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Poly- and Monoclonal Antibody-Based ELISAs for Fipronil

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An enzyme-linked immunosorbent assay (ELISA) for fipronil was developed by using polyclonal antibodies (pABs) or monoclonal antibodies (mABs), and its suitability of the determination of this analyte in spiked water samples was studied. The pABs-based assay showed I_{50} = 17.95 ppb, I_{90} = 203.40 ppb, and I_{10} = 0.066 ppb, whereas the mABs-based assay showed I_{50} = 5.99 ppb, I_{90} = 485.40 ppb, and I_{10} = 0.074 ppb. The recoveries of fipronil from tap water samples by pABs-based ELISA were 93.00−124.00% in the range of 0−500 ng/mL, and those obtained from the samples by mABs-based ELISA were 94.70−108.00%. Different types of water from pool, river, and sea were spiked at different levels (ranging form 0.1 to 10 μg/L) and were assayed by the indirect ELISA with mABs. The recoveries of fipronil by this ELISA were in the range of 80−120%. The results demonstrate that this assay is suitable for the quantitative detection of fipronil at trace levels in water samples.

KEYWORDS: Fipronil; hapten; polyclonal antibody; monoclonal antibody; ELISA

INTRODUCTION

Fipronil [5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]- 4 -[(trifluoromethyl)sulfinyl] -1 *H*-pyrazole-3-carbonitrile, $C_{12}H_4C_{l2}F_6N_4OS$), a phenylpyrazole insecticide, was discovered by Rhone-Poulenc Agro in 1987. Fipronil can be formulated as roach or ant baits, as flea and tick sprays for pets, and in granular turf products to control mole crickets (*1*, *2*). It is also used on a variety of foliar and soil insects including corn rootworm, Colorado potato beetle, and rice water weevil that attack a variety of crops, mostly corn and rice (*2*, *3*). Fipronil is also effective for locust and termite control and against insects in both larval and adult stages, as well as insects resistant to pyrethroid, organophosphate, and carbamate insecticides (*3*, *4*).

Few analytical methods have been reported for the determination of this compound (*5*). Bobe et al. (*6*) proposed a gas chromatographic (GC) method for the determination of fipronil residues in soils, and Vilchez et al. (*5*) reported the determination of fipronil by solid-phase microextraction and gas chromatography−mass (GC-MS) spectrometry. Therefore, it is necessary to develop some novel alternatives to determine and monitor the fipronil residues in environmental samples and agricultural products.

Over the past few years, enzyme-linked immunosorbent assay (ELISA) methods have been developed as attractive options for the identification or quantification of a variety of agrochemicals in water, soil, and, in some cases, agricultural produce (*7*−*9*). Most work has been done on immunoassays for insecticides, fungicides, and herbicides (*10*−*12*), but there are few works on immunoassays of the pesticide fipronil. Poly- or monoclonal antibody-based assays are simple, rapid, and sensitive methods and represent a high-throughput approach and alternative to pesticide residual analysis, although its analytical reliability and quantitative capability are sometime still in suspense due to cross-reactivity of the antibody with structurally related compounds and analogues and the susceptibility of biological reagent to ambient conditions. In this paper, to obtain a rapid, sensitive, and cost-effective immunodetermination method, the development of specific polyclonal and monoclonal antibodies to fipronil is reported, and the development of an ELISA for fiproinil and the evaluation of the assay's performance in water matrices are described.

MATERIALS AND METHODS

Reagents and Instruments. All regents and solvents were of analytical grade unless specified otherwise. Fipronil (96.00%) was provided by the Institute of Pesticide Inspectional Management of China. *N*,*N*-Dicyclohexylcarbodiimide (DCC), Tween-20, tri-*n*-butylamine, dimethyl sulfoxide (DMSO), isobutylchlorocarbonate, *N*-hydroxysuccinimide (NHS), dimethylaminopyridine (DMAP), and dimethylformamide (DMF) were obtained from Sino-American Biotechnology Co. Bovine serum albumin (BSA), ovalbumin (OVA), Freund's complete and incomplete adjuvants, goat anti-rabbit IgG−horseradish peroxidase, and tetramethylbenzidine (TMB) were all purchased from Sigma Chemical Co. Others include polyethylene goycol PEG4000 (Boehringer Mannheim), RPMI1640 (Sigma), hypoxanthine aminopterin thymidine (HAT), culture medium (GibcoBrl), and hypoxanthine thymidine (HT) (GibcoBrl).

