MACHEREY-NAGEL
Chromatography application note

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Determination of fipronil in eggs

TeLA GmbH · F. Chmelka
MACHEREY-NAGEL application department · Dr. H. R. Wollseifen

Abstract

This application note describes the determination of fipronil in eggs using dispersive SPE (QuEChERS, dSPE) for sample clean-up. The eluates from dSPE cleaning are finally analyzed by HPLC-MS/MS.

Introduction

Fipronil is a broad-spectrum insecticide that is used very effective on a large number of pests. This insecticide is often the active ingredient in flea control products for pets [1], in home roach traps or in field pest control for corn. It belongs to the phenylpyrazole chemical family and disrupts the central nervous system of the insect [2]. The insecticide fipronil was surprisingly determined in eggs from Belgium and the Netherlands in July 2017 and the amounts from 0.0031 to 1.2 mg/kg eggs were published in the European Rapid Alert System for Food and Feed [3]. According to regulation 396/2005 on maximum residue levels of pesticides, the maximum value for fipronil (sum of fipronil and sulfone metabolites) in eggs is 0.005 mg/kg [4]. The European food industry is in an uncertain position and must identify contaminated eggs and polluted egg containing products. Customers are asking for elucidation of the food scandal!

QuEChERS ("Quick, Easy, Cheap, Effective, Rugged and Safe") methodology is a common sample preparation technique in modern analysis of pesticides in food. Using this special SPE phase allows a quick and cost-efficient determination of pesticides in strongly matrix-contaminated samples by GC-MS and LC-MS.

In this application note a QuEChERS method for the determination of fipronil in eggs was developed. The method follows the recommendations introduced by Anastassiades et al. in 2003 [5]. The identification and quantification of fipronil were finally carried out by ESI mass spectrometry on a NUCLEOSHELL® Bluebird RP 18 column.

Fipronil

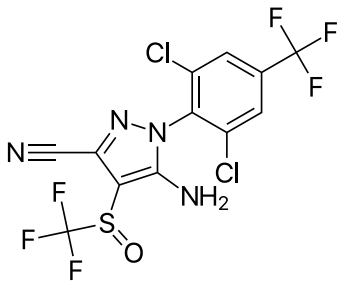

Figure 1: Compound of interest.

Sample pretreatment for dispersive solid phase extraction (dSPE)

- Weigh 10 g sample into a centrifuge tube
- Add 10 mL acetonitrile and internal standard solution
- Agitate
- Add QuECHERS-Mix I (REF 730970) and agitate
- Centrifuge
- Take an aliquot of the organic phase and subject it to an additional QuEChERS-Mix (e.g. QuEChERS-Mix III, REF 730972)
- Agitate
- Centrifuge
- Dilute sample extract 1:2 with water ultrapure before injection into LC-MS

Subsequent analysis: HPLC-MS / MS

Chromatographic conditions:

Column:
EC 100/2 NUCLEOSHELL® Bluebird RP 18, 2.7 µm
(REF 763434.20)

Eluent A: water

Eluent B: acetonitrile

Gradient: hold 20 % B for 2 min, in 9 min to 95 % B, back to 20 % B in 0.1 min, hold for 4.9 min

Flow rate: 0.4 mL/min

Temperature: 45 °C

Injection volume: 5 µL

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MS conditions:

6470 Triple Quad, ion source ESI, positive ionization mode, scan type MRM

MRM transitions

Analyte	$[M+H]^+$	Q1 (Quantifier)	Q2 (Qualifier)
Fipronil	454	290	368

Table 1: MRM transitions for the analysis of fipronil.

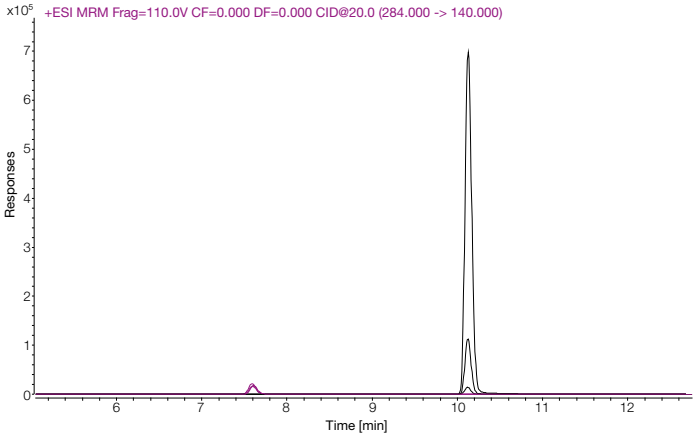

Figure 2: Chromatograms of egg sample spiked with 50 µg/kg metazachlor-d_6 (RT: 7.6 min) and 10, 100, 1000 µg/kg fipronil (RT: 10.1 min).

