Ultra-sensitive and rapid assay of neonicotinoids in honey by UHPLC-MS/MS

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Introduction

Neonicotinoids are a class of insecticides widely used to protect fields (corn, canola, soybeans…) as well as fruits and vegetables. Their systemic distribution with high efficiency against sucking insects and long residual activity made them very popular within the global pesticide market.

Recently the use of these compounds became very controversial as they were pointed as one cause of the honeybees colony collapse disorder. Since pollination is essential for agriculture, extensive studies have been conducted to evaluate the impact of neonicotinoids on bee health. Following this the European Food Safety Authority limited the use of thiamethoxam, clothianidin and imidacloprid. Some European countries have banned or restricted the use of neonicotinoids.

In order to better understand the effect of these compounds on bees and their contamination in pollen and honey, a highly sensitive assay method was necessary.

Materials and Methods

Standards and Reagents

All analytical standards were provided by Sigma-Aldrich. Internal standards (thiamethoxam-d3, imidacloprid-d4 and chlothinidin-d3) were purchased from Sigma-Aldrich.

Solvents (including water and mobile phase additives) were of ULC/MS quality (Biosolve).

Sample Preparation

Compound extraction was performed using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method with an additional dispersive Solid Phase Extraction (dSPE) step.

5 g of honey (±1%) were weighted in a 50 mL polypropylene tube. 5 µL of internal standard solution at 5 µg/mL of each compound in acetonitrile was added on honey and let dry for 10 minutes. 10 mL of ultra pure water were added and the samples were homogenized by vortex mixing for 1 minute. 10 mL of acetonitrile were then added followed by vortex mixing for 1 minute.

Salts mix (4 g $MgSO_4$, 1 g Sodium Citrate, 0.5 g Sodium Citrate sesquihydrate, 1 g NaCl ; Biotage Q0020-15V) were added. After manual shaking, samples were centrifuged at 3000 g for 5 minutes at 10 ºC.

Supernatant (6 mL) was transferred into a 15 mL tube containing 1200 mg of $MgSO_4$, 400 mg PSA and 400 mg C18 (Biotage Q0050-15V). After centrifugation at 3000 g and 10 ºC for 5 minutes the supernatant was transferred into inert glass vial for analysis (Shimadzu LabTotal 227-34001-01).

UHPLC-MS/MS Conditions

Analysis were performed using a Nexera X2 UHPLC system coupled with LCMS-8060 with Heated ESI in positive ionization (see figure 1).

Mobile phase composition was optimized to generate the highest sensitivity. Ion source parameters (gas flows, temperatures) were also optimized using the Interface Setting Support Software (Shimadzu Corp.)

Figure 1: Overview of the UHPLC-MS/MS system

Table 1: UHPLC parameters

Parameter	Value
System	Nexera X2
Column	ACE SuperC18 100 x 2.1 mm 2 µm
Column Temperature	30 °C
Mobile phases	A: Water + 0.05% ammonia B: Methanol + 0.05% ammonia
Flow rate	0.6 mL/min
Gradient	5% B to 100%B in 3 min. 100%B to 5% in 0.1 min. Total run time 6 min.
Injection volume	1 µL (POISe mode with 10 µL of water)

Table 2: MS parameters

Parameter	Value			
System	LCMS-8060			
Ionization mode	Positive HESI			
Acquisition mode	MRM			
MRM transitions	Name	MRM Quan	MRM Qual	ISTD Group
	Acetamiprid	223.1 > 126	223.1 > 56.1	2
	Clothianidin	250.1 > 169.1	250.1 > 132	3
	Imidacloprid	256.1 > 175.1	258.1 > 211.1	2
	Thiacloprid	253.1 > 126	253.1 > 90.1	1
	Thiamtehoxam	292.1 > 211.1	292.1 > 181.1	1
	Thiamethoxam-D3	295.1 > 214.05	---	1
	Imidacloprid-D4	260.1 > 179.1	---	2
	Clothianidin-D3	253.1 > 132.05	---	3
Dwell time	7 to 16 msec depending upon the number of concomitant transitions to ensure to have at least 30 points per peak (max total loop time 115 msec).			
Pause time	1 msec.			
Quadrupole resolution	Q1: Unit Q3: Unit			
Temperature	HESI: 400 °C DL: 200 °C Heater block: 400 °C			
Gaz flow	Interface: 10 L/min Nebulizer: 3 L/min Drying: 5 L/min			

