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

UCT Part Numbers

ECQUEU7-MP
Mylar pouch containing 4g $MgSO_4$, 1g NaCl, 1g $Na_3Cit \cdot 2H_2O$ and 0.5g $Na_2Cit \cdot 1.5H_2O$

CUMPSC18CT
2mL dSPE tube with 150mg $MgSO_4$, 50mg PSA and 50mg C18

SLDA50ID21-18UM
Selectra® DA UHPLC column (50 × 2.1 mm, 1.8 µm)

SLDAGDC20-18UM
Selectra® DA guard cartridge (10 × 2.0 mm, 1.8 µm)

SLGRDHLDR
Guard cartridge holder

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Summary:

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 pose a potential risk to honey bees [2]. Furthermore, neonicotinoid residues can be transferred to products derived from bees, including honey which is a popular food source [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 undergoes cleanup by dispersive-SPE (dSPE) with PSA/C18 to remove unwanted waxes, pigments and sugars. 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%.

QuEChERS 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 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 ≥3000 × *g* for 5 minutes.

Sample Clean-Up:

1. Transfer 1 ml of supernatant to a 2 mL dSPE tube (**CUMPSC18CT**).
2. Shake/vortex the sample for 30 seconds.
3. Centrifuge the samples at ≥3000 × *g* for 2 minutes.
4. Transfer 500-600 µL of purified supernatant into an autosampler vial.

LC-MS/MS Parameters:

UHPLC Conditions	
HPLC system	Thermo Scientific™ Dionex™ Ultimate™ 3000 UHPLC
MS system	Thermo Scientific™ TSQ Vantage™ (MS/MS)
HPLC column	UCT Selectra® DA, 50 × 2.1 mm, 1.8 µm (p/n: SLDA50ID21-18UM)
Guard column	UCT Selectra® DA, 10 × 2.0 mm, 1.8 µm (p/n: SLDAGDC20-18UM)
Guard column holder	p/n: SLDGRDHLDR
Column temperature	40°C
Flow rate	300 µL/min
Injection volume	2 µL

Mobile Phase Gradient		
Time (min)	**Mobile Phase A Water + 0.1% formic acid**	**Mobile Phase B Methanol + 0.1% formic acid**
0.0	95	5
1.0	0	100
4.5	0	100
4.6	95	5
7.5	95	5

MS Conditions	
Instrumentation	Thermo Scientific™ TSQ Vantage™
Ionization mode	ESI^+
Spray voltage	5000 V
Vaporizer temperature	400°C
Capillary temperature	350°C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	5 arbitrary units
Ion sweep gas	0 arbitrary units
Declustering potential	0 V
Collision gas	Argon (1.5 mTorr)
Cycle time	0.6 sec
Software	Xcalibur™ version 2.2

MRM Transitions

Compound	t_R (min)	Precursor ion	Product ion 1	Product ion 2
Dinotefuran	2.78	203.1	114.1	100.1
Nitenpyram	2.82	271.0	196.0	99.0
Clothianidin	3.07	250.0	169.0	132.0
Clothianidin-D3 (IS)	3.07	253.0	172.1	132.0
Thiamethoxam	3.14	292.0	211.0	181.0
Imidacloprid	3.33	256.0	209.0	175.1
Acetamiprid	3.45	223.0	126.0	90.0
Thiacloprid	3.62	253.0	126.0	90.0

