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Multiresidue Determination of Pesticide in Fishery Products by A Tandem Solid-Phase Extraction Technique

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ABSTRACT

In order to simplify the survey of pesticide (including carbamate, organochlorine, organophosphate and synthetic pyrethroid pesticides) residues in fishery products (including bivalve, crustacean, fish and cuttlefish), multiresidue determination methods were developed through a solid phase extraction (SPE) technique. In the present procedures, samples were extracted with acetonitrile except for bivalve and cuttlefish samples. Mixed solvent of water, acetone and acetonitrile was added to bivalve prior to homogenization, whereas water was needed in cuttlefish samples for blending. For both kinds of sample, an additional procedure of salting-out was needed during extraction. Tandem SPE cartridges of C18 and aminopropyl, using acetonitrile as the only solvent, were used to clean up extracts from either method. A total of 91 pesticides in four major pesticide groups were tested in this study. Gas chromatography (GC, equipped with electron capture detector and flame photometric detector) and high-performance liquid-chromatography (HPLC) equipped with fluorescence detector were used for analysis. The validation of the method was evaluated for each fishery product using samples spiked with all pesticide standards at three concentration levels. The results indicated percentage of recovery ranged from 60% - 120% and coefficients of variation < 20% for all but 10 of the pesticides analyzed (including 1-naphthol, 3-hydroxy carbofuran, aldicarb sulfoxide, heptachlor, trifuralin, acephate, dichlorvs, methamidophos, monocrotophos and omethoate). Residue extraction techniques described in this report are rapid and suitable for screening of pesticide residues in monitoring programs.

Key words: fishery product, pesticide, multiresidues

INTRODUCTION

Kaphalia *et al*. (1990) reported that the majority of people were indirect consumers of pesticides through food intake[(2)]. As the concern for pesticide residues in food grew, the public demanded better reassurance of chemical safety of the market food.

Pesticides used in agricultural activities can be classified into four major groups, which are organochlorine (OC), organophosphate (OP), carbamate and synthetic pyrethroids pesticides. Most OC pesticides were banned in 1970s for their long persistence in the environment[(3,4)]. Today, OC pesticide levels are still detectable in fish from various waterways[(5-8)]. Although not as persistent in the environment as OC pesticides, many pesticides in the other three groups have been identified as possible endocrine disrupters[(9-16)]. Even though most of such detrimental effects have only been observed *in vitro* and have not been verified *in vivo*, it has been receiving growing attention from the public. Therefore, it is important to assess the existence and amount of pesticide residues in fishery products.

Lipid and water contents are different among fishes[(17)]. Even for the same fish species, the lipid content could vary due to seasonal or physiological changes[(18)]. Besides, pesticides may differ in their hydrophilic or hydrophobic characteristics. These characteristics present difficulties in the extraction and clean-up of pesticide residues from different matrices. For the routine inspection of pesticide residues in fishery products, a reliable, effective, accurate, user-friendly and cost-effective method requiring minimal amounts of organic solvents is needed. In the past decade, supercritical fluid extraction, microwave-assisted extraction and pressurized liquid extraction (PLE) have been searched for meeting these requirements[(19-21)]. PLE is a relatively new technique for the extraction of OC pesticides from animal matrices. According to the results of Suchan *et al*. (2004), this method extracts fish samples quickly (5-10 min) and with much less solvent than conventional techniques such as Soxhlet extraction[(22)]. They also pointed out that the limitation of PLE application in analyzed fish samples is the maximum amount of sample that could be placed thimble. This might be drawback in case of sample with very low level of target analytes. Extraction procedure is one of the key points of residue analysis. More studies are needed for the utility of the other above-mentioned techniques in extraction of fish samples. The extraction procedure should be able to extract the compounds of

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Table 1. Ninety-one pesticides used in the study and their grouping for multi-residue determination

Type/grouping	Pesticide and its metabolite[a]
Carbamate: 20	
Group 1:	Aldicarb (3); Aldlicarb sulfoxide (3); Carbaryl (3); Carbofuran (3); Carbofuran-3-hydroxy (1); Fenobucarb (1); Isoprocarb (1); Metolcarb (3); Oxamyl (1); Thiodicarb (1);
Group 2:	1-Naphthol (3); Aldicarb sulfone (3); Bendiocarb (1); Butocarboxim (3); Carbofuran-3-keto (1); Macbal (1); Methiocarb (3); Methomyl (3); Promecarb (3); Propoxur (1).
Organochlorine and nitrogen-containing pesticides: 14	
Group 3:	Alachlor (1); Aldrin (1); Chlorobenzilate (1); *p,p'*-DDE (1); *p,p'*-DDT (1); Dieldrin (1); Endosulfan (3); Endosulfan sulfate (3); Endrin (1); Heptachlor (3); Heptachlor epoxide (3); Lindane (1); Methoxychlor (3); Trifluralin (1).
Organophosphate: 41	
Group 4:	Bromophos-methyl (3); Chlopyriphos (3); Cyanofenphos (3); Dyfoxon (1); EPN (1); Ethion (1); Ethoprophos (1); Fensulfothion (3); Isoxathion (1); Parathion (3); Parathion-methyl (3); Phorate (3); Phosalone (1); Profenofos (1); Prothiofos (3); Tokuoxon (3);
Group 5:	Carbophenothion (3); Diazinon (3); Dimethoate (3); Fenthion (3); Malathion (3); Mephosfolan (1); Methidathion (3); Phenthoate (1); Phosmet (3); Pirimiphos-methyl (3); Pyraclofos (1); Pyridaphenthion (1); Quinalphos (3); Triazophos (3);
Group 6:	Acephate (3); Bromophos-ethyl (3); Demeton-S-methyl (3); Dichlorvos (3); Fenitrothion (3); Fonofos (3); Methamidophos (3); Mevinphos (3); Monocrotophos (3); Omethoate (3); Terbufos (1).
Synthetic pyrethroids: 16	
Group 7:	Allethrin (3); Bifenthrin (3); Fenpropathrin (1); Flucythrinate (1); Tetramethrin (1);
Group 8:	Cyhalothrin (1); alpha-Cypermethrin (1); beta-Cyfluthrin (1); Esfenvalerate (1); Tralomrthrin (2);
Group 9:	Cyfluthrin (1); Cypermethrin (3); Deltamethrin (3); Fenvalerate (3); Fluvalinate (1); Permethrin (3).

