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Quality Control Products

Application Note

Analysis of Fipronil in Eggs

K. Stenerson, 8/15/2017

Background

Fipronil is a broad spectrum insecticide that is normally used to treat dogs and cats for fleas.[1] It has also been used to combat red lice in poultry, although its use with food producing animals in not permitted in the European Union (EU).[1] It is a phenylpyrazole pesticide that is classified by the World Health Organization (WHO) as moderately hazardous.[2] In early June, this pesticide was detected in eggs and chicken meat originating from Belgium, and since that time, millions of eggs have been removed from supermarket shelves throughout Europe.[3]

The maximum residue limit designated by the EU for fipronil in eggs is 5 ng/g (.005 mg/kg). Analysis to this level can be achieved using the "Quick, Easy, Cheap, Effective, Rugged and Safe" approach (QuEChERS) for extraction and cleanup. In this application, QuEChERS extraction and cleanup followed by GC/MS/MS analysis was used for the determination of fipronil in egg samples spiked at 5 ng/g.

Eggs have a relatively high fat content (approximately 10%), of which some will co-extract during the QuEChERS procedure. For a robust chromatographic analysis, a cleanup should be used which reduces these co-extracted fats. Z-Sep+, a silica sorbent functionalized with zirconia and C18, can be used to reduce fatty matrix. The zirconia interacts via Lewis acid/base interactions to retain mono and diacylglycerols, phosphoplids, and sterols. C18 retains matrix by hydrophobic interaction, thus reducing triacylglycerols as well as other fats.

Experimental

Eggs were obtained from a local grocery store and beaten until a homogeneous mixture was obtained. A 10 g sample was then weighed into a 50 mL extraction tube. Spike was added to a final concentration of 5 ng/g and allowed to equilibrate with the sample for 60 minutes. A volume of 10 mL of acetonitrile was then added, and samples were shaken for 10 minutes at 2250 rpm. The contents of a Supel™ QuE non-buffered extraction tube (55294-U) were then added to each, and the samples were shaken for 1 minute. Samples were then centrifuged at 5000 rpm for 5 minutes, and the supernatant was removed for cleanup. Cleanup was done using Supel™ QuE Z-Sep+ by depositing 1 mL of extract in a 2 mL cleanup tube or 8 mL of extract into a 15 mL cleanup tube, and shaking for 1 minute. Samples were then centrifuged at 5000 rpm for 3 minutes, and the cleaned supernatant removed for GC/MS/MS analysis. Spiked samples were quantitated against a 5-point matrix-matched calibration curve from 1 - 10 ng/mL prepared in blank egg extract. Analysis was done by GC/MS/MS following the conditions listed in **Table 1.**

Results and Discussion

Compared to no cleanup **(Figure 1)**, the Z-Sep+ sorbent reduced the background **(Figure 2)**, as seen by GC/MS scan analysis. Specifically, the cleanup reduced levels of fatty acids, fatty amide and cholesterol. The fatty acids eluted in the same retention range as fipronil, and thus have potential to interfere with low level detection. Z-Sep+ cleanup removed most of these fatty acids, resulting in a clean signal for fipronil at 5 ng/mL in the final extract **(Figure 3)**. Average recovery for spiked replicates was 94%, with an RSD of 8%, falling well within the generally acceptable ranges of 70-120% recovery and RSD < 20%.

Summary

QuEChERS extraction and cleanup can be used in the analysis of fipronil in eggs. Low level detection using GC/MS/MS was achieved with good recovery and reproducibility. Cleanup with Z-Sep+ reduced the levels of co-extracted fatty constituents, resulting in a clean analysis.

Table 1. GC/MS/MS conditions

column:	SLB®-PAHms, 30 m x 0.25 mm I.D., 0.25 µm (28340-U)
oven:	50 °C (2 min), 15 °C/min to 340 °C (10 min)
inj. temp:	250 °C
carrier gas:	helium, 1.2 mL/min, constant
detector:	MRM, 254.9/228, 350.8/254.8, 366.8/212.8
injection:	1 µL, pulsed splitless (50 psi until 0.75 min, splitter on at 0.75 min)
liner:	4 mm I.D. FocusLiner™ with taper

Figure 1. GC/MS Scan Analysis of QuEChERS Extract of Egg, Before Cleanup

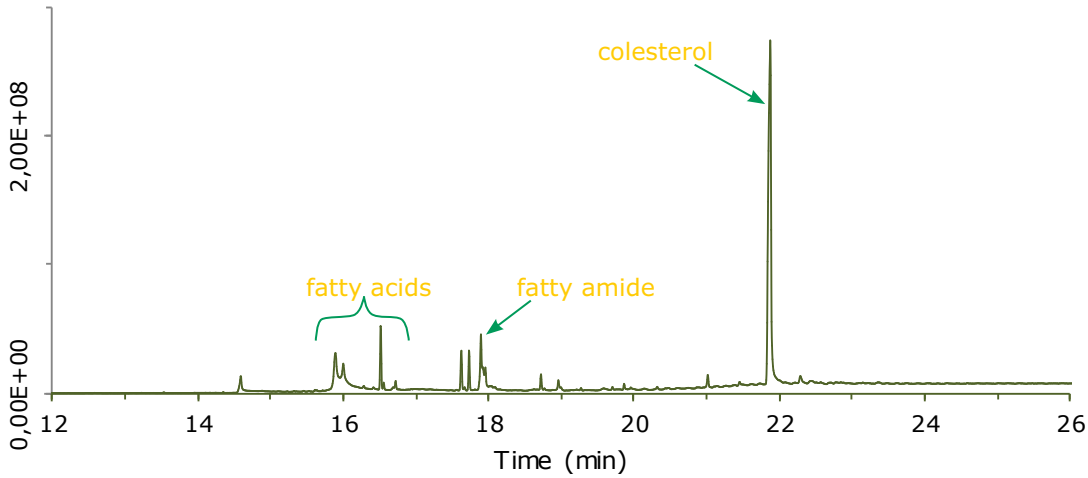

Figure 2. GC/MS Scan Analysis of QuEChERS Extract of Egg, After Cleanup With Z-Sep+

