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APPLICATION BRIEF 72483

Rapid analysis of fipronil and fipronil sulfone in eggs by liquid chromatography and triple quadrupole mass spectrometry

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Keywords

Fipronil, fipronil sulfone, eggs, LC-MS, Accucore aQ, TSQ Quantis, UltiMate 3000 RSLC

Goal

Develop a quick and simple method for the determination of fipronil and fipronil sulfone in eggs using an in-house modified QuEChERS acetonitrile extraction protocol and LC-MS/MS determination.

Introduction

Recently, it was reported that millions of eggs contaminated with the insecticide fipronil have been distributed to more than 17 countries.[1] On July 20th 2017, it was made public that in some cases the pesticide fipronil was mixed with another formulation and sprayed on chickens against ticks, fleas and lice.[1] As the determined levels were in some cases substantially higher (up to 1.2 mg/kg) than the EU MRL of 0.005 mg/kg for the sum of fipronil and fipronil sulfone,[2,3] there is a demand for quick and efficient methods for the determination of both substances in egg matrix and potentially in chicken meat.

This brief presents a quick and simple method for the determination of fipronil and fipronil sulfone in eggs using an in-house modified QuEChERS acetonitrile extraction protocol.

Experimental

Sample preparation

Egg samples purchased in a local store were extracted using the procedure described in Figure 1.

Sample preparation consumables

- 50 mL conical sterile polypropylene centrifuge tubes, P/N 339652
- 15 mL conical sterile polypropylene centrifuge tubes; P/N 339650
- Thermo Scientific™ HyperSep™ dispersive SPE Mylar pouch 4000 mg magnesium sulfate and 1000 mg NaCl, 50 pk, P/N 60105-340
- Magnesium sulfate, 99%, for analysis, anhydrous; 12198721

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- C18 pouches; P/N 60105-367-SP
- Acetonitrile LC-MS grade; A/0638/17
- Methanol LC-MS grade; M/4062/17
- Hexane for residue and pesticide analysis; 10329320
- Fisher Scientific™ Optima™ LC-MS grade water; L-16977
- Convenience kit, standard opening screw vial; P/N 60180-600

Standards of fipronil and fipronil sulfone were purchased from Lab Instruments Srl, Italy.

LC-MS conditions

Thermo Scientific™ UltiMate3000™ RSLC consisting of binary gradient pump, autosampler and thermostated column compartment was connected to a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer.

Data evaluation was performed using Thermo Scientific™ TraceFinder™ 4.1 Software.

LC-MS

Column: Thermo Scientific™ Accucore™ aQ, 100 mm × 2.1 mm; 2.6 µm; P/N 17326-102130)

Eluent: A: water
B: methanol

Injection volume: 1 µL

Column temperature: 40 °C

Table 1. Gradient used.

%B	Flow (µL/min)	Time (min)
5	300	0
5	300	0.2
70	300	1.2
100	300	3
100	300	5
5	300	5.1
5	300	8

Table 2. Mass spectrometer conditions.

Ionization mode:	Heated Electrospray (H-ESI)
Scan type:	SRM
Polarity:	Negative ion mode
Spray voltage (V):	2500
Sheath gas pressure (Arb):	30
Aux gas pressure (Arb):	6
Ion sweep gas pressure (Arb):	1
Capillary temperature (°C):	325
Vaporizer temperature (°C):	350
Cycle time (s):	0.4
Q1/Q3 resolution (FWHM):	0.7
Collision gas pressure (CID) (mTorr):	1.5
Chrom filter (sec)	6

Table 3. Selective reaction monitoring (SRM) transitions used.

