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Rapid determination of chlorinated pesticides in fish by freezing-lipid filtration, solid-phase extraction and gas chromatography–mass spectrometry

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Abstract

An analytical method has been developed for measuring 24 chlorinated pesticides in fish tissue samples. Extraction of chlorinated pesticides was carried out by ultrasonication using an acetone–*n*-hexane (5:2, v/v) mixture. Most of the lipids in the extract were eliminated by freezing-lipid filtration, prior to solid-phase extraction (SPE) cleanup. During freezing-lipid filtration, about 90% of the lipids extracted from the fish samples were easily removed without any significant losses of chlorinated pesticides. For purification, SPE using Florisil was shown to be more effective than silica. Quantification was performed using gas chromatography–mass spectrometry in the selected ion monitoring mode. Spiking experiments were carried out to determine the recovery, precision, and limits of detection (LODs) of the method. The overall recovery was above 80% in the spiked fish tissue sample at 100 ng/g level. The detection limits for chlorinated pesticides were ranged from 0.5 to 5 ppb, except for endosulfan I and II which was 20 ppb. The newly developed method is demonstrated to give efficient recoveries and LODs for detecting chlorinated pesticides spiked into fish tissue with high lipid content.

Keywords: Fish; Food analysis; Freezing-lipid filtration; Sample preparation; Pesticides; Organochlorine compounds

1. Introduction

Measuring chlorinated pesticides in biological samples is important, because some of these compounds are known as environmentally persistent pollutants that tend to accumulate in wild life due to their lipophilicity [1,2]. Moreover, these compounds can generate certain harmful effects on human as well as on aquatic life [3–5]. Continued development of analytical methodologies is needed for accurate determination of chlorinated pesticides in the environment and in biological tissues.

Many analytical methods have been reported for the measurement of pesticides to determine not only the presence of pesticides but also their concentrations in samples with precision and accuracy [6–10]. Usually, conventional approach to analyze chlorinated pesticides in biological sample involves extraction followed by a multistep purification using various adsorbents. As extraction methods, Soxhlet extraction [11], pressurized liquid extraction (PLE) [12–14], microwave-assisted extraction (MAE) [15,16], supercritical fluid extraction (SFE) [17,18] and sonication extraction (SE) [19,20] have been widely used. Recently, Soxhlet extraction that requires overnight extraction and large amount of organic solvents is being replaced by rapid extraction methods such as PLE, MAE, SFE and SE. During the extraction of chlorinated compounds in biological tissue, large quantities of lipids may get inevitably co-extracted with targets due to their high solubility in organic solvents. During instrumental analysis, lipid components tend to adsorb in GC system such as injection port and column, resulting in poor chromatographic performance. Several approaches have been developed to eliminate co-extracted lipid interferences, including liquid–liquid partitioning [21], gel permeation chromatography [22], column chromatography [22–26] and multiple cleanup methods [27,28]. However, most of these methods are time consuming and use large quantities of organic solvents to remove the fatty materials.

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Recently, solid-phase microextraction methods have been developed for the rapid determination of chlorinated pesticides in environmental samples and in biological samples [29]. In these studies, chlorinated pesticides can be effectively extracted without solvent, and cleanup procedure can be eliminated for the removal of interferences. Selective extraction of pesticides from lipid-containing sample is made possible by SFE using binary gas mixture [18]. This method provides for the minimization of extraction of lipids and leads to direct GC analysis, thereby eliminating the need to cleanup the extract.

Generally, lipid content of fish tissue in net mass is about 15%, mainly being composed of phospholipids, triacylglycerolipids, cholesterol and sterol esters [30,31]. Among these, phospholipids and triglycerolipids cannot be easily separated from non-polar analytes in organic extracts by conventional cleanup method. Basic [32] or acidic [33] treatment like saponification cannot be applied to multi-residue analysis for degrading lipids into small fatty acids, since some chlorinated pesticides are prone to degrade under extremely acidic or basic conditions [34].

There is a significant difference of melting points between lipids (below about 40 °C) and chlorinated pesticides (above about 260 °C), so that lipid components can be easily separated from chlorinated compounds. After extraction, lipids in organic extracts are precipitated as frozen form at −24 °C in the freezer, while chlorinated compounds are still dissolved in cold organic solvents. Thus, frozen-lipids can be removed just by filtering extracts. After freezing-lipid filtration, most of the remaining interference can be removed by a convenient solid-phase extraction (SPE) cartridge. To the best of our knowledge no publication has documented the use of a freezing-lipid filtration for effective removal of lipids extracted from biological matrix.

