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Determination of Traces of Fipronil and Its Metabolites in Pollen by Liquid Chromatography with Electrospray Ionization−Tandem Mass Spectrometry

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Fipronil is a pesticide suspected of having harmful effects on honey bees at microgram per kilogram levels. Considering the lack of methodology, it thus appeared to be necessary to develop a method for the determination of the lowest amounts of fipronil and its metabolites in pollen. This paper describes a new analytical method with a limit of quantification (LOQ) of 0.1 μg/kg for a representative sample weight of 5 g. In the case of a field study, this tool was used for checking the possible existence of fipronil and/or metabolites in pollen samples, but none of them contained residues higher than the LOQ. This three-step rapid method uses liquid−solid solvent extraction with mechanical grinding, followed by liquid−liquid partitioning and Florisil solid-phase extraction for the two cleanup steps. The quantification is made by liquid chromatography with electrospray ionization−tandem mass spectrometry (LC-ESI-MS/MS). Indeed, combined with an adequate sample treatment, this technique offers good sensitivity and selectivity in such a complex matrix. The method has given good recoveries of 74−104% with relative standard deviations of 5.6−18.2%.

KEYWORDS: Fipronil; metabolites; pollen; liquid chromatography; electrospray ionization; tandem mass spectrometry; mass spectrometry mechanism; method validation; normalized guidance

INTRODUCTION

Fipronil, (±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile, a phenylpyrazole insecticide, was developed by Rhone Poulenc Agro. This chemical compound, for which commercial authorization was delivered in France in 1994, is used against culture pests, clickbeetles, grasshopper, ants, fleas of pets, etc. (*1*). As a consequence, this molecule enters into the composition of numerous commercial preparations for professional agriculture, for gardening, or for veterinary treatments. It acts as a potent blocker of the γ-aminobutyric acid (GABA)-regulated chloride channel (*2*−*4*) involved in the transmission of the nervous flow. This compound may be found in pollen and the nectar of the plants issued from treated seeds such as sunflowers and maize (*5*). Nontarget pollinator insects such as honey bees collect pollen on maize and pollen and nectar on sunflowers. They bring the pollen back to their colonies, where it is stored and mixed with glandular secretions. This mix of pollens is used by bees for feeding their larvae and as winter food.

New studies have shown that the foraging activity (*6*) and the olfactory learning performance (*7*, *8*) of honey bees are affected by sublethal doses of fipronil and its metabolites in microgram per kilogram concentrations and even at lower amounts. To assess this threat, it was necessary to use a sensitive and specific analytical procedure for the determination of very low amounts of fipronil and its metabolites in sunflower and maize pollens.

Few methods have been reported for the analysis of fipronil only. Morzycka analyzed fipronil in honey bees with gas chromatography and nitrogen−phosphorus detection (NPD) (*9*). Vilchez et al. analyzed fipronil by solid-phase microextraction (SPME) with gas chromatography and simple-stage mass spectrometry in water, human urine, and soil (*10*). Both methods show a high limit of quantification, even though the latest one gave a limit of quantification (LOQ) of 30 μg/kg in soil samples. In addition, NPD and simple-stage quadrupole were not selective enough to analyze with a maximum of confidence very low amounts of analyte.

The aim of this work was to drastically lower the LOQ in pollen samples. However, in a rich and complex matrix such as pollen, the sample extraction (*9*) had to be modified, complete with adequate cleanup, and the analytical detector had to be more sensitive and selective. Furthermore, the required method must satisfy normalized criteria to give results as close as possible to reality.

