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Determination of fipronil residues in honey and pollen by gas chromatography

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Abstract

This work describes the development of the analytical methodology for determining fipronil residues in honey and pollen samples by gas chromatography with two detecting systems: electron-capture detection (GC-ECD) and mass spectrometry (GC-MS). Fipronil was extracted from honey samples by solid-phase extraction, using alumina as adsorbent and hexane-ethyl acetate (1:1, v/v) as eluting solvent, and from pollen samples by matrix solid-phase dispersion with C_{18} and acetonitrile, assisted by sonication. The developed methods gave recovery results > 90% with relative standard deviations < 6% in both matrices. The determination of residue levels was carried out with matrix matched calibration standards, to counteract the matrix effect observed, and heptachlor as internal standard. The limits of detection obtained with GC-ECD were 1 μg kg^{-1} for pollen and 0.5 μg kg^{-1} for honey and the corresponding values for GC-MS were 0.2 and 0.1 μg kg^{-1}, respectively. These methods were applied to the determination of fipronil levels in various samples of honey and pollen commercialised in Spain and no residues of this compound were detected.

Additional key words: apiculture, GC-ECD, GC-MS, pesticide residues.

Resumen

Determinación de residuos de fipronil en miel y polen por cromatografía de gases

Este trabajo describe el desarrollo de la metodología analítica para determinar residuos de fipronil en muestras de miel y polen por cromatografía de gases con dos sistemas de detección: espectrometría de masas (GC-MS) y detector de captura de electrones (GC-ECD). El pesticida fipronil se extrajo de las muestras de miel mediante extracción en fase sólida, con alúmina como adsorbente y hexano-acetato de etilo (1:1, v/v) como disolvente de elución, y de las muestras de polen por medio de la dispersión de la matriz en fase sólida, C_{18}, y extracción en baño de ultrasonidos con acetonitrilo. Los métodos desarrollados dieron recuperaciones > 90% con desviaciones estándar relativas < 6% en ambas matrices. En la determinación de los residuos, se utilizaron patrones de calibración obtenidos enriqueciendo extractos de muestras control, con la finalidad de contrarrestar el efecto matriz observado, y heptacloro como patrón interno. Los límites de detección obtenidos con GC-ECD fueron 1 μg kg^{-1} para el polen y 0,5 μg kg^{-1} para la miel y los valores correspondientes para GC-MS fueron 0,2 y 0,1 μg kg^{-1}, respectivamente. Estos métodos fueron aplicados a la determinación de fipronil en diversas muestras de miel y polen, comercializadas en España, y no se detectaron residuos de este compuesto.

Palabras clave adicionales: apicultura, GC-ECD, GC-MS, residuos de plaguicidas.

Introduction[1]

Fipronil, 5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile, is an extremely active insecticide from the phenyl pirazole family that acts disrupting the insect's central nervous system, blocking the passage of chloride ions through the gamma aminobutyric acid receptor. This compound is used worldwide to control different agricultural pests, such as lepidopterous, orthopterous, and coleopterous insects, and against ticks and mites on domestic animals. Fipronil is a systemic insecticide that may reach the

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[1] Abbreviations used: ECD (electron-capture detector), GC-ECD (gas chromatography-electron capture detection), GC-MS (gas chromatography-mass spectrometry), LOD (limit of detection), LOQ (limit of quantitation), MS (mass spectrometry), MSD (mass spectrometric detector), MSPD (matrix solid-phase dispersion), RSD (relative standard deviation), SIM (selected ion monitoring), SPE (solid-phase extraction), SPME (solid-phase microextraction).

flowers, where it can come in contact with bees when they gather pollen and nectar. The eventual ingestion of fipronil may produce bee deaths or disrupt the organization of the hives (Decourtye *et al*., 2005) and this could cause an important impact upon bees, particularly if used incorrectly.

