JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Determination of 49 Organophosphorus Pesticide Residues and Their Metabolites in Fish, Egg, and Milk by Dual Gas Chromatography−Dual Pulse Flame Photometric Detection with Gel Permeation Chromatography Cleanup

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ABSTRACT: A new method for the quantitative determination of 49 kinds of organophosphorus pesticide residues and their metabolites in fish, egg, and milk by dual gas chromatography−dual pulse flame photometric detection was developed. Homogenized samples were extracted with acetone and methylene chloride (1 + 1, v/v), and then the extracts were cleaned up by gel permeation chromatography (GPC). The response of each organophosphorus pesticide showed a good linearity with its concentration; the linearity correlation was not less than 0.99. The detection limits (S/N = 3) of pesticides were in the range of 0.001−0.025 mg kg^{-1}. The recovery experiments were performed by blank sample spiked at low, medium, and high fortification levels. The recoveries for fish, egg, and milk were 50.9−142.2, 53.3−137.2, and 50.3−139.4% with relative standard deviations (RSD, n = 6) of 2.3−24.9, 4.3−26.7, and 2.8−32.2%, respectively. The method was applied to detect organophosphorus pesticides in samples collected from the market, and satisfactory results were obtained. This quantitative method was highly sensitive and exact and could be applied to the accurate determination of organophosphorus contaminants in fish, egg, and milk.

KEYWORDS: *organophosphorus pesticides, metabolites, gel permeation chromatography cleanup, gas chromatography−pulse flame photometric detection, milk, egg, fish,*

INTRODUCTION

Organophosphorus pesticides (OPs) are typically esters of pentavalent phosphorus acids. These compounds, as the most frequently used group of insecticides, were widely used within agriculture all over the world.[1] OPs are one of the most common classes involved in poisoning because of the inhibition of acetyl-cholinesterase. According to the substituents on the P atom (methoxy, ethoxy, propoxy), the reactivation (hydrolysis) of these phosphorylated cholinesterases is very different; the components with the methoxy in the phosphorylated cholinesterase are rather quickly hydrolyzed, which results in a very low risk of accumulation in animal food. The cholinesterase coupled with the ethoxy or propoxy phosphor moiety are very slowly reactivated, and even if they are less persistent in the environment than organochlorine pesticides, they can also reach the food chain and may therefore represent risk to human health. Therefore, these substances accumulated in animal products have received more and more attention.[2−6]

Due to the contamination of feed and water, except meat and tissues,[7−11] OP residues also may occur in eggs, fishes, and milk. Some multiresidue methods for detecting OPs in the above-mentioned three types of matrix have been reported.[12−14] OPs are unstable compounds. In the bodies of animals, metabolism and degradation are very fast. Moreover, these metabolites are mostly toxic organophorus compounds.[15] To obtain the real residue levels, the primary and secondary metabolites of OPs were also selected as target compounds in the research.

As a suitable cleanup method to remove fat in animal samples, gel permeation chromatography (GPC) was adopted in this paper. Organophosphorus pesticide determination in diverse biological samples and foods is normally performed by gas chromatography coupled to mass spectrometry (GC-MS),[16] NPD,[17] FPD,[18] PFPD, and other specific detectors. Because of the requirements on the aspects of sensitivity, multiresidue detection, and specificity, dual gas chromatography−dual pulse flame photometric detection was an alternative method.

The aim of the present study was to develop a sensitive and reliable mutiresidue method for the quantitative determination of OPs in fish, milk, and egg.

EXPERIMENTAL PROCEDURES

Apparatus. GC3800 (Varian, USA), equipped with Autosampler 8400, two PFPDs (the PFPD 1 was "S" type and PFPD 2 was "P" type), and two 1177 injectors. Two columns were used: column 1, Dikma DM-5 (30 m × 0.32 mm i.d. × 0.25 μm); column 2, Agilent DB-1 (30 m × 0.32 mm i.d. × 0.25 μm). These columns had different

Received: October 28, 2011
Revised: February 2, 2012
Accepted: February 2, 2012
Published: February 2, 2012

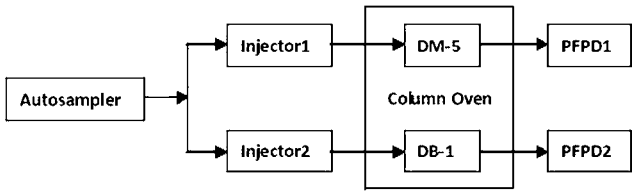

Figure 1. Flow diagram of the dual gas chromatographic columns and dual PFPD system.

polarities. The construction of the instrument is shown in Figure 1. A Bio-Beads S-X3 (300 mm × 25 mm) cleanup column (J2, USA) was used for GPC (JZ Scientific, USA). Furthermore, there are also other instruments such as a rotary evaporator (EYELA, Japan), a vortex-type mixer (Scientific Industries, USA), a nitrogen evaporator (Organomation Associates, USA), a centrifuge (Sigma, USA), and filters (0.22 μm).

Chemicals, Reagents, and Samples. Methylene chloride, acetone, cyclohexane, hexane, and ethyl acetate (HPLC grade) were purchased from Thermo Fisher Co. (Beijing, China), and cyclohexane + ethyl acetate (1 + 1, v/v) was used as mobile phase for GPC; sodium sulfate anhydrous (P.R. grade) purchased from Sigma Co. (Beijing, China) was heated at 650 °C for 4 h and kept in a desiccator; sodium chloride (analytical-reagent grade) was purchased from Beijing Chemical Industry (Beijing, China); deionized water was obtained from a Milli-Q water purification system Millipore (Bedford, MA).

