) for all analytes in the 5, 10, and 50 ppb spiked samples. Signal-to-noise ratios ranged from 117 to 22,597 for the sample set. No analytes were detected in the four test sample extracts.

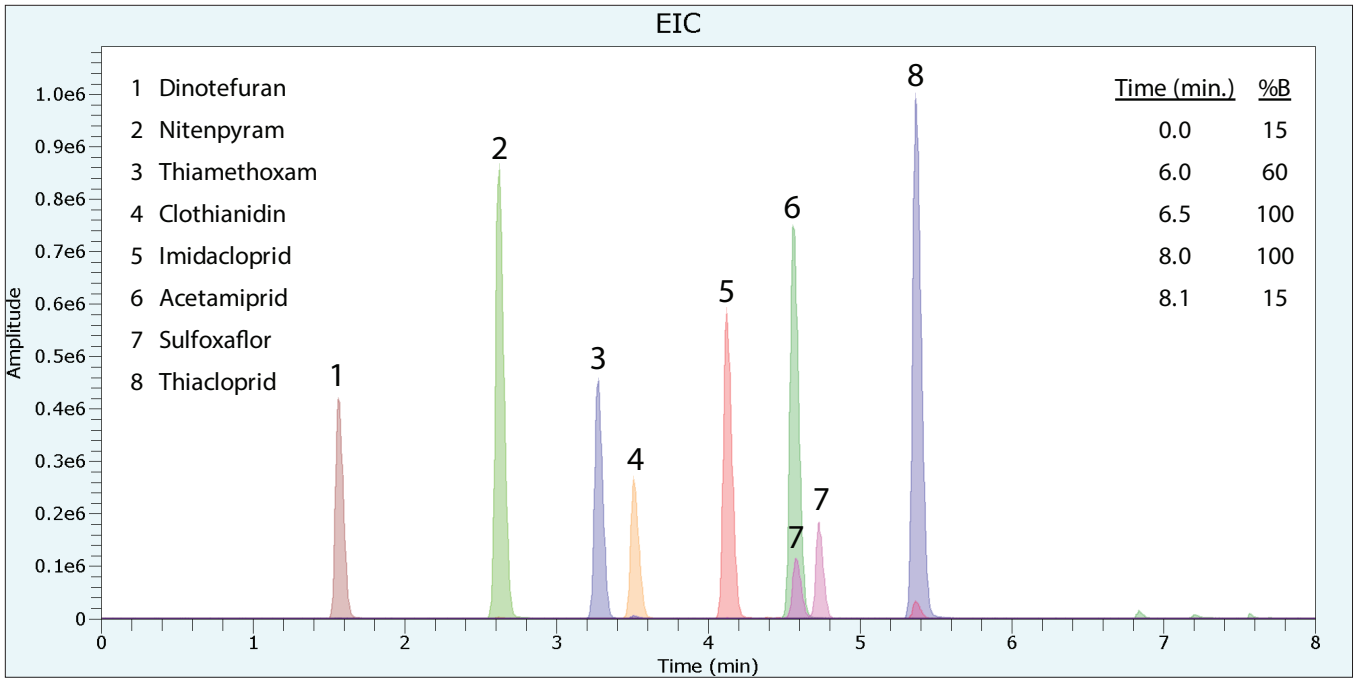

Figure 2. Extracted ion chromatogram of neat solution analytes by LC/MS.

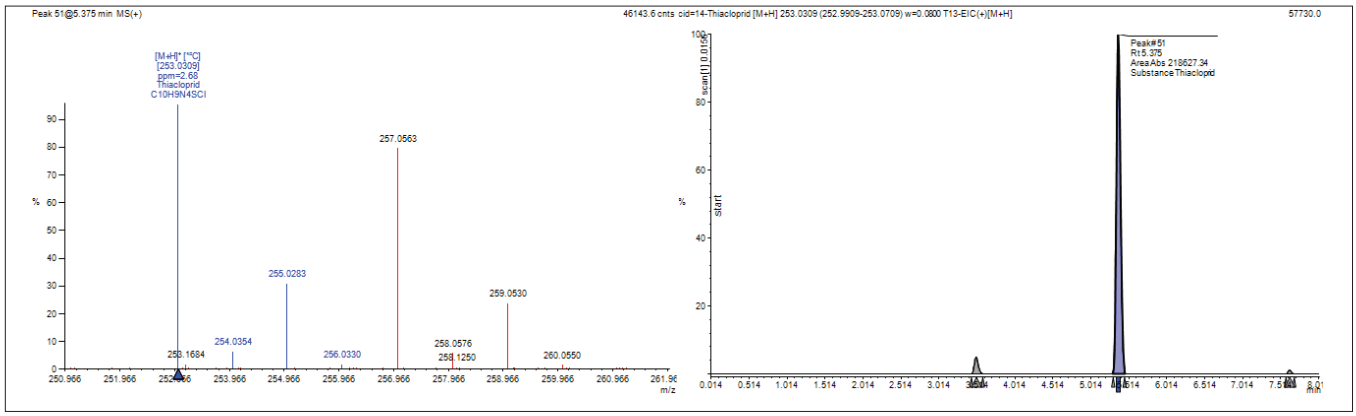

Figure 3. Example mass spectrum (left) and extracted ion chromatogram (right) of thiacloprid at 50 ppb in a honey extract sample.

