Analysis of Neonicotinoids in Honey by QuEChERS and UHPLC-MS/MS

UCT part numbers:
ECQUEU7-MP – Mylar pouch containing 4 g $MgSO_4$, 1 g NaCl, 0.5 g sodium citrate dibasic sesquihydrate and 1 g sodium citrate tribasic dehydrate
CUMPSC18CT – 150 mg $MgSO_4$, 50 mg PSA and 50 mg endcapped C18; 2 mL dSPE tube
SLDA50ID21-18UM – Selectra® DA, 50 × 2.1 mm, 1.8 µm UHPLC column
SLDAGDC20-18UM – Selectra® DA, 10 × 2.0 mm, 1.8 µm guard cartridge
SLDGRDHLDR – Guard cartridge holder

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INTRODUCTION:

Neonicotinoids are a relatively new class of insecticide that were introduced as an alternative to organophosphate, carbamate and pyrethroid insecticides. Their novel mode of action works by irreversibly binding to nicotinic acetylcholine receptors, resulting in paralysis and death of insects. Since their introduction in the 1990s the neonicotinoids have been used extensively in crop protection. However, they have recently come under increasing scrutiny over their environmental and ecological impact, especially their role in bee deaths and colony collapse disorder (CCD)[1]. It has been reported that neonicotinoid residues can accumulate in the pollen and nectar of treated plants and poses a potential risk to honey bees[2]. In addition, neonicotinoid residues can be transferred to products derived from bees, including the popular food source honey[3]. Due to their potential negative impact, the European Union recently restricted the use of three neonicotinoids (clothianidin, thiamethoxam, and imidacloprid) for a period of 2 years[4].

This application note outlines a simple, fast and cost-effective method for the determination of 7 neonicotinoid pesticides in honey. Honey is dissolved in water and extracted using a citrate-buffered QuEChERS procedure. The sample extract then undergoes cleanup by dispersive-SPE with PSA/C18 sorbent to remove unwanted waxes, pigments and carbohydrates that are present. Analysis is performed by UHPLC/MS-MS using a Selectra® DA UHPLC column. Recovery studies were carried out by spiking raw and processed honey at two concentration levels (10 and 50 ng/g). Matrix-matched calibration curves, ranging from 1-250 ng/g, were used for quantitation. The mean recovery was found to be in the range of 82 to 113%, while repeatability was less than 10%.

PROCEDURE:

Sample extraction

1. Weigh 10 g of honey sample into a 50 mL polypropylene centrifuge tube.
2. Add internal standard (*optional*).
3. Add 10 mL of deionized water and shake/vortex until the honey is dissolved.
4. Add 10 mL of acetonitrile.
5. Add the contents of the **ECQUEU7-MP** Mylar pouch and shake either by hand or mechanically for at least 1 min. For this study a SPEX® SamplePrep® 2010 Geno/Grinder® was used.
6. Centrifuge the samples at greater than 3000×g for 5 min.

Sample clean-up

1. Transfer 1ml of supernatant into a **CUMPSC18CT** dSPE tube.
2. Vortex the samples for 30 sec.
3. Centrifuge the samples at greater than 3000×g for 2 min.
4. Transfer 500-600 µL of purified supernatant into an autosampler vial.

INSTRUMENTAL:

HPLC Conditions	
Instrumentation	Thermo Scientific™ Dionex™ Ultimate™ 3000
HPLC column	UCT Selectra® DA, 50 × 2.1 mm, 1.8 µm (p/n:
Guard column	UCT Selectra® DA, 10 × 2.0 mm, 1.8 µm (p/n:
Guard column	p/n: SLDGRDHLDR
Column temp.	40°C
Flow rate	300 µL/min
Injection volume	2 µL
Autosampler	10°C
Wash solvent	methanol: water (1:1, v/v)

Time (min)	Mobile phase A water + 0.1% formic acid	Mobile phase B methanol + 0.1% formic acid
0	95%	5%
1	0%	100%
4.5	0%	100%
4.6	95%	5%
7.5	95%	5%

MS Conditions	
Instrumentation	Thermo Scientific™ TSQ
Ionization mode	ESI^+
Spray voltage	5000 V
Vaporizer	400°C
Capillary	350°C
Sheath gas	50 arbitrary units
Auxiliary gas	5 arbitrary units
Ion sweep gas	0 arbitrary units
Declustering	0 V
Q1 and Q3 peak	0.2 and 0.7 Da
Collision gas	argon
Collision gas	1.5 mTorr
Acquisition	EZ method (scheduled SRM)
Cycle time	0.6 sec
Software	Xcalibur™ version 2.2