Consequently, we developed a convenient sample treatment and analysis by LC-ESI-MS/MS, which enables the determination of fipronil and also its main biologically active metabo-

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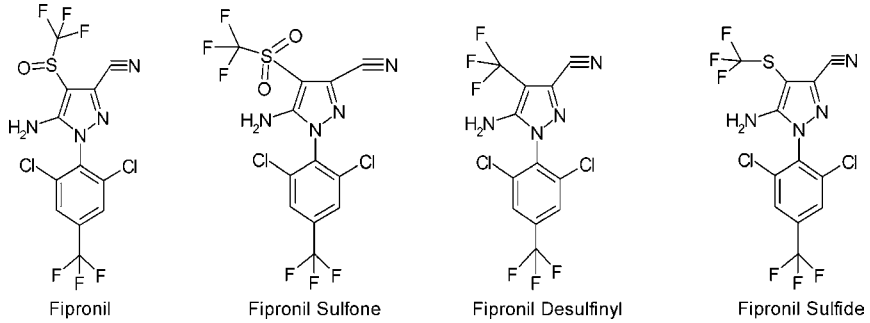

Figure 1. Molecular structures of fipronil and metabolites quantified.

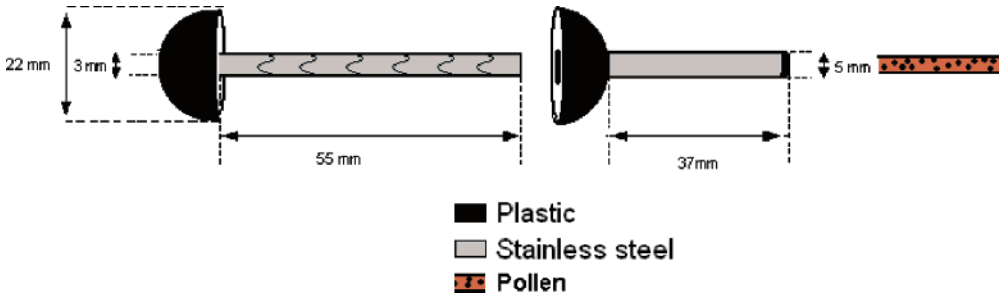

Figure 2. Outline of the manual syringe extractor.

lites: fipronil sulfone, fipronil desulfinyl (*11*), and fipronil sulfide (*12*). Their structures are shown in **Figure 1**.

This paper describes the analytical procedure developed, its validation with a French normalized guidance (*13*), and its use as a monitoring tool for multifloral pollen in the investigation of potential exposure of honey bees to fipronil. More generally, the presented method can be easily adapted for the analysis of vegetables and fruits.

MATERIALS AND METHODS

Reagents. Analytical reference standards were obtained as follows: fipronil (97.5% purity), from Sigma-Aldrich (Seelze, Germany); fipronil sulfone (99.7% purity), from Rhone-Poulenc Agro (Lyon, France); fipronil desulfinyl (98% purity), from AccuStandard (New Haven, CT); and fipronil sulfide (99% purity), from Dr. Ehrenstorfer (Augsburg, Germany). Their molecular structures are described in **Figure 1**.

Stock solutions of all standards were carefully prepared, with the use of gloves, under a suction hood by dissolving a weighed amount of insecticide into pesticide-grade acetone. The resulting solutions were stored at 4 °C in closed amber bottles.

All solvents (acetonitrile, *n*-hexane, methanol, dichloromethane, and acetone) were of analytical quality and were purchased from Fisher Bioblock Scientific (Illkirch, France).

Materials. The solid-phase material used for the solid-phase extraction was Florisil 60−100 mesh and was supplied by Fischer Bioblock Scientific. This sorbent was freshly activated by heating overnight at 180 °C and was stored in a well-closed flask. Filtration of the matrix extract was carried out on a Büchner filled with Celite 545 from Acros Organics (Fair Lawn, NJ).

Apparatus. The complete analytical instrument was a ThermoFinnigan system purchased from Thermoelectron (Courtaboeuf, France). It consisted first of a Surveyor HPLC system equipped with a dual piston quaternary low-pressure mixing LC pump, with a built-in vacuum degasser, a 20 μL loop injection, a temperature-controlled autosampler, and a column oven. The system was fitted with a stainless steel column Chromsep Polaris C18-A 100 × 3.0 mm packed with 3 μm particles provided by Varian (Genneviliers, France). The HPLC system was coupled with a triple-stage quadruple mass spectrometer TSQ Quantum using electrospray source interface (ESI) and above it, located on the front panel, a motorized divert/inject valve.