The main instruments include a mass spectrometer (HP-5988, Agilent), UV−vis spectrometer (Perkin-Elmer, Lambda 25), CO_2

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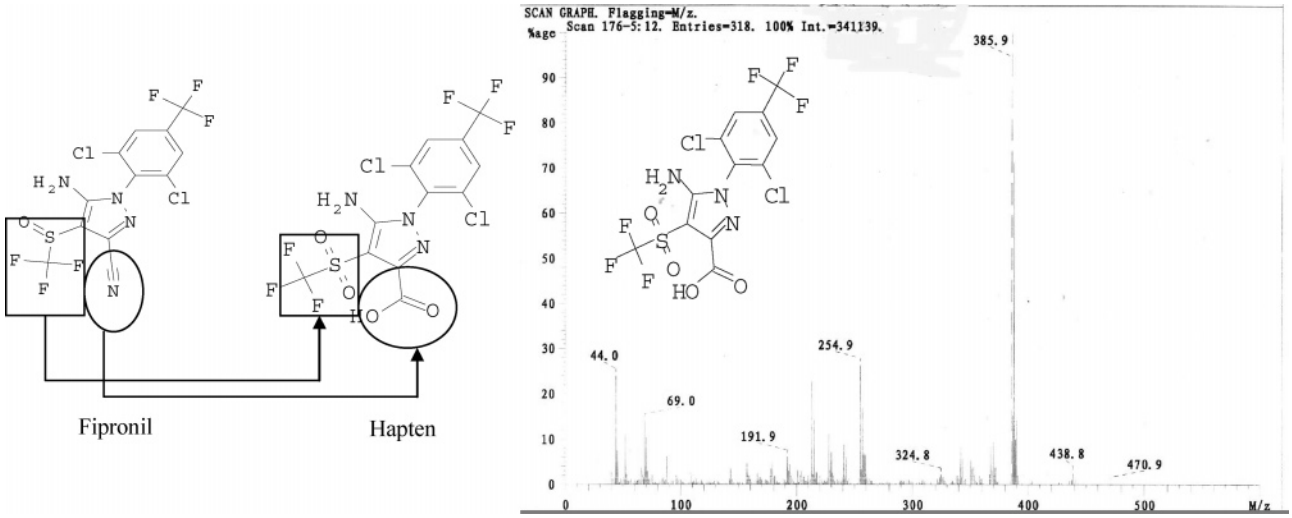


Figure 1. Structures of fipronil and hapten (fipronil analogue) and MS spectrum of the hapten.

incubators (Napco), 96-well polystyrene microplates (MaxiSorp), Multiskan EX version 1.0 (Labsystems), and Wellwash 4 MK 2 (Labsystems).

Hapten Synthesis. Fipronil and the hapten used for immunization and antigen coating are shown in **Figure 1**.The hapten (fipronil analogue, FA) mimicking the analyte (fipronil), was synthesized according to method given in ref *13* and was analyzed by MS:FAB *m*/*z*: monoisotope M_r = 470.9 (calcd for C_{12} $H_5C_{l2}F_6N_3O_4S$, 472) (**Figure 1**). In this method, hapten design is a key step in obtaining high-quality antibodies against small molecules and frequently requires considerable synthesis efforts, as it did for other small molecular compounds' immunoassays.

Preparation of Hapten−Protein Conjugates. The hapten was coupled to carrier proteins, BSA or OVA, via the active ester method (*14*). To the hapten (43 mg) dissolved in DMF (2 mL) was added a solution of NHS (10 mg) and DCC (10 mg) in DMF (1 mL). The mixture was stirred for 2 h at room temperature and filtered, and then BSA (40 mg) or OVA (30 mg) dissolved in PBS (2 mL) was added dropwise to the filtrate. The reaction mixture was stirred at 4 °C overnight. The content was dialyzed against PBS at 4 °C for 72 h. The conjugate was applied as an immunogen (hapten−BSA) or a coating antigen (hapten−OVA).