[a]Pesticide standards were supplied by: (1) Dr. Ehrenstorfer GmbH, (2) Merck, (3) Riedel-deHaen.

interest from test samples with minimal matrix interference. A clean-up step is needed for some method to remove co-extracted matrix interference.

Solid phase extraction (SPE) has been used as a reliable clean-up method for the analysis of pesticides and environmental pollutants in aquatic organisms[23-25]. We have developed a SPE method for OCs that simplifies the clean-up procedures of OC pesticide residues in fish with different lipid content[26]. In this study, we intended to develop an analytical procedure applicable in multiresidue determination of pesticides in fishery products. The application of the tandem SPE method to determine multiple classes of pesticide residues from a wider range of fishery products (including fish, shellfish and cephalopod) was described in this paper. Performance of the method was evaluated with fortified samples. The purpose of this study was to develop rapid and suitable techniques of sample preparation for enhancement screening of pesticide by multiresidues in monitoring programs to protect the health of consumers.

MATERIALS AND METHODS

I. *Pesticide Standard*

The pesticide standards tested in this study are listed in Table 1. A total of 91 pesticides from four major groups were tested. The pesticides were purchased from Dr. Ehrenstorfer GmbH, Merck or Riedel-deHaen, separately. The purity of all pesticide standards were greater than 95% except for cyfluthrin (94.50%), demeton-S-methyl (94%), permethrin (92%), phenthoate (92%), phorate (88%), pyraclofos (91.6%), tokuoxon (91%), tralomrthrin (93.50%) and triazophos (70%).

II. *Preparation of Stock Solutions of Standard Pesticides*

Ninety-one pesticides analyzed in this study were divided to nine groups as listed in Table 1. The grouping was determined by the chromatographs of the pesticides using gas chromatography (GC) and high performance liquid chromatography (HPLC). The stock solutions for carbamate and OP pesticides were prepared separately by dissolving pure pesticide standards in acetonitrile. The stock solutions of OC and synthetic pyrethroid pesticides were prepared with *n*-hexane. The stock solutions were mixed well and then serially diluted with acetonitrile or *n*-hexane depending on the pesticide type to the appropriate concentrations in mg/L. For each pesticide, there were 3 concentrations with 1/2 serial dilution added to fishery products.

III. *Materials*

All reagents were analytical grade unless otherwise mentioned. Acetone, acetonitrile (isocratic grade for chromatography), anhydrous sodium magnesium, methanol (gradient grade for chromatography) and *n*-hexane (for organic trace analysis) were purchased from Merck. Post column derivative reagents, including *o*-phthaladehyde (chromatographic grade, Part. No. O120), *o*-phthalaldehyde diluent (chromatographic grade, Cat. No. CB910) and thiofluor (chromatographic grade, Cat. No. 3700-2000), were purchased from Pickering Laboratories. Two types of

solid phase extraction cartridge, C18 cartridge (6 mL, 1000 mg) and amino propyl cartridge (6 mL, 1000 mg), were purchased from J&T Baker and Merck, respectively. DB-608 capillary column (0.53 mm i.d. × 30 m, 0.83 μm) was purchased from J & W. Carbamate analysis column (C18 4.6 × 250 mm column, 5 μm) was purchased from Pickering Laboratories (Part. No. 1846250 & 1700-0063).

IV. *Instrument*

The GC (HP Model 6890) system consisted of ^{63}Ni electron capture detector (ECD) and flame photometric detector (FPD). The HPLC (Agilent 1100 series) system consisted of post-column derivatizer (Pickering Laboratories PCX 5200) and fluorescence detector (HP1100 Series). The other apparatuses include Homogenizer (Kinematica, polytron®), solid phase extraction vacuum manifold and accessories device (J&W Scientific, SPE), rotary vacuum evaporator (Heidolph model VV2011), aspirator (EYELA A-3S; HETO SUE 300Q), refrigerated bath circulator (YIH DER BL-710) and nitrogen gas generator (NITROX, UHP0551).

V. *Instrument Condition*

For OC, OP and pyrethroids pesticides analysis, the instrument conditions were the same as the method of Sun *et al.*'s method[27]. For carbamate pesticides analysis, HPLC was used. The injection volume was 50 μL. Mobile phase combined water (solvent a) with acetonitrile (solvent b) and run with linear gradient, which a/b = 80/20 (v/v) at time 0 to a/b = 30/70 at 35 min and then equilibrated at initial conditions for 3 min, its flow-rate was 1 mL/min. Analytical column temperature was 40°C. Catalytic reactor temperature was ca 100°C. OPA-reagent flow-rate of derivatization was 0.3 mL/min. Excitation wavelength and emission wavelength of detection were 330 nm and 465 nm, respectively.

VI. *Sample Selection and Pretreatment*

The major Taiwanese fishery products with different biological characteristics were selected as target samples for method development. Common carp, pollack and sea perch were selected for varying lipid content of fish. Clam and oyster (indicator of bivalve) were selected for different salt and water contents. Crab and shrimp were selected as indicator of crustacean and cuttlefish was the indicator of cephalopod. Sample was processed according to the Pesticide Analytic Manual[17].

VII. *Extraction and Cleanup Procedure of Pesticides from Fishery Products*

Method 1. The method based on a report of Sun *et al.* was followed[26].

Method 2. Modification of method 1. An amino propyl cartridge was taken the place of florisil cartridge and connected with C18. The procedure is listed in Figure 1 (only Step 1 to Step 11). Fish and crustacean (crab and shrimp) samples were analyzed by this method.

Method 3. Modification of method 2. Step A was added (Figure 1). Bivalve (calm and oyster) samples were analyzed by this method.

