JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Development of a Broad-Specificity Monoclonal Antibody-Based Immunoaffinity Chromatography Cleanup for Organophosphorus Pesticide Determination in Environmental Samples

Zhen-Lin Xu,[†,‡] Hao Deng,[†] Hong-Tao Lei,[†] Yue-Ming Jiang,[‡] Katrina Campbell,[§] Yu-Dong Shen,[†] Jin-Yi Yang,[†] Hong Wang,[†] and Yuan-Ming Sun*[,†]

[†]Guangdong Provincial Key Laboratory of Food Quality and Safety, South China Agricultural University, Guangzhou 510642, People's Republic of China

[‡]Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, People's Republic of China

[§]Institute of Agri-Food and Land Use, School of Biological Sciences, Queen's University Belfast, Belfast BT9 5AG, Northern Ireland, United Kingdom

Supporting Information

ABSTRACT: An immunoaffinity chromatographic (IAC) method for the selective extraction and concentration of 13 organophosphorus pesticides (OPs, including coumaphos, parathion, phoxim, quinalphos, dichlofenthion, triazophos, azinphos-ethyl, phosalone, isochlorthion, parathion-methyl, cyanophos, disulfoton, and phorate) prior to analysis by high-performance liquid chromatography−tandem mass spectrometry (HPLC−MS/MS) was developed. The IAC column was prepared by covalently immobilizing a monoclonal antibody with broad specificity for OPs on CNBr-activated Sephrose 4B. The column capacity ranged from 884 to 2641 ng/mL of gel. The optimum elution solvent was 0.01 M phosphate-buffered saline containing 80% methanol. The breakthrough volume of the IAC column was found to be 400 mL. Recoveries of OPs from spiked environmental samples by IAC cleanup and HPLC−MS/MS analysis ranged from 60.2 to 107.1%, with a relative standard deviation below 11.1%. The limit of quantitation for 13 OPs ranged from 0.01 to 0.13 ng/mL (ng/g). The application of IAC cleanup coupled to HPLC−MS/MS in real environmental samples demonstrated the potential of this method for the determination of OP residues in environmental samples at trace levels.

KEYWORDS: *organophosphorus pesticides, immunoaffinity chromatography, HPLC−MS/MS, environmental samples*

INTRODUCTION

In the past 2 decades, concern about the contamination of the environment has risen because of the increasing number of pesticides detected. Regulations are well-defined in some developed countries, such as America and the European Union (EU).[1] For example, the EU has established maximum residue levels (MRLs) of 0.1 ng/mL for individual pesticides and related products and 0.5 ng/mL for total pesticides in drinking water[2] and 1−3 ng/mL for total pesticides in surface water.[3] These low MRLs have prompted the development of more sensitive analytical methods. At present, often the methods of choice for pesticide analysis are instrumental methods, such as gas chromatography with mass spectrometry (GC−MS)[4] or with tandem MS (GC−MS/MS)[5] and high-performance liquid chromatography with MS (HPLC−MS)[6] or with tandem MS (HPLC−MS/MS).[7] Most of these analytical methods include at least one step for sample pretreatment for simultaneous sample cleanup and sample enrichment (especially for trace analysis). The most commonly used sample pretreatment methods usually rely on partition in organic solvent or solid-phase extraction (SPE), which are based on hydrophobic interactions, adsorption, or ion exchange.[8] These methods often fail to remove interfering compounds from complex sample matrices because of the lack of specificity and work inefficiently for polar compounds.[9] Immunoaffinity chromatography (IAC), which is based on molecular recognition and is highly specific for a certain analyte or a group of related analytes, on the other hand, offers many advantages over traditional cleanup and concentration methods.[10] Additionally, IAC uses little or no organic solvents, and columns may be reused and easily automated.[11] Thus far, IACs for sample cleanup and concentration of food contaminants,[12] such as pesticides,[9,13−15] veterinary drugs,[11,16,17] and mycotoxins,[18,19] from different matrixes have been developed, and they are proven to be efficient and useful.

Organophosphorous pesticides (OPs) have become the most widely used pesticides in both agricultural and domestic settings since the ban on the use of organochlorine pesticides in the 1970s. There is a growing concern over the environmental contamination caused by the OP residues in recent years.[20] Previously, a monoclonal antibody (mAb) with a broad specificity to a class of *O*,*O*-diethyl and *O*,*O*-dimethyl OPs was produced and used to develop an indirect competitive

Received: February 29, 2012
Revised: May 18, 2012
Accepted: May 21, 2012
Published: May 21, 2012

ACS Publications © 2012 American Chemical Society

enzyme-linked immunosorbent assay (icELISA) for their screening.[21,22] However, the problem for a broad-specificity immunoassay is that the quantitative analysis of individual OPs is not possible because the affinity of the antibody varies between different OPs and the relative amounts of OP residues are unknown in the real samples.[23] In contrast, it was suggested that using antibodies capable of recognizing a whole class of related analytes was a better strategy than using antibodies with high specificity to one single analyte in the development of IACs.[10] Few IAC methods for the simultaneous extraction and cleanup of pesticides based on antibodies with broad specificity can be found in the literature. This paper, therefore, describes the generation of a broad-specificity mAb-based IAC as a simultaneous extraction and concentration method for OPs. The coupling efficiency, column capacity, elution condition, and breakthrough volume of the IAC were studied. The IAC was coupled to HPLC−MS/MS that was used to determine the OP residues in environmental samples.

