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Development and Validation of an Analytical Method to Determine Fipronil and its Degradation Products in Soil Samples

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Abstract The aim of this study was to develop a methodology for identifying and quantifying Fipronil and its degradation products in soil by gas chromatography–electron capture detector previously extracted using a focused ultrasound probe. This methodology was obtaining a range of recovery between 85 % and 120 %, decreasing approximately solvent used time and cost, respect to other methodologies such as bath ultrasonic, solid-phase extraction, liquid–liquid extraction and soxhlet. The method was validated in fortified matrix, presented linearity in the range of 25–400 µg kg^{-1}, and limit of detection for Fipronil and their products desulfinyl, sulfide and sulfone was 14.7, 9.8, 8.9 and 10.7 µg kg^{-1}, respectively. This process was applied to samples of agricultural soils, where two degradation products desulfinyl and sulfone were found.

Keywords Fipronil · Degradation products · GC–ECD · Focused ultrasound probe

Fipronil is an insecticide belonging to the phenylpyrazole family. It has been used in agricultural and urban environments to control a wide variety of insect pests such as locust, crickets, termites, beetles, cockroaches, fleas, etc. (Tingle et al. 2003). It is a systematic insecticide that acts by inhibiting the chloride channels activated by the gamma aminobutyric acid (GABA) (Cole et al. 1993). This insecticide shows high affinity for GABA receptors of insects, and relatively low affinity in GABA$_A$ and GABA$_C$ receptors of vertebrates, thus showing lower toxicity in mammals compared to other insecticides that block this channel, such as endosulfan and lindane (Mohamed et al. 2004).

This insecticide presents four degradation products: Fipronil sulfone, Fipronil sulfide, Fipronil desulfinyl and Fipronil amide (Fig. 1), these are generated from oxidation mechanisms, reduction, photolysis and hydrolysis, respectively (Bobé et al. 1998).

Compound persistence is evaluated by its half life ($t_{½}$). The microbial degradation of Fipronil in soil has been studied by Zhu et al. (2004), finding that the $t_{½}$ of Fipronil in non-sterile clay loam soils is 8.78–9.71 days at 35°C. Nonetheless, the $t_{½}$ observed by Tingle et al. (2003) in loam soils was 342 days.

In some countries the use of Fipronil has been banned. For example, in France, the main reason for its prohibition was bee mortality, since it presents a lethal dose 50 (DL$_{50}$) of 4–6 ng/bee and it has been demonstrated that under sublethal doses of 0.1 ng these insects show alterations in pollination and olfactory behavior (Aliouane et al. 2009).

In Mexico, Fipronil is used against plagues of locusts (*Schistocerca piceifrons piceifrons* Walker). All of the above has allowed the usage of Fipronil in a generalized manner in the northeast, central and southeast regions of Mexico, where plagues of locusts are present.

Considering the efficacy that Fipronil shows against locusts, it is important to determine the risk it can produce in aquatic and terrestrial ecosystems. Thus, it is necessary

