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Application News

SSI-LCMS-091

Liquid Chromatography Mass Spectrometry

Using UHPLC-MS-MS to analyze neonicotinoid compounds and fipronil and its metabolites

Liquid Chromatograph Mass Spectrometer

LCMS-8060

Summary: Neonicotinoid compounds are widely used as pesticides to protect farmland. Recently, significant controversy has surrounded this class of compounds because they have been linked with negative effects on bee colonies. Therefore, the European Food Safety Authority (EFSA) limited the use of neonicotinoid compounds – thiamethoxam and imidacloprid. Fipronil is a phenylpyrazole pesticide commonly used to protect corn crops. However, this compound also has the potential to affect bee health, therefore the EFSA also banned it from agricultural use.

A Nexera X2 Ultra High Performance Liquid Chromatograph (UHPLC) and LCMS-8060 triple quadrupole mass spectrometer was used to better understand the effects of these compounds on bees, pollen and honey quality.

Method: Thiamethoxam-D3, Imidacloprid-D4, and Clothianidin-D3 were used as internal standards. QuEChERS and dSPE methods were used to extract the analyte.

5 g (±1%) of honey was measured into a 50 mL centrifugation tube. 5 mL of each internal standard (5 µg/mL each in ACN) was added into the honey sample and evaporated for 10 minutes. 10 mL of water was added and vortexed for 1 minute. Then, 10 mL of ACN was added and vortexed for an additional minute.

The samples were placed on a shaking table (low) for 1 hour. A salt mixture (magnesium sulfate 4 g, sodium sulfate 1 g, sodium citrate hemihydrate 0.5 g, sodium chloride 1 g, available from Biotage) was added. After thorough shaking, the sample underwent centrifugation (3000 rpm) for 5 minutes at 10 °C. The supernatant (6 mL) was separated and placed in a 15 mL purification tube (magnesium sulfate 1200 mg, N-propyl ethylenediamine 400 mg, octadecylsilane bonded silica gel 400 mg, available from Biotage) and centrifuged (3000 rpm) again at 10 °C for 5 minutes. The supernatant was transferred into inert glass prior to analysis.

"Full flower" honey was purchased from a local supermarket for recovery analysis. Internal standards were added into a 50 ng/L honey sample. A control sample without internal standards was also created to investigate method recovery.

A blank sample without honey was processed via the extraction method to investigate matrix effect. Method recovery fell within the EU SNATE/11945/2015 range of 70% - 120%. Results can be found in Table 1.

Table 1. Percent Recovery of Analytes in Honey

Analyte	Recovery in Honey
Acetamiprid	78.8%
N-Acetamiprid-N-desmethyl	93.4%
Clothianidin	70.6%
Dinotefuran	76.5%
Fipronil	78.1%
Fipronil Sulfone	74.2%
Imidacloprid	83.2%
Nitenpyram	87.0%
Thiacloprid	82.2%
Thiamethoxam	75.6%

MRM optimization was done on the LCMS-8060. MRM transition information is listed in Table 3 and analysis conditions are listed below in Table 2.

Table 2. Chromatography and mass spectrometry analysis conditions for the honey samples

LC Conditions		MS Conditions	
Column	ACE SuperC18 (100 mm x 2.1 mm; 2µm)	Ionization Source	ESI
Column Oven Temp.	30 °C	Source Voltages	+ 1 kV (+)/ -1.5 kV (-)
Mobile Phase A	H_2O + 0.05% ammonia	Interface Temp.	400 °C
Mobile Phase B	Methanol + 0.05% ammonia	DL Line Temp.	200 °C
Flow Rate	0.6 mL/min	Heat Block Temp.	400 °C
Gradient	5-100% B (3 min) 100-5% B (0.1 min)	Nebulizing Gas	3 L/min
Total Run Time	4 min	Heating Gas	10 L/min
Injection Volume	2 µL (POISe mode. 10 µL H_2O)	Drying Gas	5 L/min