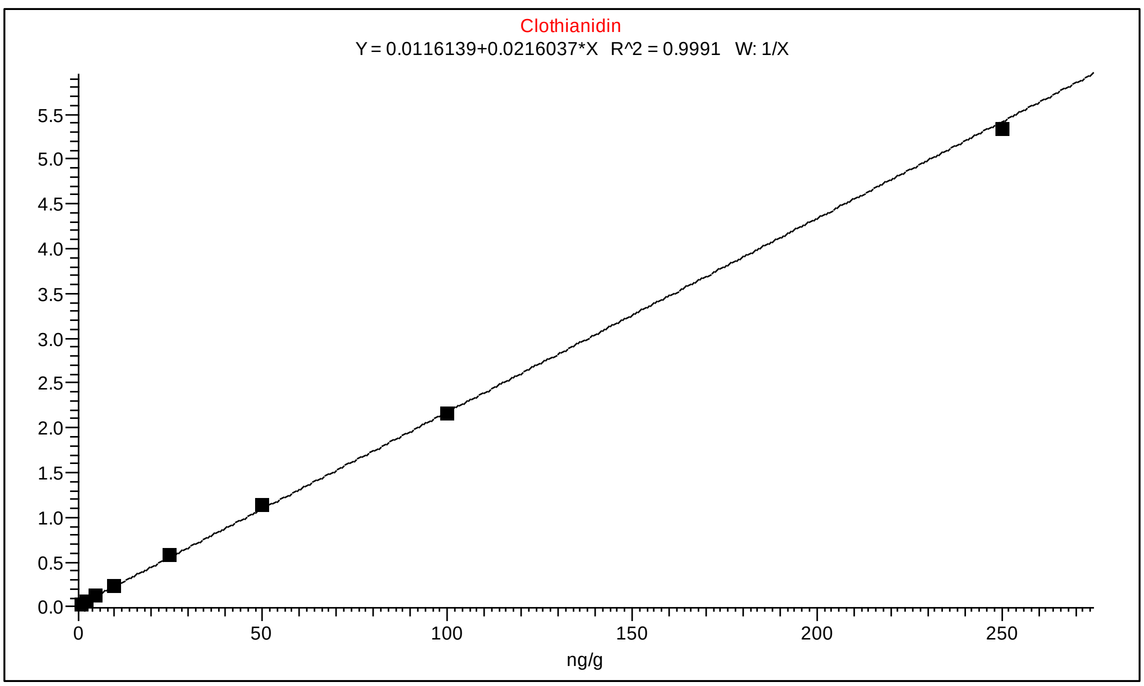

Figure 1. Example of a calibration curve (1, 2.5, 5, 10, 25, 50, 100 and 250 ng/g).

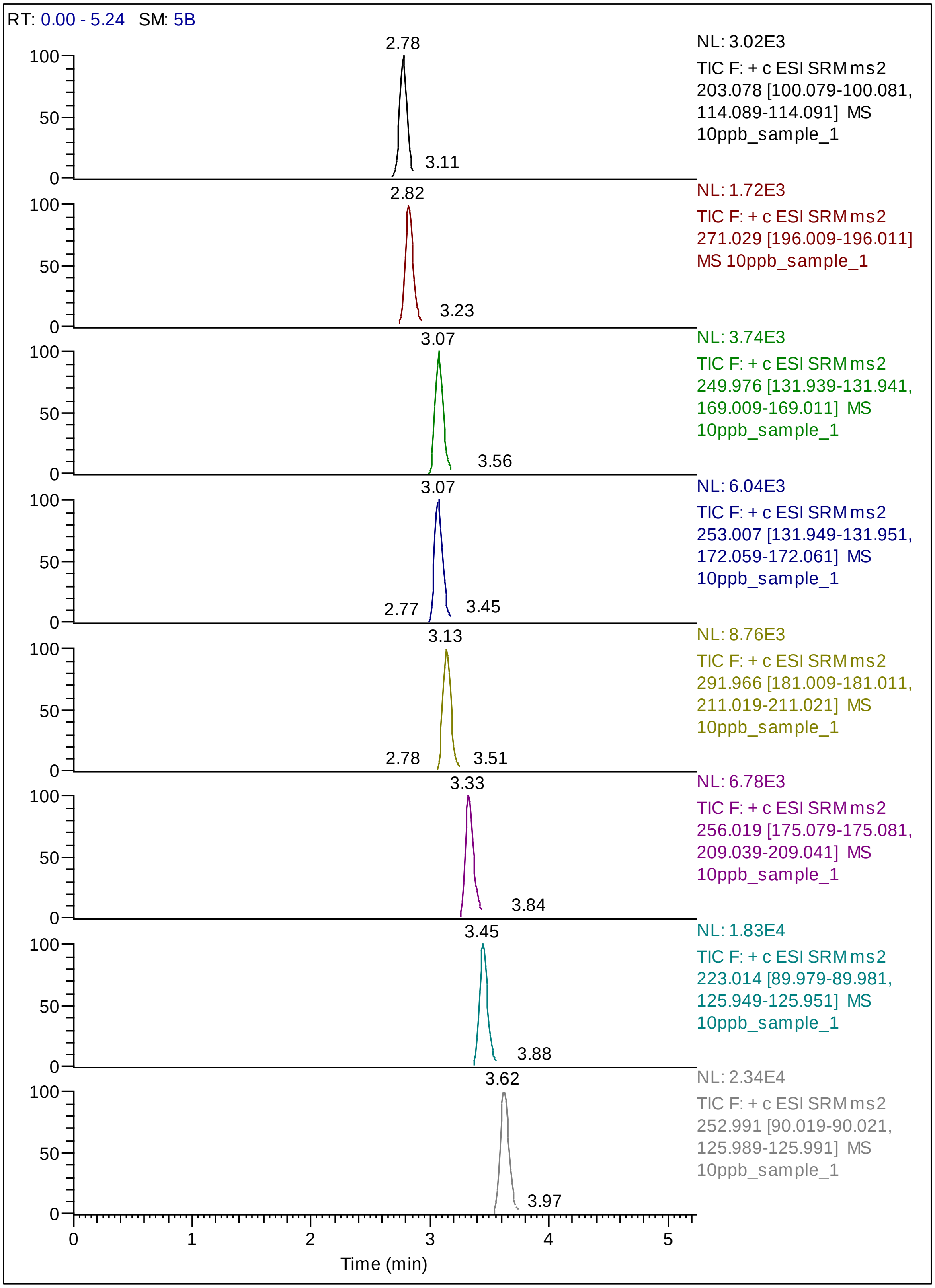

Figure 2. Chromatogram of an extracted raw honey sample fortified at 10 ng/g.

Results:

Accuracy & Precision Data for Processed Honey				
	10 ng/g		50 ng/g	
Analyte	Mean Recovery (%)	RSD (%, n=5)	Mean Recovery (%)	RSD (%, n=5)
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				
	10 ng/g		50 ng/g	
Analyte	Mean Recovery (%)	RSD (%, n=5)	Mean Recovery (%)	RSD (%, n=5)
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

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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