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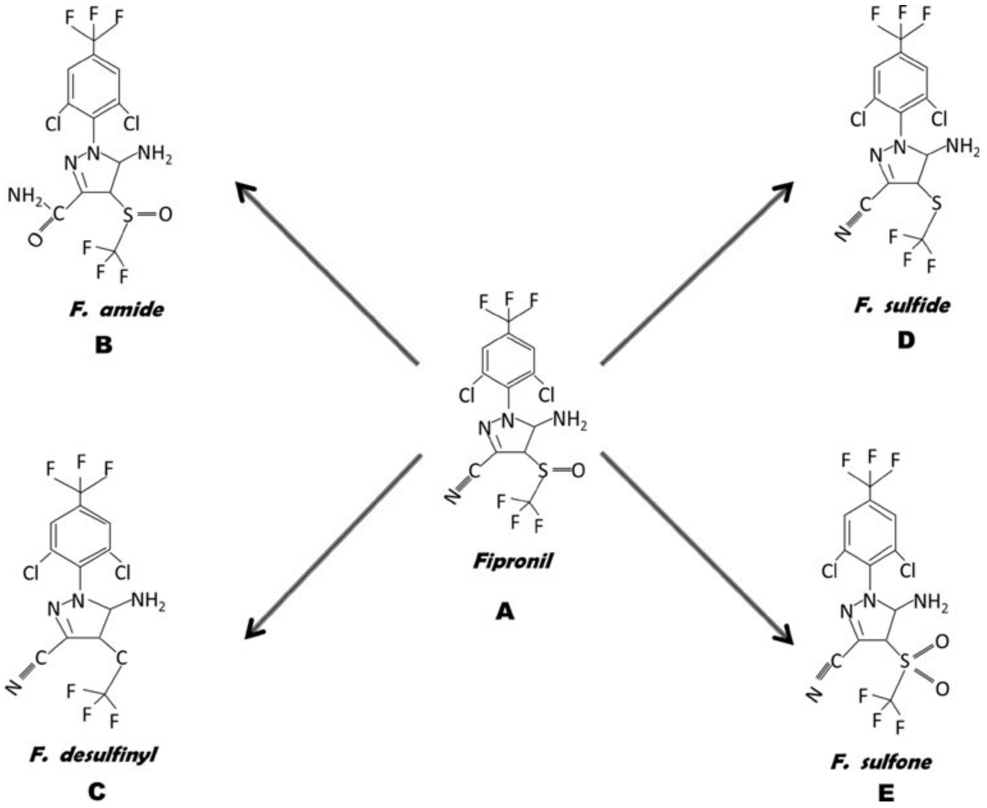

Fig. 1 Fipronil (**a**) and its degradation products: Fipronil-amide (**b**), Fipronil-desulfinyl (**c**), Fipronil-sulfide (**d**), and Fipronil-sulfone (**e**)

to know the environmental concentrations through reliable analytical methodologies.

There are various analytical methods for the determination of these compounds in soil (Bobé et al. 1998; Lin et al. 2008; Mohapatra et al. 2010). For solid samples, extraction is more complicated because direct contact between the analyte and the extraction solvent is more difficult. On the other hand, traditional techniques usually spend a long time, are tedious, require sophisticated equipment and the use of large amounts of toxic organic solvents and glassware, whereby are relatively expensive. Therefore, recent trends in sample preparation have focused on the development of methods with the following characteristics: (1) simplicity, preferably one step, (2) low cost, reduction of time and solvent consumption (3) miniaturization, reduction of sample size and (4) environmentally friendly, reduction of the use toxic organic solvents.

Recently, the ultrasonic assisted extraction (UAE) has been introduced as a new sample preparation technique for the extraction of organic compounds (PAHs, PBB, PBDEs) from liquid and solid matrixes (Sanz-Landaluze et al. 2010; Yu and Hu 2007). This technique is based on the use of ultrasonic energy to ensure a more efficient contact between the sample and the extraction solvent, allowing rapid extractions of organic compounds from liquid and solid matrixes.

The present study aims to develop and optimize a faster method using a focused ultrasound probe (FU), affordable in cost and with less waste to quantify the Fipronil insecticide and its degradation products in soil samples.

Materials and Methods

The reference standards Fipronil (94.8 %), Fipronil sulfide (99.6 %), Fipronil sulfone (100 %) and Fipronil desulfinyl (98 %) were acquired from Accustandars®. The solvents used (acetone and hexane) are of HPLC grade and were obtained from Burdick & Jackson®.

For extraction, we used an Ultrasonic Processor GEX130 (115 V 50/60 Hz, Ultrasonic Processor) equipped with a 3 mm titanium tip, mechanical agitators (Thermolyne®) and a Zymark® evaporator.

For sample preparation, we utilized polytetrafluoroethylene syringe filters (PFTE) with a pore size of 0.2 μm (Millex), industrial gases (99.99 % purity) Praxair® and florisil solid phase extraction cartridges (J.T.Baker®) of 1,000 mg/6 mL as a cleaning procedure.

