JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLES

Development of an Enzyme-Linked Immunosorbent Assay for Quantitative Determination of Quizalofop-*p*-ethyl

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Accurate quantification of quizalofop-*p*-ethyl is essential for it may do harm to humans and animals through both water and food. Currently, detection of quizalofop-*p*-ethyl mainly relies on methods such as gas chromatography, high performance liquid chromatography, and gas chromatography−mass spectrometry. Although these techniques are reliable, they are relatively expensive and time-consuming because of multistep sample cleanup. To address this, we developed a competitive indirect enzyme-linked immunosorbent assay (ciELISA) with a polyclonal antibody against quizalofop-*p*-ethyl that was generated in our lab. The IC_{50} of detection was 0.03495 μg/mL, and the lowest detection limit reached 0.00192 μg/mL. Furthermore, the method had high specificity for it did not cross-react with other structure-related compounds. When water and soil samples that were fortified with quizalofop-*p*-ethyl were analyzed by this ELISA, recoveries were in the range of 89−110% from water and 81−108% from soil. Good correlations between this immunoassay and gas chromatography data were obtained for residues of quizalofop-*p*-ethyl in water and soil. Our data indicate that this method is a convenient analytical technique for monitoring quizalofop-*p*-ethyl in waters without extraction and the extra cleanup step and in soil without the cleanup step.

KEYWORDS: Quizalofop-*p*-ethyl; hapten; polyclonal antibody; immunoassay; ELISA

INTRODUCTION

Quizalofop-*p*-ethyl {ethyl (*R*)-2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]propionate} is a member of the aryloxyphenoxypropionate group of herbicides introduced in the mid 1980s with apparent excellent herbicidal properties and low toxicity. Like other members of the aryloxyphenoxypropionate family, quizalofop-*p*-ethyl is a selective post-emergence herbicide, which was registered for use in controlling annual and perennial grass weeds in potatoes, soya beans, sugar beets, peanuts, oilseed rape, sunflowers, vegetables, cotton, flax, and other broad leafed corps (*1*−*4*). As a systemic herbicide to inhibit acetyl CoA carboxylase, quizalofop-*p*-ethyl was absorbed from the leaf surface and translocated throughout the plant, moved in both xylem and phloem from the treated foliage to the root system, and accumulated in the meristematic tissue, and thus it inhibits the biosynthesis of fatty acid.

According to the report from the WHO (III) and EPA (III), quizalofop-*p*-ethyl is toxic and further banned in European community (EC) for its toxicity, although it is widely used in many other countries (for instance, the MRL in sugar beet is 0.1 ppm in the U.S.). That quizalofop-*p*-ethyl has been detected as water contaminants and impacts the environment leading to effects on ecosystem health has been reported (*5*). Thus, a suitable analysis method, which is quick, easy, and economical for detecting its residue that might potentially contaminate water, soil, and aquatic food, is urgently needed. The current regular analysis method of quizalofop-*p*-ethyl is based on chromatography methods, including high performance liquid chromatography (HPLC), gas chromatography (GC), and gas chromatography−mass spectrometry (GC−MS), which are characterized by high precision and sensitivity (*1*, *6*−*14*). However, these methods have a few shortcomings: the procedure is time-consuming and involves toxic solvents and reagents. Sometimes, the results are inconsistent, and the need for sophisticated equipment is only available in well-equipped laboratories as well as inadequacy of on-site analysis (*15*, *16*), whereas enzyme-linked immunosorbent assay (ELISA), which has emerged from the trials to overcome the weak points of HPLC, GC, and GC-MS methods, is a simple, rapid, and cost-effective method for monitoring pesticide residues and, in particular, for high sample through-put and on-site screening analysis (*17*−*20*).

Until now, very little information is known about the development of enzyme-linked immunoassay (ELISA) for

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10.1021/jf061492n CCC: $33.50
Published on Web 10/25/2006

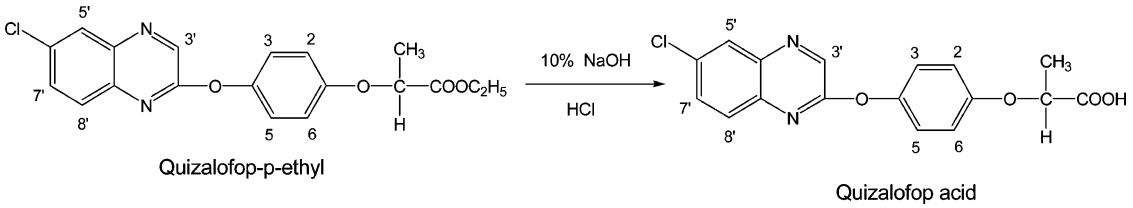


Figure 1. Synthetic route for the preparation of the hapten.

quizalofop-*p*-ethyl. In this paper, we developed an ELISA for quizalofop-*p*-ethyl, and the evaluation of the assay's performance in water and soil is described.