Table 3. MRM Transitions of the Analytes

Analyte	Polarity	Quantifier	Qualifier	IS Group
Acetamiprid	+	223.1>126.0	223.1>56.1	2
N-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>2501.0	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>126.0	253.1>90.1	1
Thiamethoxam	+	292.1>211.1	292.1>181.1	1
Thiamethoxam-D3	+	295.1>214.1	--	1
Imidacloprid-D4	+	260.0>179.1	--	2
Clothianidin-D3	+	253.1>132.1	--	3

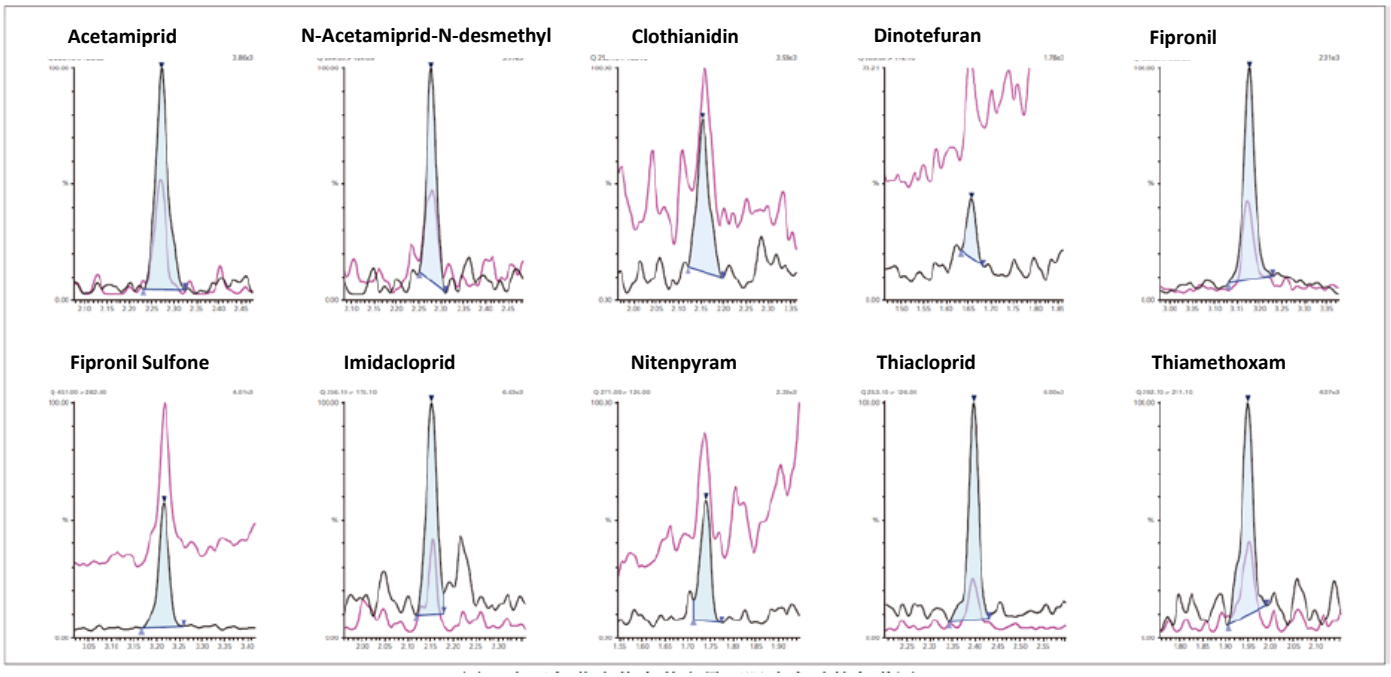


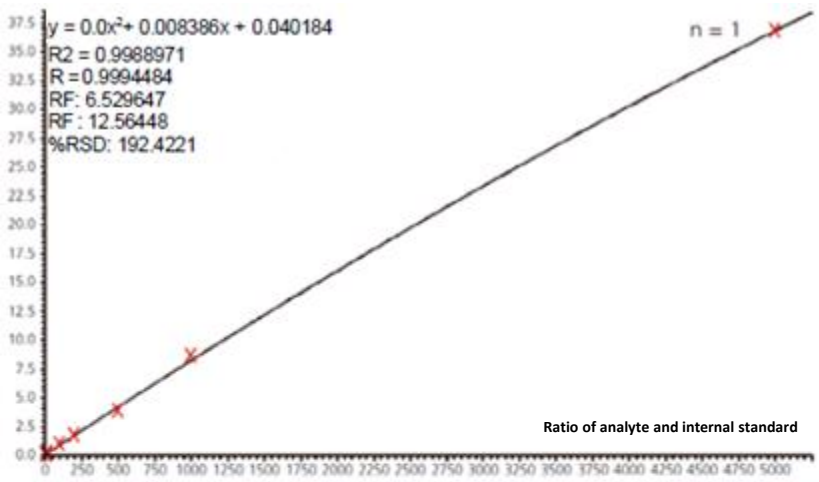
Figure 1. MRM chromatograms of each analyte at limit of quantitation (LOQ)

Calibration curves were made in the range of 0.5 pg/mL (1 fg on column) to 5 ng/mL in ACN. A sample calibration curve can be found in Figure 2.

These concentrations correlate to 1 ng/kg and 10 µg/kg in honey. Table 4 contains data for the established limit of quantitation wherein quantitation accuracy fell within 80% - 120%.

Table 4. Limit of quantitation for the analytes of interest in honey

Analyte	LOQ (µg/kg)
Acetamiprid	0.005
N-Acetamiprid-N-desmethyl	0.005
Clothianidin	0.020
Dinotefuran	0.010
Fipronil	0.001
Fipronil Sulfone	0.001
Imidacloprid	0.020
Nitenpyram	0.020
Thiacloprid	0.005
Thiamethoxam	0.005



Concentration (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

Figure 2. Calibration Curve of Acetamiprid