The purpose of the current study is to develop a new elimination method of lipids with simple SPE cleanup to quantify non-polar chlorinated compounds in high lipid containing samples by GC–MS. This developed method can be applied for the monitoring of pesticides regulated on fish in South Korea.

2. Experimental

2.1. Chemicals

Authentic chlorinated pesticides were obtained from several companies: α-, β-, γ- and δ-hexachlorocyclohexane (HCH) isomers, hexachlorobenzene (HCB), heptachlor, aldrin, dicofol, heptachlor epoxide B, *cis*- and *trans*-chlordane isomers, *trans*-chlordane, *trans*-nonachlor, endosulfan I, endosulfan II and [$^{2}H_{10}$] phenanthrene (phenanthrene-d_{10}) from Supelco (Bellefonte, PA, USA). DDE, dieldrin, endrin, DDD, DDT methoxychlor from Aldrich (Milwaukee, WI, USA) and octachlorostyrene and [$^{13}C_{6}$]-HCB from Dr. Ehrenstorfer GmbH (Augsburg, Germany). A stock standard mixture containing 24 pesticides was prepared in acetone at a concentration of 100 μg/ml, and stored at 4 °C. Working standard solution was prepared at the concentration of 0.1–5 μg/ml by volume, dilution with acetone, hexane, or methanol.

Organic solvents (hexane, acetone, methylene chloride, acetonitrile, and toluene) were of pesticide residue analysis grade (J.T. Baker, Phillipsburg, NJ, USA). Analytical grade sodium chloride was obtained from Sigma (St. Louis, MO, USA) and purified by overnight heating at 300 °C. Anhydrous sodium sulfate from E. Merck (Darmstadt, Germany) was used as the drying reagent. Before use, sodium sulfate was heated in a muffle furnace at 550 °C for 12 h. All glassware was cleaned with laboratory detergent, sequentially rinsed with distilled water, acetone, and methanol, and finally baked in an oven at 300 °C. Distilled water was obtained with a Milli-Q system (Millipore, Bedford, MA, USA). For SPE, Florisil (2 g) and silica (2 g) cartridges were purchased from Supelco. The chemical structures of the pesticides in this study were shown in Fig. 1.

For the precise analysis of chlorinated pesticides, the stock solution should be freshly prepared every month, because some of the pesticides get degraded even when stored at −4 °C in a refrigerator. Typically, about 10% of HCH isomers and methoxychlor dissolved in methanol yielded pentachlorocyclohexene isomers and bis(4,4′-dimethoxyphenyl)1,1-dichloroethylene, respectively, by the elimination of HCl molecule through reductive dechlorination. Moreover, the authentic standards should be stored in amber type bottles to protect photolytical degradation.

2.2. Equipment

Ultrasonic bath (Ultrasons Selecta) was used for the extraction of chlorinated pesticides from biota samples. The generator of ultrasonic bath has an output of 150 W and a frequency of 35 kHz. Rotary evaporator (Büchi, Swiss) was used for the concentration of organic solvents.

2.3. Sample extraction and delipidation

Samples of 10 g together with 100 ng of [$^{13}C_{6}$]-HCB were ground in a blender jar for 10 min at high speed. Extraction of chlorinated pesticides from 10 g of biota samples was carried out by ultrasonic agitation with a mixed solvent of 70 ml of acetone–*n*-hexane (5:2, v/v) for 20 min. Extract was filtered to remove traces of water with filter paper containing 5 g of sodium sulfate, and then transferred into a 250 ml round flask. The extraction was repeated one more time. Extracted solvent was dried and redissolved in 50 ml of acetonitrile that has low solubility for lipids. Acetonitrile extract was stored in the freezer at −24 °C for 30 min to freeze lipids. Most of the lipids were precipitated as pale yellow, condensed lump on glassware surface. Cold extract at −24 °C was immediately filtered with filter paper to remove frozen lipids. The precipitated lipid on glassware sur-