Method 4. Modification of method 2. Step B was added (Figure 1). Cephalopod (cuttlefish) samples were analyzed using this method.

VIII. *Method Validation*

The validity of the method was assessed by the recovery of pesticide residues from the fortified samples. Five replicates were run for each concentration level. The three average recoveries at different concentrations were used to calculate the mean recovery and inter-assay repeatability (expressed as the CV of the averages) for each pesticide. The method was judged to be invalid for a pesticide when the mean recovery fell outside the range of 60% and 120% or when the inter-assay repeatability was greater than 20%.

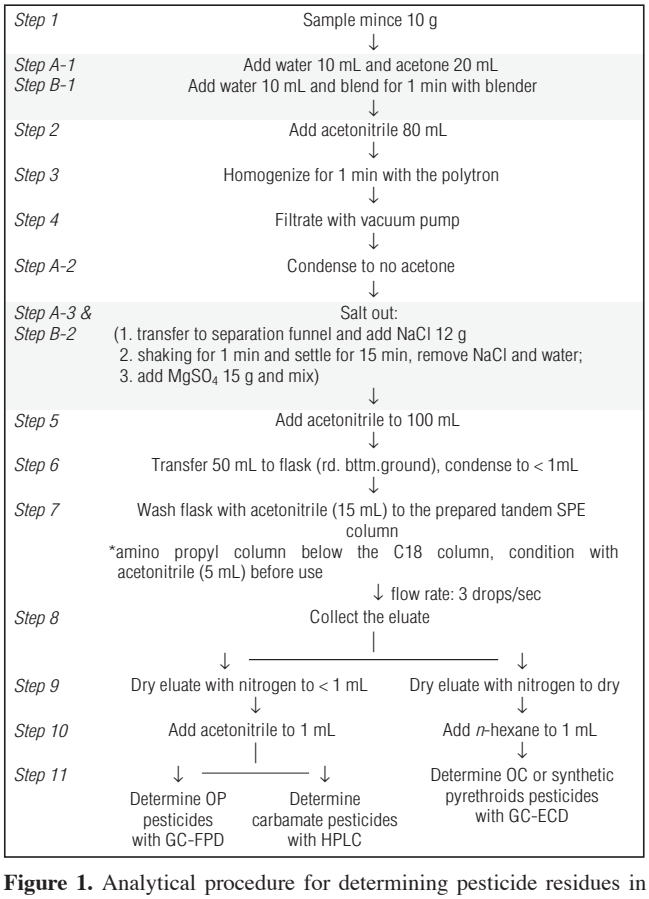

Figure 1. Analytical procedure for determining pesticide residues in fishery products. Steps 1 to 11: for all fishery products; Step A: additional procedure for bivalve; Step B: additional procedure for cephalopod.

RESULTS AND DISCUSSION

I. *Method Development*

In the beginning of the study, the analytical procedure developed by Sun *et al.*[26] was conducted. In this method, acetonitrile was the only solvent used and the extracts were cleaned by a tandem SPE column of C18 and florisil.

The analytical procedures for crab samples were as simple as that by Sun *et al.*[26]. A total of 91 pesticides belonging to four pesticide groups were spiked separately. The pesticides which recoveries were < 60% or > 120% are shown in Table 2, which shows there are four pesticides which recoveries were higher than 120%. Zero recovery was obtained from samples fortified with acephate, dichlorvs, methamidophos, monocrotophos, omethoate, and pirimiphos-methyl. Florisil used in the tandem SPE would remove pigments from samples. It would also adsorb some of the polar pesticides. Amino propyl cartridge was suggested to replace florisil for moderately polar surface

Table 2. Effect on recoveries of pesticide standards from spiked crab samples cleaned-up with different tandem cartridges

Pesticide	Max. level spiked (mg/L)	Recovery (%) of used cartridge[a]	
		C18 + florisil	C18 + amino propyl
Acephate	5	0.0	118.2 (2.6)
Dichlorvs	5	0.0	82.5 (7.8)
Endosulfan sulfan	0.1	146.1 (40.4)[b]	89.1 (5.4)
Malathion	0.5	8.3 (173.2)	79.3 (4.3)
Memeton-methyl	10	62.7 (11.2)	92.1 (3.4)
Methamidophos	3	0.0	111.5 (2.5)
Methoxchlor	0.1	163.2 (3.5)	81.4 (3.7)
Mevinphos	5	63.1 (12.8)	97.1 (4.3)
Monocrotophos	5	0.0	125.3 (19.3)
Omethoate	10	0.0	139.5 (14.8)
p,p' DDT	0.1	125.6 (2.1)	76.8 (2.8)
Pirimiphos-methyl	0.25	0.0	69.5 (5.8)
Tralomrthrin	0.5	135.1 (19.9)	79.6 (7.9)

[a]Mean of the average recoveries at three spiked levels, max. level, 1/4 of max. level, and 1/16 of max. level.

[b]Number in parenthesis is the coefficient of variation (CV, %) of the three average recoveries at three spiked levels.

Table 3. Recoveries of pesticide from samples of various fisheries products spiked with standard using the analytical procedures developed in this study