EXPERIMENTAL SECTION

Materials and Reagents. Analytical OP standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Immunizing hapten [4-(diethoxyphosphorothioyloxy)benzoic acid] and mAb with broad specificity to OPs was previously produced.[21] CNBr-activated Sephrose 4B (46−165 μm) was purchased from Pharmacia Corporation (Uppsala, Sweden). A mixed cellulose ester microporous membrane was purchased from Shanghai Xingya Purification Material Factory (Shanghai, China). All other reagents were of analytical grade and were obtained from a local chemical supplier (Yunhui Trade Co., Ltd., Guangzhou, China).

Instruments. Ultraviolet−visible (UV−vis) spectra were recorded on a UV-160A Shimadzu spectrophotometer (Kyoto, Japan). HPLC−MS/MS analysis was carried out using the 1200 series HPLC system (Agilent Technologies, Santa Clara, CA) equipped with the Agilent 6410 Triple Quad LC/MS system (Agilent Technologies, Santa Clara, CA). All aqueous solutions and buffers were prepared with water purified with a Milli-Q system (Millipore, Bedford, MA).

Preparation of the IAC Column. The IAC was prepared according to the procedure described by Li et al.,[16] with some modifications. Briefly, 1.0 g of CNBr-activated Sepharose 4B was dissolved in 5 mL of 1 mM HCl and poured into a sintered-glass funnel. The gel was washed with 200 mL of 1 mM HCl to remove the suspended protecting groups, followed by washing with 200 mL of 0.1 M $NaHCO_3$ (pH 8.3). The gel was transferred to a clean beaker with 5 mL of 0.1 M $NaHCO_3$ and mixed with 3 mL of mAb (8.4 mg/mL, predialyzed overnight in 0.1 M $NaHCO_3$). The mixture was gently stirred at room temperature for 2 h and at 4 °C overnight to allow for coupling of the mAb to Sepharose 4B. Then, the mixture was poured into a sintered-glass funnel, and the effluent was collected and measured with UV−vis spectrometry. The funnel was washed with 0.1 M $NaHCO_3$ until the UV absorbance of the eluted solution at 280 nm was zero. A volume of 10 mL of 0.1 M Tris-HCl buffer (pH 8.0) was passed through the funnel, and the gel was transferred into a clean beaker with another 10 mL of 0.1 M Tris-HCl buffer (pH 8.0). The mixture was gently stirred at 4 °C for 2 h to block the unbound sites on the CNBr-activated Sepharose 4B. The gel was washed alternately with 3 cycles of 25 mL of 0.1 M acetate buffer (pH 4.0) and 20 mL of 0.1 M Tris-HCl buffer (pH 8.0). Finally, 0.5 mL of bed volume gel was transferred to a common SPE cartridge with frits placed on both top and bottom and stored in 0.01 M phosphate-buffered saline (PBS, pH 7.4) containing 0.01% sodium azide at 4 °C until use. For comparison, a blank column without antibody immobilization was prepared in the same way as described above.

Determination of Column Capacity. The column binding capacity was determined by continuously loading each OP standard solution onto the IAC column, so that an excess amount of OP would saturate all accessible sites of the IAC column. After preconditioning with 10 mL of PBS (0.01 M, the same hereinafter), a total amount of 3000 ng of each OP in 30 mL of PBS containing 10% methanol was loaded on the column (each OP in one newly prepared column). An excess of PBS was used to wash the column. All fractions in the loading and washing steps were collected and extracted by dichloromethane[24] for HPLC−MS/MS analysis. The washing of the column using PBS was stopped when no OP residue in effluent was detected. The binding capacity of the IAC column was calculated using the following equation: column capacity (ng/gel) = $[(3000 - X)/0.5]$, where 3000 (ng) is the loading amount of each OP, X (ng) is the total amount of each OP-residue-found effluent, and 0.5 (mL) is the gel volume of one column.

