Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1146 (2007) 8–16
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Comparative study of sample preparation procedures to determine fipronil in pollen by gas chromatography with mass spectrometric and electron-capture detection☆

J.J. Jiménez*, J.L. Bernal, M.J. del Nozal, M^a.T. Martín, R. Mayo

Department of Analytical Chemistry, Faculty of Sciences, University of Valladolid, Prado de la Magdalena s/n, 47005 Valladolid, Spain

Received 21 November 2006; received in revised form 19 January 2007; accepted 25 January 2007
Available online 5 February 2007

Abstract

Several sample preparation methods have been assayed to analyze residues of fipronil in pollen at trace concentrations. Extraction with organic solvents, solid-phase extraction on either commercial cartridges or a Florisil-packed column and a matrix solid-phase dispersion, also with Florisil as a dispersing agent, have been tested. Determination of fipronil in the extracts has been carried out by GC with electron-capture and mass spectrometric detection. An extraction with acetonitrile followed by a clean-up on ODS or polymeric cartridges was the most suitable procedure to obtain acceptable recoveries and relatively simple chromatograms. The matrix-effects observed in the quantification can be corrected with a matrix-matched calibration.

Keywords: Gas chromatography; Matrix-effect; Fipronil; Pollen

1. Introduction

Fipronil is a systemic insecticide that is being used to control several pests in crops. It is considered highly toxic to the adult bee, the 50% lethal dose (LD_{50}) ranges between 0.0037 and 0.006 μg/bee [1]. Due to the fact that honey-bees can get in touch with fipronil from plants through the pollen and nectar collected in the foraging activity, it would be possible that the presence of residues of this compound could explain one of the most current problems: the declining honey-bee population. In addition, it has also been proved that fipronil at sublethal doses affects not only the feeding activity but also the ratio between the number of inactive bees and the number of active bees that reflects the restlessness of the bees visiting a contaminated feeding source [2]. Fipronil has been detected in some pollen samples after carrying out a multiresidue analysis [3], for this reason, analytical methods for the reliable identification and quantification of fipronil in pollen at concentrations about μg/kg are necessary.

Regarding this matter, fipronil can be present in human consumption products arisen from the beehive. The toxicity of fipronil in humans is not a primary concern. It has been classified as a moderately hazardous compound by the World Health Organization [4].

In the bibliography, there are a few articles devoted to the analysis of pesticide residues in pollen: methods for carbaryl [5,6], benomyl and carbendazim [7], carbaryl and parathion-methyl [8], captan and difenoconazole [9], 2-chloroethanol [10], fluvalinate [11], imidacloprid [12,13] and vinclozolin [14] have been published. Extractions are commonly made with solvents, such as acetonitrile, ethyl acetate or dichloromethane and the subsequent clean-up consists of liquid–liquid partitionings and solid-phase extractions.

In relation to the determination of fipronil in pollen, only two works have been found. One of them is based on matrix solid-phase dispersion on Florisil columns combined with an ultrasonic shaking of the columns [15]. In the other work, fipronil was detected in pollen after an extraction with acetone and purification on alumina and immunologic cartridges [3].

In the manuscripts devoted to analyze fipronil in other matrixes, such as soil, water or vegetables and, obviously, according to its chemical structure, the determination in the

☆ Presented at the 6th Meeting of the Spanish Society of Chromatography and Related Techniques, Vigo, 8–10 November 2006.

* Corresponding author. Tel.: +34 983423262; fax: +34 983423013.
E-mail address: jjimenez@qa.uva.es (J.J. Jiménez).

0021-9673/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2007.01.117

extracts is mainly done by gas chromatography (GC) with electron-capture or mass spectrometric detection [16–19].

In this manuscript, several methods (solid–liquid extraction with different solvents, solid-phase extraction on minicolumns and on Florisil-packed columns, and matrix solid-phase dispersion) are tested and compared for the extraction and clean-up of fipronil residues from pollen. The extracts are analyzed in two different chromatographs equipped with the most commonly used detection systems: electron-capture and mass spectrometry. The surprisingly high fipronil concentrations measured in a chromatograph are attributed to the influence of the matrix and corrected by a matrix-matched calibration.