Polyclonal Antibody (pAB) Production. Three male New Zealand white rabbits (FH2934, FH2962, and FH2836, about 3 kg) were immunized with hapten−BSA. Routinely, 0.5 mg for hapten−BSA dissolved in PBS was emulsified with Freund's complete adjuvant (1:1 volume ratio) and injected intradermally at multiple sites on the back of each rabbit. After 3 weeks, each animal was boosted with an additional 0.5 mg (for hapten−BSA) of the conjugate emulsified with Freund's incomplete adjuvant. Three following boostings were conducted on a biweekly interval. Each animal was bled 7−10 days after each boosting to screen antisera. After this, the last boosting was performed with an another additional (1.0 mg (for hapten−BSA) of the conjugate and physiological saline (1:1 volume ratio). Serum produced from rabbits was isolated by centrifugation (10000*g*, 10 min, 4 °C), and sodium azide was added as a preservative at a final concentration of 0.02%. Serum was then aliquotted and stored at −70 °C for use.

Monoclonal Antibody (mAb) Production. *Immunization.* Mice were immunized as for rabbits, except that 0.1 mg of the conjugate (hapten−BSA) was used and doses were divided intraperitoneally and subcutaneously. Following two boostings at fortnightly intervals, mice were bled 8 days after. Those animals with a combination of high titer and good displacement by free fipronil were rested for 6−10 weeks and boosted intraperitoneally with 0.1 mg of the conjugate (hapten−BSA); their spleens were removed for the fusion of spleen cells with Sp 2/0 myelomas.

Cell fusion. Three days after the final boosts, the mice were killed and their spleens removed. The spleen cells were fused with the myeloma cell by spinning together in the presence of 50% polyethylene glycol and 10% filter-sterilized DMSO, following the method of Kennet et al. (*15*). Growth of the fused cells (hybridomas) was aided by supplementing the routine cell culture medium containing 20% fetal calf serum with peritoneal macrophages from young mice (*16*).

Hybridoma Production. Fused cells were placed in 96-well plates at 37 °C with 8% CO_2. After 7−10 days, when growth of the hybridomas was almost confluent, culture supernatants were screened by ELISA. Clones that produced antibodies reacting with fipronil were bulked to 24-well plates and subsequently cloned twice by limiting dilution (*17*). We then bulked subclones in 25-mL tissue culture flasks, and supernatants were drawn for screening when growth was confluent and the medium was beginning to show signs of acid production. All subclones of interest were frozen in a mixture of 90% fetal calf serum at −70 °C in a Cryo freezer for 24−48 h and then transferred to liquid nitrogen.

Screening of Antisera. The titer of the serum from each animal was determined by measuring the binding of serial dilutions to microtiter plates coated with several different concentrations of the homologous coating antigens. Microtiter plates were coated with hapten−OVA in CBS and incubated for 2 h at 37 °C. The plates were washed three times with PBST (10 mM PBS containing 0.05% Tween-20, pH 7.4) and were blocked by incubation with 1% OVA in PBS (200 μL/well) for 1 h. After another washing step, 100 μL/well of antiserum previously diluted with PBS was added. After incubation for 2 h, the plates were washed. Subsequently, 100 μL/well of a diluted goat anti-rabbit IgG−horseradish peroxidase was added and incubated for 1 h, and after another washing step, 100 μL/well of a TMB solution (110 μL of 10 mg/mL TMB−DMSO and 30 μL of 0.65% H_2O_2 diluted with 10.86 mL of citrate−acetate buffer, CPBS, pH 5.5), was added. The reaction was stopped after 15 min by adding 50 μL/well of 2 M H_2SO_4, and absorbance was read at 450 nm. After each washing, the plate was banged upside down on some paper towels to remove all of the liquid.