SRM Transitions							
Analyte	**t_R (min)**	**Precursor ion**	**Product ion 1**	**CE 1**	**Product ion 2**	**CE 2**	**S-lens (V)**
Dinotefuran	2.78	203.08	114.09	12	100.08	15	50
Nitenpyram	2.82	271.03	196.01	15	99.04	15	68
Clothianidin	3.07	249.97	169.01	10	131.94	15	56
Clothianidin-D_3	3.07	253.01	172.06	11	131.95	16	64
Thiamethoxam	3.14	291.97	211.02	10	181.01	18	59
Imidacloprid	3.33	256.02	209.04	16	175.08	16	69
Acetamiprid	3.45	223.01	125.95	20	89.98	33	68
Thiacloprid	3.62	252.99	125.99	19	90.02	32	83

RESULTS:

Accuracy & Precision Data for Processed Honey				
	10 ng/g (n=5)		50 ng/g (n=5)	
Analyte	Mean Recovery (%)	RSD (%)	Mean Recovery (%)	RSD (%)
Dinotefuran	106.3	2.6	113.4	3.6
Nitenpyram	92.3	2.4	99.6	2.6
Clothianidin	105.0	2.0	113.4	3.8
Thiamethoxam	107.5	1.2	110.1	4.3
Imidacloprid	102.0	2.5	109.7	5.4
Acetamiprid	103.4	3.0	113.6	4.6
Thiacloprid	105.8	1.4	112.9	4.8

Accuracy & Precision Data for Raw Honey				
Analyte	10 ng/g (n=5)		50 ng/g (n=5)	
	Mean Recovery (%)	RSD (%)	Mean Recovery (%)	RSD (%)
Dinotefuran	100.1	5.4	93.6	2.3
Nitenpyram	91.9	5.3	95.9	4.6
Clothianidin	87.5	4.6	82.2	2.6
Thiamethoxam	87.7	5.7	85.8	4.7
Imidacloprid	101.4	4.3	98.4	3.3
Acetamiprid	87.1	8.3	91.9	7.4
Thiacloprid	87.3	1.8	89.2	9.9

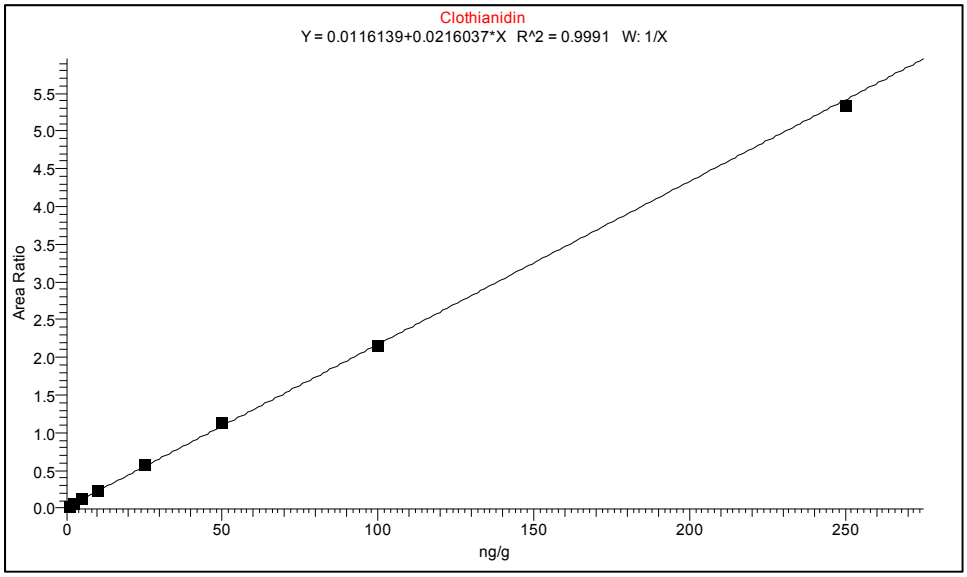

Figure 1. Example of a calibration curve (1, 2.5, 5, 10, 25, 50, 100 and 250 ng/g).

