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Short communication

Determination of fipronil by solid-phase microextraction and gas chromatography–mass spectrometry

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Abstract

A method for the determination of trace amounts of the insecticide fipronil was developed using solid-phase microextraction–gas chromatography–mass spectrometry and selected ion monitoring. Fipronil was extracted with a fused-silica fiber coated with 85 μm polyacrylate. The effects of pH, ionic strength, sample volume, extraction and desorption times as well as the extraction temperature were studied. Lindane was used as an internal standard. The linear concentration range of application was 0.3–100 ng ml^{-1} of fipronil, with a relative standard deviation of 9.5% (for a level of 50 ng ml^{-1}) and a detection limit of 0.08 ng ml^{-1}. The method was applied to check the eventual existence of fipronil above this limit in water and soil samples from Granada (Spain) as well as in human urine samples. The method validation was completed with spiked matrix samples. The method can be applied as a monitoring tool for water, soil and urine, in the investigation of environmental and occupational exposure to fipronil.

Keywords: Extraction methods; Water analysis; Environmental analysis; Soil; Fipronil; Pesticides; Lindane

1. Introduction

Fipronil, (±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethyl sulfinyl pyrazole-3-carbonitrile (Fig. 1), a recently developed phenylpyrazole insecticide (Rhône–Poulenc Agro) is used for the control of many soil and foliar insects (e.g., corn rootworm, beetles larvae, colorado potato beetles and rice water weevils) on a variety of crops [1].

Few analytical methods have been reported for the determination of this compound [2,3]. Bobé et al. [2] proposed a gas chromatographic (GC) method for the determination of fipronil residues in soils.

Actual methods for the determination of trace amounts of pesticides involve the concentration of large volumes of sample by liquid–liquid extraction (LLE) or solid-phase extraction (SPE). Solid-phase microextraction (SPME) is an alternative technique that involves direct extraction of the analytes with the use of a small-diameter optical fiber coated with a polymeric stationary phase and housed in a syringe assembly for protection [4,5]. SPME eliminates the separate concentration step from the SPE and LLE methods because the analytes diffuse directly into the coating of the SPME device and are concentrated there. This device is then transferred directly into the

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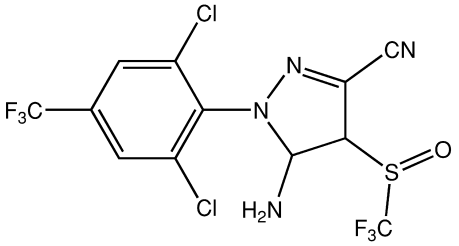

Fig. 1. Structure of fipronil.

injection port of the GC system were all the analytes are thermally desorbed and deposited at the head of the GC column.

This paper describes the application of SPME in combination with GC–mass spectrometry (MS) for the determination of fipronil in water, soil and human urine samples.

2. Experimental

2.1. Materials

All reagents were analytical-reagent grade unless stated otherwise. Water was purified with a Milli-Q plus system (Millipore). The stock standard solution (10 μg ml^{-1}) of fipronil in acetone was supplied by Dr. Ehrenstorfer (Leverkusen, Germany). It was stable for at least 6 months if stored in the dark at 4°C. Working solutions were obtained by appropriate dilutions with acetone. A solution of 10 μg ml^{-1} of lindane in methanol, stored also at 4°C, was used as an internal standard solution.

A manual fiber holder for SPME was purchased from Supelco (Bellefonte, PA, USA). Two types of fiber, 100 μm polydimethylsiloxane (PDMS) and 85 μm polyacrylate (PA) were obtained from the same manufacturer. The PDMS fiber was conditioned for 1 h at 250°C in the GC injection port and the PA fiber for 2 h at 300°C before extraction.

A magnetic stirrer/temperature-controlled oil bath (Agimatic-N, Selecta, Spain) was used during the sampling process.