Soil samples blank were taken from the Juan H. Sánchez Park in the city of San Luis Potosi, Mexico (N 22.1489, L O 101.0167). The soil was dehydrated and sterilized since Fipronil suffers a microbial degradation process (Zhu et al. 2004). We did not find any detectable levels of Fipronil or its products. For white soil, we did not work with a control of the same contaminated site since the area had been sprayed with Fipronil for a number of years, thus a soil sample of the city of San Luis Potosí was chosen. It is known that it is difficult to find a soil with the same characteristics to consider it a reference in the method.

To show the utility of the method, we obtained test samples from an agricultural area in the city of Tamuin, S.L.P., Mexico. A place affected by locusts, and Fipronil was used as insecticide since 2008 as a chemical control. We obtained 19 samples composed of a systematic sampling and focused on a surface of 1 km^2.

One gram of white soil was fortified with Fipronil, Fipronil sulfide, Fipronil sulfone, and Fipronil desulfinyl in 25, 50, 75, 100, 200, and 400 µg kg^{-1} concentrations, constituting in this manner the calibrating curve by external standard.

The extraction procedure was applied in 1.0 g of fortified soil was added to 6 mL of acetone. Subsequently, ultrasonic waves were applied for 1.5 min at 50 % power and 1 s – on and off – pulsations.

The supernatant was filtered through a PFTE syringe microdisc with a pore size of 0.2 µm, the procedure was performed by duplication and filtering, it evaporated at 1 mL with a smooth flow of nitrogen. The extraction procedure was in the same manner to real samples and white soil.

Extract from soil underwent a cleaning procedure using columns packed with florisil®. Previously, the column was conditioned with 12 mL of acetone and 12 mL of hexane. The soil extract went through the conditioned column and two washes of 6 mL of acetone were applied. Finally, it was evaporated at 1 mL with N_2 for its analysis in the GC–ECD.

Sample analysis was carried out using a gas chromatograph 6890 (Agilent) equipped with an automatic injector 7683 (Agilent) coupled to an electron capture detector (ECD). We used a capilar column DB-XLB (Agilent) 60 m long × 0.25 mm in diameter × 0.25 µm of film thickness. Helium was the carrier gas at a flow rate of 1 mL/min, the injector temperature was 210°C, the injection mode was pulsed splitless with a volume of 1 µL, the detector temperature was fixed at 300°C and N_2 was the auxiliary gas at a flow rate of 60 mL/min. The temperature program of the oven started at 100°C for 1 min, subsequently the temperature was increased according to the following speeds: 15°C/min at 240°C, 5°C/min at 255°C for 2 min, 2°C/min at 260°C for 2 min, 2°C/min at 270°C for 2 min and 5°C/min at 300°C for 10 min.

The retention times obtained for Fipronil desulfinyl, Fipronil sulfide, Fipronil and Fipronil sulfone were 14.49, 16.19, 16.57 and 18.13 min respectively (Fig. 2).

The internal quality control and method validation were performed based on the Validation Guide for Analytical Methods on the Determination of Organic Compounds at Trace Levels AOAC/FAO/IAEA/IUPAC (2000), evaluating the following parameters: linearity, sensibility, precision (repeatability and intermediate precision), recovery percentage, limit of detection, and limit of quantification.

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated with the results obtained from the triplicate of the calibration curve of each of the compounds to be determined in a concentration interval of 10 to 50 µg kg^{-1}. The LOD and LOQ were determined by a linear curve at low concentrations, adding the confidence limit of 95 % (Miller and Miller 2000; Hubaux and Vos 1970).

The linearity expressed by correlation coefficient (r) and the sensibility determined by the slope of the curve, were obtained from the average of eleven curves worked for 5 days. The percentage recovery of the method for each analyte was obtained from the average of the duplicates of the control points at three different independent concentrations of the levels of the calibration curve (35, 90 and 300 µg kg^{-1}) over 5 days of work.

Precision was obtained as repeatability and intermediate precision, evaluating the calibration curve the same day by triplicate and in five different days by duplicate, respectively.

