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Application News

No.C140

Liquid Chromatography Mass Spectrometry

Ultra-Sensitive and Rapid Assay of Neonicotinoids, Fipronil and Some Metabolites in Honey by UHPLC-MS/MS [LCMS-8060]

Neonicotinoids are a class of insecticides widely used to protect fields as well as fruits and vegetables.
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 Security Authoritiy (EFSA) limited the use of thiamethoxam, clothianidin and imidacloprid. Fipronil, a pesticide from a different chemical class, has been also banned by EFSA for maize seed treatment due to its high risk for honeybee health.
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. A method was set up using Nexera X2 with LCMS-8060.

Sample Preparation

Thiamethoxam-d3, imidacloprid-d4 and chlothianidin-d3 were used as internal standards.
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.
After incubation at room temperature for one hour with gentle shaking, a commercially available salt mix from Biotage (4 g MgSO4, 1 g Sodium Citrate, 0.5 g Sodium Citrate sesquihydrate, 1g NaCl) was added. After manual shaking, samples were centrifuged at 3000 g for 5 minutes at 10 °C.
Supernatant (6 mL) was transferred into a 15 mL tube containing 1200 mg of MgSO4, 400 mg PSA and 400 mg C18 from Biotage. After centrifugation at 3000 g and 10 °C for 5 minutes the supernatant was transferred into a LCMS certified inert glass vial for analysis (Shimadzu LabTotal 227-34001-01).

Recovery

An "all-flowers" honey from the local supermarket was extracted with or without spike at 50 ppt. A blank extract (no honey) was prepared to evaluate losses or non specific interactions. Results are presented in Table 1.
Calculated recoveries are within acceptance values 70-120 % from EU SANTE/11945/2015.

Table 1 Measured Recoveries in Honey

Compound	Recovery	Compound	Recovery
Acetamiprid	78.8 %	Fipronil sulfone	74.2 %
Acetamiprid-N-desmethyl	93.4 %	Imidaclorpid	83.2 %
Chlothianidin	70.6 %	Nitenpyram	87.0 %
Dinotefuran	76.5 %	Thiacloprid	82.2 %
Fipronil	78.1 %	Thiamethoxam	75.6 %

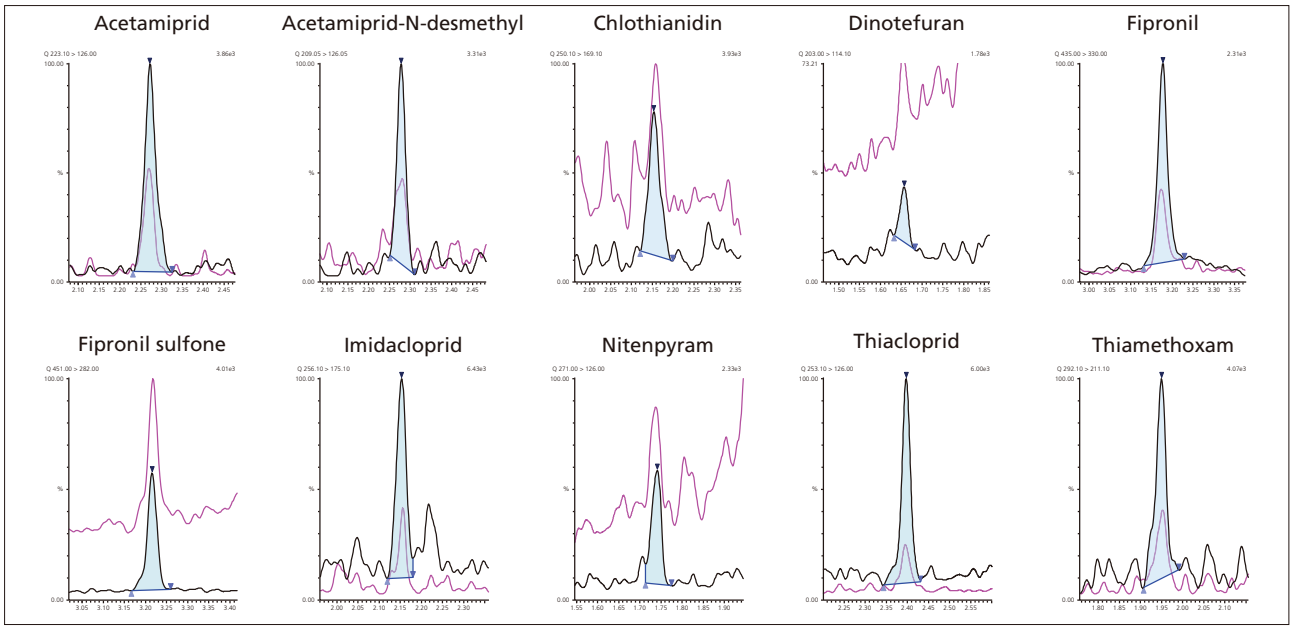


Fig. 1 Chromatogram of the Target Compounds at Their Lower Limit of Quantification