All of the pesticide standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany); the standards included 59 compounds, 10 of which were metabolites and isomers of the other 49 OPs. For stock standard solutions, 10−30 mg of individual pesticide standards was accurately weighed (accurate to 0.1 mg) into a 10 mL volumetric flask, dissolved, and diluted to volume with ethyl acetate; after mixing, the standard stock solutions were transferred to 10 mL brown glass bottles, respectively. For mixed standard solutions A and B, depending upon the properties and retention time of each pesticide, all of the standards were divided into two groups. Pesticides of group A were separated on column 1and detected by PFPD 1; pesticides of group B were separated on column 2 and detected by PFPD 2. Pesticides included in the two groups are listed in Table 1. The concentration of mixed standard solution depended upon the sensitivity of each compound for the instrument, which is shown in Table 1. It must be noted that mixed standard solutions should be stored in the dark below 4 °C and can be used for 1 month.

Sample Extraction. Yolks and whites of whole eggs were combined and blended at low speed until a homogeneous sample was obtained. Fish and milk samples were also blended to uniform states, respectively. Five grams of homogenized sample (egg sample was 2 g) was accurately weighed into a 50 mL centrifuge tube containing 2 g of sodium chloride and 2 mL of water (except milk) and was extracted with 40 mL of mixture of acetone and methylene chloride (1 + 1, v/v). Then the extracts were centrifuged for 10 min at 8000 rpm (6869*g*). The supernatants were anhydrated by passing through a glass funnel containing 6 g of sodium sulfate anhydrous. The elutes were collected and evaporated to near dryness in a water bath of 40 °C. The residues were reconstructed with a 10 mL mixture of cyclohexane + ethyl acetate (1 + 1, v/v) and set aside for cleanup.

Sample Cleanup. The above-mentioned solutions were passed through a 0.22 μm nylon filter before cleanup by GPC. The instrument conditions of GPC were as follows: flow rate, 5 mL min^{-1}; detection wavelength, 254 nm; injection volume, 5 mL. The eluted portions of 8−20 min were collected in a 100 mL evaporation flask and concentrated to 1 mL in a water bath of 40 °C on a rotary evaporator. The concentrated solutions were filtered by a 0.22 μm nylon filter again and then were provided for GC analysis.

GC Analysis. The injection models of the two injectors were splitless (30 s), and the injection volume was 1 μL. The time interval between the two injections was 1 min. The temperatures of the two injectors was set to 250 °C. The primary temperature was programmed from 60 °C (2 min) to 150 °C at 25 °C min^{-1}, then raised to 260 °C at 2 °C min^{-1}, and finally reached 290 °C (5 min) at 30 °C min^{-1}. THe carrier gas was nitrogen, purity ≥ 99.999%, and the flow rate was 1 mL min^{-1}. The combustion gas was hydrogen, with a flow rate of 14 mL min^{-1}. The flow rate of air 1 was 17 mL min^{-1} and that of air 2, 10 mL min^{-1}.

The detection system used nitrogen as makeup gas. PFPD 1 ("S" type, double S filter) conditions: temperature, 300 °C; photomultiplier tube voltage, 570 V; trigger voltage, 200 mV; amplification factor, 20; gain was used; gate delay time, 4 ms; gate width, 20 ms. PFPD 2 ("P" type, P filter) conditions: except for the photomultiplier voltage of 650 V, the other conditions were the same as those of PFPD 1.

RESULTS AND DISCUSSION

Optimization of the Cleanup Conditions of GPC. Bio-Beads S-X3 (300 mm × 25 mm) was chosen as the cleanup column, and cyclohexane + ethyl acetate (1 + 1, v/v) was used as the mobile phase; an appraisal was conducted of the GPC behavior of 59 pesticides in our laboratory. The mixed standard