To avoid extracting any wax, a manual syringe extractor (described in **Figure 2**) was used. It allowed a mechanical coring of pure enclosed pollen. This tool was made with a lathe. It is composed of stainless steel and plastic and fitted with a wire. It can be easily made by a machining factory.

Water used was ultrapurified with a Vivendi Elga Maxima system from Veolia water (Decines, France).

An MS2 shaker permitted the homogenization of the solvent extract and was purchased from IKA (Staufen, Germany). The pollen sample was pulverized with an automatic high rotation speed dispersion unit Ultra-turrax T25basic from IKA.

Organic solvents were evaporated under a N_2 flow with a Zymark TurboVap II concentration workstation produced by Zymark Center (Hopkinton, MA).

Sample Treatment. The desired amount of pollen was first extracted from the cells where it was stored by honey bees. A sample of spiked pollen (5 g) was pre-extracted in a 100 mL beaker by ultrasonication in 50 mL of acetonitrile for 1 min. It was then ground with an Ultra-turrax at 6500 rpm for 4 min. The supernatant was filtered through a Büchner with a Celite bed and collected in a 500 mL suction flask. This operation was repeated twice, but the second time, grinding was performed during 2 min and the speed was set at 9500 rpm.

This acetonitrile extract was transferred to a separatory 500 mL glass funnel; 100 mL of *n*-hexane previously saturated with acetonitrile was added. After 2 min of vigorous shaking, the acetonitrile phase was decanted into a glass sample tube of 200 mL. The extract was evaporated to dryness in a water bath adjusted to 55 °C. The inlet source of N_2 to the evaporation station was of 4.8 bar. The residue was dissolved by ultrasonication and shaken into 5 mL of *n*-hexane, and the sample tube was washed with another 5 mL.

These two volumes of n-hexane were successively loaded on the head of a chromatographic glass column filled with 7 g of freshly conditioned Florisil. After the hexane was drained to the waste, fipronil and its metabolites were eluted out with 30 mL of dichloromethane/methanol (9:1) into a clean 200 mL tube. The eluate was evaporated to dryness after the bath temperature reached 40 °C. First, 700 μL of methanol and short-time sonication were used to dissolve the residue. Second, 300 μL of deionized water was added. Finally, this 1 mL mixture was homogenized and subjected to LC-MS/MS analysis.

HPLC. Gradient elution was carried out using a binary gradient composed of solvent A (methanol) and solvent B (water) according to the following program: maintain 70% A from 0 to 6 min, linear gradient from 70 to 100% A from 6 to 9 min, maintain 100% A from 9 to 11 min, return to the initial condition from 11 to 14 min, and maintain this condition to 18 min. For all LC experiments the flow rate was 0.5

Table 1. Ions Monitored under the SRM Mode by LC-MS/MS[a] with Their Relative Intensities (Percent)

compound	precursor ion[b] (*m*/*z*)	product 1 (*m*/*z*)	product 2 (*m*/*z*)	collision energy (V)
fipronil	435	329.6 (100)	249.7 (35)	21–33
desulfinyl	387	350.8 (100)	281.7 (11)	18–40
sulfone	451	414.9 (100)	281.8 (24)	20–19
sulfide	419	261.9 (100)	382.8 (73)	40–21

[a] The compounds were quantified with both product 1 and product 2 ions. The ^{37}Cl-labeled molecule signals can also be checked as a supplementary criterion of specificity. [b] Ionized in the negative mode.

mL/min, the sample injection volume was 20 μL, and the oven and tray temperatures were controlled at 25 °C. To preserve the ESI from endogenous compounds, the LC flow was diverted to the waste from 0 to 3 min and from 6 to 18 min.