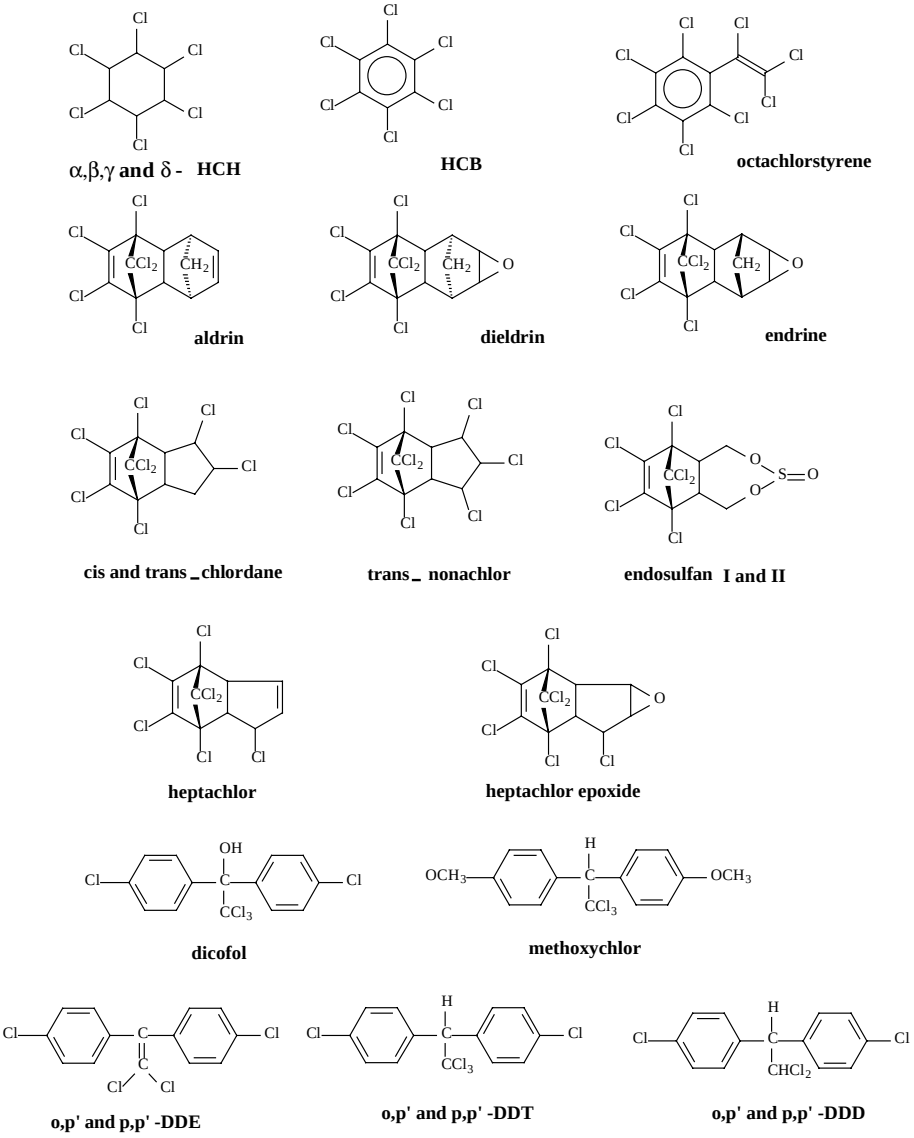

Fig. 1. Chemical structures of chlorinated pesticides investigated in this study.

face was redissolved in 50 ml of acetonitrile to perform filtration again by same procedure. The filtered extract was concentrated to 1 ml by a rotary evaporator under nitrogen atmosphere to follow Florisil-SPE procedure.

2.4. Sample clean-up

Before sample application, the cartridge was cleaned with 12 ml of *n*-hexane and air dried by positive pressure for 1 min. Another 5 ml of *n*-hexane was used to condition the cartridge. After sample application, the Florisil-SPE cartridge was air-dried for 10 min. Desorption of the pesticides, which had been preconcentrated on the Florisil sorbent, was carried out using 13 ml of acetone–*n*-hexane (1:9, v/v) mixture at a flow of 1 ml/min and collected in a 50 ml round flask. The eluate was then concentrated at 45 °C with a nitrogen stream until just the disappearance of the last drop of solution. Finally, 100 ng of phenantherene-d_{10} used as syringe internal standard was added to dried residue. The sample preparation scheme is outlined in Fig. 2.

2.5. GC–MS and fast atom bombardment (FAB) MS analysis

The sample analysis was carried out with an Agilent GC/MS (Palo Alto, CA, USA) equipped with DB-5MS fused-silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm, J&W Scientific, Folsom, CA, USA). Helium as carrier gas was used at a flow rate of 1.0 ml/min. A 1 μl sample was injected under split mode (split ratio 10:1). The temperatures of the GC injection port and the MS interface were set at 270 and 300 °C, respectively. The GC column temperature program was as follows: an