Results and Discussion

Table 1 presents the recovery percentages of three independent concentrations of the calibration curve (35, 90, 300 µg kg^{-1}), obtained values are acceptable according to the AOAC/FAO/IAEA/IUPAC guide, confirming that the focalized ultrasonic probe obtains high recovery percentages. Table 2 shows comparative results for quantifying Fipronil and its degradation products in soil between our methodology and other techniques previously reported. The last table listing extraction method, sample amount, type and volume of solvent used and time required. The volume of the solvent utilized (6 mL) represents a reduction of up to 92 %, 85 % and 98 % with respect to the methodologies of ultrasonic bath (BU) (70 mL), solid phase extraction–liquid–liquid extraction (SPE–LLE (40 mL) and soxhlet (350 mL) (Bobé et al. 1998; Lin et al. 2008; Mohapatra et al. 2010). As for the extraction time, our methodology optimizes it diminishing up to 90 % and 97.5 % with respect to BU and SPE–LLE (Bobé et al. 1998; Lin et al. 2008; Mohapatra et al. 2010) (Table 2). This is due to the action mechanism of the ultrasonic probe since it produces a cavitation phenomenon that provokes microcracks on the surface of the solid, which allows to extract compounds absorbed by the organic matter of the soil, as well as the ones adsorbed on the surface by electrostatic interaction mechanisms, with lower volume of

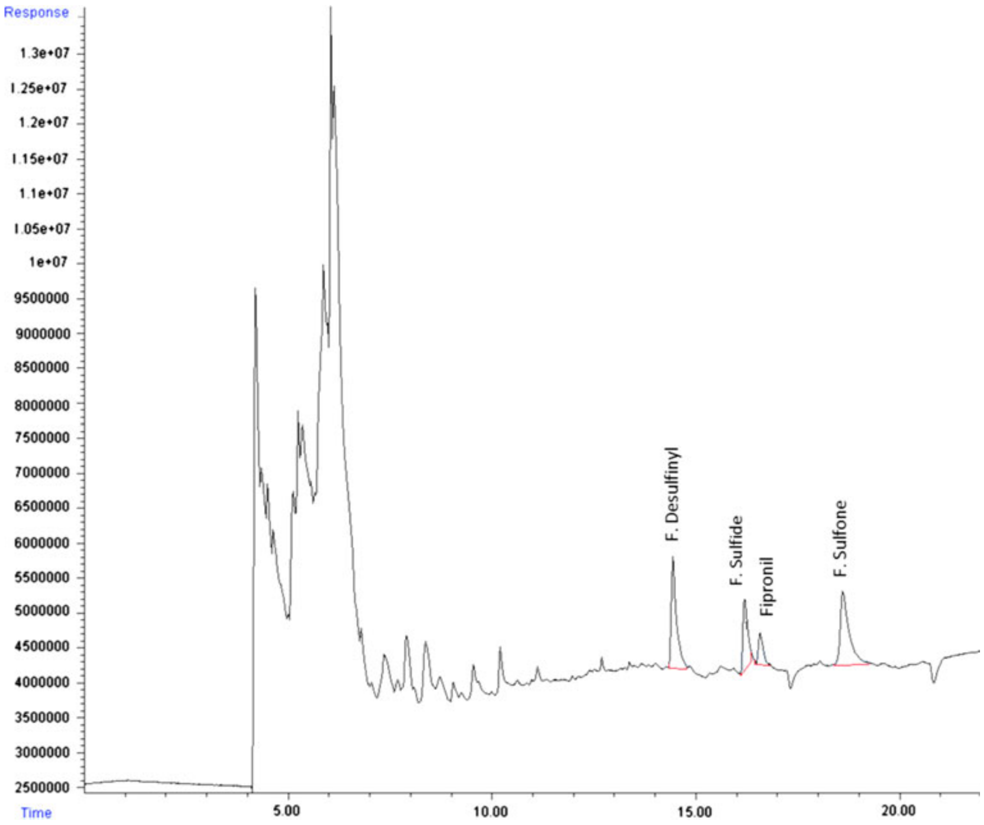

Fig. 2 Chromatogram obtained from the GC–ECD. Fortified matrix with a 25 μg kg^{-1} fipronil concentration and three degradation products