Compound	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision energy (eV)	Min dwell Time (ms)	RF lens (V)
Fipronil	Negative	434.93	249.96	27.02	21	169
Fipronil	Negative	434.93	329.99	16.56	21	169
Fipronil	Negative	434.93	398.85	10.23	21	169
Fipronil Sulfone	Negative	450.93	243.85	46.17	21	231
Fipronil Sulfone	Negative	450.93	282.00	26.49	21	231
Fipronil Sulfone	Negative	450.93	415.04	15.04	21	231

Results and discussion

The recovery of the method was tested by analysis of blank egg samples spiked with fipronil and fipronil sulfone each at 0.5, 1 and 5 ng/g (n=3 at each concentration) – Table 4. The calibration was carried out using matrix matched calibration solutions prepared at 0.1, 0.5, 1, 5, 10, 50, 100 ng/mL (corresponding to 0.2-200 ng/g in eggs) by spiking blank egg extracts with standard solutions prepared in acetonitrile – see Figure 2. The chromatographic peak overlay of quantifier and qualifier ions of both fipronil and fipronil sulfone are illustrated in Figure 3.

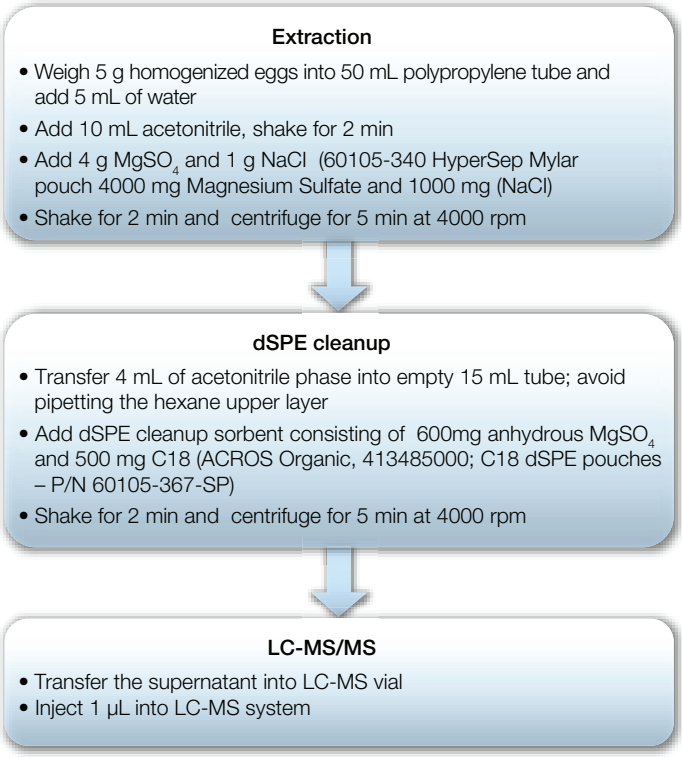

Figure 1. Sample preparation method.

The limit of quantification (LOD) of 0.5 ng/g was determined as the lowest calibration level with repeatability < 20% relative standard deviation (RSD). The limit of detection (LOD) of 0.1 ng/g was estimated using a signal to noise ratio (S/N) > 3 for a low concentration matrix matched calibration standard – see Table 5. Repeatability was tested by analysis of 6 replicates of blank egg samples spiked at 2 concentration levels - Table 5. Ion ratio stability was also tested and was determined to be < 10% - Table 6.

LC-MS/MS system robustness

The robustness of the UltiMate 3000 RSLC TSQ Quantis LC-MS/MS system was tested by analysis of 100 consecutive injections of extracts of eggs spiked at 0.5 ng/g, in one sequence without any interruption. Even after 100 injections no decrease in signal was observed, as shown in Figure 4.

Table 4. Recoveries at 3 different concentration levels.

Compound name	Recovery (%) 0.5 ng/g spike level	Recovery (%) 1 ng/g spike level	Recovery (%) 5 ng/g spike level
Fipronil	104	89	99
Fipronil sulfone	99	95	102

Table 5. Detection and quantification limits and repeatability expressed in % RSD (n=6).

Compound name	LOD [ng/g]	LOQ [ng/g]	Repeatability (%) 0.5 ng/g spike level	Repeatability (%) 5 ng/g spike level
Fipronil	0.1	0.5	8.5	6.1
Fipronil sulfone	0.1	0.5	7.7	6.4

Table 6. Ion ratio repeatability (n=6).