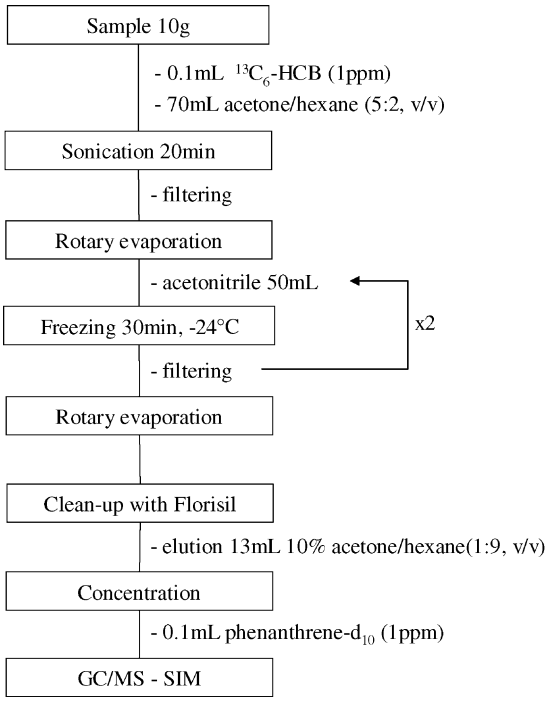

Fig. 2. Analytical procedure of chlorinated pesticides for fish tissue sample.

initial temperature 100 °C, held for 3 min, and then ramp 10 °C/min to 280 °C held for 5 min.

The mass spectrometer was run in the electron impact (EI) mode with electron energy at 70 eV. The instrumental parameters were set at the following values: 300 μA filament emission current and multiplier voltage of 2000 V. The manifold temperature was maintained at 200 °C. The mass spectrometer was operated in full scan mode between 50 and 480 amu. For the monitoring and confirmation analysis, the selected ion monitoring (SIM) mode was used and the dwell time of each ion was set at 50 ms. To improve sensitivity, selected ions used in the SIM mode are divided into seven groups, guiding by the individual pesticide retention times. The selected ion groups in SIM mode are listed in Table 1. All insecticides were identified by retention time and specific ions, and quantified by the internal standard method.

To determine the kinds of lipids extracted from fish tissue and to evaluate SPE cleanup efficiency, FAB-MS was used. FAB mass spectra were obtained with a JEOL JMS-SX102A (Akishma, Tokyo, Japan) high-resolution mass spectrometer (B/E configuration) operating at an acceleration voltage 10 kV with a mass range of 50–1500 u. Xenon and 3-nitrobenzylalcohol were used as the atom beam and matrix, respectively.

2.6. Fortified recovery studies

Either 0.1 ml of 10 μg/ml spiking standard solution or 0.5 ml of a 1 μg/ml spiking standard solution was added to 10 g fish tissue samples, which are equivalent to levels of 0.1, and 0.05 ppm in the tissue samples, respectively. Three replicates each at 0.1 ppm level, and five replicates at 0.05 ppm level were prepared. All samples were treated and analyzed using GC–MS-SIM mode described above.

Table 1
Selected ion groups of chlorinated pesticides in GC–MS-SIM analysis

Ion groups	Compounds	Time (min)	Quant. ion (*m*/*z*)	Confirm ion (*m*/*z*)
Group I	α-, β-, γ-, δ-HCH	12.0	181	183
	HCB		284	286
	$^{13}C_6$-HCB		290	292
	Phenantherene-d_{10}		188	–
Group II	Heptachlor	15.0	272	274
	Aldrin		263	265
	Dicofol		139	250
Group III	Octachlorstyrene	16.6	308	310
	Heptachlor epoxide B		353	355
Group IV	*cis*, *trans*-Chlordane	17.2	373	375
	o,*p*′-DDE		246	318
	Endosulfan I		241	237
	trans-Nonachlor		409	407
Group V	*p*,*p*′-DDE	17.85	246	318
	Dieldrin		263	265
	o,*p*′-DDD		235	237
Group VI	Endrin	18.3	263	265
	Endosulfan II		241	237
	p,*p*′-DDD		235	237
	o,*p*′-DDT		235	237
Group VII	*p*,*p*′-DDT	19.1	235	237
	Methoxychlor		227	228

3. Results and discussion

3.1. Extraction and clean-up

The sample is homogenized and extracted with a suitable solvent or a solvent mixture to remove the bulk of sample matrix and to extract chlorinated pesticides residue into the solvent. Both the selection of solvent and extraction method can be critical in obtaining a satisfactory recovery of chlorinated pesticides from the sample matrix. In this study, methylene chloride, *n*-hexane, toluene and acetone were used as ultrasonic extraction solvents to extract chlorinated pesticides from spiked fish tissue. No significant difference was found among solvents used in extraction, probably due to their good dissolving capability for chlorinated pesticides. However, large amounts of lipids were extracted when methylene chloride or *n*-hexane were used as extraction solvents. Toluene was not suitable for evaporation due to its high boiling point. When acetone or acetonitrile was used, fish tissue was aggregated during ultrasonic agitation, not enable to penetrate fish tissue. Thus, the mixed solvent of acetone–*n*-hexane (5:2, v/v) was suitable as extraction solvent.

