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Application News

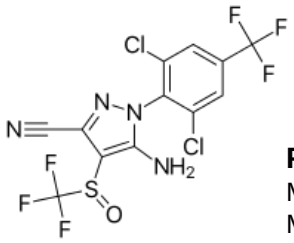
No. SCA_210_038

Liquid Chromatography Mass Spectrometry

Sensitive method for the determination of Fipronil in egg using UHPLC-MS/MS [LCMS-8060]

▪ Introduction

The broad-spectrum insecticide Fipronil from the group of phenylpyrazoles is used in many countries as a biocide and plant protection product against fleas, lice, ticks, cockroaches, mites and other insects. The use as plant protection product is restricted to seed treatment in the European Union since 2007. It is also an active compound in veterinary products fighting tick and flea infestations in dogs and cats. But its use in food-producing animals is not permitted. However, due to the illegal use as addition to the cleaning supplies used in poultry farm the eggs, egg products and meat were found to be contaminated in summer 2017 in 15 European countries.



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

▪ Sample preparation

Compound extraction was performed using a simplyfied 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 and spiked with a respective amount of Fipronil (and other pesticides using dilutions of RESTEK LC Multiresidue Pesticide Standard #5, Cat. 31976). 5 mL of acetonitrile was added and the samples were mixed vigorously. After that 2 g of $MgSO_4$ and 0.5 g of NaCl were added, samples were mixed again and centrifuged at 3000 rpm for 5 minutes. The supernatant was transferred into a glass vial.

▪ Materials and methods

Extracts were analyzed using a method set up with Shimadzus 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 (using POISe*)
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

*Performance Optimising Injection Sequence

▪ Calibration

The matrix matched calibration curve (Figure 1) was prepared according to the method described before ranging from 0.025 mg/kg to 2 mg/kg.

Control samples at 0.05 mg/kg and 0.5 mg/kg correspond to the calibration curve. Figure 2 shows a typical chromatogram of the lowest calibration point (0.025 mg/kg)

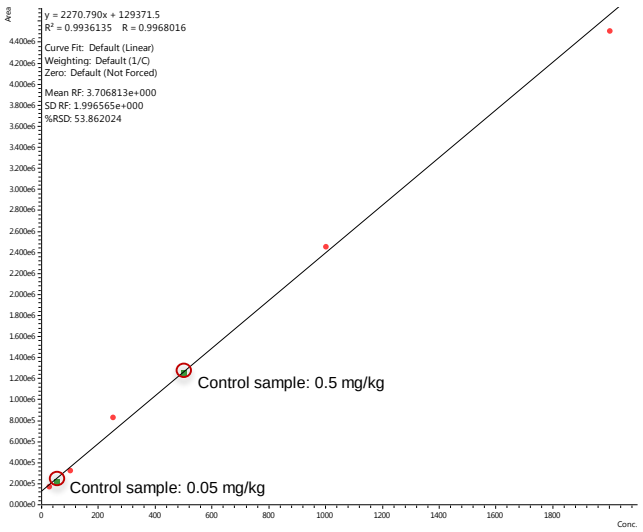


Figure 1: Calibration curve of Fipronil in egg ranging from 0.025 mg/kg to 2 mg/kg

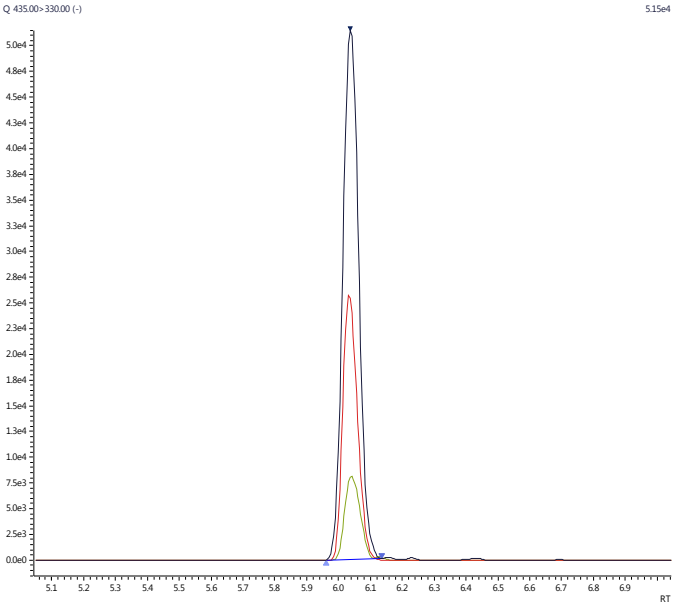


Figure 2: Chromatogram of Fipronil in egg at a concentration of 0.025 mg/kg

▪ Conclusion

By using the LC/MS/MS method package for residual pesticides V2 and a simplyfied QuEChERS sample preparation a method for the determination of Fipronil in eggs could be set up rapidly without further method development covering the calibration range from 0.025 mg/kg to 2 mg/kg.

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