MATERIALS AND METHODS

Reagents. All reagents and solvents were analytical grade. DCC, dry pyridine, Tween-20, tri-*n*-butylamine, DMSO, isobutylchlorocarbonate, NHS, and DMF were obtained from Sino-American Biotechnology Co. (Henan, China). BSA, OVA, Freund's complete and incomplete adjuvants, goat antirabbit IgG-horseradish peroxidase, and TMB were purchased from Sigma Chemical Co. (Shanghai, China). 96-Well polystyrene microplates (Maxisorp) were obtained from Nunc (Roskilde, Denmark). NaN_3 and concentrated HCl were purchased from Chemical Reagent Co. (Shanghai, China).

Buffers: 0.01 mol/L of PBS (pH 7.4, 1.42 g of Na_2HPO_4, 0.27 g of KH_2PO_4, 8.01 g of NaCl, 0.2 g of KCl in 1 L of distilled water); 0.05 mol/L of CBS (pH 9.6, 1.59 g of Na_2CO_3, 2.93 g of $NaHCO_3$ in 1 L of distilled water); PBST (0.01 mol/L of PBS plus 500 μL of Tween-20).

Pesticide Standards. For the development of enzyme immunoassays and specificity studies, the following analytical standards were obtained from the United States or the Jiangsu Branch of the National Pesticide R&D South Center, China: quizalofop-*p*-ethyl, fenoxaprop-*p*-ethyl, benazolin-ehtyl, quinclorac, cyhalofop-butyl, (*R*)-haloxyfop, and imazezethapyr. The quizalofop-*p*-ethyl standard sample was dissolved in methanol to 8 μg/mL for stock solution, which was diluted into the working range of this assay with PBS in volumetric flasks, and these solutions were used within 30 min to avoid pesticide loss through adhesion to glass surface. The organic solvent concentration in PBS is negligible.

Instruments. The NMR spectrum was obtained via an AV300 spectrometer (Bruker, Switzerland). Chemical shift values are given in parts per million (ppm) downfield from internal standard tetramethyl silane. Coupling constants are expressed in hertz, and the abbreviations s, d, t, and q represent singlet, doublet, triplet, and quartet, respectively. MS was obtained by a HP-5973 (Agilent, USA). The IR spectrum was recorded by Bruker Vertex 70FT-IR (Germany). The characterization of hapten with protein conjugate (UV spectra) was recorded on a Beckman Coulter DU 800 spectrophotometer (Fullerton, CA). ELISA plates were washed with a Wellwash 4 MK 2 (Labsystems, Finland). ELISA experiments were performed in 96-well polystyrene microplates (Maxisorp), and the absorbances were read by a Multiskan Ascent microtiter plate reader (Labsystems, Finland) at 450 nm.

Hapten Synthesis. Synthesis of the hapten was carried out as outlined in **Figure 1**. The reaction was straightforward, and the yield was high.

Quizalofop-*p*-ethyl (0.02 mol) was added to 8.4 mL of 10% NaOH (0.021 mol) liquid solution (1:1.05 mol ratio). The mixture was stirred at room temperature for 2 h and then heated to 60 °C for 1 h. The mixture was heated again to reflux for 6 h until the liquid was clear. If there were some indiscernible solids, the mixture was filtered. The filtrate was treated with concentrated HCl to adjust the pH to 1−2. Thin yellow sedimentation was collected and filtered. The residue was dried under infrared light. The hapten was obtained as a straw yellow solid.

The straw yellow solid was analyzed via NMR, IR, and MS (92% yield). ^{1}H NMR (DMSO): δ 1.52 (d, 3-CH_3), 4.85 (q, 1, CH), 6.94 (d, 2, 2-H, 6-H), 7.24 (d, 2, 3-H, 5-H), 7.73 (s, 2, 7′-H, 8′-H), 8.13 (s, 1, 3′-H), 8.88 (s, 1, 5′-H). IR (solid, KBr): cm^{-1} 3000−2622 (m), 1718 (vs), 1569 (s), 1504 (vs), 1446 (s), 1399 (s), 1213 (s), 822 (s), 1131 (s), 1001 (m). Mass spectrum found (70 eV, two most intense ions each 14 mass units above *m*/*e* 48): 50(7), 56(6), 63(5), 64(7), 76(9), 81(6), 91(10), 100(19), 109(8), 110(11), 119(5), 124(8), 136(35), 138-(11), 149(8), 150(3), 163(51), 165(17), 173(1), 179(3), 181(3), 191-(3), 192(14), 208(6), 215(5), 216(3), 217(2), 243(38), 255(12), 257(5), 271(12), 272 (11), 285(2), 287(1), 299(74), 301(34), 344(100), 346-(32).