In general, complex mixtures of several types of lipids were co-extracted during the extraction of chlorinated pes-

ticides from biological sample. Triglycerides with unsaturated fatty acids and sterol esters are the major components in fish fats [30,31]. In this study, extracted fatty materials were analyzed by FAB-MS. From FAB-MS, several triglyceride lipids with molecular weights ranging 500–800 amu were observed as major components. These compounds are less soluble in water but readily soluble in nonpolar solvents such as chloroform, methylene chloride and hexane. Thus, it is difficult to pretreat the sample extracts in order to selectively extract the interesting pesticide as well as to remove lipid-interferences from the extracts. Normally, several steps of cleanup including solvent partitioning and column cleanup were required to remove the lipid materials. In some cases, destruction of the lipid matrix by saponification method was involved to effectively eliminate the fatty matrix [33]. However, some of the chlorinated pesticides were degraded during acid or base hydrolysis step [34]. Typically, endosulfan I and endosulfan II underwent decomposition in this study and lost their sulfate group under the acidic conditions. As a consequence, about 40% of endosulfan I and II was converted into endosulfandiols and desulfated forms. Dieldrin also significantly degraded into its dechlorinated products and diol-derivative under the acidic conditions. Besides these compounds, some chlorinated pesticides were slowly hydrolyzed in aqueous basic conditions. So hydrolysis step involving extremely acidic or basic treatment should be avoided for the multi-pesticides residue analysis.

Consideration of the low melting point of triglycerides even at room temperature led to develop freezing-lipid filtration method to eliminate a large amount of lipid matrix. In this study, when acetonitrile extract by sonication method was stored in the freezer at −24 °C for 30 min, most lipid components in extract solution were precipitated as pale yellow lump, owing to their low solubility for acetonitrile. On the other hand, chlorinated pesticides with higher melting points were soluble even in cold acetonitrile solvent. The cold extract containing precipitated lipids was promptly filtered with filter paper to prevent melting lipids. To improve the extraction yield of chlorinated pesticides, freezing-lipid filtration was repeated once more. The overall extraction yield of chlorinated pesticides increased about 30% by second freezing-lipid filtration. During these processes, approximately 91.6% of lipids in extraction solution was eliminated without any significant loss of pesticides, as shown in Table 2.

After freezing-lipid filtration, the extract has still presented about 8.4% of lipids, equivalent to about 88.5 mg in 10 g of fish tissue sample. Although a significant amount of lipids was eliminated by freezing-lipid filtration method, the amount of residual lipids was three-order higher than that of the target analyte at sub-microgram level of the extract. Thus, the relatively high amounts of remaining interferences such as lipids, fatty acids, and cholesterol should be eliminated by column chromatography. In this study, Florisil and silica-SPE cartridges were tested and compared to eliminate the remaining interferences in extract. The elution patterns of chlorinated pesticides were studied on both SPE cartridges with hexane solvent. For both SPE cartridges, some pesticides such as β-, γ- and δ-HCH isomers, dicofol, heptachlor epoxide, methoxychlor, dieldrin, endrin, and endosulfan I and II isomers have shown poor elution efficiency with hexane solvent. It could be explained by the fact that these compounds are strongly retained on silica and florisil adsorbent surface. Therefore, to overcome the poor elution of these compounds, the polarity of elution solvent was increased by the addition of acetone. Most of the chlorinated pesticides were eluted within 13 ml acetone–*n*-hexane (1:9, v/v) mixture on both SPE cartridges, as shown in Fig. 3. For all chlorinated pesticides, elution recoveries on silica and Florisil-SPE were found to be more than 95%.

Table 2
Removal efficiency of lipids extracted from fish tissue sample by a freezing-lipid filtration method

	Mass (g)				R.S.D. (%)
	1	2	3	Average	
Fish tissue	10.3	10.5	10.7	10.5	1.9
Extraction lipid	0.70	1.50	1.20	1.13	30.3
Lipid after freeze and filtration	0.080	0.094	0.092	0.089	9.1
Lipid reduction ratio (%)	88.7	93.7	92.3	91.6	2.8