Table 1 Recovery percentage of Fipronil and its degradation products at low, intermediate and high concentrations in fortified matrix ± C.L. of 95 %

	Concentration (μg kg^{-1})		
	35	90	300
Fipronil desulfinyl	103.1 ± 7.6	92.4 ± 6.6	104.4 ± 6.8
Fipronil sulfide	92.8 ± 7.0	100.1 ± 7.2	103.1 ± 7.6
Fipronil	102.8 ± 8.1	97.5 ± 5.4	102.7 ± 8.8
Fipronil sulfone	113.8 ± 9.1	97.9 ± 5.9	102.4 ± 8.8

Acceptance criteria of recovery percentages for >10 μg kg^{-1} ≤ 100 μg kg^{-1} of 70 %–120 % concentrations and >100 μg kg^{-1} ≤ 1,000 μg kg^{-1} of 70 %–110 %

solvent and extraction time (Capelo et al. 2005). Ultrasounds permits the desegregation of the solid sample and particle size reduction, as a result of cavitation bubble collapse, thereby improving mass transfer of analytes into the solvent. On the other hand, each collapsing bubble can be considered as a micro-reactor in which temperatures of several and pressures higher than one thousand atmospheres are created instantaneously (Suslick et al., 1986).

The sensibility of the compounds was evaluated in matrix and system (standard in solvent), showing that Fipronil, Fipronil sulfide and Fipronil desulfinyl present a greater sensibility in the analysis performed in matrix (Fig. 3). This result can be attributed to the compounds co-extracted from the matrix that enhances the chromatographic response of the analytes in relation to the responses at system. This leads to a greater sensibility that allows a better differentiation between the obtained concentrations. The effect of the matrix on the response variation has been discussed; in this case it is increased. We postulated that the effect of the matrix occurs due to the co-extracted compounds provide protection to the analyte from thermal stress and avoid, in this manner, adsorption in active sites, such as the glass insert of the injection port in the chromatograph (Erney et al. 1993; Hajšlová et al. 1998). In the case of Fipronil sulfone, there was no difference in the response, which indicates that there is no matrix interference.

The calibration curve is linear in concentrations of 25–400 μg kg^{-1} for the four compounds. This parameter was evaluated by the correlation coefficient obtaining 0.9974, 0.9957, 0.9974 and 0.9963 for Fipronil desulfinyl, Fipronil sulfide, Fipronil and Fipronil sulfone, respectively (Fig. 3).

The LOD and LOQ were calculated for each compound by a triplicate of the calibration curves in an interval of a

Table 2 Comparison between extraction techniques

Authors	Extraction	Sample (g)	Solvent	Volume (mL)	Time (min)
Lin et al. (2008)	BU	20	Acetone: Methylene Choride 1:1	70	15
Bobé et al. (1998)	SPE/LLE	1	Acetonitrile–Acetone 70:30	40	60
Mohapatra et al. (2010)	SOXHLET	100	Acetonitrile–Acetone 70:30 Hexane–Ethyl acetate 1:1	350	NA
Developed Methodology	FU	1	Acetone	6	1.5

SPE solid phase extraction, *LLE* liquid–liquid extraction, *BU* ultrasonic bath, *FU* focalized ultrasonic probe, *NA* not available

