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Application News

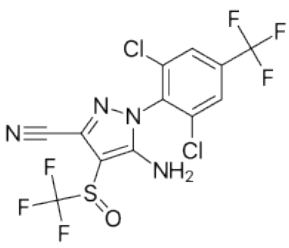
No. SCA_210_041

Liquid Chromatography Mass Spectrometry

Sensitive method for the determination of Fipronil and its metabolite Fipronil Sulfone in egg using QuEChERS sample pretreatment and LC-MS/MS detection [LCMS-8060]

▪ Introduction

Fipronil concerns a broad-spectrum insecticide from the group of phenylpyrazoles used in many countries as a biocide and plant protection product against fleas, lice, ticks, cockroaches, mites and other insects. Fipronil is an active compound in veterinary products fighting tick and flea infestations in dogs and cats. The use as plant protection product is restricted to seed treatment in the European Union since 2007. However, due to the illegal use as addition to the cleaning supplies used in chicken coops the eggs and meat might get contaminated as well.
The MRL (maximum residue levels) for Fipronil and and its metabolite Fipronil sulfone (which is classified as having similar toxicity) in eggs is set to 0.005 mg/kg by the EU (by definition the sum of fipronil and fipronil-sulfone expressed as fipronil) [1], so that there is an actual requirement for the determination of both compounds in egg matrix at a relatively low level.
This application news presents a simple method using a standard QuEChERS extraction protocol followed by LC-MS/MS detection.



Fipronil
MF $C_{12}H_4Cl_2F_6N_4OS$
MW 437,1 g/mol

▪ Sample preparation

Compound extraction was performed using a simple QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method.
5 g of egg (egg white and egg yolk) were weighted into a 50 mL polypropylene tube, diluted with 5 mL of water and spiked with a respective amount of Fipronil, Fipronil sulfone and in addition Fipronil-desulfinyl and Fipronil-sulfide (neochema, Germany).
10 mL of acetonitrile was added and the samples were mixed vigorously. After that ready to use QuEChERS extraction salts (Q-sep™ Q110, Pouch and tubes – cat. #26235, Restek) were added for sample drying and buffering. Samples were mixed again and centrifuged at 4500 rpm for 5 minutes. 1 mL of the supernatant was transferred into a dSPE tube (Q-sep™ QuEChERS dSPE – cat. #26217, Restek), shaken for 2 minutes, centrifuged, the supernatant was transferred into a glass vial and the pH was adjusted with 5% formic acid solution in acetonitrile (10 µL/mL super-natant).

▪ Materials and methods

Extracts were analyzed using a method set up with Shimadzu's LC/MS/MS Method Package for Residual Pesticides Version 2 and a Nexera X2 UHPLC system coupled to a LCMS-8060 mass spectrometer. Analysis was carried out using MRM (Multi Reaction Monitoring) mode.

LC system	Nexera X2 (Shimadzu, Japan)
Analytical column	Raptor Biphenyl™ 100 x 2.1 mm, 2.7 µm (RESTEK)
Column oven temperature	35 °C
Injection volume	2 µl
Mobile Phase A	2 mM ammonium formate + 0.002% formic acid - Water
Mobile Phase B	2 mM ammonium formate + 0.002% formic acid - Methanol

Mass spectrometer	LCMS-8060 (Shimadzu, Japan)
Interface voltage	-3 kV
Q1 resolution	Unit (0.7 Da FWHM)
Q3 resolution	Unit (0.7 Da FWHM)
Nebulizing gas flow	3 L/min
Drying gas flow	10 L/min
Heating gas flow	10 L/min
DL temperature	150 °C
Heat block temperature	300 °C
Interface Temperature	350 °C

In addition, the so-called "MRM spectrum mode" was used for analysis. Here, not only the fragments of the quantifier and the qualifiers are determined, but also a higher number (typically 6-10) of MRM fragment ions. Using this MRM spectrum mode, conventional MRM quantification is combined with a high-quality MRM product ion spectrum, which can be used in a library search routine, thus increasing the specificity and verification of results (Figures 1 and 2).