Preparation of Hapten−Protein Conjugates. *Active Ester Method (21).* The quizalofop acid hapten (68.9 mg) was dissolved in DMF (1 mL), and then a solution of *N*-hydroxysuccinimide (NHS, 69 mg) and *N*,*N*-dicyclohexylcarbodiimide (DCC, 61.8 mg) in DMF (2 mL) was added. The mixture was stirred for 12 h at room temperature, filtered, and one-half of the filtrate was added to BSA (40 mg) in 4 mL of PBS (pH 7.4) with stirring, and then the reaction mixture was stirred at 4 °C overnight. After that, the solution was dialyzed in PBS at 4 °C for 72 h and stored at −20 °C. Characterization of the conjugate was done using spectroscopy (*21*). The conjugate was applied as an immunogen (Hapten−BSA).

Mixed Anhydride Reaction (22). The hapten was coupled to OVA via a mixed anhydride reaction. The hapten (34.5 mg) and tri-*n*-butylamine (75 μL) were dissolved in dimethyl formamide (DMF, 1 mL). To this solution was added isobutylchlorocarbonate (20 μL), the formation of the mixed anhydride was allowed to proceed for 20 min, and the mixture was stirred at room temperature. The solution was then added dropwise to OVA (60 mg) in 5 mL of PBS (pH 7.4) with stirring. The mixture was stirred for 2 h and then dialyzed in PBS at 4 °C for 72 h and stored at −20 °C. Characterization was finished using spectroscopy (*21*). The hapten was covalently attached to OVA as coating antigen.

Immunization of Rabbits and Screening of Antisera. The method of producing antibody was described before (*23*); three New Zealand white rabbits (about 2 kg) were used to immunize with Hapten−BSA. The antigen (1.0 mg of Hapten−BSA) dissolved in PBS (1.5 mL) was emulsified with Freund's complete adjuvant (1:1 volume ratio) and injected intradermally at multiple sites (about 40−50 μL per site, 20−25 sites) on the back of each rabbit. Three weeks later, the animals were boosted with an additional 1.0 mg of immunogen (Hapten−BSA) that was emulsified with Freund's incomplete adjuvant (1:1 volume ratio). Three booster injections were conducted on a 2-week interval. The rabbits were bled 7−10 days later after each boosting to screen antisera. After this, the last boosting was performed with another additional 2.0 mg (immunogen Hapten−BSA) of the conjugate and physiological saline (1:1 volume ratio). The serum was isolated by centrifugation (10 000*g*, 10 min, 4 °C), and sodium azide (NaN_3) was added as a preservative at a final concentration of 0.02%. Antiserum was then aliquotted and stored at −20 °C.

The titer of the sera from each animal was screened by measuring the binding of serial dilutions to microtiter plates coated with several concentrations of Hapten−OVA. Optimal concentrations for coating antigen and antisera dilution were determined by performing two-dimensional titration experiments (*24*).

Indirect Competitive ELISA. The coated-antigen assays under the optimized conditions were performed as follows.

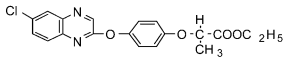
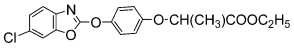
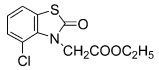
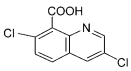
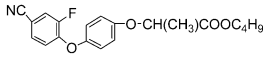
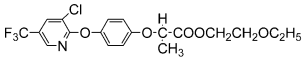
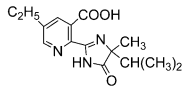
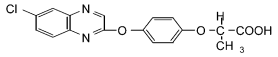
Serial dilutions of the analyte standard in PBS and the antiserum dilution (1/100 000) were individually prepared. Micro-titer plates were coated overnight at 4 °C with Hapten−OVA (2.05 μg/mL, 100 μL per well) in CBS. After the coated plates were washed three times with PBST (PBS containing 0.05% Tween-20, pH 7.4), 200 μL of blocking solution (1% OVA in PBS) was added and incubated for 1 h at 37 °C. After another washing step, 50 μL/well of inhibitor solution and 50 μL/well of the antiserum were added and shaken for 1 min, and the

