Application Brief

Food Testing and Agriculture

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Analysis of Fipronil and Metabolites in Chicken and Eggs Using Agilent QuEChERS Kit Followed with Agilent Bond Elut EMR—Lipid Cleanup by LC/MS/MS

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Abstract

This study describes a method that was developed for the quantitative analysis of fipronil and metabolites in chicken and eggs. The method used an Agilent QuEChERS extraction kit followed with Agilent Bond Elut EMR—Lipid cleanup by 6470 LC/MS/MS analysis. The method provided a reliable solution with acceptable recoveries and reproducibility for the emergent application testing request due to fipronil food contamination crisis.

Experimental

Target analytes

The four target analytes in this application include fipronil, fipronil sulfone, fipronil sulfoxide, and fipronil desulfinyl.

Instrument method

The samples were run on an Agilent 1290 Infinity II LC system consisting of an Agilent 1290 Infinity II binary pump (G7120A), an Agilent 1290 Infinity II high performance autosampler (G7167B), and an Agilent 1290 Infinity II thermostatted column compartment (G7116B). The UHPLC system was coupled to an Agilent G6470 triple quadrupole LC/MS system equipped with an Agilent Jet Stream electrospray ionization source. MassHunter workstation software was used for data acquisition and analysis.

HPLC conditions

Parameters	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 75 × 3.00 mm, 2.7 µm (p/n 697975-302)
Flow Rate	0.4 mL/min
Column Temperature	40 °C
Injection Volume	5 µL
Mobile Phase	A) Water B) MeOH
Gradient	**Time (min) / %B / Flowrate (mL/min)** 0 / 60 / 0.4 3.0 / 80 / 0.4 5.0 / 98 / 0.4 7.0 / 98 / 0.4 7.7 / 60 / 0.4
Post Time	2.5 minutes

MS conditions

Parameters	Value
Gas Temperature	250 °C
Gas Flow	7 L/min
Nebulizer	35 psi
Sheath Gas Heater	325 °C
Sheath Gas Flow	11 L/min
Capillary	0 V (POS) 3,500 V (NEG)
Data Acquisition	MRM as shown in Table 1.

Table 1. Target analytes MRM conditions.

Analyte	Polarity	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Fragmentor (V)	CE (V)
Fipronil Sulfone	NEG	450.9	415	135	15
			282	135	10
Fipronil	NEG	434.9	330	120	15
			250	120	30
Fipronil Sulfoxide	NEG	418.9	383	110	10
			262	110	30
Fipronil Desulfinyl	NEG	386.9	351	100	10
			282	100	35

Sample extraction

The following products were used for sample preparation.

- Agilent Bond Elut EN QuEChERS extraction kit (p/n 5982-5650)
- Agilent Bond Elut EMR—Lipid dSPE 15 mL tube (p/n 5982-1010)
- Agilent Bond Elut EMR—Lipid polish pouch (p/n 5982-0102)
- Agilent ceramic homogenizers for 50 mL tubes (p/n 5982-9313)
- Agilent Captiva nylon syringe filter, 0.2 µm, 13 mm (p/n 5190-5133)

Figure 1 shows the procedure.

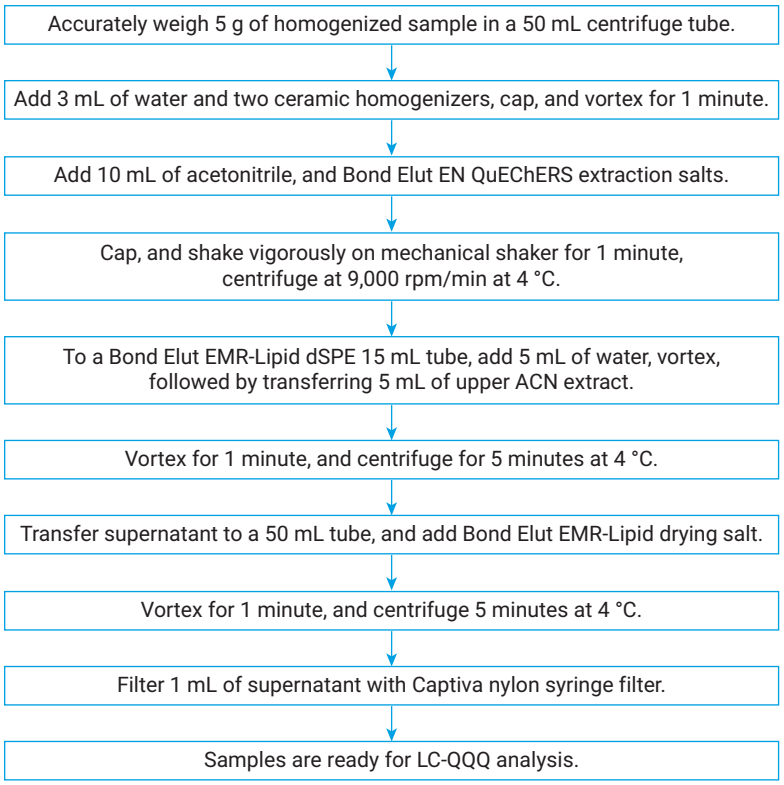

Figure 1. Sample preparation workflow chart.

Results and discussion

Table 2. Method recovery and RSDs.