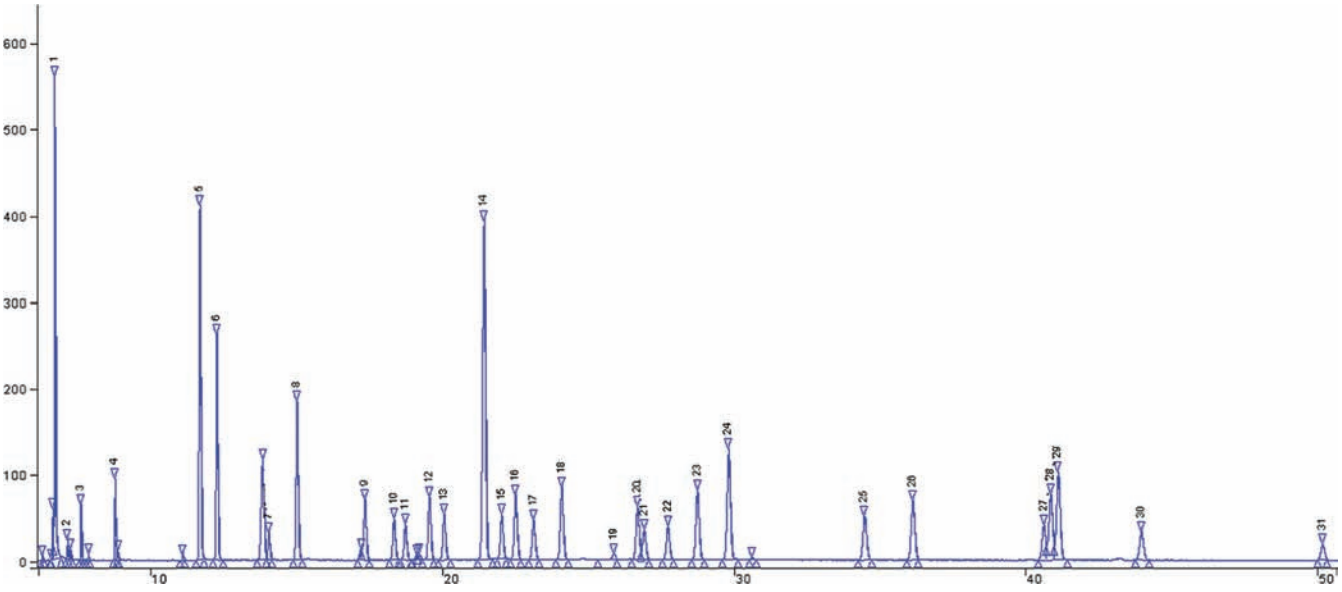

Figure 2. continued

dx.doi.org/10.1021/jf2043828 | J. Agric. Food Chem. 2012, 60, 1906−1913

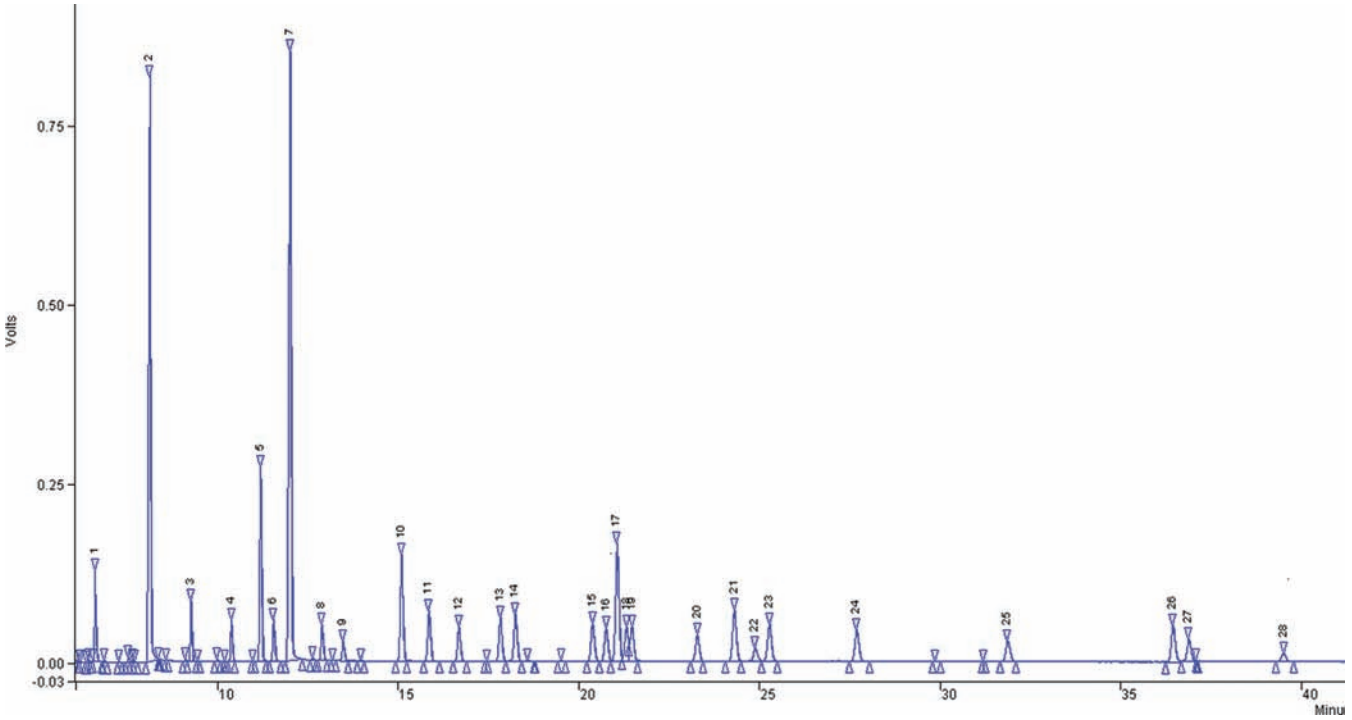

Figure 2. PFPD 1 chromatogram of 31 OPs at the middle spiked level in fish sample. Peaks: 1, methamidophos; 2, phoxim; 3, disulfoton; 4, mevinphos; 5, omethoate; 6, demeton-*S*-methyl; 7, cadusafos; 8, formothion; 9, disulfoton; 10, iprobenfos; 11, formothion; 12, phosphamidon; 13, chlorpyrifos; 14, demeton-*S*-methylsulfone; 15, phorate sulfoxide; 16, phorate sulfoxide; 17, phorate sulfone; 18, isocarbofos; 19, *trans*-fenvinphos; 20, chlofenvinphos; 21, mecarbam; 22, methidathion; 23, tetrachlorvinphos; 24, fenamiphos; 25, ethion; 26, edifenphos; 27, phosmet; 28, pyridaphenthion; 29, EPN; 30, phosalone; 31, coumaphos. PFPD 2 chromatogram of 28 OPs at the middle spiked level in fish sample. Peaks: 1, dichlorvos; 2, acephate; 3, methacrifos; 4, heptenophos; 5, demeton-*O*; 6, ethopraphos; 7, monocrotophos; 8, phorate; 9, demeton-*S*; 10, terbufos; 11, etrimfos; 12, diazinon; 13, parathion-methyl; 14, tolclofos-methyl; 15, pirimiphos-methyl; 16, malathion; 17, fenthion; 18, parathion-ethyl; 19, chlorpynfos; 20, isofenphos-methyl; 21, quinalphos; 22, vamidothion; 23, disulfoton sulfone; 24, profenofos; 25, tnazophos; 26, fenamiphos sulfoxide; 27, fenamiphos sulfone; 28, azinphos-methyl.