Optimization of Elution Conditions. A mass of 100 ng of each of coumaphos, parathion, and phorate was dissolved with 10 mL of PBS containing 10% methanol and loaded onto the IAC columns (preconditioned with 10 mL of PBS). The saturated column was washed with 20 mL of PBS and then eluted with 10 mL of various proportions of methanol (50−80%) in PBS. The elution fractions (10 mL per fraction) were collected, evaporated to dryness under a gentle stream of N_2 at 45 °C, and dissolved in 1 mL of acetonitrile. After filtration through a 0.2 μm mixed cellulose ester microporous membrane, 50 μL of the solution was used for HPLC−MS/MS analysis. The recovery (%) was calculated as follows: recovery (%) = (quantity measured/quantity loaded) × 100. The IAC column was regenerated by equilibrating with 10 mL of PBS and stored in PBS containing 0.01% sodium azide at 4 °C for further use. Unless otherwise specifically stated, the flow rate of loading, washing, and eluting was set at 1.0 mL/min in this study.

Sample Preparation and IAC Cleanup. River water samples were collected from the Zhujiang River, the largest drinking water source for the city of Guangzhou, China. The water samples were filtered to remove particles larger than 0.45 μm and then stored for further use. Soil samples were collected from the experimental fields of South China Agricultural University, Guangzhou, China. One portion (e.g., 10 g) of soil sample was distributed into a glass jar, and two portions (e.g., 20 mL) of methanol were added to the sample and vortexed for 1 min. The mixture was then sonicated for 5 min and centrifuged at 4000*g* for 10 min. The superstratum was transferred and evaporated by gently blowing N_2 to remove the solvent. The residue was dissolved with one portion (e.g., 10 mL) of PBS containing 10% methanol for further use.

For IAC cleanup, a certain volume of sample prepared as described above was loaded onto the IAC column (preconditioned with 10 mL of PBS). The saturated column was washed with 20 mL of PBS and then eluted with 5 mL of 80% methanol in PBS. The effluent was dried and submitted for HPLC−MS/MS analysis. The IAC columns were regenerated and stored as described above.

HPLC−MS/MS Conditions. HPLC−MS/MS for simultaneous determination of 13 OPs was completed by the China National Analytical Center, Guangzhou, China. The 1200 series HPLC system was used for separation of the studied pesticides on a Hypersil BDS C8 column (100 × 2.1 mm inner diameter and 2.4 μm particle size). Mobile phase A consisted of 0.2% acetic acid and 10 mmol/L ammonium acetate in water, and mobile phase B consisted of 0.2% acetic acid in acetonitrile. Mobile phases A and B were used in the following gradient profile: 0 min, 55% A and 45% B; 8 min, 10% A and 90% B; and then 8.1−14 min, 55% A and 45% B. The flow rate of the mobile phase was 0.2 mL/min, and an aliquot of 10 μL of each sample was injected into the HPLC system. Analytes were determined by ESI−MS/MS in positive mode by multiple reaction monitoring (MRM) using an Agilent 6410 Triple Quad mass spectrometer (see Table S1 of the Supporting Information). The parameters were as follows: gas temperature, 350 °C; gas flow, 12 L/min; nebulizer gas, 50 psi; and capillary voltage, 4000 V. High-purity nitrogen (>99.99%) was served as the nebulizer and collision gas.

RESULTS AND DISCUSSION

Preparation of the IAC Column. Among the solid supports used in the preparation of IAC, agarose-based

supports are one of the most common sorbents selected for both commercial and laboratory application, especially for off-line applications.[10] CNBr-activated Sepharose 4B is chemically and biologically inert, easily derivatized, and water-insoluble but hydrophilic; thus, it is favored in the preparation of IAC for organic analytes.[15−17,25] In this study, therefore, it was chosen as the solid support. To 1 g of Sepharose 4B (results in about 3 mL final volume of gel), 25.2 mg of mAb was added for immobilization on the gel. From the results of UV−vis spectrometry, about 7.1 mg of mAb was covalently coupled to 1 mL of gel. The coupling efficiency, defined as the percentage of antibody immobilized on the Sepharose gel,[26] of mAb on the solid support was found to be about 84.5%. Finally, the IAC column was constructed by packing the gel into a common SPE cartridge (0.5 mL of gel per column) with frits placed on both top and bottom.

Determination of Column Capacity. The column capacity of IAC for each OP was measured using a breakthrough volume test.[25] An excess of the OP standard solution was loaded on the column and washed with PBS until no OP was found in the effluents. The retained amount of each OP on the column was calculated by subtracting the total amount of each OP residue found in the effluent fractions from the total amount of each OP loaded. As shown in Table 1, the binding capacity of IAC for target OPs was found to be from 884 to 2641 ng/mL of gel. Table 1 also showed the cross-reactivity (CR) of the mAb with OPs determined by icELISA.[22] These results indicated that the higher the CR, the better the binding capacity of IAC obtained. To demonstrate that the OPs were specifically bound to the immobilized mAb and there is no non-specific adsorption, the control columns (without coupled antibodies) were used to measure the capacity for three selected OPs (coumaphos, parathion, and phorate). No OP was found to be absorbed on the control Sepharose 4B column.