Table 1. Effects of DMSO Concentration[a]

DMSO[b]	A_{max}	IC_{50} (μg/mL)	RSD %	R^2
0	0.93 ± 0.03	0.03495	3.4	0.99
10%	0.70 ± 0.02	0.20841	5.1	1.0
20%	0.64 ± 0.04	0.35694	2.7	0.98
30%	0.58 ± 0.02	0.41721	4.9	1.0
50%	0.45 ± 0.04	0.47336	3.6	0.99

[a] ELISA conditions: antiserum from M4743 (1:200 000), coating antigen Hapten–OVA (2.05 μg/mL). [b] Concentration of DMSO in quizalofop-*p*-ethyl standard solution; tests were replicated three times per concentration of DMSO.

Table 2. Cross-Reactivities of Related Compounds in the Quizalofop-*p*-ethyl Immunoassay

Analyte	Structure	M4743 IC_{50} (μg/ml)	M4743 CR[a] (%)
quizalofop-p-ethyl		0.03495	100
Fenoxaprop-p-ethyl		8.61	0.405
Benazolin-ehtyl		234.7	<0.1
Quinclorac		173.6	<0.1
Cyhalofop-butyl		20.78	0.167
(R)-Haloxyfop		11.59	<0.1
Imazezethapyr		>500	0.1
Quizalofop acid		0.0336	103.8

[a] Percentage of cross-reactivity = (IC_{50} of quizalofop-*p*-ethyl/IC_{50} of analyte)*100.

plates were incubated for 2 h and then washed three times. Subsequently, 100 μL/well of a diluted (1:5000) goat anti-rabbit IgG-horseradish peroxidase was added and incubated for 1 h at 37 °C. Following another washing step, 100 μL/well of a TMB solution (120 μL of 10 mg/mL TMB-DMSO and 30 μL of 0.65% H_2O_2 diluted with 10.85 mL of citrate-acetate buffer, CPBS, pH 5.5) was added. The color developments were stopped after 15 min with 2 M H_2SO_4 (50 μL/well). The absorbance was recorded at 450 nm. All experiments were conducted in triplicate or quadruplicate. Standard curves were obtained by absorbance against the analyte concentration.

Assay Optimization. The following assay parameters were investigated to optimize the assay condition. First, the effect of PBS buffer with increasing content of NaCl was studied. As described above, the antibody was diluted in PBS buffer of various ionic strengths, and the assays were conducted. Second, the effects of the solvents were also tested by preparing quizalofop-*p*-ethyl in PBS buffer containing various proportions of solvent and incubating these with antibody in PBS on the coated plate. Methanol and DMSO were tested in this way.

Cross-Reactivity. The optimized assays were applied to cross-reactivity studies by using the standard solution of the quizalofop-*p*-ethyl and other structurally related compounds (**Table 2**). Cross-reactivities (CR) were determined by dividing the IC_{50} (concentration of analyte giving 50% inhibition) of the chemical, quizalofop-*p*-ethyl, assigned to be 100% by the IC_{50} of another compound and multiplying by 100 to obtain a percent figure.

Recovery. Water and soil samples were spiked with quizalofop-*p*-ethyl standards to obtain the recoveries; concentrations used were 0.002, 0.008, 0.04, 0.1, 0.2, and 0.5 μg/mL, and each concentration was replicated three times.

Water samples spiked with quizalofop-*p*-ethyl standards could be directly analyzed by ELISA. Soil samples should be extracted with solvent but without cleanup procedure. The extraction step took place as follows.

Distilled water (20 mL) was added to a 250 mL Erlenmeyer flask containing 25 g of soil. After the mixture was stilled for 1 h, 50 mL of acetone was added, the mixture was shaken up for 1.5 h using a machine, filtered, and the filter was transferred to a separating funnel. The solution was extracted with 40 mL of petroleum ether and extracted once again with 20 mL of petroleum ether. The combined extracts were concentrated to dryness using a Turb Vap 500; the residue was redissolved with a little methanol, and then diluted with PBS in volumetric flasks. Finally, the sample was analyzed via ELISA.