2. Experimental

2.1. Material and reagents

Residue analysis grade *n*-hexane, acetonitrile, acetone, ethyl acetate, methanol and dichloromethane were supplied by Lab-Scan (Dublin, Ireland). Ultrapure water was obtained from a Milli-Q plus apparatus (Millipore, Milford, MA, USA). Fipronil and polychlorinated biphenyl (PCB) 138 certified standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Mechanical shakers were obtained from Selecta (Barcelona, Spain) and an RE-111 rotary evaporator from Büchi (Plawil, Switzerland)). A 5810R centrifuge was purchased from Eppendorf (Hamburg, Germany). For sample preparation, PTFE disposable syringe filter units, 0.50 μm pore size, were obtained from Microfiltration Systems (Dublin, CA, USA). Industrial gases (99.999% minimum purity) were supplied by Carburos Metálicos (Barcelona, Spain). For SPE, 200 mg strata X and 500 mg SDB-L polymeric sorbent cartridges and 500 mg C18M and C18E ODS cartridges from Phenomenex (Torrance, CA, USA), 500 mg phenyl cartridges from Varian (Harbor City, CA, USA), and 200 mg Oasis HLB from Waters (Milford, MA, USA) were purchased. A VacMaster sample processing station from International Sorbent Technology (Mid Glamorgan, UK) was used. Florisil of 60–100 mesh was supplied by Fisher Scientific Company (Springfield, NJ, USA) to pack glass chromatographic columns. Sodium hydroxide and hydrochloric acid were obtained from Panreac (Barcelona, Spain).

2.2. Samples and spiking

Pollen pellets collected from pollen traps placed in the beehives were supplied by the Centro Apícola Regional from Junta de Comunidades de Castilla La Mancha (Marchamalo, Guadalajara, Spain). Pollen was dehydrated by extending it on a paper sheet and leaving it for about 72 h at 25 °C. Then, it was stored in closed glass containers at room temperature and darkness until analysis. The pollen pellets were powdered in a glass mortar and spiked just before analysis. A volume of 50 μL of a solution of fipronil in acetone was added to 1 g of the powered pollen sample to achieve a concentration of 200, 7 or 0.7 μg/kg, then the sample was subjected to the sample preparation.

Pollen samples without spiking were also analyzed by the corresponding procedures to obtain blank chromatograms and ensure that the sample was free of fipronil residues.

2.3. Extraction with organic solvents

A pollen-amount of 1 g was placed in a threaded 20 mL glass tube and extracted with 10 mL of solvent by mechanical shaking for 10 min. The liquid phase was separated by centrifugation at 3500 g for 5 min and, then, collected. The sample was again extracted with 10 mL of solvent and the organic phase was also isolated and collected. The two portions collected were combined and the solvent was evaporated in a rotary evaporator at 35 °C under vacuum. Finally, the residue was dissolved with sonication in 1 mL of acetone – containing the internal standard – and passed through a PTFE filter of 0.50 μm pore size. Methanol, ethyl acetate, acetonitrile, acetone, dichloromethane and *n*-hexane were assayed as extracting solvents. These solvents were selected to cover a polarity range and according to the literature devoted to the analysis of pesticide residues in pollen.

Some liquid–liquid partitionings were used as clean-up after the solid–liquid extraction. Thus, ethyl acetate (20 mL extract) was partitioned with two portions of 50 mL of water at pH 2, 7 or 12 (adjusted with HCl or NaOH), shaking for 10 min; then, the organic phase was collected and evaporated as above-mentioned and the residue dissolved in 1 mL of acetone.

In the extractions with acetonitrile, methanol or acetone, 200 mL of water were mixed with the 20 mL extract placed in a separatory funnel and fipronil was re-extracted with two portions of 10 mL of *n*-hexane or dichloromethane, shaking for 10 min. After separating the phases, these two organic portions were combined, and as in the previous experiments, the extract was taken to 1 mL in acetone and filtered through PTFE.

2.4. Solid-phase extraction on cartridges

The extract of 1 g pollen obtained with 2 × 10 mL of acetonitrile (or methanol) was added to 200 mL water and the mixture was eluted through a cartridge at a flow-rate of about 6 mL/min; the cartridge was previously conditioned by successive elution of 10 mL of methanol and 10 mL of water, both by gravity. After that, the stationary phase was washed with 10 mL of water and the cartridges were dried with nitrogen for about 20 min. Finally, fipronil was eluted with 3 mL of methanol by gravity. Experiments have been made with cartridges containing different stationary phases: Oasis HLB, strata X, ODS, phenyl and SDB.

In some experiments made with C18M and Oasis cartridges, 100 mL of water were added to a 20 mL acetonitrile extract to vary the acetonitrile percentage in the mixture from about 9 to 17%. On the other hand, the following volumes of acetonitrile–water mixture were eluted on C18 M and Oasis cartridges: 20 mL extract + 200 mL water, 10 mL extract + 100 mL water and 5 mL extract + 50 mL water; in these latter cases, extractions were made with 2 × 10 mL of acetonitrile and an aliquot of extract was diluted with water.