Number of pesticide	Max. level spiked (mg/L)	Recovery (%)[a]							
		Bivalve		Crustacean		Fish			Cephalopod
		Clam	Oyster	Crab	Shrimp	High fat	Moderate fat	Low fat	
Carbamate									
1 *1-Naphthol*	0.25	120 (12)[b]	19 (81)	95 (5)	101 (4)	22 (142)	24 (142)	34 (141)	88 (12)
2 *3-Hydroxy carbofuran*	0.15	90 (3)	93 (5)	100 (8)	INT[c]	96 (8)	93 (8)	87 (10)	85 (7)
3 3-Keto carbofuran	0.375	82 (19)	90 (3)	84 (19)	92 (5)	96 (7)	96 (8)	73 (15)	81 (2)
4 Aldicarb	0.15	82 (5)	80 (5)	87 (2)	104 (14)	93 (5)	95 (5)	91 (7)	77 (5)
5 Aldicarb sulfone	0.2	90 (8)	90 (4)	97 (3)	90 (5)	84 (12)	97 (12)	95 (6)	84 (7)
6 *Aldlicarb sulfoxide*	0.5	89 (5)	88 (12)	103 (5)	94 (3)	2 (178)	3 (204)	24 (57)	80 (4)
7 Bendicarb	0.1	89 (8)	92 (7)	85 (5)	91 (5)	99 (12)	98 (17)	74 (20)	85 (4)
8 Butocarboxim	0.75	79 (9)	81 (6)	91 (3)	87 (6)	93 (9)	91 (9)	93 (5)	79 (3)
9 Carbaryl	0.075	91 (4)	90 (7)	91 (5)	94 (2)	97 (4)	100 (7)	85 (11)	83 (3)
10 Carbofuran	0.2	89 (4)	90 (8)	88 (6)	93 (1)	95 (4)	96 (6)	87 (9)	82 (3)
11 Fenobucarb	0.125	89 (5)	90 (6)	84 (4)	103 (17)	91 (6)	103 (14)	101 (15)	82 (9)
12 Isoprocarb	0.075	86 (4)	90 (4)	86 (4)	92 (5)	90 (3)	93 (9)	91 (6)	77 (5)
13 Macbal	0.2	84 (7)	88 (4)	86 (3)	88 (4)	90 (7)	93 (8)	88 (7)	78 (3)
14 Methiocarb	0.125	88 (8)	93 (7)	81 (3)	97 (7)	102 (6)	100 (10)	82 (13)	82 (3)
15 Methomyl	0.1	90 (7)	91 (3)	96 (4)	99 (10)	88 (13)	85 (10)	88 (6)	82 (2)
16 Metolcarb	0.1	84 (3)	89 (5)	87 (4)	86 (7)	87 (7)	88 (6)	95 (16)	78 (7)
17 Oxamyl	0.25	90 (13)	91 (6)	97 (4)	96 (3)	80 (16)	86 (6)	87 (9)	80 (12)
18 Promecarb	0.125	86 (6)	94 (6)	77 (3)	86 (6)	97 (9)	94 (9)	90 (5)	80 (3)
19 Propoxur	0.125	87 (6)	90 (5)	88 (4)	89 (5)	94 (8)	94 (8)	89 (5)	80 (6)
20 Thiodicarb	0.25	91 (7)	85 (10)	101 (6)	97 (4)	83 (10)	85 (9)	84 (18)	82 (5)
Organochlorine									
1 Alachor	0.625	85 (5)	82 (4)	74 (7)	74 (9)	103 (7)	102 (4)	97 (7)	85 (6)
2 Aldrin	0.125	61 (9)	80 (5)	62 (8)	63 (8)	104 (10)	87 (5)	71 (8)	63 (8)
3 Chlorobenzilate	1.25	92 (6)	102 (4)	83 (10)	83 (9)	96 (4)	93 (4)	87 (9)	94 (5)
4 Dieldrin	0.25	82 (6)	86 (3)	70 (3)	70 (4)	95 (3)	93 (5)	90 (7)	84 (7)
5 α-Endosulfan	0.25	80 (6)	88 (2)	70 (4)	69 (5)	96 (4)	97 (4)	103 (9)	79 (3)
6 β-Endosulfan	0.25	85 (5)	85 (6)	72 (6)	73 (6)	96 (6)	112 (7)	63 (10)	86 (4)
7 Endosulfan sulfan	0.25	100 (11)	95 (5)	91 (11)	98 (12)	86 (13)	82 (12)	79 (18)	74 (10)
8 Endrin	0.25	85 (6)	91 (3)	77 (4)	76 (4)	91 (6)	97 (5)	97 (8)	84 (4)
9 Hepta epoxide	0.125	77 (9)	86 (2)	69 (3)	69 (4)	99 (4)	90 (4)	91 (5)	81 (6)
10 *Heptachlor*	0.125	53 (17)	76 (5)	61 (5)	60 (5)	97 (9)	105 (8)	120 (9)	68 (12)
11 Lindan	0.125	73 (7)	81 (5)	61 (13)	65 (5)	112 (12)	91 (4)	82 (9)	72 (6)
12 Methoxchlor	0.25	92 (6)	110 (10)	86 (6)	87 (6)	91 (18)	64 (14)	62 (9)	94 (8)
13 p,p' DDE	0.25	88 (7)	92 (4)	80 (4)	82 (4)	95 (5)	94 (4)	97 (10)	83 (3)
14 p,p' DDT	0.25	88 (8)	100 (6)	79 (5)	80 (5)	108 (10)	62 (4)	83 (13)	87 (5)
15 *Trifuralin*	0.25	73 (10)	141 (30)	70 (7)	69 (8)	111 (18)	91 (4)	82 (13)	77 (16)

Table 3. (Continued)