solution of 59 pesticides (the concentration of each compound was about 10 μg mL^{-1}) was injected into the GPC. From 0 to 30 min, the elution of each minute was collected and analyzed by the GC system. The results showed that target compounds started elution at 8 min and stopped elution at 20 min. Results from the experiment with vegetable oil found that the lipids that interfered with the determination of peaks before 7.5 min. Therefore, the commenced collection time and stopped collection time were set at 8 and 20 min, respectively. The whole cleanup time was 25 min. According to this experimental program, the samples could be purified completely and contamination of the cleanup equipment was avoided.

Qualification and Quantification of Samples. Because of the similar chemical structures and polarities of the OPs, it is difficult to separate the 59 compounds on the same column completely. So two columns (DM-5 and DB-1) with different polarities were adopted in these experiments. Depending upon the properties and retention time of each pesticide, the compounds were analyzed in different columns. The 59 pesticide standards were injected into each of the columns to confirm their retention times, respectively. The compounds with similar polarities may have retention times close to each other on the same column; if they could not be separated on either of the columns, the compounds were divided into different groups and detected on different equipment. The chromatography is shown in Figure 2.

The mixed standard solution was diluted 10, 20, 40, 80, 100, and 200 times with *n*-hexane and used to construct calibration. The samples were quantified by an external standard method. Limits of detection (LOD, S/N = 3), linearity (r), and linear range for each compound are listed in Table 1.

Evaluation of Method Performance. Accuracy was estimated by spiking blank fish, milk, and egg samples in recovery experiments. The spiked concentrations of OPs were evaluated at high, intermediate, and low levels for each standard, as shown in Table 1. These results of average recovery and precision at six parallel tests for each of the three animal products are shown in Table 2. The recoveries for fish, egg, and milk were 50.9−142.2, 53.3−137.2, and 50.3−139.4% with relative standard deviations (RSD, n = 6) of 2.3−24.9, 4.3−26.7, and 2.8−32.2%, respectively.

Method reproducibility studies were done by injecting three replicates of the same standard solution on three different days and in the same day. Both the intraday precision and the interday precision showed RSDs below 15%.

Application to Samples. In total, 54 samples (20 milk samples, 17 egg samples, and 17 fish samples) collected from the market were analyzed for incidence by the present method. Among the 54 samples, 20 were contaminated with OPs, and the total incidence was 37%. The contaminated samples included 7 milk samples, 13 fish samples, and no egg samples; the corresponding incidences were 35, 76.5, and 0%, respectively. These data indicated that the OPs contamination was widely present in fish and milk and only slightly in eggs. A positive fish sample chromatography including a mother pesticide (dimethoate) and metabolite (omethoate) is shown in Figure 3. The contamination of fish might come from water pollution.

Table 1. Retention Time, Linear Range, Linear Equation, Correlation Coefficient, LOD, and Spiked Level of the 59 Organophosphorous Pesticides