Table 2 Analytical Conditions

System	: Nexera X2	System	: LCMS-8060
Column	: ACE SuperC18 (100 mm L. × 2.1 mm I.D., 2 µm)	Ionization	: Heated ESI
Column Temperature	: 30 °C	Probe Voltage	: +1 kV (positive ionization) / -1.5 kV (negative ionization)
Mobile Phases	: A: Water = 0.05 % ammonia B: Methanol + 0.05 % ammonia	Temperature	: Interface: 400 °C Desolvation Line: 200 °C Heater Block: 400 °C
Flowrate	: 600 µL/min		
Gradient	: 5 %B to 100 %B in 3 min 100 %B to 5 %B in 0.1 min	Gas Flow	: Nebulizing Gas: 3 L/min Heating Gas: 10 L/min Drying Gas: 5 L/min
Total Run Time	: 4 min		
Injection Volume	: 2 µL (POISe mode with 10 µL of water)		

Table 3 MS/MS Acquisition Parameters

MRM Transitions	Name	Polarity	MRM Quan	MRM Qual	ISTD
	Acetamiprid	+	223.1 > 126.0	223.1 > 56.1	2
	Acetamiprid-N-desmethyl	+	209.1 > 126.0	211.1 > 128.0	2
	Clothianidin	+	250.1 > 169.1	250.1 > 132.0	3
	Dinotefuran	+	203.0 > 114.0	203.0 > 87.0	1
	Fipronil	-	435.0 > 330.0	435.0 > 250.0	3
	Fipronil sulfone	-	451.0 > 415.0	451.0 > 282.0	3
	Imidacloprid	+	256.1 > 175.1	258.1 > 211.1	2
	Nitenpyram	+	271.0 > 126.0	271.0 > 225.0	3
	Thiacloprid	+	253.1 > 126	253.1 > 90.1	1
	Thiamethoxam	+	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

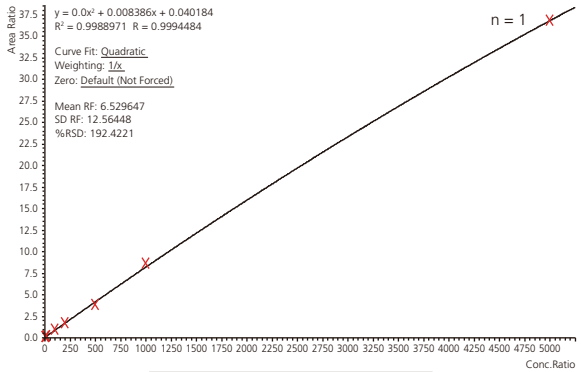
Dwell Time	: 3 to 34 msec depending upon the number of concomitant transitions to ensure to have at least 30 points per peak (max total loop time 140 msec).
Pause Time	: 1 msec
Quadrupole Resolution	: Q1: Unit Q3: Unit

Calibration

Calibration curves were prepared in acetonitrile to obtain final concentrations ranging from 0.5 pg/mL (1 fg on column) to 5 ng/mL. These concentrations corresponds to 1 ng/kg and 10 µg/kg in honey, respectively.
For each compound, the lower limit of quantification was selected to give an accuracy between 80-120 % (see table 4).
A typical calibration curve is shown in Fig. 2.

Table 4 Limits of Quantification in Honey

Compound	LOQ (µg/kg)	Compound	LOQ (µg/kg)
Acetamiprid	0.005	Fipronil sulfone	0.001
Acetamiprid-N-desmethyl	0.005	Imidacloprid	0.020
Chlothianidin	0.020	Nitenpyram	0.020
Dinotefuran	0.010	Thiacloprid	0.005
Fipronil	0.001	Thiamethoxam	0.005



Standard (µg/kg)	Accuracy (%)
0.005	106
0.010	97.2
0.020	95.6
0.100	107
0.200	98.4
0.500	91.5
1.000	104
5.000	99.9
10.000	100

Fig. 2 Calibration Curve of Acetamiprid

Real Samples Analysis

Nine honey samples purchased at the local supermarket or used as raw materials in cosmetics (orange tree honey) were assayed as unknowns.
All tested honeys showed concentrations far below the authorized maximum residue limit. But thanks to the very high sensitivity reached, even low concentrations of neonicotinoids were quantified. Results are presented in table 5.
A representative chromatogram of a sample honey is shown in Fig. 3.