Table 1. CR of the mAb for OPs and the MBC of IAC for OPs

analyte	IC_{50} (ng/mL)	CR (%)	MBC[a] (ng/mL of gel)
hapten[b]	28.31	100	ND[c]
coumaphos	0.19	18621.0	2641
parathion	1.53	1856.6	1854
phoxim	7.82	372.0	1436
quinalphos	15.17	191.8	1676
dichlofenthion	20.56	149.5	1561
triazophos	29.14	104.9	1278
azinphos-ethyl	38.35	87.8	1438
phosalone	67.67	53.0	1358
isochlorthion	87.19	33.3	1172
parathion-methyl	197.96	13.0	1226
cyanophos	213.06	11.1	969
disulfoton	246.29	10.1	1012
phorate	265.20	9.4	884

[a]MBC = maximum binding capacity. [b]Hapten is the immunizing hapten [4-(diethoxyphosphorothioyloxy)benzoic acid].[21] [c]ND = not determined.

Optimization of Elution Conditions. The most common strategies used for the elution of analytes from the IAC included reducing the pH to 2 or 3, using water-miscible organic solvent or chaotropic agents, or a combination of these conditions.[10] In most of the reported off-line procedures, desorption was achieved with a high percentage of organic solvent (methanol was mostly used) mixed with water or buffer.[14−17,25,27] In this study, therefore, different concentrations of methanol (50−80%) in PBS were first chosen as the elution solvent. PBS was chosen because an appropriate ionic strength can alter the column conditions, which can disrupt the ionic bonds, the dielectric constant, and the hydrophobic interactions.[17,28] To define the methanol content under which the desorption takes place, a step elution was applied to the IAC column loaded with 100 ng of coumaphos, parathion, and phorate in 10 mL of PBS containing 10% methanol. These three analytes were selected because they showed the highest CR (coumaphos), medium CR (parathion), and lowest CR (phorate) with the proposed mAb (Table 1). As seen from Figure 1, the continuous elution with 50, 60, 70, and 80% methanol in PBS gave recoveries of about 85, 94, and 98% for coumaphos, parathion, and phorate, respectively. The relatively low recovery obtained for coumaphos should be due to the high affinity of the mAb for coumaphos (IC_{50} of 0.19 ng/mL; Table 1). Because the increasing of the methanol content in the elution solvent might cause damage to the antibody binding ability, it was decided not to use a higher methanol content (>80%) in the elution solvent. The recoveries for parathion and phorate using step elution indicated that 80% methanol in PBS was enough to desorb all of the OPs (except for coumaphos) bound to the immunosorbent with desired recoveries. Therefore, 80% methanol in PBS was used as the elution solvent in further studies.

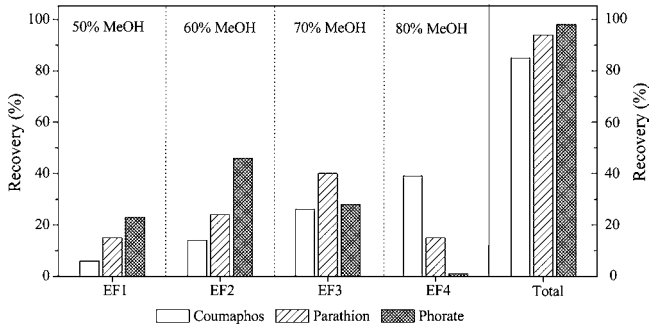

Figure 1. Step elution of coumaphos, parathion, and phorate with different methanol concentrations in the elution buffer (PBS). The IAC is loaded with 100 ng of analytes (10 ng/mL, 10 mL of PBS containing 10% methanol) and washed with 20 mL of PBS. Elution was performed with 10 mL of 50% MeOH/PBS, followed by 10 mL of 60% MeOH/PBS, 10 mL of 70% MeOH/PBS, and finally, 10 mL of 80% MeOH/PBS. Each elution fraction (EF, 10 mL) was collected for HPLC−MS/MS analysis.