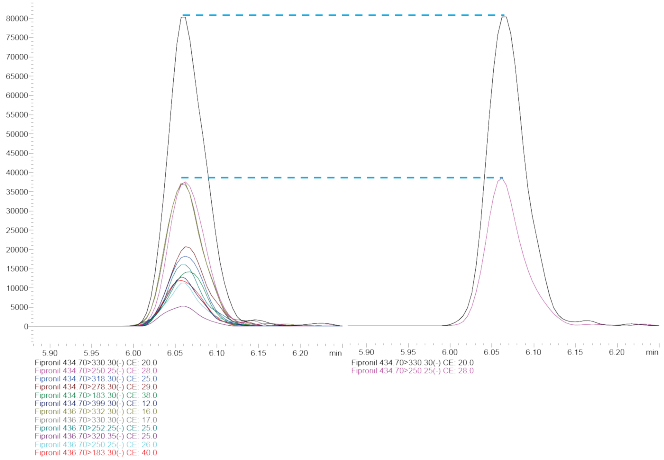


Figure 2: The figure shows MRM chromatograms for Fipronil, one recorded with the usual 2 fragment ions, and compared with a method with higher number (12) of fragment ions which, despite this fact, have the same sensitivity.

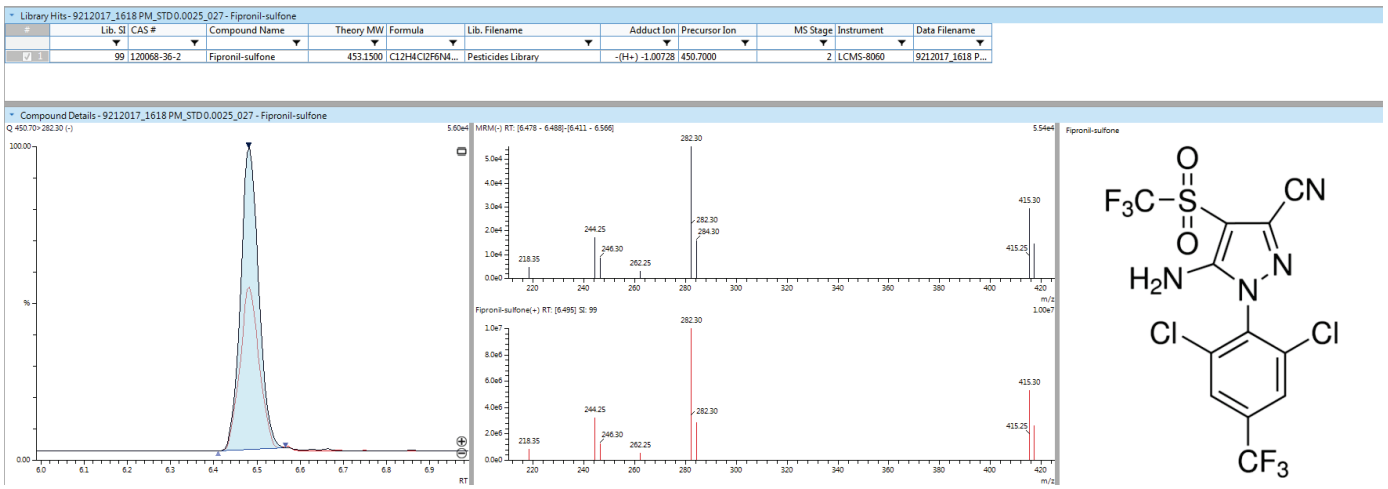


Figure 1: Result of the library search, presented with LabSolutions Insight Screening software

▪ Calibration

The matrix matched calibration curve (Figure 3-6) was prepared according to the method described before ranging from 0.0005 mg/kg to 0.05 mg/kg.

Control samples at 0.001 mg/kg and 0.01 mg/kg correspond to the calibration curve.

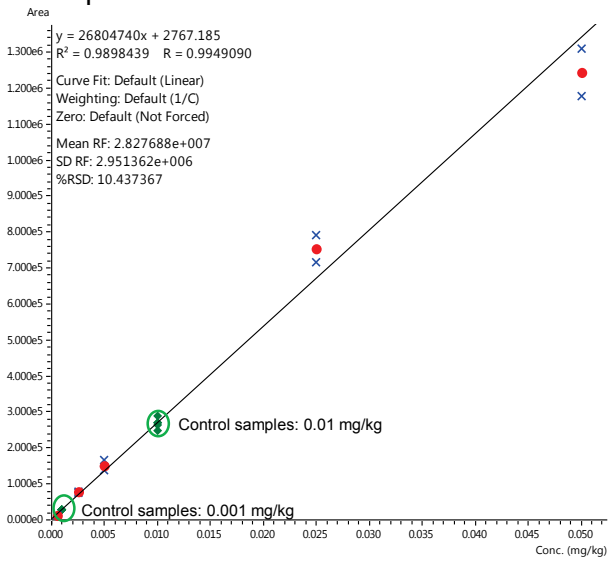


Figure 3: Calibration curve of Fipronil in egg ranging from 0.0005 mg/kg to 0.05 mg/kg