Analytical methods for the determination of fipronil are rather scarce in the available scientific literature. Fipronil has been mainly analysed in environmental samples such as soil and water. The extraction of fipronil from environmental samples, vegetables and pollen has been performed using liquid-liquid partitioning (Jennings *et al.*, 2002; Chauzat *et al.,* 2006). Alternative techniques such as, solid-phase extraction (SPE) (Ngim *et al*., 2000) and solid-phase microextraction (SPME) (Vílchez *et al*., 2001) have also been applied. Matrix solid-phase dispersion (MSPD) is an extraction technique that has been used in the determination of fipronil in honeybees (Morzycka, 2002). These techniques, SPE and MSPD, were firstly applied by our laboratory for the extraction of fipronil from honey and pollen samples, respectively (Sánchez-Brunete *et al*., 2005a).

Analysis of fipronil has been generally performed by gas chromatography with different selective detectors as electron-capture detectors (ECD) (Bobé *et al*., 1998; Ramesh and Balasubramanian, 1999; Pei *et al.*, 2004) and mass spectrometry (MS) (Morzycka, 2002; Jiménez *et al*., 2007).

The aim of this study was to develop the analytical methodology for the determination of fipronil in honey and pollen samples, based on solid-phase extraction (SPE) and MSPD, respectively. Fipronil levels were determined by gas chromatography coupled with mass spectrometry and with electron-capture detection, as an alternative detection system usually available in analytical laboratories due to its low price. Method validation was evaluated in terms of linearity, precision, recovery and limit of detection. The developed methods were applied to determine fipronil residue levels in honey and pollen commercialised in Spain.

Material and Methods

Standards and reagents

Fipronil and heptachlor (99% purity) standards were obtained from Reidel-de Haën (Seelze, Germany). Residue analysis grade ethyl acetate, hexane, methanol, and acetonitrile were supplied by Scharlab (Barcelona, Spain). A Milli-Q water purification system from Millipore (Bedford, MA, USA) was used to provide ultrapure water. Silica Bondesil-C_{18}, particle diameter of 40 μm, was purchased from Scharlab (Barcelona, Spain) and anhydrous sodium sulfate, reagent grade, was obtained from Merck (Darmstadt, Germany). Florisil, a magnesium silicate adsorbent, 150-250 μm (60-100 mesh) research grade, was obtained from Merck (Germany). The Florisil adsorbent was heated for 12 h at 140ºC before use.

A 500 μg mL^{-1} stock solution of fipronil was prepared by dissolving 0.050 g in 100 mL of ethyl acetate and stored at 4°C. An intermediate standard solution (5 μg mL^{-1}) was prepared by appropriate dilution of the stock solution. A set of working solutions at concentrations ranging from 0.01 to 1 μg mL^{-1} were prepared by dilution of the corresponding intermediate standard solution and stored at 4°C. The internal standard was prepared by dissolving heptachlor in ethyl acetate to make a 500 μg mL^{-1} solution.

In order to test the matrix enhancement effect and to obtain a correction function for fipronil, calibration solutions in neat solvent and in blank matrix extracts, instead of pure solvent, were prepared. The analyte range of concentrations in both cases were the same, 10 to 500 μg L^{-1}. The internal standard, heptachlor, was added to all calibration solutions. A 2 μL volume of these solutions was injected in GC-MS and GC-ECD, obtaining calibration curves in pure solvent and in matrix matched standards.

Honey and pollen samples

Various Spanish commercial honeys from two different botanical origins, orange [*Citrus sinensis* (L.) Osbeck] and rosemary (*Rosmarinus officinalis* L.) were purchased. Five orange honeys were from the Mediterranean citrus region and five rosemary honeys from the province of Guadalajara. Moreover, six samples of raw citrus honey were collected from the beekeepers in the Valencian Community. Ten pollen samples of different commercial brands were purchased in local supermarkets. All these samples were stored at 4°C until analysis.

Extraction procedure

Honey

Honey was heated in a water bath at 50°C for 15 min to facilitate its manipulation. A 10 g amount of honey,

placed in a Sovirell tube, was dissolved in 10 mL of water-methanol (7:3, v/v) and homogenized on a Vortex mixer (Selecta, Madrid, Spain) until complete dissolution. Recovery samples were spiked with a mixture of fipronil and the internal standard, heptachlor, in ethyl acetate, to give final concentrations in the range of 0.5 to 50 μg kg^{-1} of fipronil and 25 μg kg^{-1} of heptachlor, and the fortification solvent was allowed to evaporate before the corresponding extraction step.