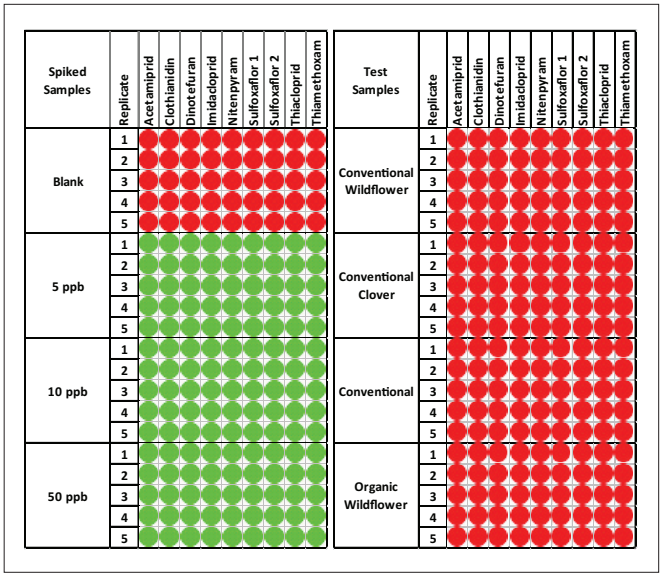

Figure 4. AxION Solo screening analysis of spiked (left) and test honey extracts (right).

Table 5. Signal-to-noise ratios of EICs in screening analysis.

Analyte	5 ppb	10 ppb	50 ppb
Acetamiprid	1449	3309	19154
Clothianidin	117	215	1564
Dinotefuran	1142	2365	12762
Imidacloprid	835	1799	10723
Nitenpyram	258	736	3166
Sulfoxaflor 1	159	260	1361
Sulfoxaflor 2	182	301	2164
Thiacloprid	1527	3846	22597
Thiamethoxam	359	701	4821

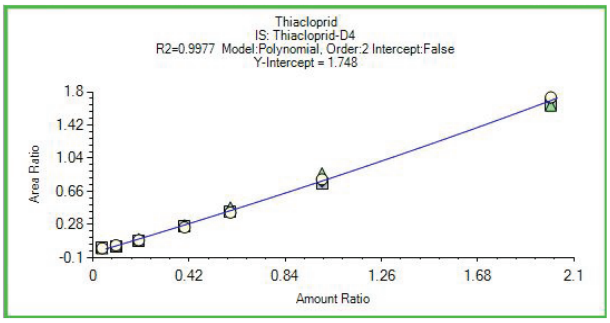

Figure 5. Calibration curve for thiacloprid in honey matrix.

Table 6. Recovery, imprecision, and linearity of five analytes in honey (n=5).

		5 ppb		10 ppb		50 ppb	
Analyte	r^2	Recovery	%CV	Recovery	%CV	Recovery	%CV
Acetamiprid	0.9983	113.3	4.9	104.4	4.2	108.5	7.0
Dinotefuran	0.9924	115.6	6.5	101.6	8.9	106.2	12.3
Imidacloprid	0.9985	109.1	7.1	101.2	4.4	107.4	2.1
Thiacloprid	0.9977	108.2	4.7	98.5	8.5	99.8	1.2
Thiamethoxam	0.9905	113.0	7.8	94.1	6.8	107.9	3.6

Calibration curves were generated from honey samples spiked at 2, 5, 10, 20, 30, 50, and 100 ppb levels. Clothianidin, nitenpyram, and sulfoxaflor calibration curves had r^2 values less than 0.99 and were not used for quantitative analysis. Calibration curves for acetamiprid, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam were applied to the three level spiked samples. Figure 5 shows an example calibration curve for thiacloprid. Recoveries and imprecision were determined and are shown in Table 6. These results demonstrate acceptable recoveries (94.1% to 115.6%) and imprecision (1.2 to 12.3 %CV) using an acceptability criteria of 70 to 120% recovery and a CV of less than 20%.

Conclusion

The results presented in this study show the feasibility of UPLC®/TOF MS analysis for the detection of nAChR inhibitors in honey. Good specificity of detection was obtained using accurate mass measurement, isotope pattern, and expected retention time. Optimization of sample preparation may improve quantitation of selected analytes.

References

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