Number of pesticide	Max. level spiked (mg/L)	Recovery (%)[a]							
		Bivalve		Crustacean		Fish			Cephalopod
		Clam	Oyster	Crab	Shrimp	High fat	Moderate fat	Low fat	
Organophosphate									
1 *Acephate*	0.125	278 (40)	101 (8)	126 (5)	108 (5)	0	0	88 (3)	171 (14)
2 Bromophos-ethyl	0.125	98 (5)	97 (4)	77 (9)	92 (4)	94 (8)	98 (11)	94 (10)	81 (4)
3 Bromophos-methyl	0.25	91 (7)	87 (2)	80 (8)	93 (5)	93 (8)	98 (9)	92 (10)	85 (9)
4 Carbophenothion	0.125	95 (7)	83 (8)	77 (12)	98 (9)	101 (9)	100 (6)	109 (13)	89 (14)
5 Chlopyriphos	0.25	98 (15)	91 (7)	75 (6)	97 (5)	96 (5)	106 (15)	101 (11)	94 (15)
6 Cynofenphos	0.25	93 (6)	94 (10)	85 (8)	103 (6)	103 (8)	96 (3)	102 (7)	85 (3)
7 Diazinon	0.125	92 (8)	97 (6)	79 (9)	91 (6)	94 (5)	91 (7)	98 (9)	83 (8)
8 *Dichlorvs*	0.125	77 (6)	88 (19)	77 (3)	46 (9)	55 (26)	47 (22)	37 (33)	67 (4)
9 Dimethoate	0.125	118 (18)	82 (4)	105 (8)	104 (8)	98 (6)	97 (7)	97 (5)	91 (9)
10 Dyfoxon	0.125	96 (18)	101 (16)	96 (4)	79 (5)	61 (9)	67 (7)	63 (19)	77 (9)
11 EPN	0.25	90 (8)	90 (9)	83 (5)	103 (6)	109 (6)	106 (11)	104 (12)	87 (8)
12 Ethion	0.125	99 (12)	94 (12)	78 (9)	102 (6)	119 (14)	106 (12)	88 (10)	88 (3)
13 Ethoprophos	0.125	89 (11)	95 (13)	83 (5)	91 (5)	99 (6)	91 (12)	96 (10)	77 (2)
14 Fenitrothion	0.125	96 (5)	94 (6)	93 (5)	97 (4)	108 (14)	110 (15)	104 (8)	80 (4)
15 Fensulfothion	0.125	96 (7)	95 (4)	112 (8)	100 (6)	119 (5)	101 (7)	108 (10)	90 (2)
16 Fenthion	0.125	94 (13)	91 (6)	84 (6)	96 (5)	103 (6)	99 (8)	102 (17)	89 (12)
17 Fonofos	0.125	87 (7)	92 (11)	76 (7)	88 (14)	94 (18)	84 (8)	91 (9)	77 (7)
18 Isoxthion	0.25	106 (7)	108 (6)	96 (8)	113 (7)	107 (11)	108 (6)	111 (8)	98 (8)
19 Malathion	0.25	83 (20)	86 (10)	81 (9)	93 (4)	108 (18)	94 (9)	100 (13)	89 (10)
20 Memeton-methyl	0.25	78 (8)	78 (19)	99 (6)	100 (7)	82 (16)	92 (8)	91 (9)	80 (7)
21 Mephosfolan	0.125	101 (11)	87 (3)	105 (8)	105 (7)	100 (10)	102 (14)	97 (9)	89 (5)
22 *Methamidophos*	0.075	104 (10)	89 (9)	118 (7)	107 (5)	0	0	88 (15)	90 (6)
23 Methidathion	0.125	104 (6)	84 (8)	96 (12)	104 (4)	93 (11)	99 (15)	90 (9)	91 (10)
24 Mevinphos	0.125	90 (7)	93 (5)	95 (7)	88 (14)	66 (14)	65 (11)	80 (12)	80 (5)
25 *Monocrotophos*	0.125	117 (7)	101 (3)	169 (29)	145 (17)	71 (16)	101 (19)	99 (13)	91 (8)
26 *Omethoate*	0.25	118 (8)	90 (14)	140 (18)	135 (17)	0	0	49 (40)	96 (20)
27 Parathion	0.125	89 (8)	91 (10)	84 (6)	96 (5)	97 (5)	104 (12)	110 (10)	89 (12)
28 Parathion-methyl	0.25	93 (6)	94 (9)	92 (4)	99 (5)	95 (11)	100 (7)	95 (9)	84 (6)
29 Phenthoate	0.25	94 (9)	105 (9)	74 (16)	90 (3)	102 (4)	94 (5)	107 (16)	92 (6)
30 Phorate	0.125	72 (7)	93 (16)	77 (6)	93 (7)	94 (3)	92 (4)	93 (14)	89 (16)
31 Phosalone	0.25	106 (6)	104 (7)	109 (17)	101 (6)	100 (8)	106 (7)	100 (8)	95 (7)
32 Phosmet	0.25	108 (8)	93 (4)	103 (17)	97 (5)	108 (4)	109 (7)	103 (9)	99 (17)
33 Pirimiphos-methyl	0.125	81 (13)	84 (7)	73 (13)	90 (3)	97 (6)	89 (6)	97 (12)	87 (12)
34 Profenophos	0.25	89 (7)	98 (8)	86 (6)	95 (5)	92 (9)	94 (10)	90 (8)	89 (8)
35 Prothiofos	0.25	91 (8)	86 (10)	91 (33)	92 (5)	95 (12)	90 (7)	96 (14)	82 (5)
36 Pyidaphenthion	0.25	98 (5)	98 (5)	102 (12)	102 (9)	103 (6)	116 (19)	107 (11)	95 (13)
37 Pyraclofos	0.5	106 (4)	95 (3)	99 (11)	108 (7)	111 (5)	116 (11)	103 (5)	95 (6)
38 Quinalphos	0.125	94 (6)	117 (10)	82 (15)	93 (4)	95 (14)	100 (10)	97 (5)	83 (5)
39 Terbufos	0.25	89 (5)	85 (12)	70 (3)	84 (6)	91 (7)	86 (5)	92 (8)	74 (8)
40 Tokuoxon	0.25	90 (9)	86 (8)	84 (7)	95 (5)	99 (9)	94 (5)	90 (9)	88 (3)
41 Triazophos	0.125	98 (6)	115 (7)	93 (9)	106 (8)	97 (13)	104 (10)	103 (8)	90 (5)
Synthetic pyrethroids									
1 Allethrin	0.5	106 (11)	99 (12)	95 (14)	99 (7)	120 (11)	113 (5)	95 (10)	87 (4)
2 Alphamethrin	1	80 (13)	77 (7)	76 (8)	90 (4)	107 (8)	117 (7)	62 (10)	96 (13)
3 Betacyfluthrin	1	92 (5)	83 (7)	81 (8)	95 (3)	112 (12)	117 (7)	62 (15)	78 (11)
4 Bifenthrin	0.5	88 (4)	86 (9)	87 (16)	79 (5)	76 (7)	79 (5)	109 (10)	79 (6)
5 Cyfluthrin	1	109 (8)	84 (10)	91 (8)	89 (2)	109 (6)	117 (14)	116 (8)	97 (8)
6 Cyhalothrin	0.5	89 (5)	80 (5)	81 (9)	92 (3)	115 (15)	118 (10)	74 (11)	93 (11)
7 Cypermethrin	1	91 (7)	83 (8)	87 (3)	92 (3)	112 (7)	101 (5)	101 (6)	92 (6)
8 Deltamethrin	1	92 (14)	97 (15)	86 (5)	89 (3)	117 (14)	115 (9)	87 (4)	99 (10)
9 Esfenvalerate	1	93 (5)	88 (6)	81 (7)	94 (3)	103 (7)	116 (7)	63 (7)	84 (7)
10 Fenpropathrin	0.5	93 (3)	86 (7)	100 (13)	97 (3)	105 (7)	114 (9)	114 (9)	106 (17)
11 Fenvalerate	1	89 (12)	86 (13)	86 (4)	88 (3)	114 (8)	112 (12)	79 (10)	114 (12)
12 Flucythrinate	1	118 (12)	115 (18)	105 (12)	111 (8)	110 (5)	116 (3)	116 (9)	101 (4)
13 Fluvalinate	1	72 (8)	85 (9)	86 (3)	87 (3)	108 (5)	102 (4)	95 (4)	90 (6)
14 Permethrin	5	86 (6)	85 (7)	86 (5)	91 (3)	102 (4)	106 (4)	98 (5)	91 (5)
15 Tetramethrin	1	97 (8)	82 (8)	104 (16)	94 (17)	110 (5)	113 (6)	109 (8)	75 (11)
16 Tralomrthrin	1	95 (6)	94 (9)	85 (9)	98 (4)	119 (9)	117 (7)	64 (9)	109 (13)