Nine different types of honey were purchased as unknown samples. No honey sample exceeded acceptable neonicotinoid levels. However, trace amounts of neonicotinoids, fipronil and its metabolites were still detected by this sensitive method.

Quantitation results are shown in Table 5 and MRM chromatograms of the liquid Pyrenees honey are shown in Figure 3.

Table 5. Neonicotinoids, Fipronil and its metabolites in Unknown Honey Samples (µg/kg)

Honey	Acetamiprid	N-A0.34cetamiprid-N-desmethyl	Clothianidin	Dinotefuran	Fipronil
Creamy Provence Honey	--	--	0.20	--	0.010
Creamy Italian Honey	0.15	--	0.17	--	--
Liquid Pyrenees Honey	0.38	--	0.043	0.020	--
Creamy French-Spanish Honey	0.27	--	0.047	0.020	--
Liquid Thyme Honey	--	--	--	--	--
Creamy Lemon Tree Honey	1.7	--	0.15	0.033	--
Liquid Orange Honey	1.2	--	0.62	--	--
Milky Nectar	0.14	--	0.055	0.39	--
Liquid Nectar	0.34	--	0.11	0.010	--

Honey	Fipronil Sulfone	Imidacloprid	Nitenpyram	Thiacloprid	Thiamethoxam
Creamy Provence Honey	--	0.052	0.005	--	--
Creamy Italian Honey	--	0.00	--	--	--
Liquid Pyrenees Honey	--	--	0.015	0.004	--
Creamy French-Spanish Honey	--	0.032	--	--	--
Liquid Thyme Honey	--	--	--	--	--
Creamy Lemon Tree Honey	--	--	0.020	--	--
Liquid Orange Honey	--	0.024	0.018	--	--
Milky Nectar	--	--	0.016	--	--
Liquid Nectar	--	--	0.006	--	--