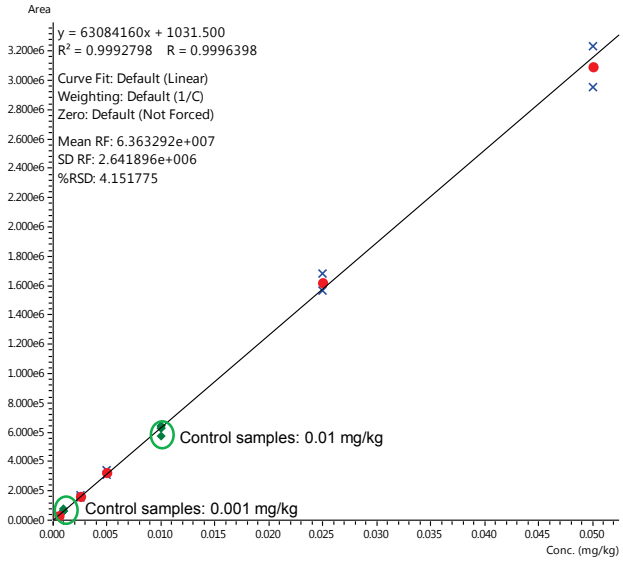


Figure 4: Calibration curve of Fipronil-sulfide in egg ranging from 0.0005 mg/kg to 0.05 mg/kg

[1] EU Comission Regulation 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 in regards to maximum residual levels for amitrole, dinocap, fipronil, flufenacet, pendimethalin, propyzamide and pyridate in or on certain products.

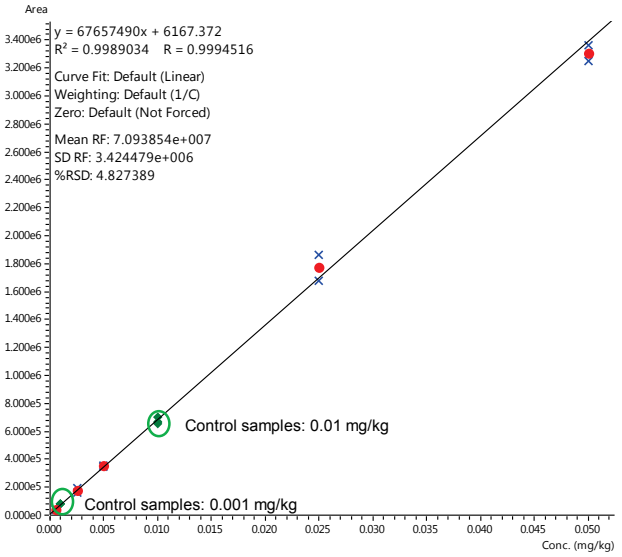


Figure 5: Calibration curve of Fipronil-sulfone in egg ranging from 0.0005 mg/kg to 0.05 mg/kg

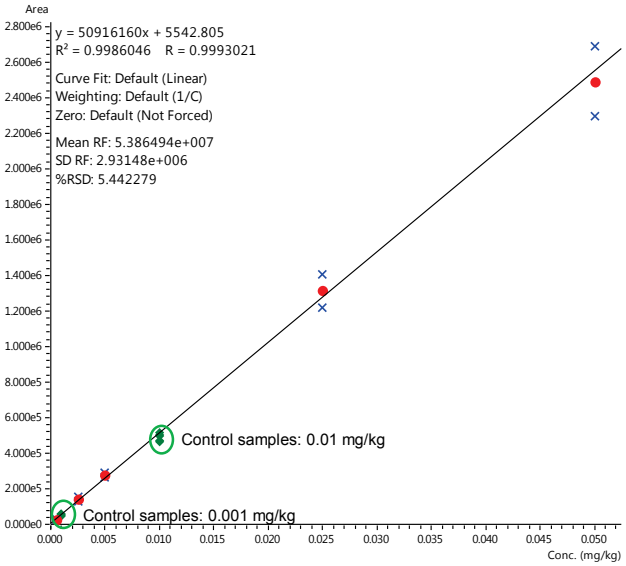


Figure 6: Calibration curve of Fipronil-desulfinyl in egg ranging from 0.0005 mg/kg to 0.05 mg/kg

▪ Conclusion

By using the LC/MS/MS method package for residual pesticides V2 and a QuEChERS sample preparation a method for the determination of Fipronil and Fipronil-sulfone in eggs below the requested MRL of 0.005 mg/kg could be set up rapidly without further method development.

Shimadzu Europa GmbH

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