[a]Mean of the recoveries at three spiked levels, max. level, 1/4 of max. level, and 1/16 of max. level.
[b]Number in parenthesis is the coefficient of variation (CV, %) of five average recoveries at three spiked levels.
[c]INT: matrix interference identified.

character[28]. Such modification in tandem cartridges improved the pesticide recoveries of fortified samples (recovery rate ranged from 69.5% to 139.5%, Table 2). A brief description of the analytic procedure is shown in Figure 1 (only Step 1 to Step 11 were necessary).

To analyze bivalve samples (clam and oyster), sample was combined with acetonitrile and then homogenize with the polytron, firstly. The mixture of sample and the solvent was too sticky to be homogenized, probably due to high water and salt contents of bivalves. Therefore, modification of the polarity and salinity of the mixture was required. For this purpose, water and acetone were added to the sample along with acetonitrile prior to homogenization. After homogenization, an additional procedure of salting-out was needed in order to enhance the extraction of organic components from water layer. A brief description of the extraction procedure for pesticide residues analysis in bivalve is shown in Figure 1 (including Step A).

Different from bivalve samples, mantle muscle of cephalopod was too hard to be minced. Adding water and blending the muscle pellets were required in sample preparation. The same salting-out procedure just described was also necessary (Figure 1, including procedure of Step B) for extraction.

II. *Method Validation*

The performance of the method was evaluated for different fishery products by samples fortified with grouped pesticides at three concentration levels. The results indicated that the method was valid for most pesticides analyzed in this study. However, there were ten pesticides, 1-naphthol, 3-hydroxy carbofuran, aldicarb sulfoxide, heptachlor, trifuralin, acephate, dichlorvs, methamidophos, monocrotophos and omethoate, that gave poor results in validation evaluation (i.e., recovery rate <60% or >120%, or CV >20%) (Table 3). Unexpectedly high recoveries were found for some pesticides in certain samples (e.g. trifuralin in oyster, acephage in clam, crab and cuttlefish, monocrotophos in crab and shrimp, and omethoate in crab and shrimp). The recoveries of 1-naphthol, aldicarb sulfoxide and omethoate from fish were lower than the acceptable level irrespective of their lipid content. It was indicated that lipid content was not the major factor influencing the performance of the analysis. Acephate and its metabolite methamidophos were not detected from fish samples with high and moderate lipid content. The HPLC chromatogram of shrimp control sample showed a peak appearing at the same retention time as 3-hydroxy carbofuran that could interfere with the analysis (Figure 2).

The method detection limits of the four kinds of pesticides were determined at signal-to-noise ration (S/N) of 3 under the developed method by fortified the lowest concentration, which were 1/16 of maximum concentration (same as Table 3), of standard pesticide to sample. Figure 3 shows that the method detection limit (MDL) for bivalve, crustacean and cephalopod were below 5 ng/g for analyzing the most pesticides, and below 10 ng/g for fish of organophosphate and synthetic pyrethroids pesticides. The MDL for fish of carbamate and organochlorine pesticides were 5-34 and 4-100 ng/g, respectively.

CONCLUSIONS

The accurate determination of pesticide residues in fishery products is important in safeguarding the public health and monitoring environmental pollution. Many methods have been reported for the detection of pesticides residues in fish or other seafood[25,26,29-34]. Manipulation of the partition of fatty matrix and pesticide in solvent is always an important concern. According to the AOAC method the OC in high-fat samples is extracted and partitioned with acetonitrile and petroleum ether followed by a florisil column to remove residual oil[35]. These procedures are time consuming[36]. Schenck and Schenck *et al.* suggested determination of OC pesticide residues in fatty fish and nonfatty fish with different solid-phase extraction cleanup procedure[31,32]. Their methods give good cleanup efficiency, as well as save time and solvent. However, the suitability of applying these methods to fish depends on the fat content and gives low recoveries for some pesticides.