2.2. Instrumentation

A Hewlett-Packard system consisting of a 5890 GC system fitted with a splitless injector for the HP-1 fused-silica capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness), a 5971 mass spectrometer, a HP-UX Chemsystem computer and the proprietary software was used. A silanized narrow-bore injector liner (0.75 mm I.D.) for the SPME injections was installed and the fiber was inserted into this injector using the splitless mode with the split closed for 3 min. The injector temperature was set at 250°C and the transfer line temperature was 260°C. The oven temperature was held at 75°C for 3 min, then heated to 250°C at a heating rate of 30°C min^{-1}. The temperature was held at 250°C for 3 min. The carrier gas was helium (purity 99.999%) at a flow-rate of 2 ml min^{-1}. The mass spectrometer detector was tuned by maximum sensitivity autotune. The following m/z values were fixed in the electron impact ionization mode by single ion monitoring (SIM): 369, 367 and 213 for fipronil and 181, 219 for lindane.

2.3. Sample treatment

2.3.1. Water

Water samples were filtered through a cellulose acetate filter (Millipore HAWP 04700, pore size 0.45 μm) and collected in glass bottles previously cleaned with HCl and washed with deionized water and stored at 4°C.

2.3.2. Soil

Air dried soil (0.5 g) was extracted in an ultrasonic bath with 1.5 ml of acetonitrile in a tube test for 60 min. The extract was filtered through a Whatman No. 1 filter paper, diluted to 5 ml with deionized water in a volumetric flask, and stored at 4°C.

2.3.3. Human urine

Urine samples (150 ml) were obtained from fasting healthy men, centrifuged for 10 min at 3800 rpm, filtered through a Minisart-plus syringe filter (0.2 μm pore size, Supelco) and stored at 4°C.

2.4. Basic procedure for determination of fipronil in water, soil and human urine samples

2.4.1. Water samples

A 3-ml volume of water sample containing between 0.3 and 100 ng ml^{-1} of fipronil, 3 μl of lindane internal standard solution and 1 ml of 0.16 *M* borate buffer solution (pH 9.5) were placed into a vial together with a magnetic stirrer and sealed. Magnetic agitation was performed by 10 min. The PA fiber was introduced carefully directly into the solution and stirred at 1400 rpm for 30 min at 60±2°C. After the extraction, the fiber was directly exposed to the hot injector of the gas chromatograph for 5 min and the chromatogram was registered.

Calibration graphs were constructed using solutions of fipronil of known concentrations. Lindane was used as internal standard in order to normalize the chromatographic system performance.

2.4.2. Soil samples

A 2-ml volume of the diluted extract (see Section 2.4), 3 μl of lindane internal standard solution and 2 ml of 0.08 *M* borate buffer solution (pH 9.5) were placed in a vial. Then the procedure was identical to that for water (see Section 2.4.1).

2.4.3. Human urine samples

A 2-ml volume of treated urine (see Section 2.4), 3 μl of lindane internal standard solution and 2 ml of 0.08 *M* borate buffer solution (pH 9.5). Then the procedure was identical to that for water (see Section 2.4.1).

3. Results and discussion

3.1. Optimization of conditions for SPME

The more adequate fiber was found by comparing the extraction behavior on two commercial SPME fibers, PDMS and PA, at different pH values obtained with phosphate buffer and borate buffer solutions. The 85 μm polyacrylate PA fiber showed the highest extraction performance at pH 9.5 (Fig. 2). The PA fiber was thus chosen to perform the rest of the experiments. A 0.04 *M* concentration of the borate buffer (pH 9.5) was selected to obtain an adequate buffering capacity.