Analyte	Spiking Level (µg/kg)	Chicken		Eggs	
		Recovery (%)	RSD% (n = 3)	Recovery (%)	RSD% (n = 3)
Fipronil desulfinyl	1	97.0	5.4	96.0	6.3
	5	101.5	4.0	91.1	1.2
	20	91.3	4.7	97.1	4.5
Fipronil sulfone	1	94.4	3.2	99.7	3.7
	5	101.6	3.5	98.7	5.4
	20	98.2	5.3	91.2	4.5
Fipronil sulfide	1	97.9	3.9	94.6	5.3
	5	101.2	2.1	99.9	6.1
	20	92.4	3.7	101.5	5.5
Fipronil	1	94.2	4.5	86.4	4.1
	5	99.8	4.2	97.8	5.1
	20	96.0	5.5	93.8	3.3

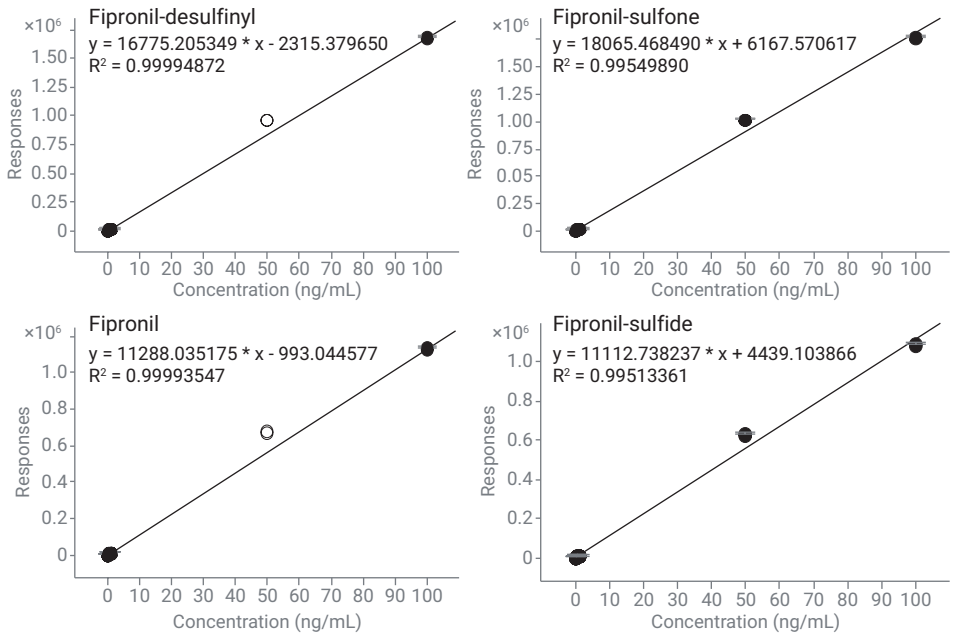

Figure 2. Calibration curves for chicken samples.

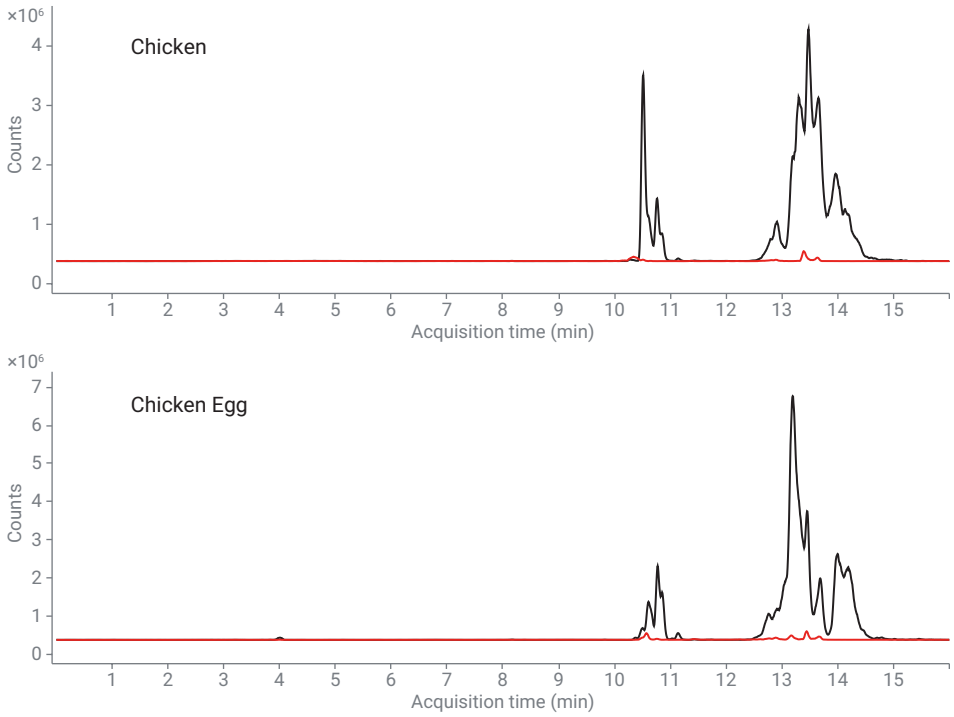

Figure 3. Phospholipids (PLs) removal from sample matrix. Black chromatogram: sample PLs profile prepared with traditional C18+PSA cleanup; red chromatograms: sample PLs profiles prepared with Bond Elut EMR-Lipid cleanup.

Conclusion

A new method using the QuEChERS extraction kit followed with Bond Elut EMR-Lipid cleanup is established for the fast and reliable analysis of fipronil and metabolites in chicken and eggs using LC/MS/MS. The method provided excellent analytes recovery and reproducibility, efficient matrix cleaning, and a simplified workflow.

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