Gas Chromatographic Analysis. *Water Samples*. Sodium chloride (5 g) was added to a separating funnel containing 50 mL of water sample, and the solution was extracted once with 50 mL of dichloromethane for 2 min and twice with 25 mL of dichloromethane for 2 min. The combined extracts were concentrated to nearly dry using a Turbo Vap 500 and redissolved with hexane−acetone (9:1 v/v) to the volume of 2 mL. Cleanup was then performed on a Florrisil column. After the column was prewet with 5 mL of hexane, 2 mL of water extracts was added. The vial was rinsed with 3 mL of hexane−acetone (9:1 v/v), and the rinsate was also added to the column. The column was eluted with 10 mL of hexane−acetone (9:1 v/v). All of the eluate was collected and concentrated to 2 mL for GC-NPD analysis.

Soil Samples. The extraction step is the same as described in the Recovery section, but the extracts have to be cleaned using the Florrisil column before GC-NPD analysis.

Assay validation was conducted with an HP-5890 GC equipped with an NPD and a 30 m × 0.25 mm i.d. (0.25 μm) HP-5 column. The injector was operated at 300 °C. An HP model 6890 series autoinjector was used to inject 1 μL of sample. The oven temperature was programmed from 70 to 280 °C at 25 °C/min and held for 7 min. Nitrogen was used as a carrier gas (velocity of flow: 1 mL/min).

RESULTS AND DISCUSSION

Hapten Synthesis. Because the primary goal of this study was to develop a specific immunoassay for quizalofop-*p*-ethyl, the design of the immunogen hapten became a key step for obtaining high-quality antibodies against this small molecule and frequently required considerable synthesis efforts. Because it is desirable to immunize rabbits with an antigen that exposes the most unique portions of the target analyte for antibody development, like the immunoassay of other small molecular weight compounds, in terms of our experiences, we have synthesized the hapten needed for immunization and coating antigen, which keeps nearly the same geometry as the quizalofop-*p*-ethyl, with a high yield of 92%.

Identification of Artificial Antigens. Figure 2 shows the spectra of hapten, BSA, OVA, Hapten−BSA, and Hapten−OVA recorded from 200 to 400 nm. The shape of the three curves is distinct. Although Hapten−BSA and Hapten−OVA have a peak at 286 and 284 nm, respectively, their values are obviously different from those of hapten, BSA, and OVA. For example, the peak (300−400 nm) value of hapten−protein (BSA or OVA) is less than the hapten but larger than carrier proteins, indicating these conjugates were obtained successfully.

Screening and Selection of Antisera. The antisera of three rabbits were first screened against Hapten−OVA using a two-dimensional titration method. Titers of the three rabbits are M4736 (1:40 000), M4740 (1:80 000), and M4743 (1:800 000); we used the antisera of M4743 in the following experiments.

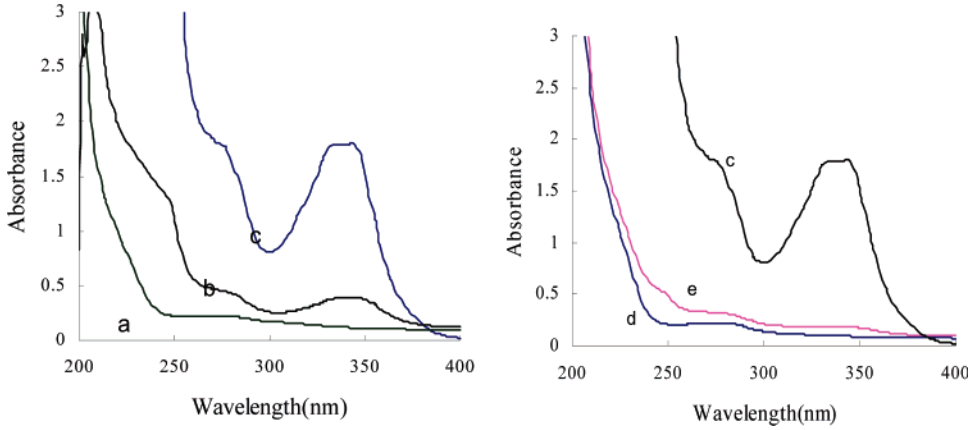


Figure 2. Ultraviolet absorbance spectra of BSA, OVA, Hapten, Hapten–BSA, and Hapten–OVA. (**a**) BSA, (**b**) Hapten–BSA, (**c**) Hapten, (**d**) OVA, (**e**) Hapten–OVA.