Mass Spectrometry. Prior to tuning with fipronil and metabolites, the mass scale and electron multiplier on the mass spectrometer were calibrated in negative ion mode using a solution of 1,3,6-polytyrosine.

The mass spectrometer parameters for the LC-MS/MS analysis of fipronil and metabolites were found. Mass analysis was performed in the negative ion polarity using selected reaction monitoring (SRM) scan mode. The negative ion polarity settings were −3000 V for ESI needle voltage, a 40 arbitrary unit sheath gas pressure, a 30 arbitrary unit auxiliary gas pressure, a 350 °C ion capillary temperature, a 4 V source collision-induced dissociation offset, and 1.5 mTorr for the second-quadrupole collision gas (Ar) pressure.

The optimized collision energy was tuned for each SRM transition as presented in **Table 1**.

Instrument (ILOQ) and Estimated Method Limit of Quantification (EMLOQ). The ILOQ (micrograms per liter) is treated as the minimum concentration of pure analyte that can be reliably quantified by the LC-MS/MS system used in this study. The ILOQ of fipronil and its metabolites were estimated through the statistics of the linearity validation.

The EMLOQs for each compound were calculated from the ILOQ as follows:

$$\text{EMLOQ}\ (\mu\text{g/kg}) = \frac{\text{ILOQ} \times 1 \times 0.85}{M}$$

The term 1 refers to the final 1 mL of mixture subjected to analysis, and 0.85 represents the density of the solvent 7:3 methanol/water mixture. $M \leq 5$ is the mass of the sample analyzed (g). For a standard weight of 5 g the EMLOQ is equivalent to the LOQ of the global method.

RESULTS AND DISCUSSION

LC-MS/MS Conditions. A gradient system (methanol and water) was applied to try to separate the fipronil and its metabolites as peaks almost independently. Fipronil, which has a log $P_{o/w} = 4.0$ (*14*), is poorly water soluble. As a result, its hydrophobic character induces a high retention on the C_{18} phase LC column. In addition, the final residue needed to be dissolved in a first volume of pure methanol. Consequently, the gradient elution started with a proportion of 70% of methanol and 30% of water. The details of the optimized gradient conditions are described under Materials and Methods. **Figure 3** shows typical LC-MS/MS chromatograms of pollen fortified before extraction.

ESI is widely applied for the analysis of pesticides (*15*). Coupled with tandem mass spectrometry, the precursor ions formed by the ESI can be selected in the first quadrupole. Afterward, they collide in the second quadrupole under a low argon pressure. Finally, the product ions are selected. The double mass selection enables a highly specific quantification in SRM scan mode. The conditions of the mass spectrometer were optimized, and the appropriate SRM transitions were determined by flow injection analysis (FIA) with the syringe pump of the individual solutions of the pesticides (100 μg/L). The tuned values are shown under Materials and Methods. The quantitation was carried out with both transitions per compound. The specificity was obtained by following the two specific fragmentations for each compound. For fipronil, the first fragment, at *m*/*z* 329.6, is due to the loss of both HCl and CF_3. The second fragment at *m*/*z* 249.7 is due to the loss of HCl, CF_3, C_2N_2, and CO. For the desulfinyl, the first fragment, at *m*/*z* 350.8, is due to the loss of HCl. The second fragment, at *m*/*z* 281.7, is due to the loss of both HCl and CF_3. For the sulfone, the first fragment, at *m*/*z* 414.9, is due to the loss of HCl. The second fragment, at *m*/*z* 281.7, is due to the loss of both HCl and SO_2CF_3. For the sulfide, the first fragment, at *m*/*z* 382.8, is due to the loss of HCl. The second fragment, at *m*/*z* 261.9, is due to the loss of HCl, CF_3, and C_2N_2. The signals of the ^{37}Cl-labeled molecules can be checked as a supplementary criterion of specificity.