The optimum volume of the elution solvent was also determined by loading with 100 ng of coumaphos, parathion, and phorate in 10 mL of PBS containing 10% methanol. As shown in Figure 2, the three analytes were nearly not detected in the collected loading fraction and the collected washing fractions, which indicated that the analytes were retained completely by the immunosorbent. About 76, 93, and 95% of the loaded coumaphos, parathion, and phorate, respectively, were eluted in the first five fractions (5 mL). Although the increasing of the elution solvent volume (9 mL) can give recovery of about 83% for coumaphos, it also resulted in wasting time on evaporating the elution fractions by N_2 drying. Therefore, 5 mL was used as the optimum volume of the elution solvent.

dx.doi.org/10.1021/jf300896z | J. Agric. Food Chem. 2012, 60, 5847−5852

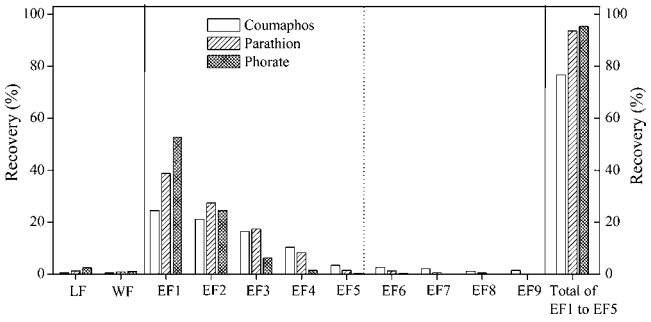

Figure 2. Elution profile of IAC of coumaphos, parathion, and phorate with 80% MeOH/PBS. The IAC is loaded with 100 ng of analytes (10 ng/mL, 10 mL of PBS containing 10% methanol), washed with 20 mL of PBS, and eluted with 5 mL of 80% MeOH/PBS. The loading fraction (LF, 10 mL), washing fraction (WF, 20 mL), and eluting faction (EF, 1 mL) were collected for HPLC–MS/MS analysis.

Determination of the Breakthrough Volume. The breakthrough volume is the volume at which analytes are no longer retained because of insufficient recognition or overloading.[29] In this study, 300 ng of coumaphos, parathion, and phorate was applied to determine their breakthrough volumes on the IAC because of the maximum binding capacity (MBC) of IAC for all analytes ranging from 884 to 2641 ng/mL of gel (equal to about 400–1300 ng per column). The results in Table 2 showed that up to 400 mL of volume can be applied without an obvious loss of recovery for the three analytes. The larger volume was loaded, and the higher sensitivity was obtained for these analytes. However, when the loading volume was increased, the run time for sample loading was increased. The total run time for columns loaded with 400 mL of sample was approximately 6.7 h. In practice, the loading volume can be chosen according the need for sensitivity.

Table 2. Recoveries of Three Analytes Using Different Loading Volumes (300 ng Applied)[a]

	loading volume (mL)				
analyte	10	50	100	200	400
coumaphos (%)	75	76	73	75	72
parathion (%)	96	92	94	95	92
phorate (%)	98	101	97	96	93

[a]The analytes were dissolved in OP-free river water samples.

Analysis of Spiked Samples. OP-free environmental river samples and soil samples were spiked with OPs at three concentrations, pretreated with IAC cleanup, and analyzed by HPLC–MS/MS. The results of recoveries are shown in Table 3. The mean recovery for coumaphos ranged from 67.4 to 80.1% from spiked river water samples and from 65.2 to 76.9% from spiked soil samples. The mean recovery for other OPs ranged from 82.3 to 107.1% from spiked river water samples and from 78.9 to 103.5% from spiked soil samples. The relative standard deviation (RSD) for all samples was between 3.6 and 11.1%. These data indicated good repeatability of the sample extraction, cleanup, and analysis methods.

The limit of quantitation (LOQ) of HPLC–MS/MS for 13 OP standards was as follows (ng/mL): coumaphos (2.5), parathion (2.5), phoxim (1), quinalphos (1), triazophos (1), dichlofenthion (5.0), azinphos-ethyl (1), phosalone (1), isochlorthion (10), parathion-methyl (2.5), cyanophos (50), disulfoton (10), and phorate (5). According to the results of the breakthrough volume test, a 10–400-fold enrichment of these analytes in samples can be obtained after the IAC cleanup. This would indicate that the LOQ for these analytes could reach 0.01–0.13 ng/mL, which would fulfill the demand of MRLs for pesticides in drinking water and surface water set by the EU.

Analysis of Real Samples. A total of 10 river water samples were collected from different sites of the Zhujiang River, and a total of 10 soil samples were collected from the farmland near the South China Agricultural University. A volume of 100 mL for water samples or 100 g for soil samples were treated with IAC cleanup and analyzed by HPLC–MS/MS. No OP residues were detected in the river water samples. However, two soil samples (sample 4, collected from a paddy land, and sample 7, collected from vegetable land) were detected with trace amounts of parathion-methyl (0.06 ng/mL) and phorate (0.24/ng mL), respectively. Although the use of