Indirect Competitive ELISA. Antigen coating and antibody concentrations for the competitive assays were optimized. Also, the tolerance of the ELISA to methanol used to dissolve pesticides was tested for assay optimization. The coated-antigen assays under the optimized conditions were performed as follows. Serial dilutions of the analyte standard in 10% MeOH−PBS were individually added to antiserum previously diluted with PBS (1:1 volume ratio), and they were incubated overnight at 4 °C. The mixture with a dilution of antiserum was called the analyte-antiserum. The following day, microtiter plates were coated with hapten−OVA (100 μL/well) in CBS and incubated for 2 h at 37 °C. The plates were washed three times with PBST (10 mM PBS containing 0.05% Tween-20, pH 7.4) and were blocked by incubation with 1% OVA in PBS (200 μL/well) for 1 h. After another washing step, 100 μL/well of the analyte-antiserum was added. After incubation for 2 h, the plates were washed. Subsequently, 100 μL/well of a diluted goat anti-rabbit IgG−horseradish

Table 1. Summary of Titers of Antisera[a]

	boost				
antiserum	first	second	third	fourth	fifth
FH2934[b]	1:8000	1:12000	1:12800	1:25600	1:25600
FH2962	1:3600	1:9600	1:12800	1:12800	1:12800
FH2836	1:800	1:3200	1:4800	1:4800	1:4800

[a] The titer of antiserum is defined as the antiserum dilution that gave 2.1 times the absorbance of the control serum. The coating antigen was hapten–OVA with 2 μg/mL. [b] The antiserum was used in all following experiments.

Table 2. Summary of Titers of Antisera[a]

	boost				
antiserum	first	second	third	fourth	fifth
FM6672	1:4000	1:8000	1:12800	1:25600	1:25600
FM6673	1:3600	1:9600	1:12800	1:19200	1:25600
FM6674[b]	1:8000	1:1200	1:12800	1:25600	1:51200
FM6675[c]	1:400	1:1600			

[a] The titer of antiserum is defined as the antiserum dilution that gave 2.1 times the absorbance of the control serum. The coating antigen was hapten–OVA with 2.5 μg/mL. [b] The antiserum was used in all following experiments. [c] FM6675 was dead after the second boost.

peroxidase was added and incubated for 1 h, and after another washing step, 100 μL/well of a TMB solution (110 μL of 10 mg/mL TMB−DMSO and 30 μL of 0.65% H_2O_2 diluted with 10.86 mL of citrate−acetate buffer, CPBS, pH 5.5), was added. The reaction was stopped after 15 min by adding 50 μL/well of 2 M H_2SO_4, and absorbance was read at 450 nm. After each washing, the plate was banged upside down on some paper towels to remove all of the liquid.

RESULTS AND DISCUSSION

Identification of Artificial Antigens. The spectra of hapten, BSA, OVA, hapten−BSA, and hapten−OVA were recorded from 200 to 400 nm to identify artificial antigens. The shape of three curves was distinct. Hapten−BSA and hapten−OVA had a peak at 291 and 297 nm, respectively. Their values were different from those of hapten, BSA, and OVA. The peak value of hapten−protein (BSA or OVA) was larger than that of the hapten and less than that of carrier proteins. All of this indicates that the hapten and carrier protein might be coupled successfully. In this study, it was regretful that other techniques such as MALDI-TOF were not used to make sure the conjugation was successful. However, the antisera of high titers from those artificial antigens could sufficiently support the hapten−protein conjugation.

Antibodies. *Polyclonal Antibodies.* The antisera of three rabbits were screened against hapten−OVA using a two-dimensional titration method. The results of the titration experiments are shown in **Table 1**. The two-dimensional titration of the antisera FH2934 and FH2962 against hapten−OVA (1:4000, 2 μg/mL) revealed that the absorbance was around 1.000 when the dilution of antisera was 1:1600 and 1:3200. Because the titer of FH2934 antiserum was higher than those of others, this antiserum was used in all of the following experiments. With optimized combination, a standard curve for fipronil was obtained by plotting inhibition rate versus the concentration of fipronil ($I = 78.65 + 16.41$ Log C, $r = 0.9821$). The assay showed $I_{50} = 17.95$ ppb, $I_{90} = 203.4$ ppb, and $I_{10} = 0.06556$ ppb.

Monoclonal Antibodies. Four mice were immunized, and the antisera of three mice were screened against hapten−OVA using a two-dimensional titration method. In general, serum with a titer in excess of 1:25600 was obtained after five immunizations, and so fusions were done (**Table 2**). The spleen cells from FM6674 were fused with the myeloma cells (SP20), and a wide range of mABs was obtained from the fusion (**Figure 2**). Mouse FM6674 yielded 12 hybridomas from 96 wells. With optimized combination, a standard curve for fipronil was obtained by plotting inhibition rate versus the concentration of fipronil ($I = 33.70 + 20.96$ Log C, $r = 0.9864$). The assay showed $I_{50} = 5.990$ ppb, $I_{90} = 485.400$ ppb, and $I_{10} = 0.074$ ppb. Compared with the assay with polyclonal antibodies, the sensitivity was higher.