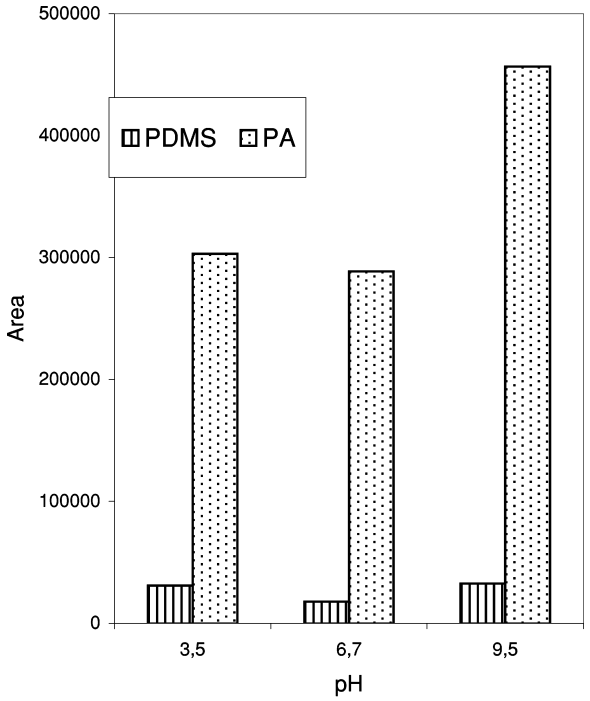

Fig. 2. Comparison of two commercial SPME fibers for extraction efficiency at different pH values.

Earlier, the role of ionic strength was investigated using sodium chloride. Signal intensities of fipronil increased by 20% when a 250 g l^{-1} concentration of NaCl was used. Deposits of the salt on the surface of the fiber and in the injection port liner of the GC system and crystallization onto the fiber damaging the fiber coating when pulled into the septum piercing needle as well as breaking of the fiber precluded the use of NaCl. Thus, in spite of reduced signal intensities further measurements where made without using salt.

The optimum stirring rate was determined in the range between 300 and 1600 rpm. Extraction increases with increased stirring speed. We chose a stirring speed of 1400 rpm.

The effect of temperature was monitored by extracting samples of 10 ng ml^{-1} of fipronil at different temperatures. Fig. 3 shows a clear increase in the amount of analyte adsorbed when temperature

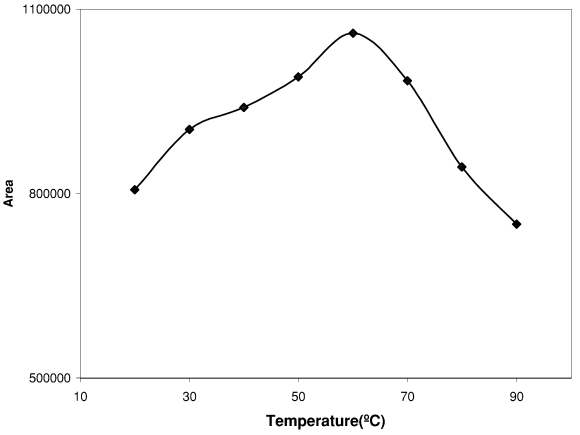

Fig. 3. Influence of temperature on SPME.

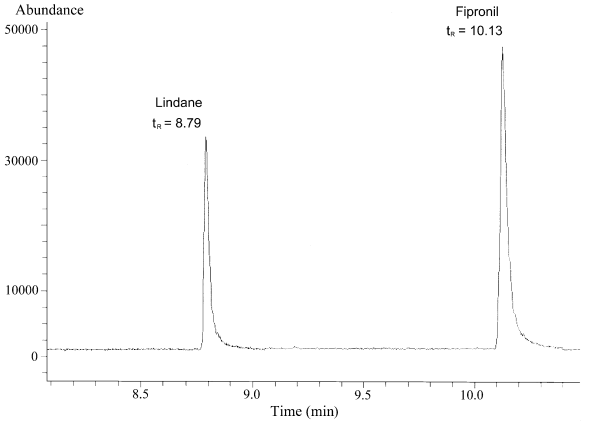

Fig. 4. Typical chromatogram obtained in the SIM mode. A 10 ng ml^{-1} concentration of fipronil, treated as indicated in the analytical procedure.

increases to about 60°C but a decrease above this temperature. There are two experimental parameters related with temperature which help to explain these results [6]. Extraction is basically limited by mass transfer with higher efficiency the higher the temperature; however, absorption is an exothermic process and when the temperature is increased the overall effect above a certain temperature is negative. Extractions were carried out at 60±2°C.