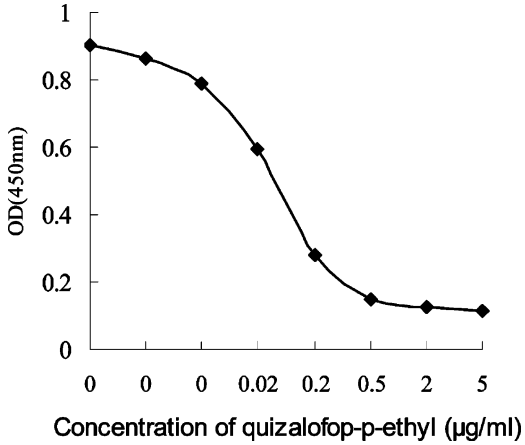


Figure 3. Standard curves of quizalofop-*p*-ethyl by indirect ELISA. Coating antigen: 2.05 μg/mL Hapten–OVA for M4743.

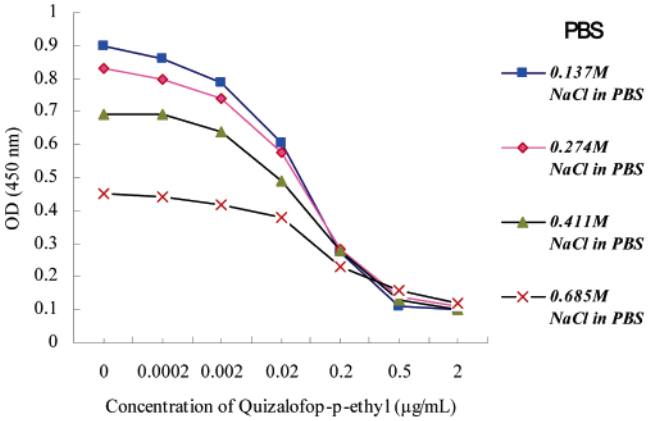


Figure 4. ELISA competition curves of quizalofop-*p*-ethyl prepared in PBS buffer at various ionic strengths. Reagent concentrations: coating antigen, 2.05 μg/mL; antiserum (M4743), 1/200 000. Each curve represents the average of five replicates.

The two-dimensional titration of the antisera M4743 against Hapten−OVA revealed that when the absorbance was around 1.000 the dilution of M4743 antiserum was 1/200 000 against 2.05 μg/mL Hapten−OVA. This combination was used to perform the following ELISA method.

Affinity of Polyclonal Antibody. With the optimized combination, a standard curve for quizalofop-*p*-ethyl was obtained by OD (450 nm) versus concentration of quizalofop-*p*-ethyl (**Figure 3**). The assay showed that the IC_{50} was 0.03495 μg/mL and the lowest detection limit (IC_{10}) was 0.00192 μg/mL. According to the linear portion of the sigmoidal curve, the working range was among the concentrations of 0.002−0.5 μg/mL for quizalofop-*p*-ethyl.

Assay Optimization. To identify potential interferences from aqueous environmental samples, the ionic strength on ELISA performance was studied in this research. When analyte was dissolved in PBS buffer at different ionic strengths, a significant effect upon the IC_{50} was detected (**Figure 4**). A higher salt concentration (the concentration of NaCl in PBS) in the assay system resulted in lower $OD_{450\ nm}$ values and higher IC_{50} values. Research has demonstrated that the $OD_{450\ nm}$ values at salt concentrations of 0.411 and 0.685 mol/L in PBS decreased by approximately 22% and 47%, respectively, from the OD values at the salt concentration of PBS. As compared to the IC_{50} values, PBS has the lowest IC_{50}, and it increased with the salt concentration of PBS. Because the salt concentrations can affect antibody binding, PBS, which was commonly used, is the best salt concentration to improve assay performance in this assay.

For solvent optimization, we tested methanol and DMSO because both are common solvents used in immunoassay to improve analyte solubility. As observed in our experiments, methanol slightly influenced the quizalofop-*p*-ethyl assay sensitivity and absorbance, and the IC_{50} values, which varied depending upon the different concentrations of the methanol, are small (**Figure 5**). When PBS did not contain methanol, the IC_{50} was the lowest, which was about 0.9 times that of PBS containing 10% methanol. However, DMSO significantly influenced the assay sensitivity and absorbance (**Table 1**); when there is about 10% DMSO in the system, the IC_{50} is 6 times higher than that without DMSO. Considering the solubility of quizalofop-*p*-ethyl in PBS and the influences on IC_{50} values after adding solvent to PBS, methanol and DMSO were not added in the following experiments.