Sample Treatment. An analytical procedure for the determination of pesticide traces in a complex matrix such as pollen requires extraction from the solid matrix and adequate cleaning of the extract before chromatographic analysis. In the present method the weighed amount of sample is 10-fold higher than in former methods (*9*, *10*), in which the weights were 0.5 g. This represents a real advantage because it is more representative of the entire sample.

Extraction. In the present study, it was first necessary to extract the target compounds from the pollen.

Dissolution in a polar solvent such as water was tested, but it gave an emulsion very difficult to manipulate afterward. As a consequence, it was necessary to help the extraction with mechanical grinding. Effectively, the pollen needs to be pulverized to facilitate the liberation of the compounds. Acetonitrile was tested and enabled a comfortable and efficient grinding of the matrix. In addition, as reported in ref *10*, this solvent provided good recoveries.

Cleanups. Lipids are present in pollen, and their proportion can reach 10% (*16*). These interferents can seriously disturb the analysis. The traditional liquid−liquid partitioning (LLP) with *n*-hexane saturated with acetonitrile permits the removal of lipid coextractives. The LLP was repeated three times with pure solvents, and 100% of each compound was at each time recovered. Thus, this first cleanup step did not induce any loss of our analytes.

Moreover, we speculated that some other polar interferents could be removed. Therefore, Florisil was applied to carry out a second cleanup step. The conditions of its application needed to be determined. The experiments were performed in duplicate. The elution profile of fipronil and its metabolites with mixtures of hexane, dichloromethane, and methanol is listed in **Table 2**.

All of the analytes were retained when the dichloromethane proportion was <20%. Fipronil's metabolites were all eluted when the dichloromethane was increased to 100%, whereas the whole amount of fipronil needed 10% of methanol to be completely eluted.

Second, to find the minimum volume of elution, successive additions of 10 mL of 0:9:1 *n*-hexane/dichloromethane/methanol were applied until nearly the total amount of fipronil and metabolites was eluted. The experiments were performed in duplicate, and the successive recoveries are displayed in **Table 3**.

The elution volume was consequently reduced from 50 to 30 mL.