Based on the elution patterns of chlorinated pesticides for both SPE cartridges, the removal efficiency of interferences after freezing-lipid filtration was compared. Total ion chromatograms of blank fish tissue samples purified by silica- and Florisil-SPE cartridges are shown in Fig. 4. When used silica SPE, several interferences were detected in total ion chromatogram (upper trace in Fig. 4). The main interferences were hexadecanoic acid (retention time at 16.00 min), octadecanoic acid (retention time at 17.70 min), and cholesterol (retention time at 20.12 min). Moreover, the baseline had significantly high retention time of 16 to 22 min, not enabling to obtain sufficient sensitivity for pesticides in this range of retention time. On the other hand, when extract was purified by Florisil-SPE, fatty acids were effectively eliminated, as shown with lower trace of Fig. 4. However, cholesterol was not sufficiently removed by silica- or Florisil-SPE purifications. Although significant amount of cholesterol was still observed in total ion chromatogram, this compound did not interfere with other pesticides as it was eluted late in the chromatogram. Moreover, the appearance of several interferences detected in GC–MS-scan mode could be greatly reduced in GC–MS-SIM mode by the selection of specific ions, as shown in Fig. 5. Compared with total ion chromatograms (Fig. 4) obtained by GC–MS-scan mode, there were no significant interference peaks in the SIM chromatogram (Fig. 5B). However, the baseline between retention times of 16 and 20 min in the chromatogram (Fig. 5A) is still high due to interferences. Therefore, the purification method of extract was selected to be Florisil-SPE with elution of acetone–*n*-hexane (1:9, v/v) mixture.

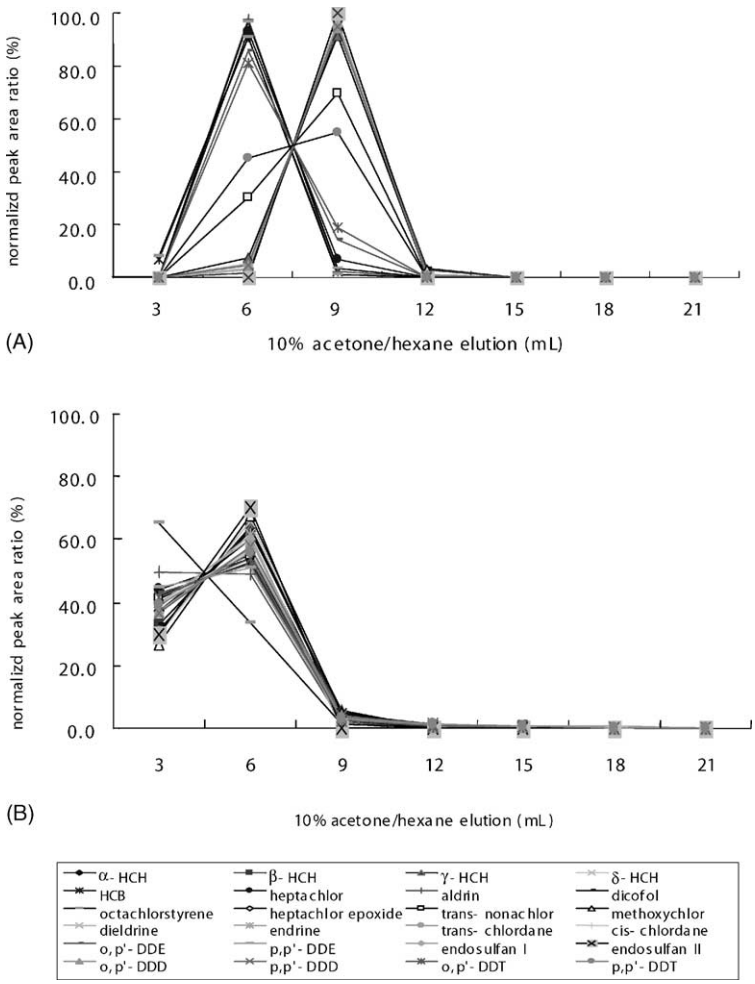

Fig. 3. Elution patterns of chlorinated pesticides on (A) silica and (B) Florisil-SPE cartridge with acetone–*n*-hexane (1:9, v/v).

3.2. Method application

As in the method developed and described above, 100 ng of internal standard and 1000 ng of chlorinated pesticides standards were spiked into a 10 g of blank fish tissue sample. The mixed sample was extracted, purified, and analyzed by the methods employed. Typical SIM chromatogram obtained from control fish tissue extract using freezing-lipid filtration