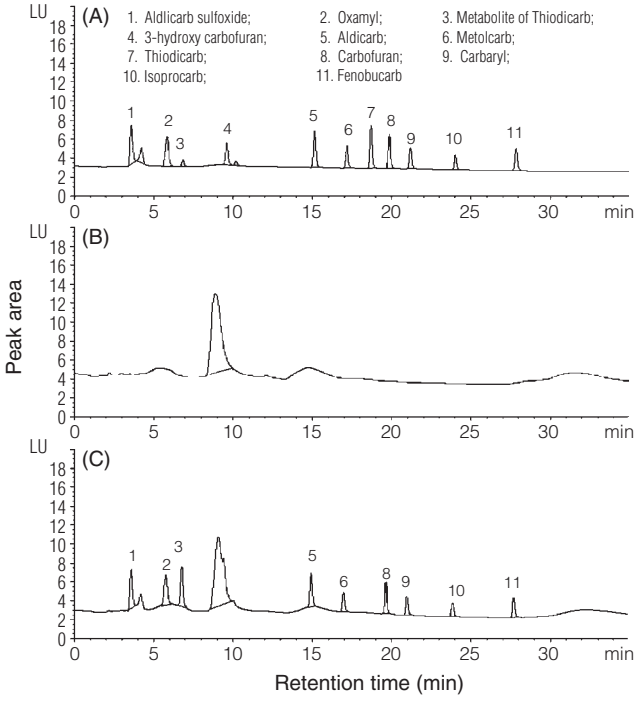

Figure 2. (A) Chromatograms of the grouped pesticide standards, (B) blank control of shrimp, and (C) shrimp sample spiked with grouped pesticides. Analysis condition are as follows — column: Pickering C18 (4.6 × 250 mm, 5 μm); column temperature: 40°C; injection volume: 50 μL; mobile phases: water (a) and acetonitrile (b); gradient of mobile phase: a/b (80:20) to a/b (30:70) in 35 min then 3 min hold at a/b (30:70); flow rate of mobile phase: 1 mL/min; post column derivitization: 1000°C; OPA-reagent flow rate, 0.3 mL/min; detector: fluorescence, Ex. 330 nm, Em. 465 nm.

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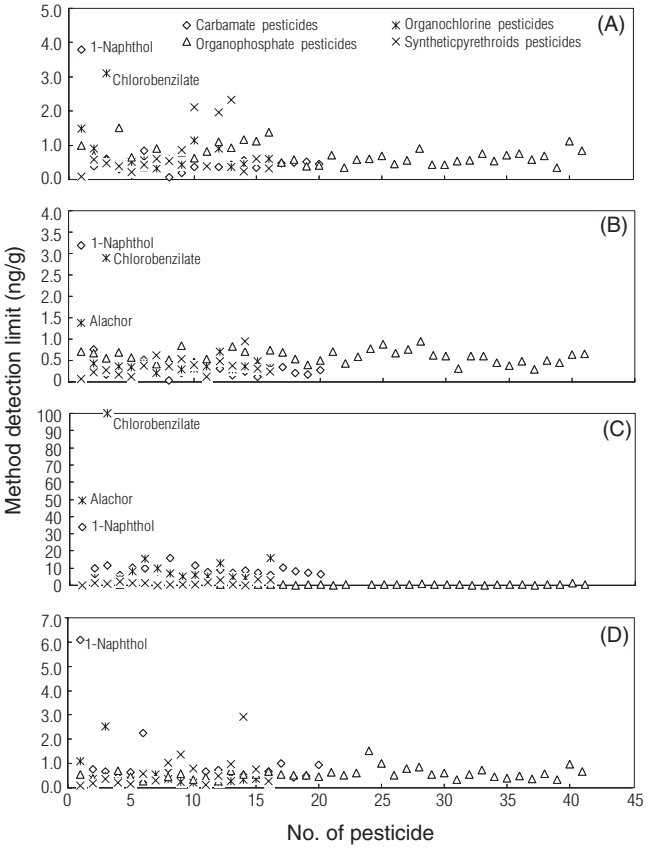

Figure 3. Method detection limit of each kinds of pesticide of various fishery products following the analytical procedures developed in this study. A: bivalve (clam); B: crustacean (crab); C: fish (carp); D: cephalopod. Number of pesticides are as listed in Table 3.

For analysis of OP pesticides residues in aquatic organisms (i.e. phytoplankton, crustaceans and fish), Hernandez *et al*. have developed an automated procedures based on normal phase LC[33], but the use of LC column for two months does affect the retention of pesticides and lipids. There is no detailed research in cephalopod.

Residue extraction techniques described in this report allow rapid extraction of multi-residues with high recovery. The proposed procedure provides a simple way of residue analysis for both polar and non-polar pesticides in fishery products differing in lipid, water or salt contents, thereby simplify the survey task of pesticide residues in aquatic organisms or marketable fishery products.