						spiked level of milk and eggs[b] (mg/kg)		
no.[a]	compound	retention time (min)	linear range (mg/L)	correlation coefficient (r)	LOD (mg/kg)	low	middle	high
1	methamidophos	6.609	0.1−2	0.9968	0.002	0.02	0.2	0.8
2	phoxim	6.801	1−10	0.9990	0.024	0.25	2.5	10
3	disulfoton sulfoxide	7.534	0.05−0.5	0.9990	0.002	0.005	0.05	0.2
4	mevinphos	8.699	0.025−0.5	0.9999	0.001	0.005	0.05	0.2
5	omethoate	11.568	0.625−5	0.9937	0.020	0.05	0.5	2
6	demeton-*S*-methyl	12.157	0.125−2.5	0.9998	0.005	0.025	0.25	1
7	cadusafos	13.913	0.02−2	0.9967	0.001	0.01	1	4
8	dimethoate	14.880	0.1−2	0.9998	0.002	0.004	0.4	1.6
9	disulfoton	17.207	0.05−1	0.9997	0.003	0.01	0.1	0.4
10	iprobenfos	18.198	0.05−1	0.9994	0.002	0.01	0.1	0.4
11	formothion	18.837	0.1−1	0.9997	0.003	0.01	0.1	0.4
12	phosphamidon	19.705	0.2−2	0.9984	0.006	0.02	0.2	0.8
13	chlorpyrifos-methyl	20.143	0.05−1	0.9981	0.002	0.02	0.2	0.8
14	demeton-*S*-methylsulfone	21.536	0.8−8	0.9962	0.020	0.08	0.8	3.2
15	fenitrothion	22.163	0.05−1	0.9990	0.002	0.01	0.1	0.4
16	phorate sulfoxide	22.640	0.8−8	0.9997	0.016	0.08	0.8	3.2
17	phorate sulfone	23.237	0.1−1	0.9998	0.002	0.01	0.1	0.4
18	isocarbofos	24.135	0.01−1	0.9961	0.002	0.05	2	0.5
19	*trans*-chlofenvinphos	25.694	0.125−2.5	0.9997	0.008	0.025	0.25	1
20	*cis*-chlofenvinphos	26.670	0.25−2.5	0.9953	0.025	0.025	0.25	1
21	mecarbam	27.040	0.05−1	0.9988	0.005	0.001	0.1	0.4
22	methidathion	27.857	0.05−1	0.9998	0.004	0.01	0.1	0.4
23	tetrachlorvinphos	28.833	0.1−2	0.9999	0.008	0.04	0.4	1.6
24	fenamiphos	29.891	0.25−5	0.9981	0.025	0.05	0.5	2
25	ethion	34.592	0.05−1	0.9990	0.003	0.01	0.1	0.4
26	edifenphos	36.252	0.125−2.5	0.9998	0.009	0.025	0.25	1
27	phosmet	40.643	0.04−4	0.9961	0.019	0.02	2	8
28	pyridaphenthion	40.874	0.2−4	0.9995	0.005	0.09	0.9	3.6
29	EPN	41.208	0.25−5	0.9988	0.006	0.05	0.5	2
30	phosalone	44.015	0.1−2	0.9987	0.010	0.02	0.2	0.8
31	coumaphos	50.243	0.2−2	0.9937	0.005	0.02	0.2	1
32	dichlorvos	6.767	0.0025−0.5	0.9989	0.001	0.005	0.05	0.2
33	*acephate*	8.362	0.625−5	0.9976	0.006	0.05	0.5	2
34	methacrifos	9.656	0.025−0.5	0.9965	0.001	0.005	0.05	0.2
35	heptenophos	10.842	0.05−0.5	0.9991	0.001	0.005	0.05	0.2
36	demeton-*O*	11.715	0.125−2.5	0.9983	0.002	0.025	0.25	1
37	ethoprophos	12.099	0.025−0.5	0.9963	0.002	0.005	0.05	0.2
38	monocrotophos	12.497	0.8−8	0.9916	0.011	0.08	0.8	3.2
39	phorate	13.515	0.025−0.5	0.9971	0.001	0.005	0.05	0.2
40	demeton-*S*	14.129	0.125−2.5	0.9968	0.005	0.05	0.5	2
41	terbufos	15.832	0.1−2	0.9982	0.002	0.02	0.2	0.8
42	diazinon	16.627	0.05−1	0.9981	0.002	0.01	0.1	0.4
43	etrimfos	17.495	0.05−1	0.9971	0.003	0.01	0.1	0.4
44	parathion-methyl	18.651	0.05−1	0.9968	0.002	0.02	0.2	0.8
45	tolclofos-methyl	19.133	0.05−1	0.9962	0.002	0.01	0.1	0.4
46	pirimiphos-methyl	21.288	0.05−1	0.9961	0.003	0.01	0.1	0.4
47	malathion	21.669	0.05−1	0.9970	0.004	0.01	0.1	0.4
48	fenthion	21.973	0.15−3	0.9974	0.003	0.03	0.3	1.2
49	parathion-ethyl	22.243	0.05−1	0.9995	0.002	0.02	0.2	0.8
50	chlorpyrifos	22.414	0.05−1	0.9999	0.002	0.02	0.2	0.8
51	isofenphos-methyl	24.256	0.05−1	0.9980	0.004	0.01	0.1	0.4
52	vamidothion	25.867	0.1−1	0.9985	0.016	0.01	0.1	0.4
53	disulfoton sulfone	26.271	0.02−2	0.9952	0.005	0.04	0.4	1.6
54	quinalphos	27.023	0.1−2	0.9975	0.007	0.005	0.05	0.2
55	profenofos	28.745	1.0−10	0.9905	0.017	0.025	0.25	1
56	triazophos	35.395	0.1−2	0.9976	0.009	0.02	0.2	0.8
57	fenamiphos sulfoxide	37.669	0.1−22	0.998	0.006	0.02	0.2	0.8
58	fenamiphos sulfone	37.975	2.0−20	0.9993	0.012	0.4	4	16
59	azinphos-methyl	40.644	0.2−2	0.9976	0.006	0.02	0.2	2

[a]Samples labeled 1−31 belonged to group A; samples labeled 32−59 were detected by PFPD 2. [b]The spiked levels of eggs were 2.5 times the same levels of milk and fish.

dx.doi.org/10.1021/jf2043828 | J. Agric. Food Chem. 2012, 60, 1906−1913

Table 2. Results of the Recoveries (rec) of Blank Spiked Samples (n = 6)