Table 5 Honey Samples Results (concentrations in µg/kg)

Honey	Acetamiprid	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
1. Provence creamy	---	---	0.20	---	0.010
2. Italy creamy	0.15	---	0.17	---	---
3. Pyrenees liquid	0.38	---	0.043	0.020	---
4. French-Spanish creamy	0.27	---	0.047	0.020	---
5. Thyme liquid	---	---	---	---	---
6. Lemon tree creamy	1.7	---	0.15	0.033	---
7. Orange tree liquid	1.2	---	0.62	---	---
8. Flowers creamy	0.14	---	0.055	0.39	---
9. Flowers liquid	0.34	---	0.11	0.010	---

Honey	Dinotefuran	Nitenpyram	Acetamiprid-N-desmethyl	Fipronil	Fipronil sulfone
1. Provence creamy	---	0.052	0.005	---	---
2. Italy creamy	---	0.040	---	---	---
3. Pyrenees liquid	---	---	0.015	0.004	---
4. French-Spanish creamy	---	0.032	---	---	---
5. Thyme liquid	---	---	---	---	---
6. Lemon tree creamy	---	---	0.020	---	---
7. Orange tree liquid	---	0.024	0.018	---	---
8. Flowers creamy	---	---	0.016	---	---
9. Flowers liquid	---	---	0.006	---	---

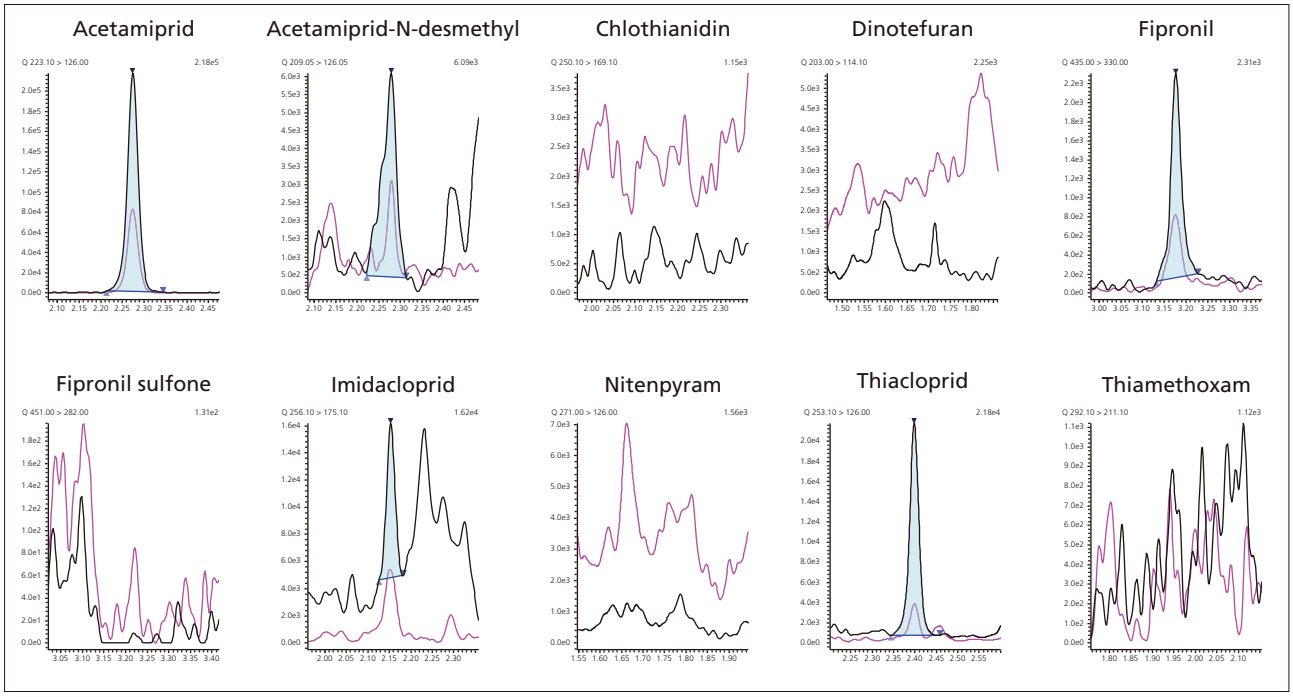


Fig. 3 Chromatogram of a Sample Honey (Pyrenees)

Stability

The thyme honey sample with no detectable target compound was spiked at 50 ng/kg with all compounds prior to extraction. The extract obtained was then consecutively injected 150 times in the system.
The results presented in Fig. 4 show excellent stability of the signal even at these low concentrations. This demonstrates that the excellent sensitivity can be maintained over long series of real sample analysis thanks to the ion source ruggedness.