Table 3. Recoveries of OPs from Spiked River Water Samples and Soil Samples with IAC Cleanup and HPLC–MS/MS Analysis

		river water		soil	
analyte	spiked level[a] (ng/mL or ng/g)	recovery (%)	RSD[b] (%)	recovery (%)	RSD (%)
coumaphos	0.2, 1, 5	67.4–80.1	3.8–7.7	65.2–76.9	5.5–10.6
parathion	0.2, 1, 5	85.2–101.3	4.5–6.8	79.1–95.6	4.7–6.8
phoxim	0.2, 1, 5	89.9–96.5	5.1–6.0	84.6–92.1	5.9–10.4
quinalphos	0.2, 1, 5	84.3–98.1	6.8–9.9	85.3–94.7	6.4–11.1
dichlofenthion	0.2, 1, 5	91.1–105.4	4.3–10.6	78.9–89.6	4.6–8.7
triazophos	0.2, 1, 5	86.8–95.3	5.5–8.1	83.5–103.5	3.5–7.3
azinphos-ethyl	0.2, 1, 5	82.7–104.1	3.7–9.6	84.6–95.7	4.7–8.8
phosalone	0.2, 1, 5	86.9–101.4	5.6–10.3	90.5–96.3	5.2–9.6
isochlorthion	0.2, 1, 5	87.1–93.4	6.7–8.0	79.8–98.2	4.8–8.6
parathion-methyl	0.2, 1, 5	84.5–95.9	5.8–9.8	88.6–94.8	3.8–10.0
cyanophos	1, 5, 10	88.3–107.1	4.3–7.2	84.5–99.2	7.4–9.5
disulfoton	0.2, 1, 5	82.3–94.5	3.6–8.3	80.3–96.7	6.5–10.3
phorate	0.2, 1, 5	86.1–95.7	4.5–9.1	87.2–101.6	5.8–9.1

[a]The spiked concentration is in units of ng/mL for water samples and ng/g for soil samples. A total of 100 mL of sample volume was loaded for OPs spiked at 0.2 ng/mL (ng/g) and cyanophos spiked at 1 ng/mL (ng/g). A total of 20 mL of sample volume was loaded for OPs spiked at 1 ng/mL (ng/g) and cynaophos spiked at 5 ng/mL (ng/g). A total of 10 mL of sample volume was loaded for OPs spiked at 5 ng/mL (ng/g) and cyanophos spiked at 10 ng/mL (ng/g). [b]RSD = relative standard deviation (n = 2).

dx.doi.org/10.1021/jf300896z | J. Agric. Food Chem. 2012, 60, 5847–5852

parathion-methyl was completely forbidden in China since 2004,[30] its residue in environmental samples and agricultural samples was detected.[30,31] Phorate was prohibited to be used in the vegetable fields according to announcements issued by the Chinese Ministry of Agriculture.[32] However, this prohibition was not seriously put into practice in vegetable production in China because its residue has been detected in agricultural samples.[33] Therefore, further investigation for these residues in environmental samples would be required.

Column Reusability. One obvious advantage of IAC is that it can be reused several times without apparent loss in recoveries if the initial capacity was high enough. Actually, most antibodies are tolerant of the organic solvent to some extent without significant loss of activity, and the IAC column can be easily regenerated with PBS solution.[25] In this study, the reusability of the IAC was evaluated by loading 100 ng of coumaphos, parathion, and phorate in river water samples (10 ng/mL), following 20 cycles of use in 30 days. The recovery curves for three analytes are presented in Figure 3. The results

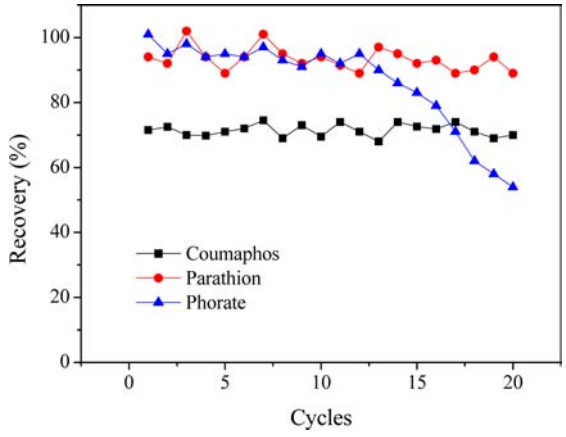

Figure 3. Variation of recovery for coumaphos, parathion, and phorate with IAC cleanup in 20 cycles of use over 30 days. The IAC is loaded with 100 ng of analytes (10 ng/mL, 10 mL of OP-free river water), washed with 20 mL of PBS, and eluted with 5 mL of 80% MeOH/PBS.

indicated that, for coumpahos and parathion, a stable recovery was obtained within 20 repeated usages, while for phorate, this was decreased significantly after 15 usages. With the increasing times of use of the IAC, the MBC was reported to decrease gradually for analytes.[16,17] The loss of recovery for phorate after 15 usages was due to the MBC for phorate being below 100 g per column. These results indicated that the proposed IAC columns can be reused at least 15 times at the loading level of 100 ng for all analytes.