Polypropylene columns (5 mL, 5 cm × 10 mm i.d., from Becton-Dickinson, Madrid, Spain) with a Teflon frit (1 cm diameter and 20 μm pore, Supelco, Madrid, Spain) at the bottom end were filled with 1 g of C_{18}, the columns were placed on a multiport vacuum manifold (Supelco Visiprep, Madrid, Spain) and preconditioned with 3 mL of acetonitrile and 5 mL of water. The honey solution was transferred to a column, the Sovirell tube washed with 3 mL of water-methanol (7:3, v/v) that were also transferred to the column and the eluate discarded. Fipronil retained in the solid phase was eluted with 2 × 5 mL of hexane-ethyl acetate (1:1, v/v), and the eluates collected in 10 mL graduated tubes. The collected combined extract was concentrated with a gentle stream of air in a fumehood to a 1 mL volume and a small amount of anhydrous sodium sulfate was added to dry the concentrated extract, which was stored at 4°C until analysed by GC-MS and GC-ECD. The chromatographic standards were prepared using blank extracts. These blank extracts were fortified with the corresponding mixture of fipronil and heptachlor standard solution.

Pollen

Pollen (4 g), finely ground in a mill, was placed in a glass mortar containing 3 g of Florisil. This mixture was then covered with an additional amount of Florisil (1 g) and gently blended with a glass pestle with circular motion to yield a homogeneous powdered material. Recovery samples were previously fortified with 0.5 mL of fipronil and heptachlor standard mixture, to give final concentrations in the range of 0.5 to 50 μg kg^{-1} of fipronil and 25 μg kg^{-1} of heptachlor, and the fortification solvent was allowed to evaporate before the corresponding extraction step.

The blended sample was transferred to a polypropylene column (20 mL, 10 cm × 20 mm i.d.) with 2 cm diameter Whatman No. 1 filters (Maidstone, UK) at the bottom end and closed with one-way stopcocks. Mortar and pestle were rinsed with 10 mL of acetonitrile, the solvent poured into the extraction column and the column placed for 10 min extraction in an ultrasonic water bath (Raypa, Spain) at room temperature. The water level in the bath was previously adjusted to equal the extraction solvent level inside the columns. After extraction, the columns were placed on a multiport vacuum manifold where the solvent was filtered and collected in graduated tubes. The extract was concentrated with a gentle stream of air to a volume around 5 mL and samples were extracted again with another 5 mL of acetonitrile (10 min). The combined total extract collected in a 10 mL graduated tube was concentrated with a gentle stream of air to a final volume of 2 mL. This extract was stored overnight in the freezer at –20°C to make the lipids precipitate. The cold extract was immediately filtered through a 0.45 μm nylon filter (Millipore, Ireland) to remove lipids and the filtered extract was then analysed by gas chromatography with the two detection systems. Chromatographic standards were prepared spiking blank sample extracts with a mixture of fipronil and the internal standard.

Chromatographic analysis

Gas chromatography with electron-capture detection (GC-ECD)

The analysis of fipronil was performed with an HP 5890 Series II gas chromatograph (Waldbronn, Germany) equipped with an automatic injector Model HP 7673, an HP 3365 integrator, and a ^{63}Ni ECD. A HP-1 capillary column (30 m × 0.25 mm i.d. and 0.25 μm film thickness), supplied by Agilent (Madrid, Spain), was employed. Fipronil was determined in the following conditions:

— Carrier gas: helium at a flow-rate of 1 mL min^{-1}.

— Injection port temperature 270°C.

— Injection volume: 2 μL volume was injected splitless, with the valve closed for 1 min, in a double-taper glass liner with a nominal volume of 800 μL.

— Detector temperature 300°C.

— Column temperature: 80°C (1 min) programmed at 15°C min^{-1} to 270°C (10 min). The total analysis time was 22.67 min.