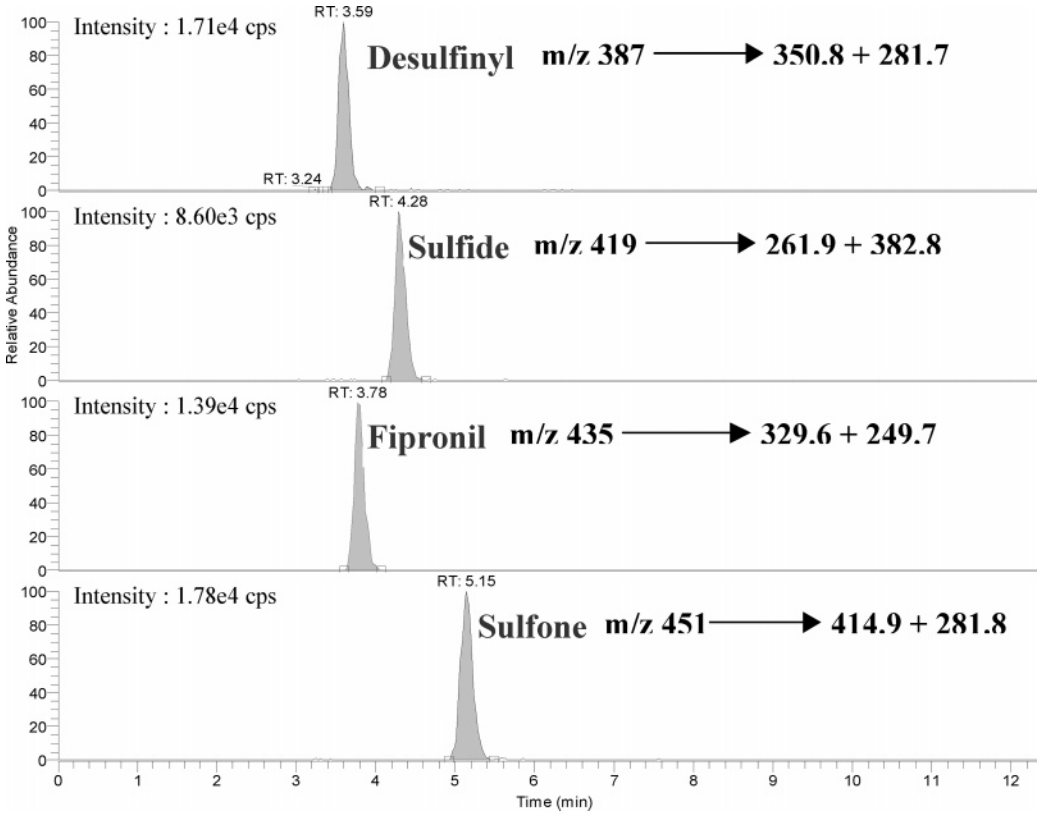

Figure 3. LC-MS/MS chromatograms obtained from initially fortified pollen sample (0.1 μg/kg).

Table 2. Elution of Fipronil and Its Metabolites from Florisil Sorbent Column ($n = 2$)

elution mixture, *n*-hexane/dichloromethane/ methanol (v/v/v, 50 mL)	elution rate from (%)			
	fipronil	desulfinyl	sulfide	sulfone
1:0:0	0	0	0	0
9:1:0	0	0	0	0
8:2:0	0	0	0	0
2:8:0	29	99	99	95
0:1:0	94	99	99	100
0:9:1	100	99	99	100

Table 3. Recoveries for Successive Elution Volumes Added ($n = 2$)

eltuion mixture, *n*-hexane/dichloromethane/ methanol (0:9:1)	elution rate (%)			
	fipronil	desulfinyl	sulfide	sulfone
first 10 mL	80	85	86	81
second 10 mL	18	13	13	18
third 10 mL	2	0	0	0

Method Validation. Method validation was carried out according to the French NF V 03-110 guidance (*15*) for the validation of quantitative analysis methods of agricultural foods.

Linearity and Limits of Quantification. The linearity study was used, on the one hand, to validate a linear dynamic range and, on the other hand, to statistically determine the ILOQ for each component.

To carry out this study, five repetitions of six levels of concentration ranging from 0.1 to 20 μg/L were prepared and injected. All of the compounds showed a very satisfying linearity with all of the correlation coefficients as good as 0.9999.

Fisher's test (*13*) was realized, and it enabled both the adequacy and the range of the linear model.

The ILOQ was estimated by using the parameters of the calibration function $y = a + bx$ and obtained via ILOQ = $[a + 10S(a)]/b$, where $S(a)$ is the intercept standard deviation and b is the slope of the regression line. The parameters of the linearity calibrations, the ILOQs, and the calculated LOQs (according to the formula under Materials and Methods) are displayed in **Table 4**.

Table 4. Linearity Calibrations,[a] ILOQs and LOQs for Fipronil and Metabolites (Five Calibrations Separately Prepared and Injected)

compound	intercept	slope	correlation coefficient	ILOQ (μg/L)	LOQ (μg/kg)
fipronil	−14317.9	273837.2	0.9999	0.3	0.05
desulfinyl	−6614.4	62387.0	0.9999	0.4	0.07
sulfide	−4105.4	62695.8	0.9999	0.4	0.07
sulfone	−9751.9	113446.7	0.9999	0.5	0.09

[a] These data were used to establish the linearity with a confidence level of 99% (Student's and Fisher's tests).

For all of the compounds the limit of quantification chosen was 0.1 μg/kg and the limit of detection was set to 0.05 μg/kg. This value is superior to the calculated values to enhance the confidence in the final result at such low levels.