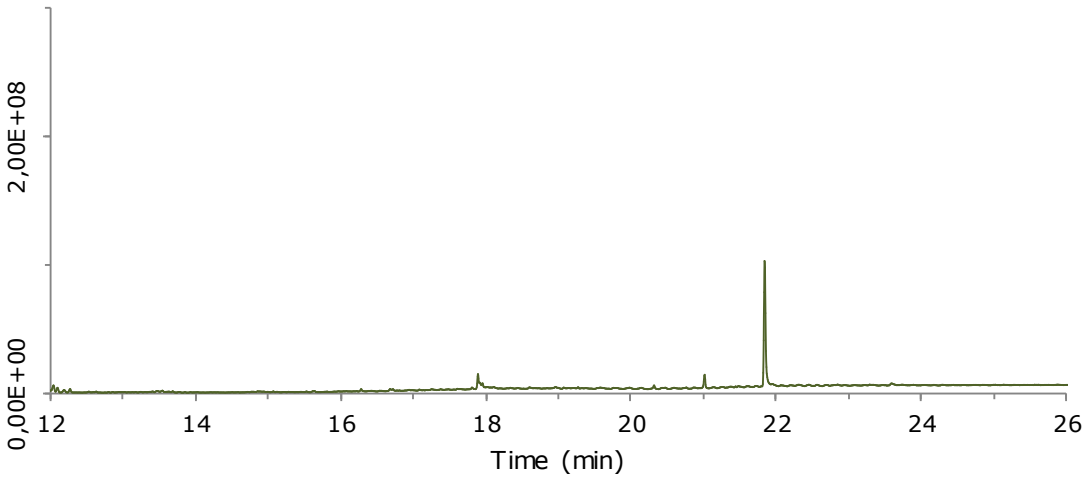

Figure 3. TIC of Fipronil from QuEChERS Extract of Egg Spiked at 5 ng/g, After Cleanup With Z-Sep+

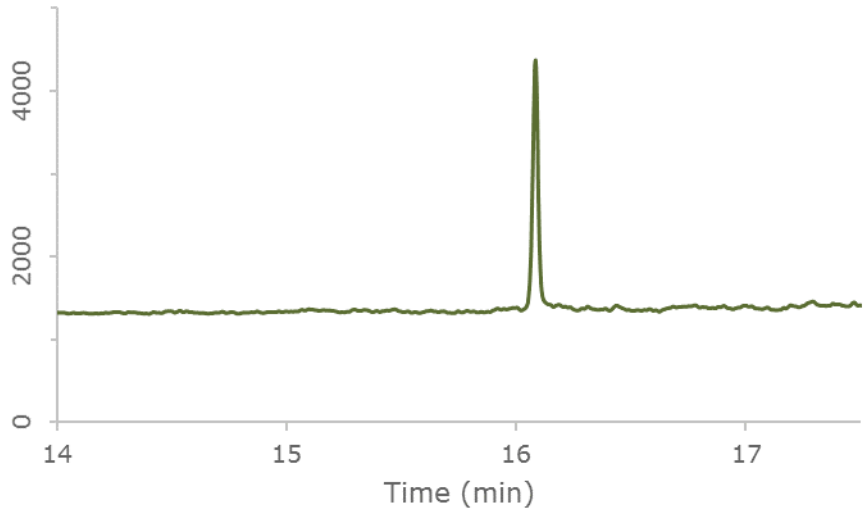

Table 2. Recovery and Reproducibility for Analysis of Fipronil From Eggs Spiked at 5 ng/g.

Fibronil	4.7	94%	8%

References

1. Fipronil Egg Scandal: What We Know. bbc.com, 8/11/2017 (accessed 8/15/17).
2. The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 2009; International Program on Chemical Safety, World Health Organization, 2010.
3. 6 Countries Warned of Eggs Contaminated with Pesticide. CBS news.com, 8/8/2017 (accessed 8/15/17).

Featured Products

Description	Cat. No.
SLB®-PAH ms, 30 m x 0.25 mm I.D., 0.25 µm	28340-U
Empty 50 mL tubes for extraction	55248-U
Supel™ Que nonbuffered extraction tube #1	55294-U
Supel™ QuE Z-Sep + Tube, 2 mL	55408-U
Supel™ QuE Z-Sep + Tube, 15 mL	55486-U
QuEChERS shaker and rack starter kit, 115V	55278-U
QuEChERS shaker and rack starter kit, 230V	55438-U
Fipronil, TraceCERT®, certified reference material	16785-50mg
Fipronil, Pestanal®, analytical standard	46451-100mg

Related Products

Description	Cat. No.
FocusLiner™, 4 mm ID with taper (for Agilent®)	2879901-U
LB-2 molded septa, 11 mm	28676-U
Inlet Liner O-Rings for Agilent® GC	21003-U
Gold-plated inlet seal for Agilent® GC	23318-U
Capillary column nut for Agilent® MS	28034-U
Low Adsorption Vials w/inserts, amber. PTFE/ Silicone septa	29663-U
SupraSolve® Acetonitrile for GC/MS	1.00665.1000

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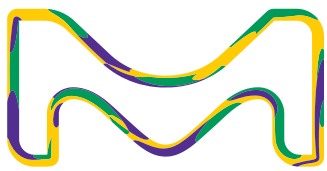

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