	fish						milk						egg					
	low		intermediate		high		low		intermediate		high		low		intermediate		high	
compound	rec (%)	RSD (%)	rec (%)	RSD (%)	rec (%)	RSD (%)	rec (%)	RSD (%)	rec (%)	RSD (%)	rec (%)	RSD (%)	rec (%)	RSD (%)	rec (%)	RSD (%)	rec (%)	RSD (%)
methamidophos	79.9	9.3	62.5	13.3	77.1	14.3	67.4	18.6	85.4	20.0	71.7	17.4	88.3	7.0	60.4	28.6	80.4	7.6
phoxim	65.5	11.0	82.333	7.6	62.9	7.3	80.12	9.2	53.4	9.9	92.5	16.1	78.5	12.9	69.4	13.8	79.4	20.7
disulfoton sulfoxide	80.9	10.4	59.1	24.0	85.1	16.0	75.6	15.1	86.9	19.3	106	3.7	42.3	14.9	76.6	30.1	95	21.6
mevinphos	74.2	20.4	56.3	4.8	88.0	6.7	102.5	20.9	82.0	27.1	82	27.1	87.2	29.9	74.7	4.4	91	13.7
omethoate	85.5	5.9	77.7	6.2	54.7	6.6	61.9	8.9	93.5	14.3	83	16.1	71.1	5.6	66.1	11.7	66.6	22.5
demeton-*S*-methyl	87	8.2	65.7	4.8	105.9	7.3	82	18.1	96.3	16.4	102.8	14.2	137	12.8	104.6	17.1	88.1	11.6
cadusafos	74.9	3.4	87.5	6.9	87.5	6.9	73.8	17.0	60.3	27.0	60.3	27.0	70.5	22.8	49.9	4.9	79.8	12.9
dimethoate	75.7	7.5	64.4	5.4	80.8	8.6	66.6	7.9	91.8	6.5	72.6	16.5	68.2	7.0	72.1	66.7	86.5	8.3
disulfoton	67.3	11.3	51.7	4.2	75.3	7.2	84.8	14.1	80.3	26.7	80.3	26.7	85.4	31.5	71.5	5.3	87.7	15.7
iprobenfos	80.3	11.5	68	21.1	111.4	7.5	100.7	13.1	92.8	8.9	126.9	5.1	81.6	10.7	108.9	22.9	108.9	22.9
formothion	88.1	8.1	68.8	3.4	58	28.5	84.1	18.1	105.6	17.6	111.7	10	125	11.5	111.9	8.8	94	8
phosphamidon	65.7	9.0	53.4	3.2	62.2	19.9	53.3	24	97.9	21.7	86.5	9.9	104.1	9.3	94.5	16.8	92.8	8.5
chlorpyrifos-methyl	67.1	7.0	61.2	4.0	72.9	5.2	78	15.2	95.6	14.5	91.5	21.6	94	10.1	98.8	11.5	105.8	21.8
demeton-*S*-methylsulfone	56.5	4.5	57.5	24.9	56.5	19.7	78.5	10.2	88.0	11.6	73.2	10.4	76.1	11.0	101.9	15.4	71.0	25.4
fenitrothion	76.6	4	60.9	2.8	64.7	5.2	75.4	14.4	94.4	12.8	91.6	17.0	74.7	9.2	76.1	10	98.7	7.4
phorate sulfoxide	78.8	7.7	77.1	4.6	97.7	6.2	76.2	11.5	89.7	7.7	84.2	34.2	68.2	9.3	89.1	19.4	67	24.6
phorate sulfone	54.6	7.3	62.7	3.2	59	13.3	77.4	17.1	100.1	17.4	101.8	12.2	99.2	11	100.1	9.9	102	5.1
isocarbofos	65.4	22	60.9	12.7	80.9	12.7	73.5	21.9	71.2	24.1	71.2	24.1	73.4	12.3	54.7	13.8	75.9	11.1
trans-chlofenvinphos	65.3	7.2	61.5	4.5	77.7	8.4	75.4	12.1	92.2	9.0	85.1	23.7	62.8	8.2	85.5	22.3	81.9	10
cis-chlofenvinphos	69.1	4.9	57.2	3.0	56.3	12	79.6	15.4	97.6	15.7	93.5	19.7	81.5	9.8	96.7	14.5	115.6	8.1
mecarbam	64.8	16.5	52.2	23.4	104.6	7.2	74.2	11.9	79.4	7.4	116.9	8.9	51.9	7.2	71.2	19	84.3	7.3
methidathion	65.6	16.8	59.1	4.3	142.2	8.4	70.5	13.0	74.3	8.2	62.8	5.6	82.5	9.8	74.8	11.8	91.2	17.9
tetrachlorvinphos	78.1	29.8	64.1	2.7	138.1	7.3	36.8	8.0	49.1	25.5	49.4	2.3	77.7	21.0	55.4	5.4	77.6	6.4
fenamiphos	80.2	11.9	59	10.4	98.6	16.0	57.2	21.6	50.2	5.3	73.9	8.6	67.5	19	56.1	4.5	63	4.1
ethion	65.5	7.8	63.8	3.4	132	9.8	76.4	4.2	75.4	24.5	142.9	11.5	65.2	3.3	107.1	21.7	107.1	21.7
edifenphos	61.7	14.5	58.9	4.4	138.4	9.0	67.2	18.1	70.9	15	62.7	16.6	68.6	12	69.9	6.9	81.2	4.1
phosmet	79.7	6.4	58.2	7.3	81.4	5.7	88.7	15.1	65.1	29.2	65.1	29.2	81.5	27.5	68.6	6	65.2	23.9
pyridaphenthion	70.7	14.9	67.4	4.9	87.8	10.8	55.5	5.4	83.1	9.2	109.2	6.3	126.9	11.2	65.4	10.6	55.8	9.1
EPN	64.4	22.7	62.9	6.5	57.4	11.9	65.9	23.8	52.4	4.7	88.6	7.1	67.7	16.2	59.5	19.1	70.9	13.2
phosalone	97.5	8.0	66.7	10.6	57.9	3.4	63.6	16.1	50.2	5.9	59.8	18.6	59.9	18.1	56	7.0	50.3	17.2
coumaphos	83.1	17.0	58.3	14.7	86.6	27.5	62.5	14.9	47.5	7.3	79.7	22.5	63.7	17.1	74.2	18.3	55.5	3.6
dichlorvos	53.4	10	63.1	8.7	84.7	17.9	79.7	22.4	63.4	16.4	60.7	26.1	66.3	19.8	57	22.8	38.6	14.5
acephate	66.6	17.2	51.5	11.6	69.5	11.7	66.4	16.3	64.2	4.5	64.2	4.5	71.3	29.1	53.2	10.2	64.8	21.0
methacrifos	76.0	10.9	58.9	6.4	77.2	13.1	83.1	16.2	88.7	14.5	102.7	15.3	142.6	14.8	89.3	24.4	64.4	11.4
heptenophos	65.1	8.8	65.2	6.3	52.4	13.7	78.2	16.8	86.8	12.6	102.7	21	120.8	14.2	95.2	21.5	83.7	7
demeton-*O*	83.6	6.2	58.2	5.4	62.1	5.8	65.4	10.2	88.9	16.7	81	16.4	78.1	7.4	76.5	11	92.7	17.4
ethoprophos	71.1	10.5	52.2	5.1	67.6	13.0	55.8	21.2	86.1	17.2	83.2	17.8	120.6	10.8	89.8	23	67.1	8.1
monocrotophos	71.1	9.9	88.4	5.0	63.1	23.2	73.3	28.4	90.5	19.6	72.7	12.8	110.2	10	87.4	19	61.4	9.5
phorate	72.2	11	60	6.3	73.8	19.5	75.4	18.1	90.8	16.5	89.6	12.7	139.4	13.1	107.1	15.1	100.1	19
demeton-*S*	71.7	10.4	60.7	5.2	57.4	12.6	78.5	18.7	93.7	17.2	105.8	13.1	123.6	13.1	100.4	14.7	86.5	11.2