Cross-Reactivities. The assay specificity was evaluated by obtaining competitive curves for several structurally related compounds as competitors, calculating their IC_{50}, and comparing these IC_{50} values with those of quizalofop-*p*-ethyl. CR values for each compound were given in **Table 2**. In all cases, the antiserum did not recognize other structurally related analytes, while any change of quizalofop-*p*-ethyl dramatically reduced the recognition and influenced the interaction. The lack of cross-reaction between quizalofop-*p*-ethyl and other structurally related compounds suggests that the antibody is very specific for the quizalofop-*p*-ethyl. Comparing the structure of quizalofop-*p*-ethyl with that of fenoxaprop-*p*-ethyl, they have similar side chains but different heterocyclic structures, and there was

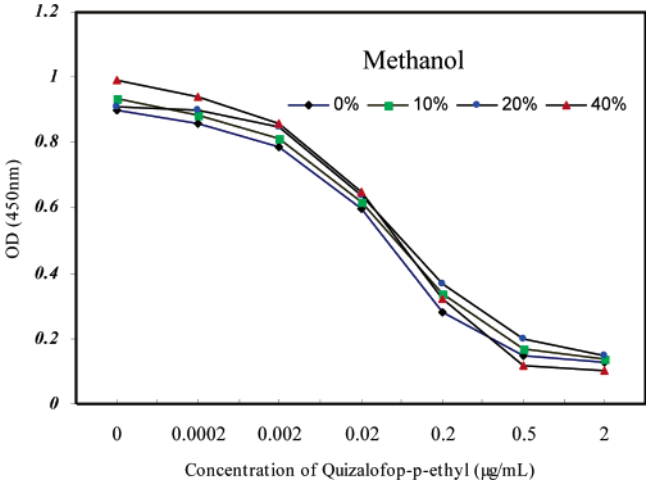


Figure 5. ELISA competition curves of quizalofop-*p*-ethyl prepared in PBS buffer containing various concentrations of methanol. Reagent concentrations: coating antigen, 2.05 μg/mL; antiserum (M4743), 1/200 000. Each curve represents the average of three replicates.

Table 3. Spike Recoveries in Water[a] and Soil

samples	spiked concentration (μg/mL)	ELISA recovery[b] (%)	ELISA RSD (%)	GC-NPD recovery (%)	GC-NPD RSD (%)
water	0.002	108.88	5.3	ND[c]	
	0.008	105.38	4.8	ND	
	0.04	106.50	5.6	101.75	2.6
	0.10	90.14	2.3	118.4	4.1
	0.20	89.02	3.9	91.0	3.3
	0.50	97.92	1.7	99.4	3.5
	1.0	101.73	6.2	94.6	2.1
soil	0.002	103.00	4.1	ND	
	0.008	91.75	4.6	ND	
	0.04	107.75	2.2	104.5	3.7
	0.10	83.57	3.0	92.6	4.0
	0.20	90.45	3.4	97.1	2.3
	0.50	91.74	2.6	94.0	2.1
	1.0	81.58	3.3	93.8	2.9

[a] Spiked water samples were directly analyzed without previous treatment and dilution. [b] Data obtained from at least three replications. [c] Not detected.

no cross-reaction, so we believe that the quinoxaline ring of quizalofop-*p*-ethyl plays an important role in immunoreaction.

Finally, a cross-reactivity experiment was conducted for the hapten, quizalofop acid, which was used for preparing antigens. A high inhibition was detected (**Table 2**). Similar phenomena have also been reported for other small molecules, for example, (*S*)-bioallethrin and dioxin immunoassays (*25*, *26*).

Analysis of Spiked Water and Soil Samples. Water samples were fortified with quizalofop-*p*-ethyl at different concentrations (ranging from 0.002 to 0.5 μg/mL) and assayed by the indirect ELISA without previous treatment. Soil samples were extracted with solvent and analyzed by the indirect ELISA without cleanup. Interestingly, good correlation between spiked and ELISA-measured quizalofop-*p*-ethyl was obtained from linear regression analysis. All recoveries of quizalofop-*p*-ethyl analyzed by this ELISA were in the range of 89−110% from water samples and 81−108% from soil samples (**Table 3**). The results demonstrate that this ELISA is suitable for the quantitative detection of quizalofop-*p*-ethyl in water and soil contamination monitoring that requires high sensitivity.

Relationship between ELISA and GC-NPD Data for Water and Soil Samples. The sensitivity of the gas chromatography method for quizalofop-*p*-ethyl is relatively low. The detection limit that was calculated from a signal-to-noise ratio of 3:1 was 5 ppb for quizalofop-*p*-ethyl, somewhat higher than that of the immunoassay. The detection range for quizalofop-*p*-ethyl in water and soil was from 0.04 to 1 μg/mL.