Compound name	Ion ratio repeatability (%) 0.5 ng/g spike level	Ion ratio repeatability (%) 5 ng/g spike level
Fipronil	9.1	4.4
Fipronil sulfone	5.8	1.8

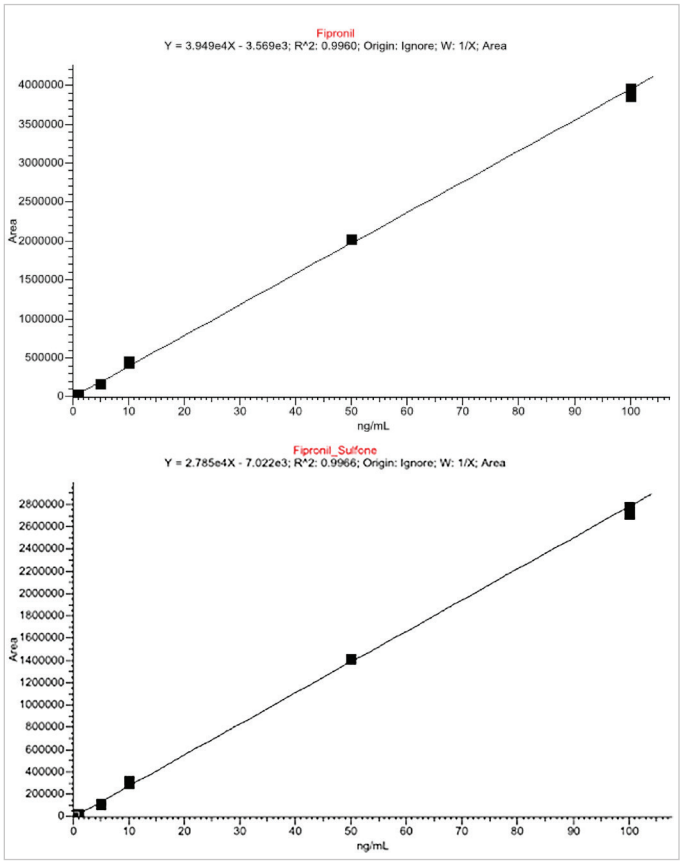

Figure 2. Matrix matched calibration curve, ranging from 0.1 to 100 ng/mL; (corresponding to 0.2-200 ng/g in eggs).

Conclusion

The study demonstrates the suitability of the of the UltiMate 3000 RSLC TSQ Quantis LC-MS/MS system for the analysis of fipronil and fipronil sulfone in egg extracts. The limit of quantification and identification of 0.0005 mg/kg for each of fipronil and fipronil sulfone is 5x below the EU statutory MRL for sum of fipronil and fipronil sulfone and the results are in full compliance with the SANTE11945/2015 analytical quality control guidelines for pesticides.[4] Sample preparation, using a modified QuEChERS protocol, takes around 15 min and allows routine high throughput analysis. Using Accucore aQ column technology, the run time could be shortened to 8 min while maintaining excellent separation efficiency and robustness. The study proves very good robustness of the system allowing uninterrupted analysis of up to 100 egg samples without the need for any system maintenance.