dx.doi.org/10.1021/jf2043828 |J. Agric. Food Chem. 2012, 60, 1906–1913

Table 2. continued

	fish						milk						egg					
	low		intermediate		high		low		intermediate		high		low		intermediate		high	
compound	rec (%)	RSD (%)	rec (%)	RSD (%)	rec (%)	RSD (%)	rec (%)	RSD (%)	rec (%)	RSD (%)	rec (%)	RSD (%)	rec (%)	RSD (%)	rec (%)	RSD (%)	rec (%)	RSD (%)
terbufos	74.2	9.6	57.4	4.3	62.1	18.4	80.5	20.4	100.1	19	105.1	11.7	135.1	12.6	105.1	13	78.9	6.9
diazinon	68.8	14.6	51.1	12.4	97.6	7	66.2	14.5	59.1	32.7	59.1	32.7	71	30.0	55.2	9.4	67.4	23.3
etrimfos	62.5	5.2	70.9	10.9	79.5	9.4	60.1	21.4	111.7	15.7	111.7	15.7	110.7	27.2	73.8	13.5	87	15
parathion-methyl	85.8	7.5	67.7	4.1	56.5	29.3	79.4	18.3	103.6	18.5	111.9	7.6	119.6	11.0	109.4	9.0	112.9	10.4
tolclofos-methyl	65.5	29.0	99.0	16.9	84.4	14.7	65.2	17.9	71.7	25.5	71.7	5.5	75.3	23.1	57.4	27.9	49.9	7.7
pirimiphos-methyl	50.9	8.9	72.5	5.0	100.3	6.3	71.3	12.1	72.1	7.7	59.2	4.6	60.6	8.5	67.1	12	81.1	3.9
malathion	74.3	7.5	67.3	3.2	63.9	4.1	75.2	14.1	95.2	16.7	92.7	15.5	80.6	9.6	91.8	13.2	99.7	12.2
fenthion	94.8	8.6	66.1	3.3	74	17.2	76.5	16.6	104.2	15.8	110.4	19.7	93	9.6	102.1	10.3	126.3	11.5
parathion-ethyl	73.7	9.0	70	4.6	89.2	10.9	69.3	22.5	61.9	3.3	56.8	6.4	95	11.7	67.1	12.9	83.5	11.1
chlorpyrifos	66.9	7.8	65.9	2.3	85.4	6.7	78.3	14.5	98.5	13.8	96.5	23.4	84.4	9.6	96.7	12.8	104.4	8.1
isofenphos-methyl	69.8	7.8	68.0	4.5	100.6	7.8	75.3	12	88.8	6	84.1	23.5	59.8	9.2	85.7	21.8	110.8	15.8
vamidothion	67	3.7	64.5	4.4	120.8	6.9	72.1	11.7	84.7	6.5	75.2	36.7	55.2	9.8	82.9	23.9	72.0	24.3
disulfoton sulfone	69	9.2	62.9	5.9	116.3	9.1	76.6	11.1	84.9	4.3	73.3	4.7	50.7	8.7	78	28.1	97.0	11.0
quinalphos	73.4	5.3	66.8	3.7	66.5	5.2	87.3	15.9	84.8	25.8	84.8	25.8	84.2	27.2	77.6	2.8	96.7	10.4
profenofos	89.1	9.1	60.8	7	90.8	9.1	68.6	16.7	65.8	16.7	61.7	14.1	62.2	11.8	68.5	8.5	55.5	11.7
triazophos	71.5	13.2	110.2	24.7	135.4	5.2	100.3	12.8	137.2	29.7	120	12.4	121.3	13.7	112.7	21.2	122.7	24.2
fenamiphos sulfoxide	65.1	25.1	69.1	7.9	94.7	21	65.5	27.7	58.2	8.3	54.5	8.0	56.7	14	60.5	9.3	80.3	7.1
fenamiphos sulfone	64.9	23.4	60.4	5.7	26.7	25.6	66.5	25.7	57.7	6.4	48.8	8.0	68.6	12	58.9	7.8	52.7	10.3
azinphos-methyl	88.0	10.6	67.7	11.5	58.3	25.8	65.1	16.8	77.9	9.0	72.3	6.6	66.6	23.8	59.9	17.1	84.2	9.8