REFERENCES

1. Norse, E. A. 1993. Global Marine Biological Diversity: A Strategy for Building Conservation into Decision Making. 32 ed. p. 383. Island Press. Covelo, CA.
2. Kaphalia, B. S., Takroo, R., Mehrotra, S., Nigam, U. and Seth, T. D. 1990. Organochlorine pesticide residues in different Indian cereals, pulses, spices, vegetables, fruits, milk, butter, deshi ghee, and edible oils. J. AOAC Int. 73: 509-512.
3. EHC. 1979. DDT and its derivatives. In "Environmental Health Criteria 9". World Health Organization. Geneva.
4. EHC. 1989. DDT and its derivatives - environmental aspects. In "Environmental health criteria 83". World Health Organization. Geneva.
5. Antunes, P. and Gil, O. 2004. PCB and DDT contamination in cultivated and wild sea bass from Ria de Aveiro, Portugal. Chemosphere 54: 1503-1507.
6. Chan, H. M., Chan, K. M. and Dickman, M. 1999. Organochlorines in Hong Kong Fish. Marine Poll. Bull. 39: 346-351.
7. Berg, V., Polder, A. and Skaare, J. U. 1999. Organochlorines in deep-sea fish from the Nordfjord. Chemosphere 38: 275-282.
8. Sapozhnikova, Y., Bawardi, O. and Schlenk, D. 2004. Pesticides and PCBs in sediments and fish from the Salton Sea, California, USA. Chemosphere 55: 797-809.
9. Maiti, P. K. and Kar, A. 1997. Dimethoate inhibits extrathyroidal 5'-monodeiodination of thyroxine to 3,3',5-triiodothyronine in mice: the possible involvement of the lipid peroxidative process. Toxicol. Letters 91: 1-6.
10. Johnson, D. E., Seidler, F. J. and Slotkin, T. A. 1998. Early biochemical detection of delayed neurotoxicity resulting from developmental exposure to chlorpyrifos. Brain Res. Bull. 45: 143-147.
11. Roy, T. S., Andrews, J. E., Seidler, F. J. and Slotkin, T. A. 1998. Chlorpyrifos elicits mitotic abnormalities and apoptosis in neuroepithelium of cultured rat embryos. Teratology 58: 62-68.
12. El-Gohary, M., Awara, W. M., Nassar, S. and Hawas, S. 1999. Deltamethrin-induced testicular apoptosis in rats: the protective effect of nitric oxide synthase inhibitor. Toxicology 132: 1-8.
13. Silvestroni, L. and Palleschi, S. 1999. Effects of organochlorine xenobiotics on human spermatozoa. Chemosphere 39: 1249-1252.
14. Chatterjee, S., Dasmahapatra, A. K. and Ghosh, R. 2001. *Heteropneustes fossilis* (Bloch). Comp. Biochem. Physiol. C 129: 265-273.
15. Grünfeld, H. T. and Bonefeld-Jorgensen, E. C. 2004. Effect of *in vitro* estrogenic pesticides on human oestrogen receptor α and β mRNA levels. Toxicol. Letters 151: 467-480.
16. Vinggaard, A. M., Hnida, C., Breinholt, V. and Larsen, J. C. 2000. Screening of selected pesticides for inhibition of CYP19 aromatase activity *in vitro*. Toxicol. *in Vitro*. 14.
17. FDA. 1999. FDA 2905a (6/92). Multiresidue method. In "Pesticides Analytical Manual". Vol. 1. U. S. A.
18. Mendez, E. and Gonzalez, R. M. 1997. *Merluccius hubbsi*. Food Chem. 59: 213-217.
19. Conte, E., Milani, R., Morali, G. and Abballe, F. 1997. Comparison between accelerated solvent extraction and

traditional extraction methods for the analysis of the herbicide diflufenican in soil. J. Chromatogr. A 765: 121-125.

20. Camel, V. 2000. Microwave-assisted solvent extraction of environmental samples. Trends Anal. Chem. 19: 229-248.
21. Dean, J. R. and Xiong, G. 2000. Extraction of organic pollutants from environmental matrices: selection of extraction technique. Trends Anal. Chem. 19: 553-564.
22. Suchan, P., Pulkrabová, J., Hajšlová, J. and Kocourek, V. 2004. Pressurized liquid extraction in determination of polychlorinated biphenyls and organochlorine pesticides in fish samples. Analytica Chimica Acta. 520: 193-200.
23. Kohler, P. W. and Su, S. Y. 1986. Analysis of some organochlorine pesticides in seafood samples by solid-phase extraction - gas chromatography. Chromatographia 21: 531-537.
24. Molto, J. C., Albelda, C., Font, G. and Manes, J. 1990. Solid-phase extraction of organochlorine pesticides from water samples. Int. J. Environ. Anal. Chem. 41: 21-26.
25. Walker, C. C., Lott, H. M. and Barker, S. A. 1993. Matrix solid-phase dispersion extraction and the analysis of drugs and environmental pollutants in aquatic species. J. Chromatogr. 642: 225-242.
26. Sun, F., Lin, F. Y., Wong, S. S. and Li, G. C. 2000. Determination of organochlorine and nitrogen-containing pesticide residues in fish with different fat content. J. Food Drug Anal. 8: 103-111.
27. Sun, F., Lin, F. Y., Wong, S. S. and Li, G. C. 2003. The screen of organophosphorus, organochlorine and synthetic pyrethroid pesticide residues in beef fat by tandem solid-phase extraction technique. J. Food Drug Anal. 11: 258-265.
28. Schenck, F. J., Callery, P. S., Gannett, P. and Daft, J. R. 2000. How dry is dry? Isolation of pesticide residues from foods by salting-out. In "The 114th AOAC International Meeting and Exposition. Conference". Philadelphia, PA. (Poster Presentation)
29. Hernandez, F., Serrano, R., Beltran, J. and Lopez, F. J. 1996. Comparison of cleanup techniques for simple method for analysis of selected organophosphorus pesticide residues in molluscs. J. AOAC Int. 79: 123-131.
30. Marquis, P. J., Hanson, R. L., Larsen, M. L. and Devita, W. M. 1994. Analytical methods for a national study of chemical residues in fish. II. Pesticides and polychorinated biphenyls. Chemosphere 29: 509-521.
31. Schenck, F. J. 1996. Screening of nonfatty fish for organochlorine pesticide residues by solid-phase extraction cleanup: interlaboratory study. J. AOAC Int. 79: 1215-1219.
32. Schenck, F. J., Calderon, L. and Podhorniak, L. V. 1996. Determination of organochlorine pesticide and polychlorinated biphenyl residues in fatty fish by tandem solid-phase extraction cleanup. J. AOAC Int. 79: 1209-1214.
33. Hernandez, F., Serrano, R., Pitarch, E. and Lopez, F. J. 1998. Automated sample clean-up procedure for organophosphorus pesticides n several aquatic organisms using normal phase liquid chromatography. Analytica Chimica Acta 374: 215-229.
34. Serrano, R., Lopez, F. J., Noig-Navarro, A., Hernandez, F., Serrano, R., Lopez, F. J., Roig-Navarro, A. and Hernandez, F. 1997. Automated sample clean-up and fractionation of chlorpyrifos, chlorpyrifos-methyl and metabolites in mussels using normal-phase liquid chromatography. J. Chromatogr. 778: 151-160.
35. AOAC. 1995. Official Methods of Analysis. 16th ed. 970.52. AOAC International. Arlington, VA, U. S. A.
36. Gillespie, A. M. 1995. Multicolumn solid-phase extraction cleanup of organophosphorus and organochlorine pesticide residues in vegetable oils and butterfat. J. AOAC Int. 78: 431-437.