Results

Calibration

Calibration curves were prepared in acetonitrile to obtain final concentrations ranging from 2.5 pg/mL (2.5 fg on column) to 5 ng/mL. These concentrations corresponds to 5 ppt and 10 ppb in honey, respectively. A typical calibration curve is shown in figure 2.

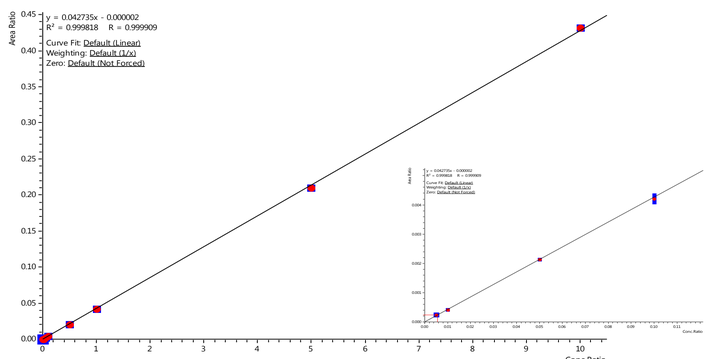


Figure 2: Calibration curve of clothianidin

Standard (ng/g)	Accuracy (%)
0.005	110
0.010	96.0
0.050	100
0.100	98.4
0.500	97.0
1.000	99.0
5.000	98.4
10.000	101

Recovery

Eight different honeys from the local supermarket were extracted with or without spike at 0.1 ppb. Each extract was injected 5 times to evaluate repeatability in matrix samples. A blank extract (no honey) was prepared to evaluate losses or non specific interactions. Results are presented in Table 3.

Table 3: Recovery results

	No matrix extract	Provence creamy honey	Italian creamy honey	Pyrenees liquid honey	French-Spanish creamy honey	Thyme liquid honey	Lemon tree creamy honey	Flowers creamy honey	Flowers liquid honey
ACETAMIPRID									
Mean (ng/g)	0.1073	0.1055	0.1489	0.201	0.159	0.105	0.505	0.180	0.194
%RSD	**1.1%**	**1.0%**	**0.9%**	**0.8%**	**1.4%**	**1.1%**	**0.5%**	**0.4%**	**0.9%**
Conc. in raw sample (ng/g)	0	0	0.032	0.082	0.064	0	0.394	0.032	0.081
% Recovery	**107%**	**106%**	**117%**	**119%**	**94.6%**	**105%**	**111%**	**147%**	**114%**
CLOTHIANIDIN									
Mean (ng/g)	0.1048	0.098	0.102	0.104	0.102	0.101	0.100	0.101	0.103
%RSD	**2.7%**	**4.6%**	**3.1%**	**1.2%**	**1.5%**	**3.0%**	**3.3%**	**4.7%**	**2.9%**
Conc. in raw sample (ng/g)	0	0	0	0	0	0	0	0	0
% Recovery	**105%**	**98.2%**	**102%**	**104%**	**102%**	**101%**	**100%**	**101%**	**103%**
IMIDACLOPRID									
Mean (ng/g)	0.093	0.091	0.117	0.101	0.095	0.091	0.121	0.097	0.122
%RSD	**1.4%**	**3.0%**	**1.8%**	**2.9%**	**2.4%**	**1.1%**	**1.8%**	**1.5%**	**2.4%**
Conc. in raw sample (ng/g)	0	0	0.0248	0.0048	0.0035	0	0.0219	0	0.0222
% Recovery	**93.0%**	**91.2%**	**92.5%**	**96.1%**	**91.7%**	**91.1%**	**98.7%**	**97.2%**	**100.0%**
THIACLOPRID									
Mean (ng/g)	0.096	0.093	0.095	0.097	0.102	0.100	0.118	0.157	0.110
%RSD	**2.1%**	**1.1%**	**0.6%**	**2.8%**	**0.9%**	**0.9%**	**1.1%**	**1.5%**	**0.9%**
Conc. in raw sample (ng/g)	0	0	0	0	0	0	0.0059	0.0505	0.0012
% Recovery	**96.4%**	**93.4%**	**94.7%**	**93.4%**	**98.4%**	**100%**	**112%**	**107%**	**108%**
THIAMETHOXAM									
Mean (ng/g)	0.104	0.100	0.103	0.101	0.099	0.099	0.097	0.095	0.097
%RSD	**0.9%**	**1.3%**	**1.5%**	**0.8%**	**1.1%**	**0.9%**	**1.3%**	**1.0%**	**1.1%**
Conc. in raw sample (ng/g)	0	0	0.0022	0	0	0	0	0	0
% Recovery	**104%**	**100%**	**101%**	**101%**	**98.6%**	**98.5%**	**96.9%**	**95.5%**	**97.1%**