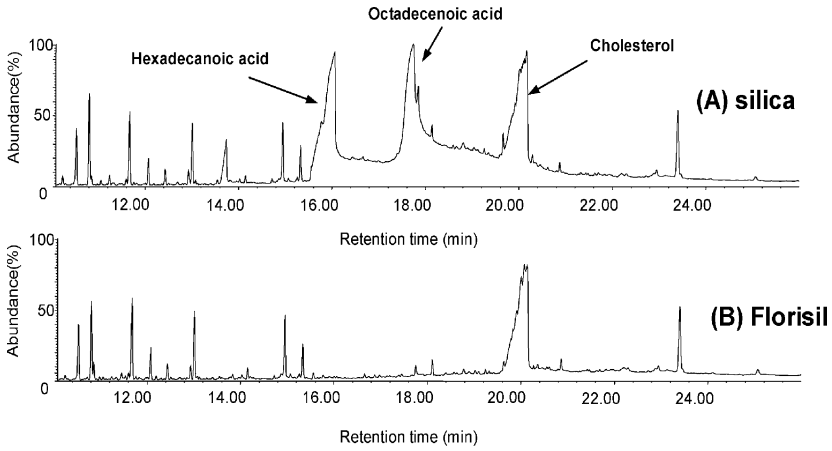

Fig. 4. Total ion chromatograms of blank fish extracts purified by silica-SPE (A) and Florisil-SPE (B) cartridges after freezing-lipid filtration, and analyzed by GC–MS-scan mode.

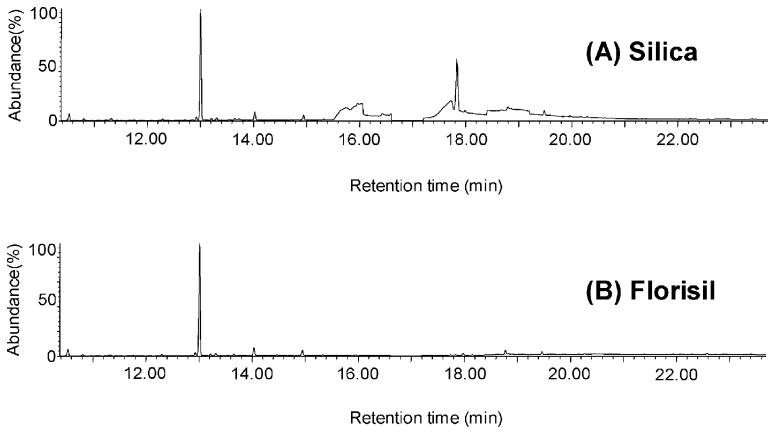

Fig. 5. SIM chromatograms of blank fish extracts purified by silica-SPE (A) and Florisil-SPE (B) cartridges after freezing-lipid filtration, and analyzed by GC–MS-SIM mode.

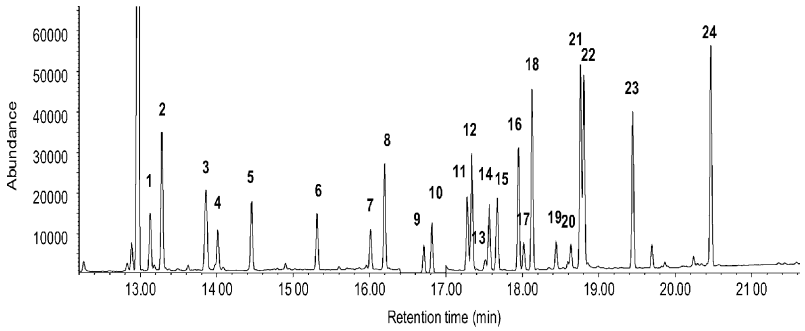

Fig. 6. SIM chromatogram of chlorinated pesticides in spiked fish sample after freezing-lipid filtration, Florisil-SPE, and analyzed by GC–MS-SIM mode. Peak identities: 1, α-HCH; 2, HCB; 3, β-HCH; 4, γ-HCH; 5, δ-HCH; 6, heptachlor; 7, aldrin; 8, dicofol; 9, octachlorstyrene; 10, heptachlor epoxide; 11, *trans*-chlordane; 12, *o,p′*-DDE; 13, endosulfan I; 14, *cis*-chlordane; 15, *trans*-nonachlor; 16, dieldrin, 17, *p,p′*-DDE; 18, *o,p′*-DDD; 19, endrin; 20, endosulfan II; 21, *p,p′*-DDD; 22, *o,p′*-DDT; 23, *p,p′*-DDT; 24, methoxychlor.

and Florisil-SPE cartridge is shown in Fig. 6. No significant interferences were observed in SIM chromatogram. Twenty-four chlorinated pesticides at 100 ppb level were successfully detected with excellent sensitivity.

To plot calibration curves, 0.1 μg of [$^{13}C_6$]-HCB and chlorinated pesticide standards in amount of 0.05, 0.1, 0.2, 1.0, and 2.0 μg were spiked into a 10 g control fish tissue, and the relative ratios of peak areas of chlorinated pesticides versus [$^{13}C_6$]-HCB were determined. The calibration curve was generated by a least-squares linear regression analysis of the pesticides/[$^{13}C_6$]-HCB response ratio versus the amount of spiked chlorinated pesticides. The correlation coefficient for each chlorinated pesticide was higher than 0.992.