Specificity. According to the guidance (*13*), to show that the evaluated method satisfies the criterion of specificity, an experiment schedule needed to be followed. The schedule aims at checking the absence of interferences (matrix effects), which could influence the response. The experiment consists of pure standard additions from 0.1 to 10 μg/kg. The amounts added were of the same quantity order as initially quantified in the 10 pollen extracts chosen. The results of these measures are presented in **Table 5**.

For each analyte, a graph was plotted using the amount added versus the amount recovered.

The specificity was acceptable for each because the overlap line $y = b + ax$ was equivalent to the line $y = x$ as demonstrated

Table 5. Pollen Sample Extracts Initially Quantified and Analyzed after Additions of Pure Standard Amounts

fipronil (μg/kg)			
initial amount in extract	amount added	quantified after addition	amount recovered
0.31	0.10	0.39	0.08
0.44	0.20	0.58	0.14
0.64	0.40	1.06	0.42
0.95	0.80	1.72	0.77
1.32	1.00	2.36	1.04
2.66	2.00	4.29	1.64
4.62	4.00	8.81	4.20
5.67	5.00	10.85	5.18
8.30	8.00	16.96	8.65
11.23	10.00	21.50	10.27

Table 6. Mean Recoveries of Pollen Samples Fortified at Four Different Levels and Relative Standard Deviations (RSD) ($n = 5$)

fortification level (μg/kg)	recovery (%)	RSD (%)
fipronil in fortified pollen		
0.1	80	17.3
0.5	93	18.2
1	87	12.1
5	89	13.6
desulfinyl in fortified pollen		
0.1	78	9.9
0.5	93	9.8
1	104	10.2
5	75	14.5
sulfide in fortified pollen		
0.1	74	9.3
0.5	97	5.6
1	85	10.1
5	80	6.8
sulfone in fortified pollen		
0.1	86	9.1
0.5	89	10.7
1	94	9.0
5	74	8.8

by a statistical test (Student's test) (*13*). It showed a slope *A* equivalent to 1 and an intercept point *B* equivalent to 0.

The specificity was obtained with the chromatogram retention time and the two characteristic fragmentations of the active ingredient.

Accuracy and Repeatability. Furthermore, the accuracy and the precision (mentioned in this work as the repeatability) of the method have been considered in accordance with the guidance. Twenty samples of blank pollen were fortified and extracted at four different levels following the operative mode described.

The accuracy was measured by calculating the recovery, and the repeatability was studied by calculating the relative standard deviation (RSD). **Table 6** shows the accuracy and repeatability results.

Application to Analysis of Field Samples. A study aiming at checking the possible presence of traces of fipronil and/or metabolites in pollen from different apiaries was carried out. These apiaries were located in the Indre département (the administrative unit surrounding the town of Chateauroux in the center of France). Six different apiaries, all exposed to sunflower and maize cultures, were visited, first before the honey flow (on June 22, 2005) and second after the flowering (on July 22, 2005).

In each apiary, five hives randomly selected were sampled. All 60 samples were sent to our laboratory by a means of refrigerated transport. Among the analyzed set of samples, 58 of them showed no traces of either fipronil or its metabolites. Two samples showed a positive result for fipronil and its photodegradation product desulfinyl, but not higher than the LOQ of 0.1 μg/kg.

Conclusion. The method developed allows the determination of traces of fipronil and its metabolites in pollen. It was validated according to a normalized guidance. The LOQ of the global method is low and adapted for checking the eventual presence of traces of fipronil and/or its biologically active ingredients in pollen. It is suitable for an important and representative initial sampling of 5 g.

This method was applied to pollens sampled for a field study. In these samples, neither the fipronil nor any of its metabolites were quantified, even though fipronil and its desulfinyl metabolite were detected (>LOD) in two of them.

Finally, the present method has been adapted to analyze honey bees and their larvae (not shown) and can be easily adapted to more general fruit and vegetable samples.

LITERATURE CITED

(1) *Index Phytosanitaire ACTA 2006*, 42nd ed.; Couteux, A., Lejeune, V., Ed.; Association de coordination Technique Agricole: Paris, France, 2006; p 167.