Cross-Reactivity (CR). The assay specificity was evaluated by obtaining competitive curves for several structurally related compounds as competitors, estimating their respective I_{50} values, and comparing the data with fipronil I_{50}. CR values for each compound are given in **Table 3**. The results indicate that the antisera did not recognize some structurally related analytes. Contrary to the conventional analytical methods (GC-MS) for fipronil (*5*), ELISA still has drawbacks that may give an overestimated value because of the CR; however, ELISA offers considerable advantages over the conventional analytical procedure because of the ease of handling (no special skills necessary) and extraction, relatively fast measurement, high sample turnover, low quantification limit, and acceptable costs. With these important and attractive features, it is speculated that the ELISAs developed in this study may contribute to the routine monitoring of fipronil in our environment and agroproducts, although no agroproduct samples have been tested in this study. Those complementary studies are intriguing.

Currently, most interests in residue analysis particularly focus on the simultaneous analysis of parent compound and its metabolites. Manclus et al. (*18*) developed immunoassays for the analysis of chlorpyrifos and its major metabolite 3,5,6-trichloro-2-pyridinol in the aquatic environment. It was reported that fipronil may degrade or metabolize to its sulfone, sulfide, amide, and desulfinyl products. Therefore, specificity tests with those degradates may have practical application importance; it is known that those degradates are considerably toxic to some fishes and other species (*2*). To avoid the repetition, all data

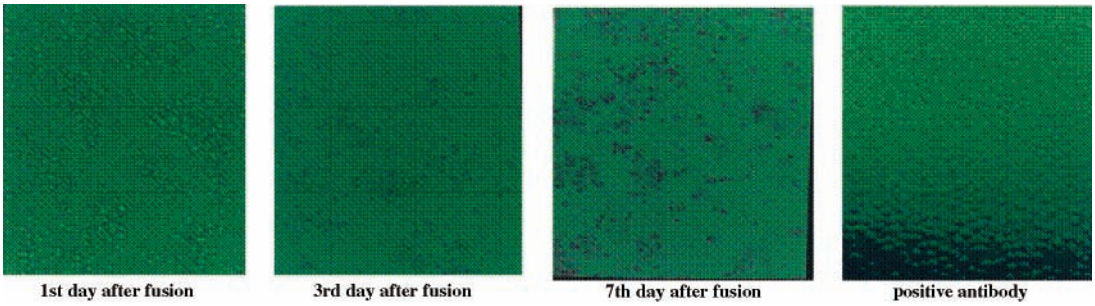
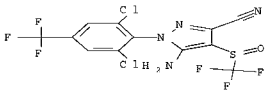
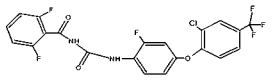
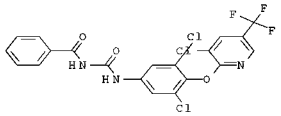
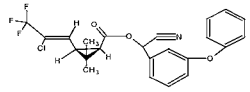
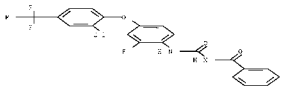
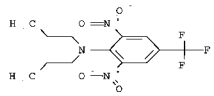


Figure 2. Cloning of hybridoma.

Table 3. Cross-Reactivities of Related Compounds in the Fipronil Immunoassay

Analyte	Structure	I_{50}(ppb)		CR(%) [a]	
		pABs	mABs	pABs	mABs
Fipronil		16.80	5.99	100	100
Flufenoxuron		NA [b]	4320	NA [b]	0.16
Chlorfluazuron		6268	>10000	2.280	<0.0599
Cyhalothin		NA [b]	2509	NA [b]	0.24
Flufenoxuron		5197	NA [b]	3.233	NA [b]
Trifluralin		>10000	894	--	0.67

[a] Percentage of cross-reactivity = (I_{50} of fipronil/I_{50} of analyte) × 100. [b] NA, not analyzed.

regarding specificity tests with those degradates and studies on the fates of fipronil in water and soils by the ELISA will be published in another paper.