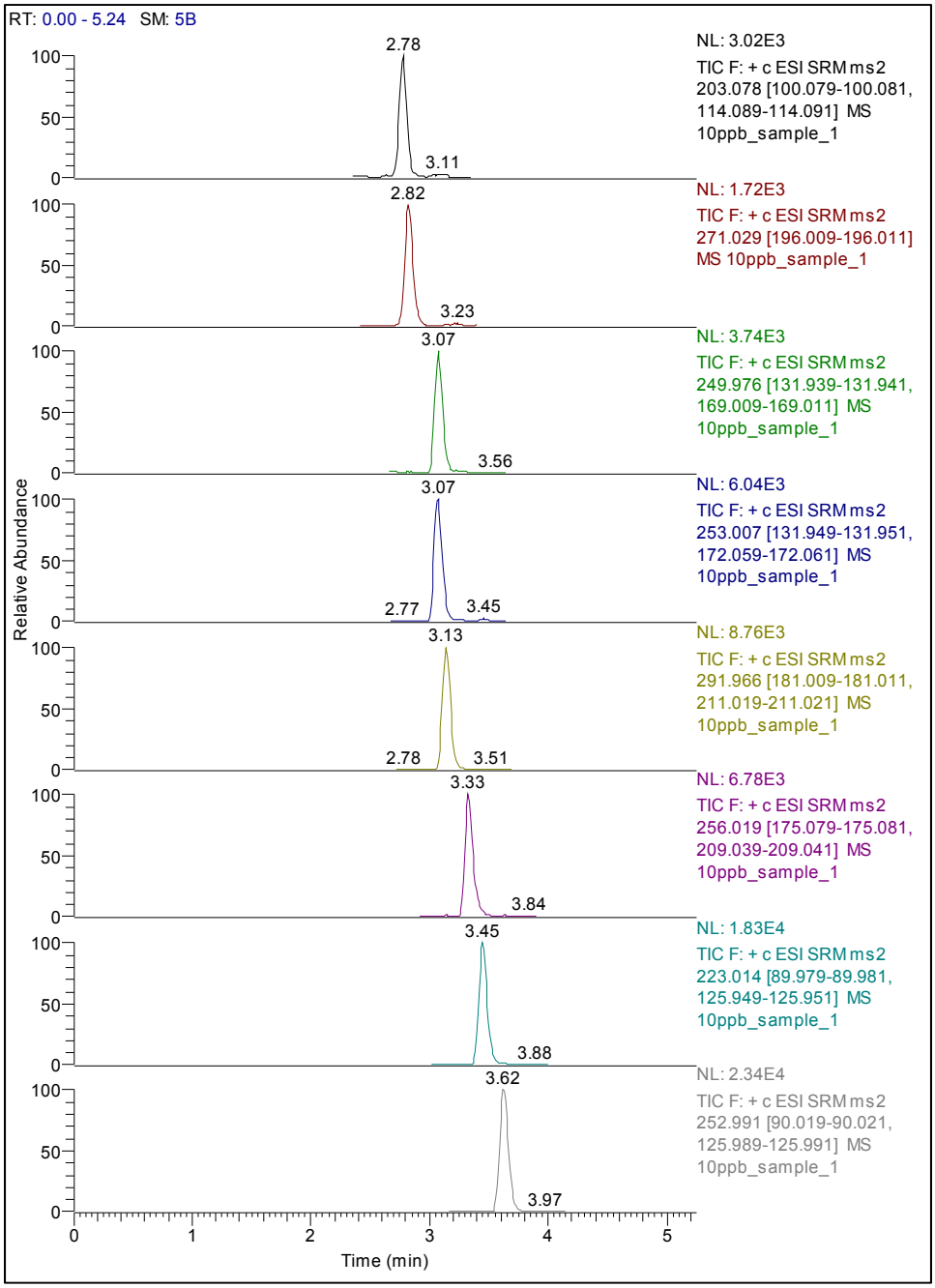

Figure 2. Chromatogram of an extracted raw honey sample fortified at 10 ng/g.

REFERENCES:

[1] C. Lu, K. M. Warchol, R. A. Callahan, Bulletin of Insectology, 67,125-130, 2014.
[2] T. Iwasa, N. Motoyama, J. T. Ambrose, R. M. Roe, Crop Protection, 23, 371–378, 2004.
[3] M. P. Galeano, M.Scordino, L. Sabatino, *et al.,* International Journal of Food Science, vol. 2013, Article ID 863904, 7 pages, 2013.
[4] Commission Regulation (EU) No 485/2013, Official Journal of the European Union, L 139, 12-26, 2013.

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