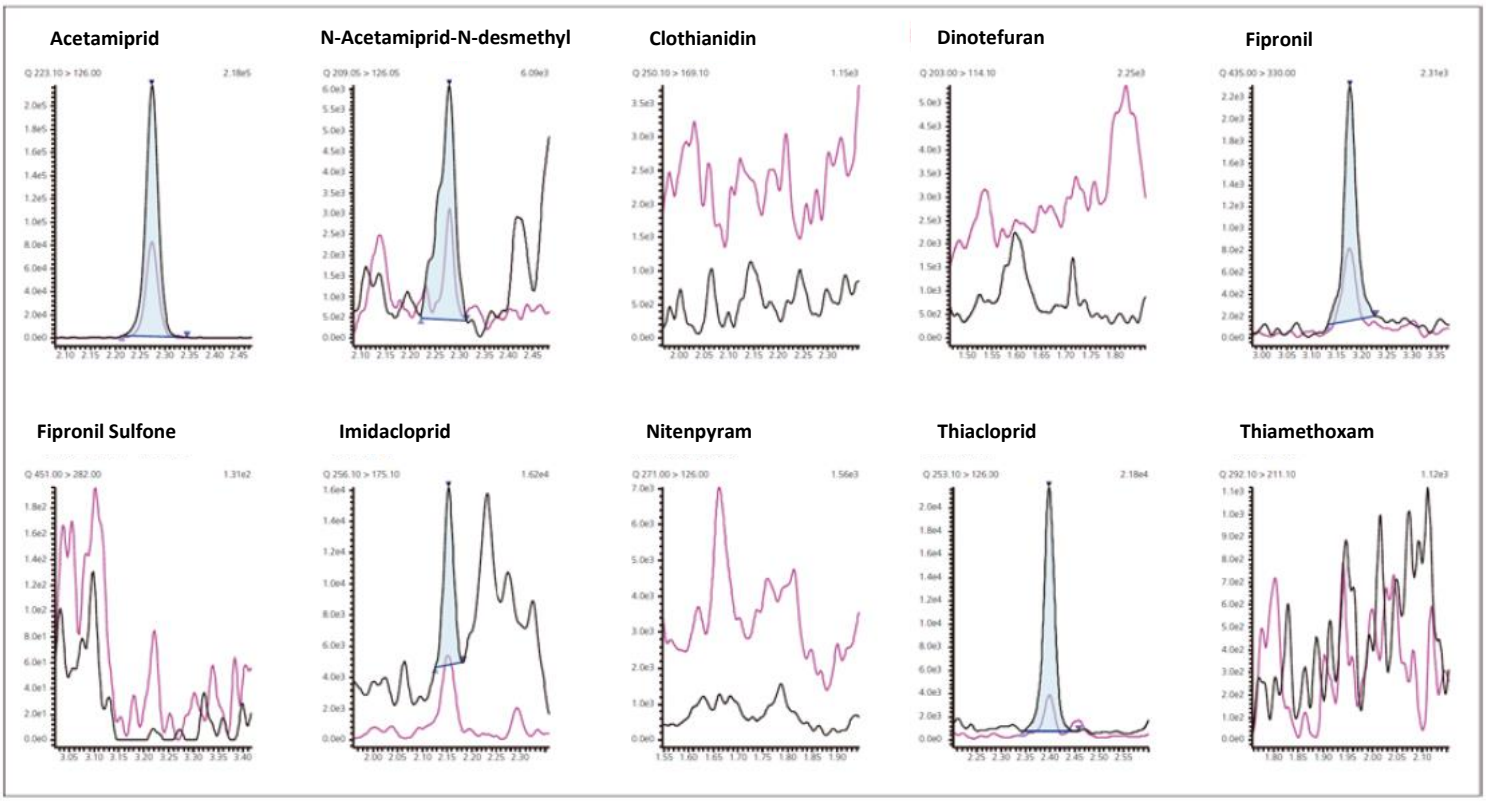


Figure 3. MRM chromatograms of liquid Pyrenees Honey

Spiked liquid thyme honey (50 ng/kg) was used for a reproducibility study. Results of 150 replicates were obtained and shown in Figure 4. Even at low levels, highly reproducible results were obtained.