Calibration curve

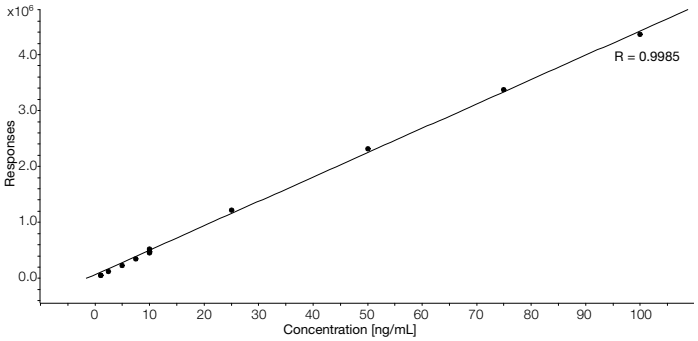

Figure 3: Calibration curve in concentration range between 1 ng/mL and 100 ng/mL.

Recovery rates

Sample pretreatment	Recovery rate in %
dSPE	92–100

Table 3: Recovery rate for presented dispersive solid phase extraction method.

Method validation

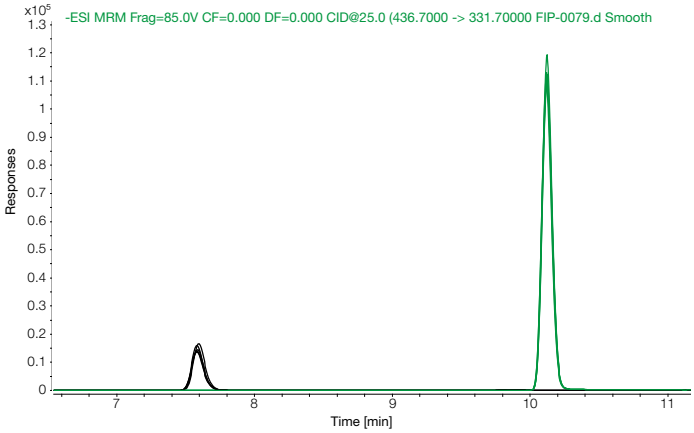

Figure 4: Overlay of 5 chromatograms of egg sample each spiked with 50 µg/kg metazachlor-d_6 (RT: 7.6 min) and 100 µg/kg fipronil (RT: 10.1 min).

Conclusion

The results show that the determination of fipronil from eggs could be carried out successfully with all the tested products. By using QuEChERS methodology it was possible to recover more than 92 % of fipronil from eggs. A correction with the internal standard metazachlor-d_6, which was chosen because of its structural similarity, was not necessary. The sample preparation method was validated by extracting 5 samples that were spiked with 50 µg/kg metazachlor-d_6 and 100 µg/kg fipronil. Figure 4 shows that the chromatograms of these extracts are congruent. The QuEChERS methodology leads to analytical results with very high reproducibility. The identification and quantification of fipronil in the cleaned sample extracts were carried out by ESI mass spectrometry on an EC 100/2 mm, 2.7 µm NUCLEOSHELL® Bluebird RP 18 column. The chromatographic results are presented in figure 2. The chromatograms show the results of the extraction of egg samples spiked with 10, 100 and 1000 µg/kg fipronil. With regard to the linearity of quantification by mass spectrometry detection, a calibration curve was performed in the concentration range between 1 ng/mL to 100 ng/mL. The response of standard solutions with concentrations higher than 50 ng/mL fipronil presents saturation effects. In summary the presented application describes a quick and convenient method for the determination of fipronil in eggs.

References

1. M.W. Dryden, T. M. Denenberg, S. Bunch, Veterinary Parasitology, Volume 93, Issue 1, 1st November 2000, Pages 69-75.
2. D. Hainzl, L.M. Cole, J.E. Casida, Chemical research in toxicology, 1998 - ACS Publications.
3. https://webgate.ec.europa.eu/rasff-window/consumers/?event=getListByCountry&country=DE
4. REGULATION (EC) NO 396/2005 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 23rd February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.
5. M. Anastassiades, S. J. Lehotay, D. Stajnbaher, F. J. Schenck, J. AOAC Int. 86 (2003), 412–431.

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Additional information

The following applications regarding “Determination of fipronil in eggs” and further applications can be found on our online application database at *www.mn-net.com/apps*

SPE:	MN Appl. No. 306550
HPLC:	MN Appl. No. 128250

Product information

The following MACHEREY-NAGEL products have been used in this application note:

REF 763434.20, EC 100/2 NUCLEOSHELL® Bluebird RP 18, 2.7 µm
REF 730970, CHROMABOND® QuEChERS extraction Mix I
REF 730972, CHROMABOND® QuEChERS clean-up Mix III
REF 730223, CHROMABOND® centrifuge tubes with screw cap, 50 mL
REF 702293, Screw neck vials N 9, 1.5 mL
REF 702107, N 9 PP Screw cap, yellow, center hole, silicone white / PTFE red

Acknowledgment

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