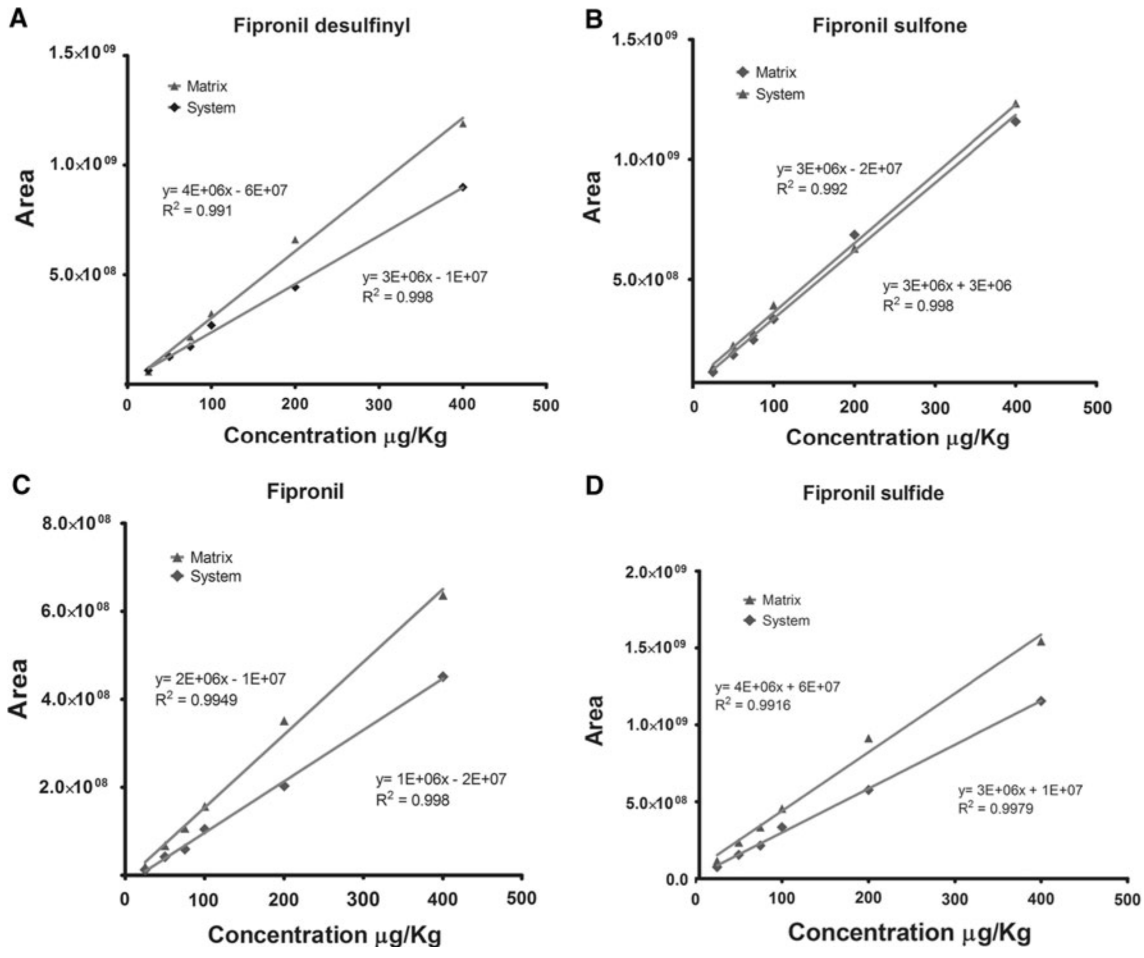

Fig. 3 Calibration curves in matrix and system (solvent) for **a** Fipronil desulfinyl, **b** Fipronil sulfone, **c** Fipronil and **d** Fipronil sulfide

lower concentration level (10–50 μg kg^{-1}). The value was obtained by the slope method, which consists in measuring the variation in the lowest zone of the curve (where greater uncertainty exists) to determine the response value that is differentiable from the target. This is calculated with the value of the intercept (yB) plus the deviation that estimates random errors in the direction of the intercept ($S_{y/x}$), corresponding yB + 3 $S_{y/x}$ to the LOD and yB + 10 $S_{y/x}$ to the LOD. The 95 % confidence limit of the slope variation in the interpolation zone is added to the obtained value, thus the final value contemplates the largest uncertainty in the lowest zone of the curve and ensures analyte presence and quantification with acceptable precision. The results are shown in Table 3 (Hubaux and Vos 1970; Miller and Miller 2000).

Other authors show lower LOD and LOQ values for Fipronil and its derivatives, however they do not indicate what calculation method they used, and report the same value for all analytes (Lin et al. 2008; Mohapatra et al. 2010).