Extraction time profiles were studied extracting samples of 10 ng ml^{-1} of fipronil and monitoring the GC area counts as a function of exposure time. Equilibrium was not attained even after 120 min. For quantitative analysis it is not necessary for the analytes to have reached equilibrium, but only for sufficient loading onto the fiber and reproducible extraction times [7,8]. A 30-min extraction time was adopted, even though fipronil had not reached equilibrium at this point, because analytical sensitivity was thought to be good enough.

To study the carryover effect, blanks were run after extractions of 50 ng ml^{-1} of fipronil. No signals were obtained when a 5-min desorption time was chosen, which ensured a complete desorption of fipronil.

We investigated the effect of the sample volume on the amount of fipronil extracted from the sample onto the PA fiber. The sample volume profile was studied by monitoring the GC area counts as a function of sample volume. The response increased around 20% when the water volume increased from 1.5 to 4 ml while larger sample volumes did not produce a significant increase in the response. The rest of the experiments were carried out at a 4 ml sample volume. Fig. 4 shows a typical chromatogram obtained under the above mentioned conditions.

3.2. Analytical parameters

The calibration graph for the samples treated according to the procedure described previously, monitored using the SIM mode, is linear for the concentration range 0.3–100 ng ml^{-1} ($r=0.9986$). The lack-of-fit test [9] was used to check the linearity of the calibration graph. The test was performed by comparing the variability of the current model residuals to the variability between observations at replicate values of the independent variable x. Since the P-value obtained ($P=0.65$) is greater that 0.10, the linear model appears to be adequate for the observed data. Two replicates were used for each of six prepared standards to obtain the calibration graph. The equation for the calibration graph was $y=0.0318x-0.0306$.

The detection limit was calculated by comparing the signal-to-noise ratio (S/N) of the lowest detectable concentration to a $S/N=3$. The detection limit found was 0.08 ng ml^{-1}. An $S/N=10$ was applied for the calculation of the quantification limit. The quantification limit found was 0.27 ng ml^{-1}.

The precision was measured for fipronil concentrations of 2, 10, 50 and 75 ng ml^{-1} by performing 10 independent determinations. The relative standard deviations (RSDs) were 11.2, 9.8, 9.5 and 9.3%. These values are commonly found in SPME studies [10].

3.3. Application and validation of the proposed method

3.3.1. Analysis of water samples

We tried to find fipronil in ground water from the Santa Maria farm, near Granada and in tap water from the city of Granada city. We did not find fipronil above our detection limit.

Validation of the proposed method for water samples was carried out on spiked samples (final fipronil concentrations of 5.0 ng ml^{-1} for tap water and 2.5 ng ml^{-1} for ground water) using the standard addition methodology [11], whereby three experiments are required to obtain the data set necessary to obtain the proposed statistical protocol: (a) standard calibration (SC): as described above; (b) standard addition calibration (AC): which is obtained by addition of continuous variations of standard at constant sample volume; (c) Youden calibration (YC). The Youden method [12] involves calibration curves established with continuous variations of sample. In this curve, the value that corresponds to sample volume ''zero'' is not included. A difference between the intercepts of the curves SC and YC indicates a bias component due to sample matrix effect. Linear regression analysis was applied.

The parameters obtained from these three checks are reported in Table 1. Student's *t*-test shows the similarity of the representative values of slope of SC and AC. On the other hand, the non-significant value of the intercept in the YC reveals the absence of matrix effect. Finally, the trueness of the results is verified by comparison, using a *t*-test, of the means of the analyte concentrations obtained from SC and AC graphs. *P*-Values obtained are greater than 5%, hence it is inferred that our method is true because of the similarity of the results for the analyte contents calculated from SC and AC graphs which are not significantly different (see Table 1).