(2) Cole, L. M.; Nicholson, R. A.; Casida, J. E. Action of phenylpyrazole insecticides at the GABA-gated chloride channel. *Pestic. Biochem. Physiol.* **1993**, *46* (1), 47−54.

(3) Mohamed, F.; Senarathna, L.; Percy, A.; Abeyewardene, M.; Eaglesham, G.; Cheng, R.; Azher, S.; Hittarage, A.; Dissanayake, W.; Sheriff, M. H. R.; Davies, W.; Buckley, N. A.; Eddleston, M. Acute human self-poisoning with the *N*-phenylpyrazole insecticide fipronil—a GABA(A)-gated chloride channel blocker. *J. Toxicol. Clin. Toxicol.* **2004**, *42* (7), 955−963.

(4) Sammelson, R. E.; Caboni, P.; Durkin, K. A.; Casida, J. E. GABA receptor antagonists and insecticides: common structural features of 4-alkyl-1-phenylpyrazoles and 4-alkyl-1-phenyltrioxabicyclooctanes. *Bioorg. Med. Chem.* **2004**, *12* (12), 3345−3355.

(5) Aajoud, A.; Raveton, M.; Aouadi, H.; Tissut, M.; Ravanel, P. Uptake and xylem transport of fipronil in sunflower. *J. Agric. Food. Chem.* **2006**, *54*, 5055−5060.

(6) Colin, M. E.; Bonmatin, J. M.; Moineau, I.; Gaimon, C.; Brun, S.; Vermandere, J. P. A method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. *Arch. Environ. Contam. Toxicol.* **2004**, *47* (3), 387−395.

(7) Decourtye, A.; Devillers, J.; Genecque, E.; Le, M. K.; Budzinski, H.; Cluzeau, S.; Pham-Delegue, M. H. Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*. *Arch. Environ. Contam. Toxicol.* **2005**, *48* (2), 242−250.

(8) El Hassani, A. K.; Dacher, M.; Gauthier, M.; Armengaud, C. Effects of sublethal doses of fipronil on the behavior of the honeybee (*Apis mellifera*). *Pharmacol. Biochem. Behav.* **2005**, *82* (1), 30−39.

(9) Morzycka, B. Simple method for the determination of trace levels of pesticides in honeybees using matrix solid-phase dispersion and gas chromatography. *J. Chromatogr. A* **2002**, *982* (2), 267−273.

(10) Vilchez, J. L.; Prieto, A.; Araujo, L.; Navalon, A. Determination of fipronil by solid-phase microextraction and gas chromatography−mass spectrometry. *J. Chromatogr.* A **2001**, *919* (1), 215−221.

(11) Hainzl, D.; Cole, L. M.; Casida, J. E. Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. *Chem. Res. Toxicol.* **1998**, 1529−1535.

(12) Beeler, A. B.; Schlenk, D. K.; Rimoldi, J. M. Synthesis of fipronil sulfide, an active metabolite, from the parent insecticide fipronil. *Tetrahedron Lett.* **2001**, *42* (32), 5371−5372.

(13) Analysis of agri-foodstuffs—intralaboratory procedure for an alternative method compared to a reference method—case of quantitative analysis methods. In *NF V 03*-110; Association Francaise de Normalisation: Paris, France, 1998; pp 1−40.
(14) *The Pesticide Manual*, 10th ed.; Tomlin, C. D. S., Ed.; British Crop Protection Council: Surrey, U.K., 1994; p 463.
(15) Slobodnik, J.; van Baar, B. L. M.; Brinkman, U. A. T. Column liquid chromatography−mass spectrometry: selected techniques in environmental applications for polar pesticides and related compounds. *J. Chromatogr. A* **1995**, *703* (1−2), 81−121.
(16) Sommerville, D. C. Lipid content of honey bee-collected pollen from south-east Australia. *J. Exp. Agric.* **2006**, *45* (12), 1659−1661.

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