Recoveries of Fipronil from the Spiked Tap Water. Fipronil is commonly and extensively used for the control of many pests on a variety of crops or animals (*1*); thereby, it will ineluctably appear in the environment. The reported solubilities for fipronil are 2.0−2.4 ppm (*2*). The reported half-life for fipronil under aerobic aquatic conditions was about 14.5 days. The hydrolysis data suggest that hydrolysis will not be a major degradative pathway for fipronil at typical environmental pH values. Therefore, it is necessary to determine or monitor the fipronil in water. To make sure the application for water samples of the ELISA developed in this research and to study the spike recovery, local tap water (Nanjing, China) was fortified with fipronil at different levels (ranging from 0 to 500.00 ng/mL) and was assayed by the indirect ELISA with pABs and mABs.

Table 4. Spiked Recoveries in Tap Water[a,b]

	pABs ELISA			mABs ELISA		
spiked concn (ng/mL)	detected (ng/mL)	CV (%)	recovery (%)	detected (ng/mL)	CV (%)	recovery (%)
0	ND[c]			ND		
0.50	0.62	4.7	124	0.52	8.30	104.00
5.00	5.25	1.3	105	5.40	4.00	108.00
50.00	46.5	3.6	93	47.00	3.90	94.70
500.00	OFR[d]			492.00	4.20	98.30

[a] Spiked water samples were directly analyzed without pretreatment and dilution. [b] Data obtained from three determinations. [c] ND, not detected. [d] OFR, out of range.

The recoveries of fipronil from tap water samples by pAB-based ELISA were in the range of 93−124%, whereas those obtained from the samples by mAB-based ELISA were 94−108% (**Table**

Table 5. Recovery of ELISA[a] for Fipronil in the Spiked Waters[b,c]

sample	pH	concn (μg/L)	detected value (μg/L)	recovery rate (%)
tap water	7.02	10	9.47	94.7
		1	1.08	108.0
		0.1	0.11	110.0
mineral water	7.84	10	10.36	103.6
		1	1.06	106.0
		0.1	0.083	83.0
Mill-Q water	7.01	10	9.86	98.6
		1	0.95	95.0
		0.1	0.086	86.0
pool water	7.32	10	10.02	100.2
		1	1.08	108.0
		0.1	0.094	94.0
river water	6.95	10	10.56	105.6
		1	1.09	109.0
		0.1	0.11	110.0
paddy field water	7.89	10	10.72	107.2
		1	1.12	112.0
		0.1	0.087	87.0
underground water	8.06	10	10.36	103.6
		1	1.06	106.0
		0.1	0.092	92.0
seawater	6.83	10	8.65	86.5
		1	1.16	116.0
		0.1	0.82	82.0

[a] Antisera, monoclonal antibodies. [b] Spiked water samples were directly analyzed after the SPE procedure. [c] Data obtained from three determinations.

4). Two assays indicate that both are suitable for the analysis of fipronil in water samples.

Analysis of Fipronil in Water Samples by mAB-Based ELISA. To detect fipronil in water samples at a sub parts per billion levels, a rapid and efficient SPE procedure was developed. The C_{18} column has been considered to be the primary choice for the extraction of nonpolar or semipolar pesticides from natural water sources (*19*, *20*). On the basis of the assays by pAB- and mAB-based ELISAs, different types of water were spiked at different levels (ranging from 0.1 to 10 μg/L) and assayed by the indirect ELISA with mABs. The recoveries of fipronil by this ELISA were in the range of 80−120% (**Table 5**). The recovery values were obtained from the standard solution prepared with an assay buffer. These data well match requirements of residue analysis. The results demonstrate that this assay is suitable for the quantitative detection of fipronil at trace levels in water samples. The limit of detection (LOD) for water samples by the ELISA, established as the analyte concentration showing 90% of the maximum response (10% inhibition), was <0.08 μg/L. (The pAB-based assay showed $I_{10} = 0.066$ ppb, whereas the mAB-based assay showed $I_{10} = 0.074$ ppb.) A study (*5*) showed that the linear concentration range of application was 0.3−100 μg/L of fipronil, with a relative standard deviation of 9.5% and a detection limit of 0.08 μg/L. These indicate the advantage and innovation of the ELISA developed in this report over other methods (*4*, *5*).