dx.doi.org/10.1021/jf2043828 |J. Agric. Food Chem. 2012, 60, 1906–1913

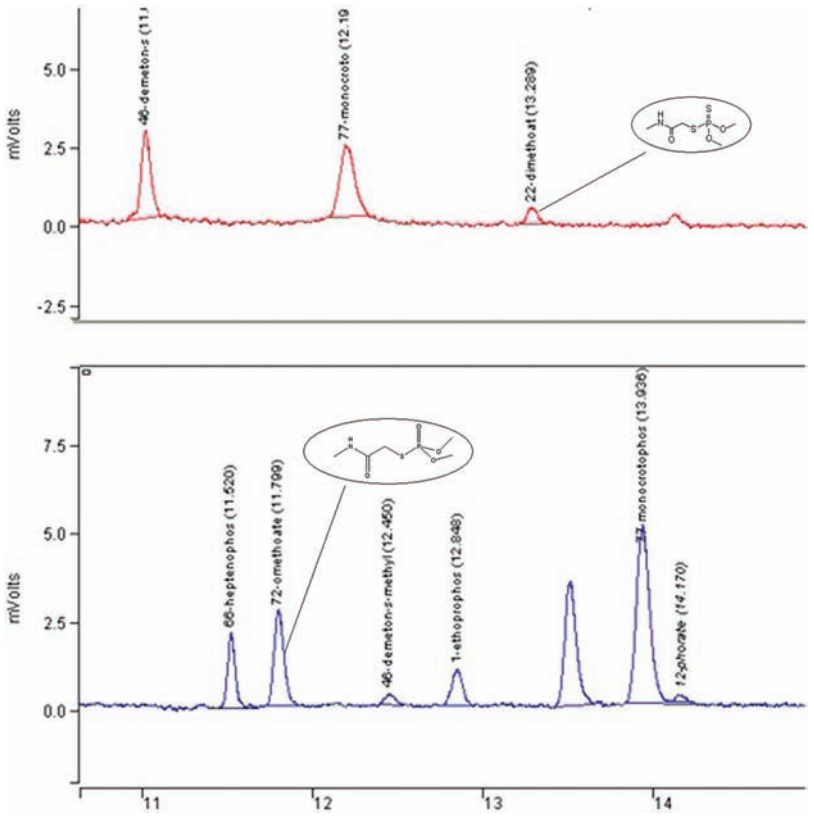

Figure 3. Positive fish sample chromatogram.

OPs were widely used in agriculture, and a large block of the pesticides might get into rivers, lakes, and other water bodies by precipitation. On the other hand, feed and water contamination may be indirect reasons for pesticide residues in milk. In addition, some pesticides, such as dichlorvos and ethoprophos, are used as veterinary medicine in the livestock industry, which also can cause residues in milk.

There were 18 OPs detected among the 59 species, 7 of which had been detected in milk samples; meanwhile, 17 species had been detected in fish samples. Methamidophos, dichlorvos, ethoprophos, and dimethoate were the most common contaminants in milk. At the same time, dichlorvos, ethoprophos, methacrifos, terbufos, heptenophos, and monocrotophos were the most common contaminants in fish. The levels of OPs in milk and fish ranged from 0.006 to 0.071 mg $kg^{-1}$and from 0.016 to 0.120 mg kg^{-1}; dimethoate and disulfoton sulfoxide residue levels were highest in milk and fish, respectively. It should be pointed that the residue level of fenamiphos sulfone (0.057 mg kg^{-1}) in a fish sample has surpassed the MRL (0.05 mg kg^{-1}) of CAC.

The method proposed for 59 OPs determination in milk, fish, and egg is based on GPC cleanup followed by dual gas chromatography−dual pulse flame photometric detection analysis. The method has proven to work at the parts per billion level required for the control of MRLs in animal products. It was demonstrated to have several advantages including high sensitivity, good selectivity, and specificity.

Overall, the detection rates of the eight most common contaminants were highest, but the pollution levels were low. The reason may be that 7 of them were compounds containing methoxy and their metabolic rate was high. The components containing ethoxy are relatively slowly hydrolyzed, so disulfoton sulfoxide and fenamiphos sulfone (in structure) are the contaminants with highest levels. The results indicated that no sample in the present study showed high levels of OPs contamination and the majority of samples in this study were safe. However, due to the accumulation function, it might result in a higher exposure of disulfoton sulfoxide and fenamiphos sulfone OPs for some individuals.

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Funding

We gratefully acknowledge financial support from the Science Research Foundation of Ministry of Health of the People's Republic of China (Grant 200902009).

Notes

The authors declare no competing financial interest.

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