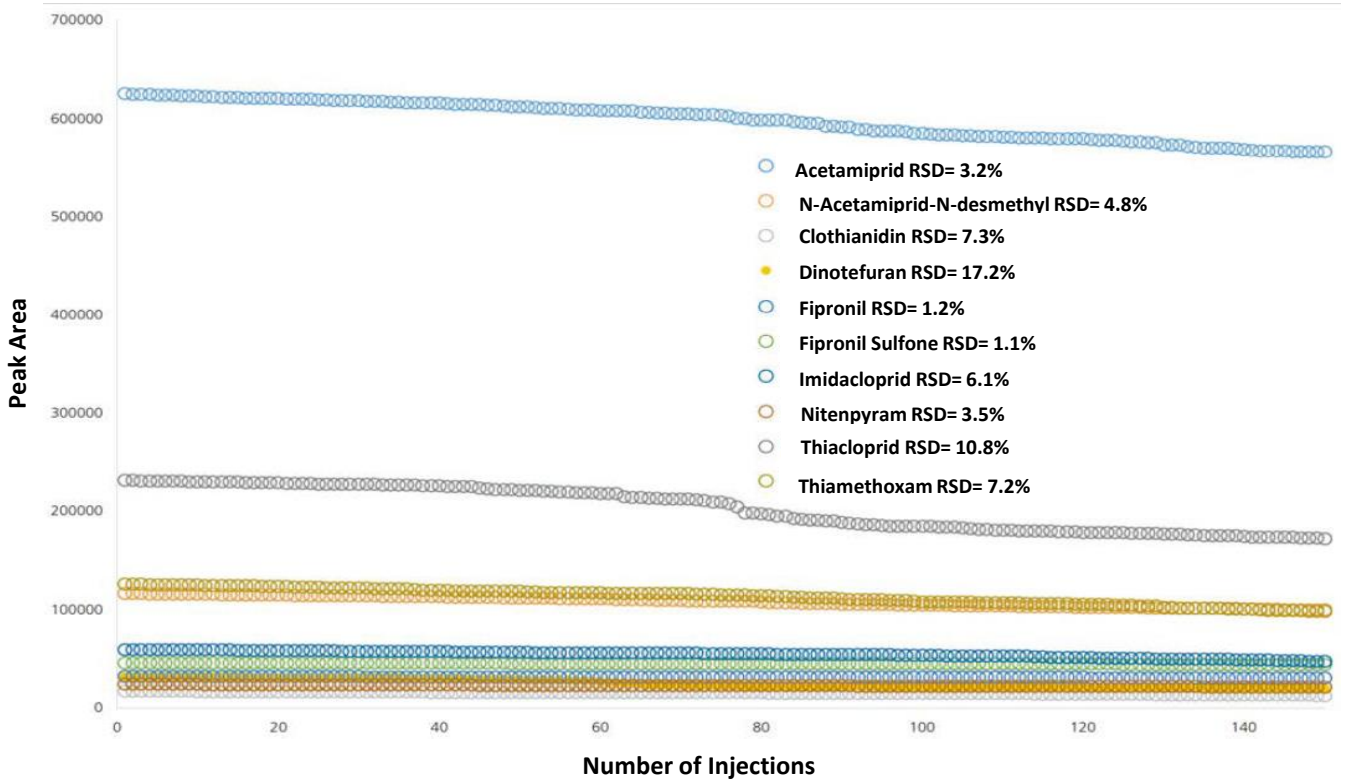


Figure 4. Reproducibility results of neonicotinoids, fipronil and its metabolites in liquid thyme honey

Conclusion: A sensitive method was developed on the LCMS-8060 to analyze neonicotinoids, fipronil and its metabolites. The sample preparation step included high recovery. This sensitive method was capable of detecting residual pesticides present in honey samples that met regulation levels. Reproducible results were obtained after a long period of usage with multiple injections. This method can benefit the investigation of pesticide levels and its correlation to bee health. With minor adjustments to the method, the quality of pollen and/or honey can be effectively analyzed.

Please note that this document was translated and summarized into English from Chinese (C140; LAAN-A-LM-E111).

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ULTRA FAST MASS SPECTROMETRY

LCMS-8030 LCMS-8040 LCMS-8050 LCMS-8060 LCMS-2020 LCMS-IT-TOF

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