Water and soil samples were spiked with quizalofop-*p*-ethyl and analyzed by ELISA and GC-NPD. All recoveries from water samples were >89% and from soil samples were >81% of the spiked values (**Table 3**). Spike recoveries of both ELISA and GC-NPD methods were also compared, and a good correlation between quizalofop-*p*-ethyl concentration measured by the GC-NPD and ELISA was obtained from linear regression analysis (**Figure 6**). The regression equations are $y = 0.9183x + 0.023$ ($R^2 = 0.9975$) for water samples and $y = 1.1417x - 0.0139$ ($R^2 = 0.996$) for soil samples. The results demonstrate that this immunoassay was suitable for the quantitative detection of quizalofop-*p*-ethyl at trace levels in water and soil.

Conclusions. Polyclonal antibody against quizalofop-*p*-ethyl was raised to the novel hapten conjugated to BSA. The polyclonal antibody M4743 against quizalofop-*p*-ethyl showed high sensitivity and specificity, and the developed IC-ELISA with antibody (M4743) could detect 0.03945 μg/mL of quizalofop-*p*-ethyl as IC_{50}, and the lowest limit was 0.00192 μg/mL. The indirect ELISA could recover 0.002−0.5 μg/mL of quizalofop-*p*-ethyl in water samples with the recovery ratio of 89−110% and in soil samples in the range of 81−108%. Good correlations between this immunoassay and gas chromatography data were obtained for residues of quizalofop-*p*-ethyl in water and soil. These results indicated that IC-ELISA with quizalofop-*p*-ethyl could precisely measure the concentration of the pesticide residue in water without treatment in advance and in soil samples at low levels without the cleanup procedure.

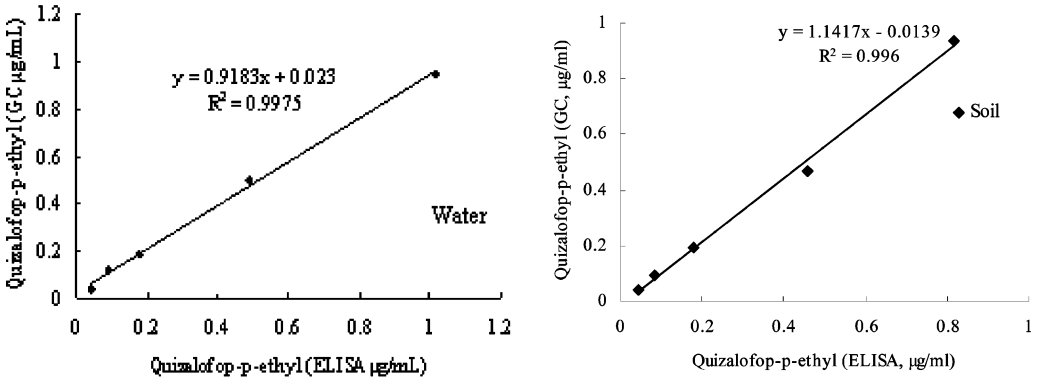


Figure 6. Relationship between quizalofop-*p*-ethyl levels as measured by ELISA and GC for water and soil samples.

ABBREVIATIONS USED

ELISA, enzyme-linked immunoassay; GC, gas chromatography; GC−MS, gas chromatography−mass spectrometry; OD, optical densities; DCC, *N*,*N*-dicyclohexylcarbodiimide; DMSO, dimethyl sulfoxide; NHS, *N*-hydroxysuccinimide; DMF, dimethyl formamide; BSA, bovine serum albumin; OVA, ovalbumin; TMB, tetramethylbenzidine; PBS, phosphate-buffered saline; CBS, carbonate-buffered saline; PBST, phosphate-buffered saline containing 0.05% Tween-20; CPBS, citrate-acetate buffer; CR, cross-reactivities; IC_{50}, concentration of analyte giving 50% inhibition.

ACKNOWLEDGMENT

We would like to thank Dr. Pingsheng Liu (UT Southwestern Medical Center, Dallas, TX) for his critical reading of and corrections to this manuscript. We are grateful to X. J. Liu, Y. Liu, and F. Cheng (Food Safety & Service Center, Jiangsu Academy of Agricultural Sciences, Nanjing) for assistance with production of the antibody. Thanks also go to S. C. Xu and C. L. Li (Jiangsu Pesticide Research Institute, Nanjing) for assistance with the NMR studies.

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Received for review May 26, 2006. Revised manuscript received September 10, 2006. Accepted September 19, 2006.

JF061492N