When the broadly specific monoclonal antibody was covalently coupled for 13 OPs on CNBr-activated Sepharose 4B and the immunosorbents were packed into a SPE cartridge, the IAC column for selective extraction and concentration of OPs was prepared and applied to environmental samples prior to analysis by HPLC–MS/MS. Efficient desorption of bound OPs was achieved with 80% ethanol in PBS. The column was evaluated with regard to capacity, recovery, breakthrough volume, and reusability. The obtained results show that IAC coupling with HPLC–MS/MS can be used for quantitative extraction, concentration, and determination of OPs from environmental samples at trace levels.

■ ASSOCIATED CONTENT

Supporting Information

Conditions for MS in MRM mode (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: +86-20-8528-3925. Fax: +86-20-8528-0270. E-mail: ymsun@scau.edu.cn.

Funding

This work was supported by the Special Fund for Agro-Scientific Research in the Public Interest of China (201003008-08) and the National Basic Research Program of China (973 Program, 2012CB720803).

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Quintana, J.; Martí, I.; Ventura, F. Monitoring of pesticides in drinking and related waters in NE Spain with a multiresidue SPE–GC–MS method including an estimation of the uncertainty of the analytical results. *J. Chromatogr., A* **2001**, *938*, 3–13.

(2) European Economic Community (EEC). *EEC Drinking Water Guidelines*; EEC: Brussels, Belgium, 1980; 80/779/EEC Number L229/11-29, p 1.

(3) Fielding, M.; Barceló, D.; Helweg, A.; Calassi, S.; Torstensson, L.; van Zoonen, P.; Wolter, R.; Angeletti, G. *Water Pollution Research Report 27*; Commission of the European Communities: Brussels, Belgium, 1992.

(4) Ballesteros, E.; Parrado, M. J. Continuous solid-phase extraction and gas chromatographic determination of organophosphorus pesticides in natural and drinking waters. *J. Chromatogr., A* **2004**, *1029*, 267–273.

(5) Gamón, M.; LlEó, C.; Ten, A.; Mocholí, F. Multiresidue determination of pesticides in fruit and vegetables by gas chromatography/tandem mass spectrometry. *J. AOAC Int.* **2001**, *84*, 1209–1216.

(6) Hernández, F.; Sancho, J. V.; Pozo, O. J. Critical review of the application of liquid chromatography/mass spectrometry to the determination of pesticide residues in biological samples. *Anal. Bioanal. Chem.* **2005**, *382*, 934–946.

(7) Alder, L.; Greulich, K. Fast multiresidue screening of 300 pesticides in water for human consumption by LC–MS/MS. *Anal. Bioanal. Chem.* **2008**, *391*, 183–197.

(8) Picó, Y.; Fernández, M.; Ruiz, M. J.; Font, G. Current trends in solid-phase-based extraction techniques for the determination of pesticides in food and environment. *J. Biochem. Biophys. Methods* **2007**, *70*, 117–131.

(9) Sanchez, F. G.; Diaz, A. N.; Herrera, R. G.; Jose, L. P. S. Development and characterisation of an immunoaffinity chromatographic column for the on-line determination of the pesticide triclopyr. *Talanta* **2007**, *71*, 1411–1416.

(10) Hennion, M.-C.; Pichon, V. Immuno-based sample preparation for trace analysis. *J. Chromatogr., A* **2003**, *1000*, 29–52.

(11) Holtzapple, C. K.; Buckley, S. A.; Stanker, L. H. Immunosorbents coupled on-line with liquid chromatography for the determination of fluoroquinolones in chicken liver. *J. Agric. Food Chem.* **1999**, *47*, 2963–2968.

(12) Senyuva, H. Z.; Gilbert, J. Immunoaffinity column clean-up techniques in food analysis: A review. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2010**, *878*, 115–132.

(13) Prince, A. E.; Fan, T. S.; Skoczenski, B. A.; Bushway, R. J. Development of an immunoaffinity-based solid-phase extraction for diazinon. *Anal. Chim. Acta* **2001**, *444*, 37–49.

(14) Chuang, J. C.; Emon, J. M. V.; Jones, R.; Durnford, J.; Lordo, R. A. Development and application of immunoaffinity column