3.3.2. Analysis of soil samples

The proposed method was applied to the de-

Table 1
Statistics for the determination of fipronil in water samples

Parameter	Tap water			Ground water		
	SC	AC	YC	SC	AC	YC
Calibration						
n	12	10	8	12	10	8
a	0.06	1.721	−0.0025	0.067	0.729	−0.0367
b	0.337	0.342	0.336	0.269	0.267	0.284
Syx	0.093	0.056	0.099	0.045	0.048	0.034
Sp		0.063			0.042	
t(*b*)		0.525			0.032	
		P=28%			*P*=61%	
b_p		0.336			0.267	
a′	0.087	1.732		0.084	0.729	
YB			−0.089			−0.101
Analysis						
C_{sample} (ng ml^{-1})	5.08	5.15		2.67	2.77	
t(*c*)	0.836 (*P*=38%)		1.24 (*P*=26%)			

SC, Standard calibration; AC, standard addition calibration; YC, Youden calibration; *n*, number of measurements; *a*, intercept; *b*, slope; *Syx*, regression standard deviation; *Sp*, pooled standard deviation of SC and AC; *t*(*b*), statistic for slope; *bp*, pooled slope of AC and SC; *a*′, corrected intercept; YB, Youden blank; *t*(*c*), statistic for analyte content.

termination of fipronil in two soil samples from the fertile plain of Granada.

Although soil is a matrix which does not allow one a direct extraction of fipronil by SPME the sample can be extracted with polar solvents to extract the target compound from soil. Then, SPME can be used to concentrate the analyte and to determine it by GC–MS.

Methanol, acetone, acetonitrile and water were tested as solvents in ultrasonic extraction of fipronil in soils as a previous step to SPME. Acetonitrile provided the highest recovery from soil but fixing of the analyte on the fiber requires mixtures of acetonitrile and water. Acetonitrile–water (15:85, v/v) was established as a good compromise.

The validation for spiked soil samples was tested by using a one-sample test (Student's *t*-test) [13]. Soil samples were fortified with different levels of fipronil. Quantification of fipronil concentrations was completed by the standard additions method. The *P*-values calculated, in all cases, are greater than 0.05 and therefore the null hypothesis might be accepted (Table 2). The detection limit was 9 μg kg^{-1}.

Table 2
Results of assays to check the accuracy of the proposed method for fipronil in spiked soil samples (concentration in μg g^{-1}) and human urine samples (concentration in ng ml^{-1})

Sample	Concentration		*t*	*P* (%)
	Spiked	Found[a]		
Soil 1	0.05	0.047±0.004	1.91	11.5
	0.10	0.092±0.013	1.51	19.2
	0.30	0.286±0.026	1.29	25.3
Soil 2	0.05	0.046±0.005	1.81	12.9
	0.10	0.096±0.008	1.22	27.6
	0.30	0.284±0.022	1.82	12.8
Human urine 1	5	5.01±0.53	0.04	97.1
	15	14.70±0.62	1.20	28.3
	30	30.03±3.11	0.02	98.5
Human urine 2	5	4.83±0.38	1.08	32.9
	15	14.49±1.01	1.23	27.4
	30	28.98±1.63	1.53	18.5

P Value of the one-sample comparison test.
[a] Average value±standard deviation of six determinations.

3.3.3. Analysis of human urine samples

Validation for spiked human urine samples was carried out by using a one-sample test (Student's *t*-test) [13]. Urine samples were fortified with different levels of fipronil. Quantification of fipronil concentrations was completed by the standard additions method. The *P*-values calculated, in all cases, are greater than 0.05 and so the null hypothesis might be accepted (Table 2). The detection limit was 0.7 ng ml^{-1}.

4. Conclusions

A simple and practical GC–MS method in combination with SPME for the determination of the pesticide fipronil in water, soil and human urine samples is presented. Maximum responses were obtained using an 85 μm PA fiber, 30 min immersion time, pH 9.5 and 60°C. In view of its simplicity and sensitivity, it is recommended for the quantification of fipronil in water and soil samples in environmental studies, as well as for eventual detection in human urine in forensic or toxicological studies.

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