Chlorinated pesticides-spiked fish tissue sample was repeatedly analyzed to determine the recovery, reproducibility and detection limits of the method. The mean and the relative standard deviations (R.S.D.) of the recovery, calculated as the measured amount divided by the spiked amount are listed in Table 3. As indicated in Table 3, recoveries of pesticides are between 78.25 and 115.24%, and the relative standard deviations are 2.99–14.44%, with an average of 88.1%. In view of their recoveries and removal of interference, freezing-lipid filtration and Florisil-SPE cleanup is effective for the reliable confirmation and quantitation analysis of chlorinated pesticides.

The detection limits of some pesticides were around 0.5–2.5 ppb using two abundant ions in SIM mode, except for endosulfan I and II, aldrin, and endrin, whose detection limits were 20 and 5 ppb, respectively, at signal-to-noise ratio of 5. Especially, the base peak *m/z* 66 of aldrin, *m/z* 100 of heptachlor, *m/z* 81 of heptachlor epoxide B, *m/z* 79 of dieldrin, and *m/z* 81 of endrin could not be distinguished from interferences. These lower mass ions can be easily produced from fatty species, possibly present in the sample matrix. Thus, the higher mass ions with second abundance instead of lower mass ion could be monitored to improve specificity for these pesticides, but with slightly reduced sensitivity. The instrumental detection limits of these compounds increased by about two times, using higher mass ions instead of lower mass ions in SIM mode. In many cases of GC–MS analysis, the detection limits could decrease by adjusting the sample volume, cleanup efficiency and injection mode.

Table 3
Recoveries and detection limits of chlorinated pesticides in fish tissue sample

No.	Compounds	Recovery ± R.S.D. (%)	MDL (ng/g)	IDL (ng/g)
1	α-HCH	94.73 ± 1.16	2.0	0.2
2	HCB	91.21 ± 3.18	1.0	0.05
3	β-HCH	95.88 ± 5.56	2.0	0.1
4	γ-HCH	87.79 ± 2.99	5.0	0.2
5	δ-HCH	90.51 ± 4.79	2.0	0.1
6	Heptachlor	91.83 ± 6.45	2.5	0.2
7	Aldrin	90.15 ± 9.33	5.0	0.1
8	Dicofol	90.93 ± 8.55	1.0	0.05
9	Octachlorstyrene	80.08 ± 10.35	5.0	0.1
10	Heptachlor epoxide B	84.71 ± 8.37	2.0	0.1
11	*trans*-Chlordane	84.31 ± 10.81	2.0	0.1
12	*o*,*p*′-DDE	82.59 ± 8.78	1.0	0.05
13	Endosulfan I	97.23 ± 14.44	20.0	1.0
14	*cis*-Chlordane	82.81 ± 7.90	2.0	0.1
15	*trans*-Nonachlor	82.41 ± 8.88	2.0	0.1
16	Dieldrine	86.61 ± 7.62	1.0	0.2
17	*p*,*p*′-DDE	86.89 ± 7.37	5.0	0.05
18	*o*,*p*′-DDD	85.75 ± 10.25	0.5	0.05
19	Endrine	115.24 ± 3.85	5.0	0.5
20	Endosulfan II	82.90 ± 7.76	20.0	1.0
21	*p*,*p*′-DDD	90.04 ± 10.17	0.5	0.05
22	*o*,*p*′-DDT	78.65 ± 4.48	0.5	0.1
23	*p*,*p*′-DDT	81.87 ± 3.02	0.5	0.2
24	Methoxychlor	78.25 ± 8.25	0.5	0.1
Internal standard	[$^{13}C_6$]-HCB	82.57 ± 4.39		

4. Conclusion

A rapid extraction, freezing-lipid filtration and GC–MS measurement method was developed and used to measure chlorinated pesticide levels in a biota sample. The freezing-lipid filtration combined with Florisil-SPE cartridge enabled efficient removal of lipids extracted from fish sample without any significant loss of chlorinated pesticides. Compared with the other column clean-up methods for removing lipids extracted from biota sample, the freezing-lipid filtration showed higher throughput and was easy to handle, while generating equivalent analytical results. Hence, the method offered a rapid screening tool with high sensitivity and accuracy for the determination of chlorinated pesticides in fish tissue, based on GC–MS-SIM and two internal standards. Spike and recovery studies in fish tissue validated the analysis of chlorinated pesticides, particularly at fortifications most applicable to the fish tissues that were analyzed. Future work will examine further applications of the method to analyze other environmental pollutants such as polychlorinated biphenyls and dioxins, and in other type of biological samples in the presence of high level of lipids.

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