Gas chromatography coupled with mass spectrometry (GC-MS)

An Agilent 6890 gas chromatograph (Waldbronn, Germany), equipped with an automatic injector Model

Table 1. Retention times (t_R), target ion (T), qualifier ions (Q_1, Q_2), and abundance ratio of qualifier ion/target ion (Q_1/T, Q_2/T)[a]

Compound	t_R ECD (min)	t_R MS (min)	T	Q_1	Q_2	Q_1/T (%)	Q_2/T (%)
Heptachlor[b]	10.76	10.43	272	274	337	99.4	33.5
Fipronil	11.73	11.57	367	369	255	90.6	72.7

[a] Q/T (%) are the results of abundance values of the qualifier ion (Q_1, Q_2) divided by the abundance of the target ion. [b] Internal standard.

HP 7683 and an inert mass spectrometric detector (MSD), Model HP 5973 was used in the analysis of fipronil. A fused silica capillary column ZB-5MS, 5% phenyl polysiloxane as nonpolar stationary phase (30 m×0.25 mm i.d. and 0.25 µm film thickness), from Phenomenex (Torrance, CA), was used. Operating conditions of the gas chromatograph were as follows:

— Injection: 280°C ; pulsed splitless (pulsed pressure 310 kPa for 1.5 min), 2 µL.

— Carrier gas: helium at a flow-rate of 1.0 mL min^{-1}.

— Column temperature: 80°C (1 min) then programmed at 15°C min^{-1} to 270°C (10 min). The total analysis time was 22.67 min and the equilibration time 2 min.

The MSD was operated in the following conditions:

— Ionisation mode: electron impact ionisation (70 eV).

— Ion source temperature 300°C.

— Quadrupole temperature 150°C.

— Interface temperature 280°C.

— Electron multiplier (EM) voltage: 100 V above autotune.

— Solvent delay 5 min.

The analysis was carried out with selected ion monitoring (SIM) using one target and two qualifier ions. The abundance of the selected ions was determined by injection of a fipronil standard solution under the same chromatographic conditions using full-scan with the mass/charge ratio ranging from 60 to 500 m/z. Calibration standards were prepared in blank matrix extracts, in order to counteract the matrix effect observed. Table 1 lists the retention times, the target and qualifier ions and their qualifier to target abundance ratios of both fipronil and the internal standard, heptachlor. The SIM program used to determine and confirm fipronil residues in pollen and honey samples is indicated in Table 2. Fipronil was confirmed by its retention time, the identification of the target and qualifier ions and the determination of the qualifier-to-target ratios. The retention time had to be within ±0.3 min of the expected time and the qualifier-to-target ratios had to be within a 20% range for positive confirmation.

Results

Extraction of fipronil from pollen and honey samples

The extraction of fipronil from pollen samples was carried out by MSPD. In the optimisation of this method several adsorbents and solvents of different polarity were assayed. Florisil, alumina and C_{18} were tested and Florisil was the adsorbent chosen as it gave cleaner extracts with minimal chromatographic interferences. The next step was to optimise the amount of Florisil needed to obtain good recovery results. An amount of adsorbent from 3 to 6 g was assayed and the best recovery results were obtained with 4 g. Extraction of fipronil with solvents of different polarity, such as acetonitrile, ethyl acetate and hexane, was also studied. Large amount of lipids were co-extracted when ethyl acetate or hexane were used and somewhat low recoveries of fipronil were obtained with both solvents. Aceto-

Table 2. SIM (selected ion monitoring) program used to analyse Fipronil in honey and pollen

Group	Time (min)	Compound	m z^{-1}	Dwell time (ms)	Scan rate (cycles s^{-1})
1	5.00	Heptachlor[a]	272, 274, 337	100	2.86
2	10.90	Fipronil	255, 367, 369	100	2.86

[a] Internal standard.

nitrile was found to be the most efficient solvent as cleaner extracts with good recoveries were achieved. The low solubility of lipids in acetonitrile allowed the precipitation of these compounds when the extract was kept in the freezer at –20°C and subsequently the filtered extract can be analysed directly by GC. A subsequent freezing of this extract was carried out and as no more lipids precipitated, a second freezing of the extract was not considered necessary.