chromatography for atrazine in complex sample media. *Anal. Chim. Acta* **2007**, *583*, 32−39.
(15) Chen, Y.; Wang, X.; Wang, J.; Tang, S. Preparation of acetochlor antibody and its application on immunoaffinity chromatography cleanup for residue determination in peanuts. *J. Agric. Food Chem.* **2009**, *57*, 7640−7643.
(16) Li, C.; Wang, Z.; Cao, X.; Beier, R. C.; Zhang, S.; Ding, S.; Li, X.; Shen, J. Development of an immunoaffinity column method using broad-specificity monoclonal antibodies for simultaneous extraction and cleanup of quinolone and sulfonamide antibiotics in animal muscle tissues. *J. Chromatogr., A* **2008**, *1209*, 1−9.
(17) Zhao, S.; Li, X.; Ra, Y.; Li, C.; Jiang, H.; Li, J.; Qu, Z.; Zhang, S.; He, F.; Wan, Y.; Feng, C.; Zheng, Z.; Shen, J. Developing and optimizing an immunoaffinity cleanup technique for determination of quinolones from chicken muscle. *J. Agric. Food Chem.* **2009**, *57*, 365−371.
(18) Garcia-Villanova, R. J.; Cordón, C.; Paramás, A. M. G.; Aparicio, P.; Rosales, M. E. G. Simultaneous immunoaffinity column cleanup and HPLC analysis of aflatoxins and ochratoxin A in Spanish bee pollen. *J. Agric. Food Chem.* **2004**, *52*, 7235−7239.
(19) MacDonald, S. J.; Chan, D.; Brereton, P.; Damant, A.; Wood, R. Determination of deoxynivalenol in cereals and cereal products by immunoaffinity column cleanup with liquid chromatography: Inter-laboratory study. *J. AOAC Int.* **2005**, *88*, 1197−1204.
(20) Tankiewicz, M.; Fenik, J.; Biziuk, M. Determination of organophosphorus and organonitrogen pesticides in water samples. *Trends Anal. Chem.* **2010**, *29*, 1050−1063.
(21) Xu, Z.-L.; Shen, Y.-D.; Zheng, W.-X.; Beier, R. C.; Xie, G.-M.; Dong, J.-X.; Yang, J.-Y.; Wang, H.; Lei, H.-T.; She, Z.-G.; Sun, Y.-M. Broad-specificity immunoassay for *O*,*O*-diethyl organophosphorus pesticides: Application of molecular modeling to improve assay sensitivity and study antibody recognition. *Anal. Chem.* **2010**, *82*, 9314−9321.
(22) Xu, Z.-L.; Zeng, D.-P.; Yang, J.-Y.; Shen, Y.-D.; Beier, R. C.; Lei, H.-T.; Wang, H.; Sun, Y.-M. Monoclonal antibody-based broad-specificity immunoassay for monitoring organophosphorus pesticides in environmental water samples. *J. Environ. Monit.* **2011**, *13*, 3040−3048.
(23) Degelmann, P.; Wenger, J.; Niessner, R.; Knopp, D. Development of a class-specific ELISA for sulfonylurea herbicides (sulfuron screen). *Environ. Sci. Technol.* **2004**, *38*, 6795−6802.
(24) Tse, H.; Comba, M.; Alaee, M. Method for the determination of organophosphate insecticides in water, sediment and biota. *Chemosphere* **2004**, *54*, 41−47.
(25) Qiao, Y.; Yang, H.; Wang, B.; Song, J.; Deng, A. Preparation and characterization of an immunoaffinity chromatography column for the selective extraction of trace contraceptive drug levonorgestrel form water samples. *Talanta* **2009**, *80*, 98−103.
(26) He, J.; Hou, X.; Jiang, H.; Shen, J. Multiresidue analysis of avermectins in bovine liver by immunoaffinity column cleanup procedure and liquid chromatography with fluorescence detector. *J. AOAC Int.* **2005**, *88*, 1099−1103.
(27) Thomas, S. D.; Li, Q. X. Immunoaffinity chromatography for analysis of polycyclic aromatic hydrocarbons in corals. *Environ. Sci. Technol.* **2000**, *34*, 2649−2654.
(28) Firer, M. A. Efficient elution of functional proteins in affinity chromatography. *J. Biochem. Biophys. Methods* **2001**, *49*, 433−442.
(29) Ballesteros, B.; Marco, M. Basic principles of the use of immunoaffinity chromatography for environmental analysis. *Food Technol. Biotechnol.* **1998**, *36*, 145−155.
(30) Na, T.; Fang, Z.; Zhanqi, G.; Ming, Z.; Cheng, S. The status of pesticide residues in the drinking water sources in Meiliangwan Bay, Taihu Lake of China. *Environ. Monit. Assess.* **2006**, *123*, 351−370.
(31) Bai, Y.; Zhou, L.; Wang, J. Organophosphorus pesticide residues in market foods in Shaanxi area, China. *Food Chem.* **2006**, *98*, 240−242.
(32) Jin, F.; Wang, J.; Shao, H.; Jin, M. Pesticide use and residue control in China. *J. Pestic. Sci.* **2010**, *35*, 138−142.
(33) Wang, L.; Liang, Y.; Jiang, X. Analysis of eight organophosphorus pesticide residues in fresh vegetables retailed in agricultural product markets of Nanjing, China. *Bull. Environ. Contam. Toxicol.* **2008**, *81*, 377−382.