LITERATURE CITED

(1) Tomlin, C. *The Pesticide Manual: A World Compendium*, 11th ed.; British Crop Protection Council: Croydon, U.K., 1997.

(2) U.S. Environmental Protection Agency. New Pesticide Fact Sheet. PB96-18151.6; EPA737-F-96-005; U.S. EPA Office of Prevention, Pesticides and Toxic Substances: Washington, DC, 1996.

(3) Kidd, H.; James, D. *The Agrochemicals Handbook*, 3rd ed.; Royal Society of Chemistry Information Services: Cambridge, U.K., 1991.

(4) Bobe, A.; Coste, C. M.; Coope, J. Factors influencing the adsorption of fipronil on soils. *J. Agric. Food Chem.* **1997**, *45*, 4861−4865.

(5) 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*, 215−221.

(6) Bobe, A.; Cooper, J.; Coste, C. M.; Muller, M. A. Behavior of fipronil in soil under Sahelian Plain field conditions. *Pestic. Sci.* **1998**, *52*, 275−281.

(7) Hammock, B. D.; Gee, S. J.; Cheung, P. Y. H.; Miyamoto, T.; Goodrow, M. H.; Van Emon, J.; Seiber, J. N. *Utility of Immunoassay in Biotechnology*; Greenhalgh, R., Roberts, T. R., Eds.; Blackwell Scientific: Otttawa, Canada, 1987; pp 309−316.

(8) Knopp, D. Application of immunological methods for the determination of environmental pollutants in human biomonitoring: a review. *Anal. Chim. Acta* **1995**, *311*, 383−392.

(9) Xu, D. M.; Yu, X. Y.; Liu, Y. Q.; Feng, J. T.; Pan, L. G.; Liu, X. J.; He, J.; Zhang, X. Development of an enzyme-linked immunosorbent assay for podophyllotoxin. *Int. Immunopharmacol.* **2005**, *5*, 1583−1592.

(10) Vanderlaan, M.; Watkins, B. E.; Stanker, L. Environmental monitoring by immunoassay. *Environ. Sci. Technol.* **1988**, *22*, 247−254.

(11) Hock, B. Enzyme immunoassays for pesticide analysis. *Acta Hydrochim. Hydrobiol*. **1993**, *21*, 71−83.

(12) Xu, D.; Zhang, X. Enzyme immunoassay in pesticide science: a comprehensive review and prospect. China Pestic. Sci. Admin. **2004**, *25* (9), 27−31.

(13) Bobe, A.; Meallier, P.; Cooper, J.; Coste, C. M. Kinetics and mechanisms of abiotic degradation of fipronil (hydrolysis and photolysis). *J. Agric. Food Chem.* **1998**, *46*, 2834−2839.

(14) Weetall, H. H. Covalent coupling method for inorganic support materials. *Methods Enzymol.* **1976**, *44*, 139−148.

(15) Kennet, R. H.; Davis, K. A.; Tung, A. S.; Kilman, N. R. Hybrid plasmocyta production: fusion with adult spleen cells, monoclonal spleen ragments, neonatal spleen cells and human spleen cells. *Curr. Topics Immunol.* **1978**, *81*, 77−91.

(16) Campbell, A. M. *Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*; Elsevier: Amsterdam, The Netherlands, 1986; pp 265−276.

(17) Harlow, E.; Lane, O. *Antibodies: A Laboratory Manual*; Cold Spring Harbor Laboratory: New York, 1988; pp 726−748.

(18) Manclus, J. J.; Montoya, A. Development of immunoassays for the analysis of chlorpyrifos and its major metabolite 3,5,6-trichloro-2-pyridinol in aquatic environment. *Anal. Chim. Acta* **1995**, *311*, 341−348.

(19) Junk, G. A.; Richard, J. J. Organics in water: solid-phase extraction on a small scale. *Anal. Chem.* **1988**, *60*, 451−454.

(20) Durhan E. J.; Lukasewycz, M. T.; Amato, J. R. Extraction and concentration of non-polar organic toxicants from effluents using solid-phase extraction. *Environ. Toxicol. Chem.* **1990**, *9*, 463−466.

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