The extraction of fipronil from honey samples was carried out by SPE using C_{18} as adsorbent and 10 mL of hexane-ethyl acetate (1:1, v/v) as eluting solvent. No interferences that could hinder the determination of fipronil were observed and good recovery results were obtained. Therefore, this was the method applied to determine fipronil in honey samples.

Gas chromatographic determination

Fipronil levels were determined by gas chromatography-mass spectrometry with selected ion monitoring (GC-MS-SIM) and by gas chromatography with electron-capture detector (GC-ECD). Heptachlor was added as internal standard to compensate for possible losses in sample preparation or variations in the analytical procedure.

When standards were prepared by spiking blank honey and pollen samples with known amounts of fipronil, higher peak areas than those recorded for the same pesticide concentration in neat solvent were obtained. Therefore, the quantification of fipronil residues was carried out using fortified blank samples, previously analysed to confirm the absence of co-extracted interferences. Figure 1 shows representative chromatograms of honey and pollen samples obtained with ECD.

Method validation

The linearity of the method was determined by means of five-point calibration curves using blank honey and pollen extracts fortified in the range from 10 to 500 μg L^{-1} containing the internal standard at a concentration of 250 μg L^{-1}. The MS and ECD responses were linear in the concentration range assayed with determination coefficients higher than 0.998 for both honey and pollen samples. The calibration equations for ECD detection were $y = 0.8099x - 2.75 \cdot 10^{-3}$ (honey) and $y = 0.9507x - 7.83 \cdot 10^{-4}$ (pollen), whereas for the mass spectrometric detection the calibration equations were $y = 1.2x - 4.7 \cdot 10^{-3}$ (honey) and $y = 1.9x - 7.7 \cdot 10^{-2}$ (pollen).

The repeatability of the chromatographic analysis was determined with a sample spiked at 50 μg L^{-1} in GC-MS and GC-ECD. This sample was injected 10 times with an automatic injector and the relative standard deviation (RSD) values obtained for the retention time were 0.06% for ECD and 0.02% for MS, whereas for peak areas the RSD was 5.2% for ECD and 9.1% for MS. The repeatability of the whole analytical method was also determined by replicate analysis of a fortified sample during different days. Five replicate samples of honey and pollen fortified at 5 μg kg^{-1} were analysed during successive days. The repeatability of the method, expressed as RSD was < 8% for honey and 10% for pollen samples.

Stock standard and working solutions were found to be stable when stored at 4°C, at least 3 months and one week, respectively. Moreover, the stability of a fortified blank sample kept in the autosampler for 24 h was assayed and differences from a freshly prepared sample were lower than 4%.

The specificity of the proposed method was assessed by analysing blank honey and pollen samples. The absence of background peaks, above a signal to noise

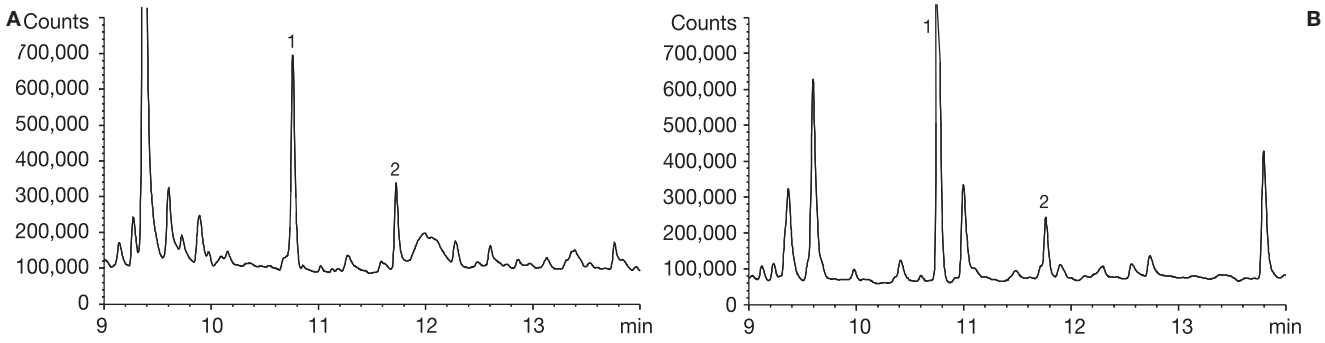

Figure 1. GC-ECD chromatograms of (A) a pollen sample fortified at 5 μg kg^{-1} and (B) an orange honey sample fortified at 10 μg kg^{-1}. Peak identification: 1 is the internal standard (heptachlor) and 2 is fipronil.