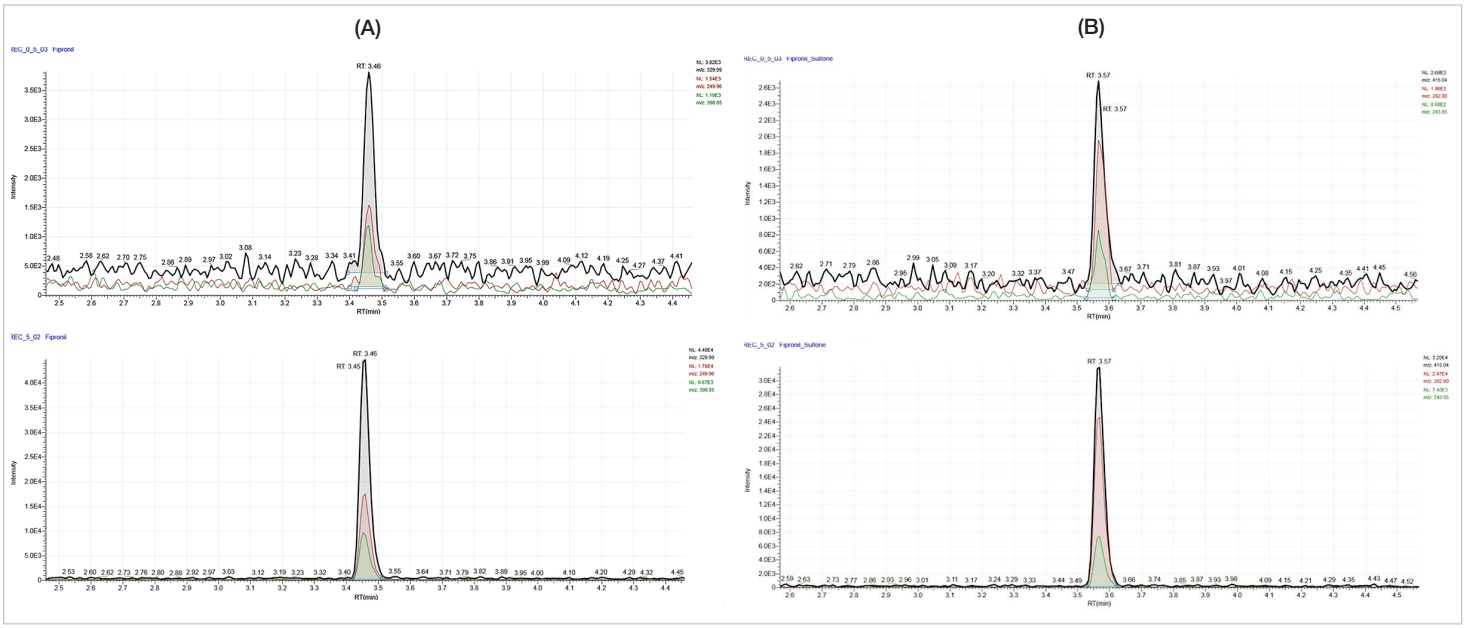

Figure 3. Quantitative and qualifier ions of fipronil (a) and fipronil sulfone (b) at 0.5 and 5 ng/g in egg matrix.

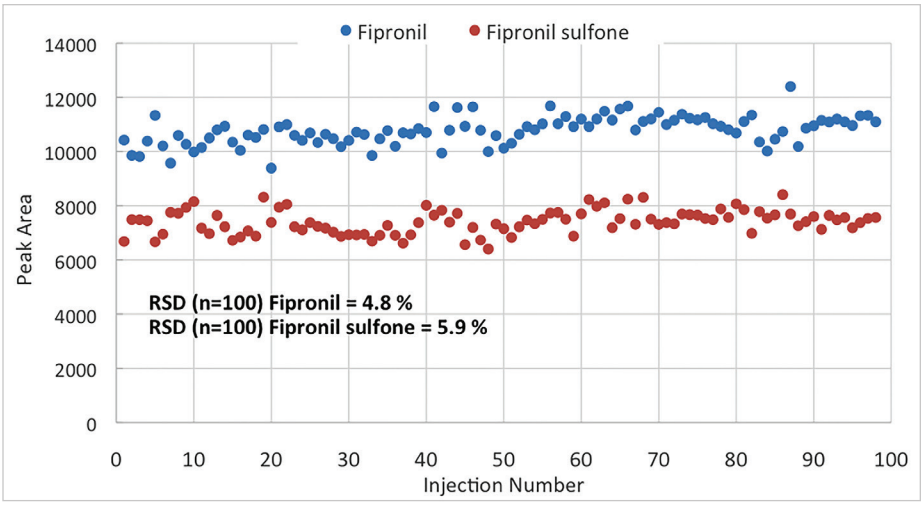

Figure 4. Long term signal stability.

References

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2. Commission Regulation (EU) No 1127/2014 of 20 October 2014 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for amitrole, dinocap, fipronil, flufenacet, pendimethalin, propyzamide, and pyridate in or on certain products Text with EEArelevance; https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_11945.pdf
3. https://webgate.ec.europa.eu/rasff-window/portal/?event=notificationDetail&NOTIF_REFERENCE=2017.1065
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