Precision was evaluated as repeatability, by three independent curves in a day and intermediate precision with

Table 3 Limit of detection (LOD) and limit of quantification (LOD)

Compound	LOD (μg kg^{-1})	LOQ (μg kg^{-1})
Fipronil desulfinyl	9.8	19.8
Fipronil sulfide	8.9	17.9
Fipronil	14.7	22.4
Fipronil sulfone	10.7	21.5

The LOD and the LOQ were determined by a linear curve at low concentrations, plus upper confidence limit

Table 4 Precision expressed as repeatability and intermediate precision

Concentration (μg kg^{-1})	Repeatability		Intermediate precision	
	RDS Interval Fipronil total[a]	Aceptable RDS	RDS interval fipronil total[a]	Aceptable RDS
25	6.2–10.96	13.93	12.06–18.46	18.58
50	14.29–18.62	12.55	4.72–12.71	16.74
75	0.72–5.06	11.81	3.68–15.41	15.75
100	4.19–4.58	11.31	5.36–10.91	15.08
200	3.14–3.58	10.19	9.57–11.57	13.58
400	6.75–7.89	9.18	3.07–8.13	12.24

[a] Fipronil desulfinyl, Fipronil sulfide, Fipronil and Fipronil sulfone

two independent curves for 5 days, with a total of 11 independent readings of each point on the curve (Table 4). The variation coefficients were evaluated obtaining values within the acceptance criteria. This shows that the results by this methodology are reliable in different working days (Horwitz 1982).

To verify the utility of the method, soil samples from a surface previously sprayed for a year and a half were used. The concentrations of Fipronil desulfinyl were below the LOD, while for Fipronil sulfone, seven samples were below the LOD, four samples below the LOQ and in one sample the concentration was 25.97 μg kg^{-1}. To this day, there are no reports showing that, under the values of the limits presented in this work, ecotoxicological damage has been observed.

Agricultural landscapes, including paddies, play an important role in maintaining biodiversity, but this biodiversity has been under the threat of toxic agro-chemicals. Our knowledge about how aquatic communities react to, and recover from, pesticides, particularly in relation to their residues, is deficient, despite the importance of such information for realistic environmental impact assessment of pesticides.

Fipronil has a high selectivity, potency and low persistence and it has widely used to control locusts in Mexico (SENASICA 2012). However, Fipronil is highly toxic to non-target insects included honeybees and aquatic organisms, this is a major concern due may involve an ecological risk, as occurred in the U.S. with the decline of populations of crustaceans and in France with the mass death of honeybees (Bedient et al. 2005; Chauzat et al. 2006).

In Mexico, Fipronil utilization should be subject to careful review due to high risk of water pollution and toxicity of not target insects. For example, in aquatic communities of experimental rice fields, is well-documented Fipronil toxicity at 90 μg kg^{-1} per 7 days of exposure (Hayasaka et al., 2012). Therefore, it not seems unwise to use an insecticide Fipronil-based without environmental monitoring in regions or countries where it has not been used before.

The photodegradation and oxidation products, Fipronil desulfinyl and Fipronil sulfone respectively, are the most persistent and less selective among arthropods and mammals than the parent compound (Hainzl et al. 1998). From them, the only one that was observed in soil samples was Fipronil sulfone. There is not enough evidence that supports toxicological damage on insect or human species at this concentration; however, the present work shows suggestion, that Fipronil degradation products manifest its persistence on soil with characteristics from the Huasteca Potosina, thus studies that support these results need to be performed.

The use of this new analytical technology produces reliable results in the determination of compounds at trace levels. The extraction technique shows that it is efficient in a shorter time of analysis, lower volume of extraction and decreases sample manipulation. These advantages carry as well, error reduction in sample treatment. The use of FU for extraction has shown to be an attractive analytical alternative compared to other methods such as SPE, LLE, BU, where larger volumes of solvents are used. In addition, due to the low cost, FU is a viable procedure for many laboratories.

The results show that this methodology could be utilized for evaluation of pollutants on soil, hence providing future lines of investigation in the environmental assessment of toxic chemicals.

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