Real Sample Analysis

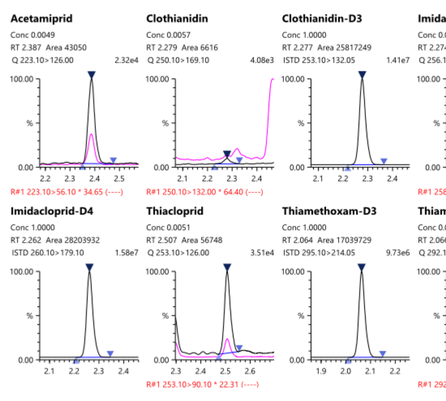
The eight samples used for recovery experiments were assayed as unknowns. Thanks to the very high sensitivity reached, even low concentrations of neonicotinoids were quantified. Results are presented in table 4.

Table 4: Honey sample results (concentrations in ng/g)

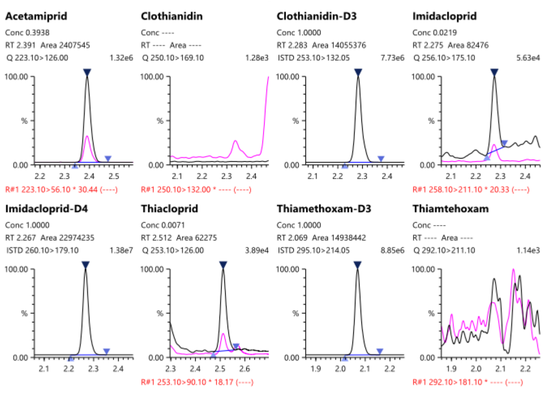
Honey	Acetamiprid	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
Provence creamy	---	---	---	---	---
Italy creamy	0.032	---	0.0248	---	0.0022
Pyrenees liquid	0.082	---	0.0048	---	---
French-Spanish	0.064	---	---	---	---
Thyme liquid	---	---	0.0035	---	---
Lemon tree creamy	0.394	---	0.0219	0.0059	---
Flowers cremy	0.032	---	---	0.0505	---
Flowers liquid	0.081	---	0.0222	0.0012	---

Chromatograms

Calibration standard at 0.0025 pg/mL



Lemon tree sample



Conclusion

A method for ultra sensitive assay of neonicotinoids in honey was set up. The sample preparation was simple but provided excellent recoveries, whatever is the honey type. The injection mode used prevented the use of tedious evaporation/reconstitution or dilution steps.

The sensitivity obtained enabled assay in real samples at very low levels far under the regulated residue levels.

This method can be a very efficient support tool to better understand the impact of neonicotinoids on honey bee colonies.