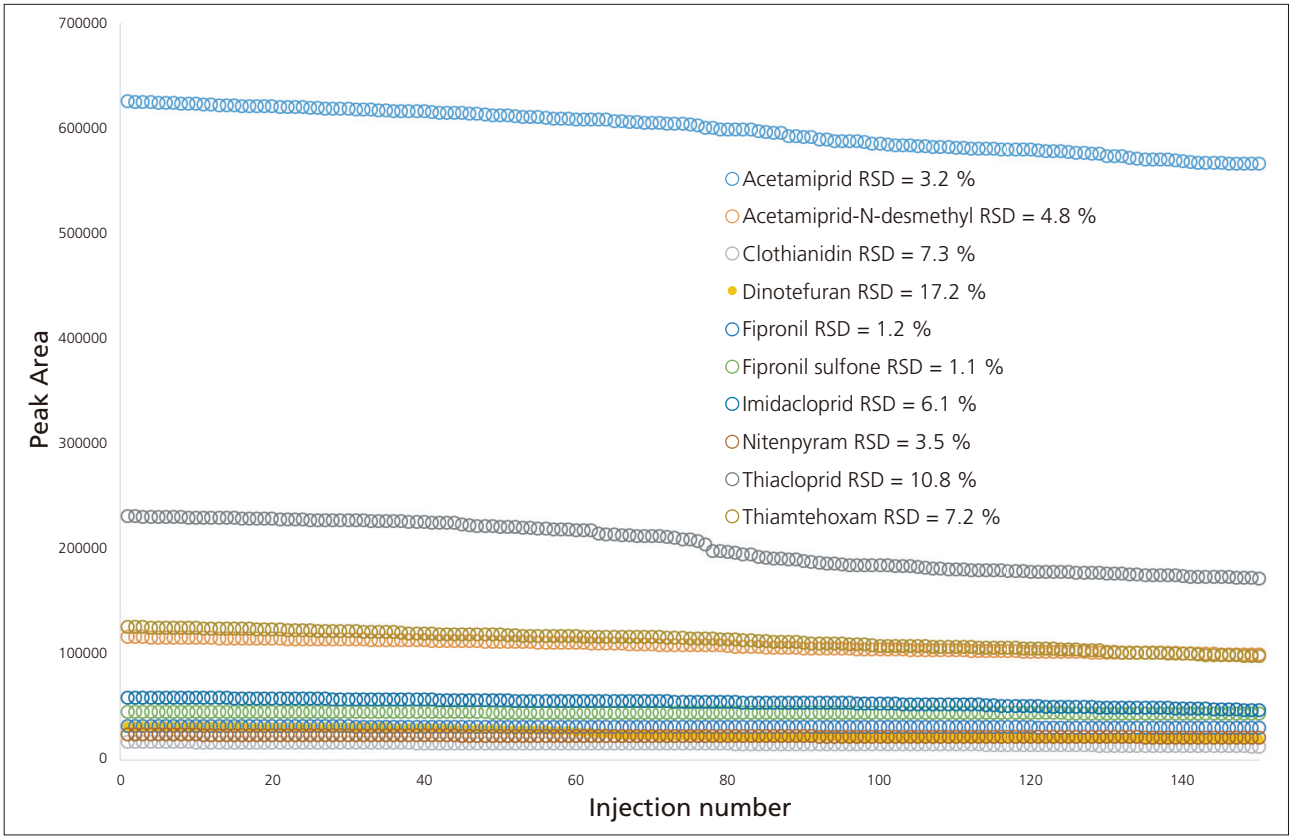


Fig. 4 Stability of Peak Areas in Real Honey Samples

Conclusion

A method for ultra sensitive assay of neonicotinoids in honey was set up. The sample preparation was simple but provided excellent recoveries. The injection mode used prevented the use of tedious evaporation/reconstitution or dilution steps.
Thanks to the high sensitivity obtained enabled assay in real samples at very low levels far under the regulated residue levels. Furthermore, even at low measured concentrations, the system demonstrated its stability after long analytical series of real samples.
This method can be a very efficient support tool to better understand the impact of neonicotinoids on honey bee colonies and could be easily transposed to pollen or bee samples.

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