Table 3. Recoveries (%) of fipronil in honey[a] from two different botanical origins

Fipronil added (μg kg^{-1})	Rosemary		Orange	
	GC-MS	GC-ECD	GC-MS	GC-ECD
50	101.4 ± 2.8	99.3 ± 2.3	97.9 ± 3.6	101.3 ± 2.9
20	95.0 ± 4.9	99.1 ± 2.8	97.2 ± 2.9	99.4 ± 4.6
10	99.6 ± 4.7	97.6 ± 4.3	99.7 ± 5.2	99.6 ± 0.8
5	99.4 ± 4.6	98.9 ± 4.1	97.0 ± 4.4	102.6 ± 1.9
1	90.2 ± 3.1	99.1 ± 3.4	94.1 ± 3.3	100.6 ± 2.1
0.5	96.3 ± 2.5	ND[b]	97.7 ± 4.9	ND[b]

[a] Results are the mean of four replicates ± SD. [b] ND: not determined, concentration below the quantitation limit.

ratio of 3, at the retention time of fipronil, showed that no interferences occurred.

Tables 3 and 4 show the recovery results obtained for fipronil in honey and pollen samples using the two detection systems. Before extraction, honey samples were fortified at 50, 20, 10, 5, 1 and 0.5 μg kg^{-1} and pollen samples at 50, 20, 10, 5 and 2.5 μg kg^{-1} by adding 0.5 mL of the appropriate working standard solution containing fipronil and the internal standard. Four sample replicates at each fortification level were prepared. When calibration standards were prepared in neat solvents recoveries of fipronil higher than 120% were obtained, therefore, as indicated above, the quantification of fipronil was carried out with fortified blank samples.

The recovery of fipronil through the method ranged from 90 to 104% in MS and 97 to 103% in ECD. The precision of the method, obtained as the RSDs of analyte recoveries, is good, < 5% for ECD and < 6% for MS. The ions used for quantification are shown in Table 1.

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined by considering a value 3 or 10 times, respectively, the background noise obtained for blank samples. These limits were based on a 2 μL injection from the final extract of 10 g of honey or 4 g of pollen samples. The detection limit of fipronil in honey was 0.1 μg kg^{-1} in MS and 0.5 μg kg^{-1} in ECD and for pollen samples it was 0.2 μg kg^{-1} and 1 μg kg^{-1} in MS and ECD, respectively. Figure 2 shows the GC-MS chromatograms of a honey and pollen samples fortified at 0.5 and 1 μg kg^{-1}, respectively.

The developed method was finally applied to the analysis of several samples of honey and pollen commercialised in Spain as well as honey collected directly from beekeepers of the Valencian Community. No detectable residues of fipronil residues were found in all of the samples analysed.

Discussion

Honey and pollen are complex matrices containing a wide variety of organic compounds like lipids, pigments and sugars that may interfere in the determination of pesticide residues. Sample preparation is therefore, an important step in the analysis of these compounds at trace level.

The extraction of fipronil from honey is based on a multiresidue method previously developed in our laboratory for the simultaneous determination of pesticides by GC-MS after SPE of the sample dissolved in a water-methanol mixture (Albero e*t al*., 2004). The

Table 4. Recoveries (%) of fipronil in pollen[a] of two different commercial brands

Fipronil added (μg kg^{-1})	Brand A		Brand B	
	GC-MS	GC-ECD	GC-MS	GC-ECD
50	99.5 ± 4.8	100.2 ± 4.5	102.5 ± 2.2	100.8 ± 3.2
25	101.4 ± 4.3	101.6 ± 2.8	103.9 ± 3.9	101.7 ± 1.6
10	101.3 ± 3.7	102.7 ± 2.8	99.9 ± 1.7	99.8 ± 4.3
5	98.3 ± 4.8	97.4 ± 3.5	101.6 ± 3.1	99.0 ± 2.7
2.5	90.6 ± 5.7	ND[b]	100.7 ± 4.4	ND[b]

[a] Results are the mean of four replicates ± SD. [b] ND: not determined, concentration below the quantitation limit.

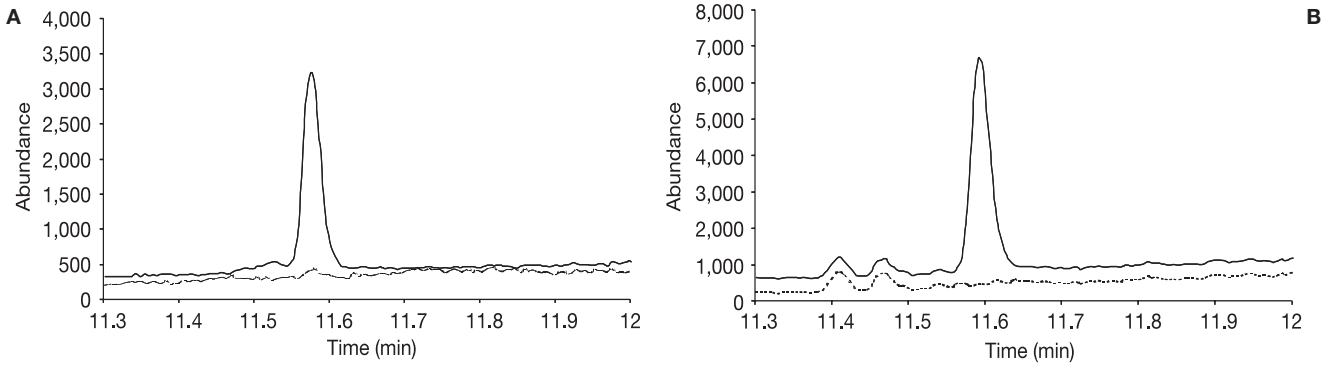

Figure 2. Representative GC-MS-SIM chromatograms of (A) a honey sample fortified at 0.5 µg kg^{-1} and (B) a pollen sample fortified at 1 µg kg^{-1}, the dotted lines correspond to unfortified blank samples.

results obtained in the present work show that this method can be used for the analysis of fipronil in honey with both detection systems, ECD and MS, with good recoveries and detection limits. In the case of pollen, SPE is not applicable as a solution of this matrix can not be obtained. Therefore, an alternative method was developed based on the dispersion of pollen on Florisil (matrix solid-phase dispersion, MSPD), extraction with acetonitrile and subsequent separation of lipids by cold precipitation and filtration. Both sample preparation methods, SPE and MSPD, are miniaturised methods that have the advantage of using a low amount of sample and low volumes of organic solvents, producing good and reproducible recoveries.

The detection systems employed, ECD and MS, were adequate for the determination of fipronil at trace levels without interferences from other compounds. Nevertheless, a response enhancement was observed when fipronil was injected in matrix extracts in comparison with the response obtained in neat solvents. This can be explained by a matrix effect that improves the transfer of analytes from the injection port to the column, enhancing the chromatographic response. There are different methods to overcome this matrix effect (Sánchez-Brunete *et al.,* 2005b). The use of matrix matched standards represents an adequate way of correcting the response enhancement due to the matrix effect, as it has been shown in this work.

The limits of detection obtained were lower in MS than in ECD, because ECD is not as sensitive and selective as mass spectrometry in the selected ion monitoring mode. Nevertheless, although MS has in addition the possibility of confirming pesticide residues by their mass spectra, the use of ECD has the advantage of its low price and maintenance costs, being an economic alternative detector widely used for halogenated compounds.

In summary, the proposed procedures, MSPD for pollen and SPE for honey, allow to perform the extraction and clean-up of extracts in a single process, requiring only a low volume of organic solvents. Fipronil residues were determined by capillary gas chromatography with electron-capture detection and mass spectrometry, providing a simple and rapid procedure for the determination of this compound in pollen and honey samples with good reproducibility and low detection limits.

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